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# Research to Guide the Use of Lampricides for Controlling Sea Lamprey

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***ABSTRACT.*** *This paper is one of a series supported by the Great Lakes Fishery Commission that describe research priorities related to each facet of the sea lamprey control program (assessment, pheromones, barriers and trapping, sterile males and lampricides). The specific focus of this paper is research needs related to the use of lampricides. To that end, we first provide a brief history of the lampricide control program and its processes and operations emphasizing the progress that has been made over the last 50 years. We then articulate research priorities for the continued improvement of lampricide use under four major categories; improving the effectiveness of lampricide treatments, improving the understanding of the effects of lampricides on non-target species, gaining a better understanding the mode(s) of toxic action of lampricides, and how they differ between lamprey and non-target species, and finally, initiatives designed to find new and more effective methods of applying existing lampricides and to develop new lampricides, based on new knowledge of chemical vulnerabilities unique to larval sea lamprey. Research priorities are summarized at the end of the paper and sources of additional information concerning lampricide research are provided.*

***INDEX WORDS:*** *Lampricides, sea lamprey control.*

## INTRODUCTION

The sea lamprey, *Petromyzon marinus*, is the major invasive species in the Great Lakes. Mitigating its negative effects on other fish has led to one of the largest control programs for a vertebrate pest. This paper examines the use of lampricides to kill sea lampreys at the larval stage. The purposes of this paper are 1) to describe the origins of the control program and its evolution, 2) to review current processes and procedures, and 3) to identify research needs to guide the use of lampricides. Meeting these research needs will improve lampricide treatment effectiveness, reduce the impacts of lampricides on non-target species, and improve understanding of the mechanisms of toxicity and selectivity of lampricides. The latter may lead to new, more effective, ways to apply existing lampricides or to the development of new lampricides.

## BACKGROUND

### History of the Lampricide Control Program

Although sea lampreys are thought to be indigenous to Lake Ontario (Waldman *et al.* 2004, Bryan *et al.* 2005), and were first officially observed spawning in a Lake Ontario tributary in 1835 (Lark 1973), the widening and deepening of the Welland Canal from 1913 onward led to their invasion of the remaining Great Lakes. Sea lampreys were found in Lake Erie by 1921, in Lakes Michigan and Huron by 1932, and in Lake Superior by 1946 (Holeck and Mills 2004). Sea lamprey abundance rose sharply soon after their arrival in each of the upper Great Lakes, followed by precipitous declines in host species, most notably lake trout (*Salvelinus namaycush;* Smith and Tibbles 1980, Eshenroder *et al.* 1992).

From its beginnings in the 1940s, sea lamprey control has evolved into a program of integrated

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pest management that includes application of the lampricides, TFM and Bayluscide®, to tributary streams (Brege *et al.* 2003), operation of low-head barrier dams (Lavis *et al.* 2003), trapping of adults (Mullett *et al.* 2003), and release of sterile male sea lampreys (Twohey *et al.* 2003).

The Great Lakes Fishery Commission (GLFC) was established in 1955 "to formulate and implement a comprehensive program for eradicating or minimizing the sea lamprey populations" and "to formulate a research program . . ." (GLFC 1955). Early control efforts (1940 to 1960) used mechanical and electrical barriers to block adult sea lampreys from upstream spawning areas. However, these barriers were abandoned because they did not reduce adult sea lampreys as expected and resulted in substantial mortality of non-target fishes (Smith and Tibbles 1980). By 1950 it was concluded that the most effective control would be to target stream-dwelling non-parasitic larvae (ammocoetes) as they are relatively sedentary and several generations are in the stream concurrently (Applegate 1950). Consequently, a search for a selective larval lampricide began in 1951. Over the next 7 years more than 6,000 chemicals were screened for selective toxicity and 3-trifluoromethyl-4-nitrophenol (TFM) was identified as the most effective (Applegate *et al.* 1961). A chemical control program was implemented in 1958 starting with Lake Superior tributaries (Applegate *et al.* 1961), extending to Lake Michigan and Lake Huron in the 1960s, Lake Ontario in 1972, and Lake Erie in 1986. The search for selective lampricides continued and in 1963 the molluscicide niclosamide (Bayluscide®) was found to be selectively toxic to ammocoetes, in mixtures with TFM as well as alone (Howell *et al.* 1964). Formulated for bottom release, niclosamide is used to treat slow moving waters where TFM is largely ineffective and as a survey tool to detect and collect ammocoetes in lentic areas. Because of its irritant properties, niclosamide stimulates lampreys to come out of their burrows. Chemical control was very successful and by 1978 had, for example, reduced the catches of spawning sea lampreys in Lake Superior by 92% (Christie and Goddard 2003). Chemical treatments eliminated the spawning runs in many smaller streams, but the larger tributaries require on-going treatment. Presently about 173 streams are treated at least once every 5 years (Brege *et al.* 2003).

## Recent Changes to the Program

Prior to 1980 the emphasis of the lampricide treatment program was eradication of sea lamprey using a treatment philosophy centered on achieving good double lethal concentrations (i.e., two times Minimum Lethal Concentration (MLC); Brege *et al.* 2003). MLC is defined as the concentration of TFM or a TFM/niclosamide mixture that produces 99.9% mortality of sea lamprey larvae during a 9 hour exposure. However, concerns over impacts to non-target organisms, release of pesticides into the environment, and increasing lampricide cost prompted the GLFC, in its 1992 Vision Statement, to "reduce reliance on lampricides" by the year 2000 by the "development and use of alternate control techniques" (GLFC 1992).

In December 1995, the Lampricide Control Task Force was established to improve the efficiency of lampricide control by maximizing sea lampreys killed in treatments while minimizing lampricide use, costs, and impacts on stream/lake ecosystems. The Task Force was also asked to define lampricide control options for near- and long-term stream selection and target setting (Klar and Young 2004).

By 1999, TFM usage was reduced from an average of 52,904 kg/y during 1977–1989 to 34,120 kg/y during 1995–1999. This 36% reduction was achieved by implementing the following (Brege *et al.* 2003):

1. Reducing the number of streams treated and treating at a lower discharge resulted in 26% of the reduction.
2. Use of niclosamide; addition of 1% by weight of niclosamide to a TFM treatment block reduced the quantity of TFM required by up to 50% while retaining selectivity to sea lamprey. Use of niclosamide produced significant cost savings, protected some non-target species (Boogaard *et al.* 2003), and was responsible for 7% of the reduction.
3. Use of a pH/alkalinity model to predict TFM toxicity (Bills *et al.* 2003); this accounted for 26% of the reduction.
4. Targeting TFM concentrations at or near MLC. This was achieved through computer aided treatment planning and was responsible for 28% of the reduction.
5. Single block treatment. Adopted for large dendritic streams which previously required numerous treatments. This strategy

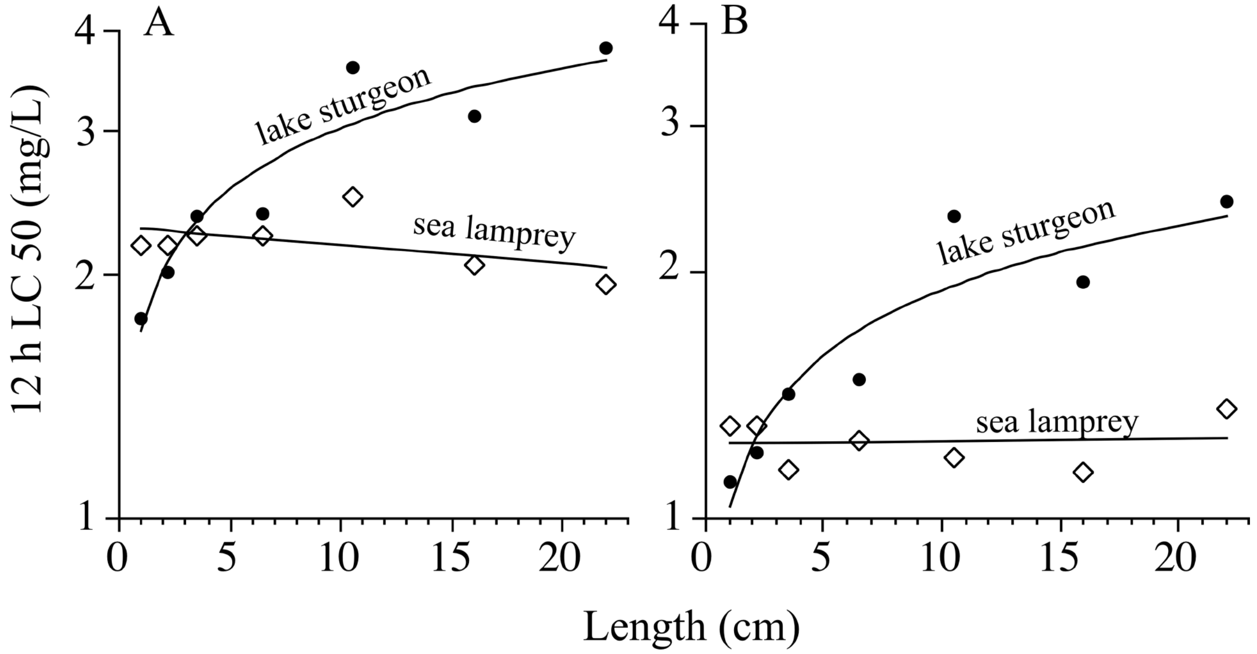

*FIG. 1. Toxicity (12 h LC50 - concentration required to produce 50% mortality in 12 h) of (A) TFM or (B) TFM + 1% niclosamide (in mg/L as TFM) to juvenile lake sturgeon* (**Acipenser fulvescens**) ***and larval sea lamprey in laboratory exposures at pH 8. Note that lake sturgeon < 1 cm long are more sensitive to TFM than sea lamprey. Note also that TFM + 1% niclosamide is slightly more selective for lamprey than TFM alone. Graphs drawn from data in Boogaard*** **et al.** ***(2003).***

requires numerous personnel and aided in 4% of the reduction.

The most recent changes in treatment philosophy have emphasized the protection of sensitive and/or endangered species. One such species is the lake sturgeon (*Acipenser fluvescens*) which in its early life stages (< 10 mm length) is actually more sensitive to TFM than sea lamprey larvae of similar size (Fig. 1). This concern led to the adoption of a "sturgeon protocol" which stipulates that target lampricide concentrations must not exceed 1.0 × MLC in streams where larval sturgeon are known to be present (1.2 × MLC when niclosamide is applied in combination with TFM).

### Selection of Streams for Lampricide Treatment

Prior to 1995, streams were selected for lampricide treatment based primarily on larval sea lamprey presence, catch-per-unit-effort, and length-frequency distribution of larvae (Slade *et al.* 2003). This approach proved to be not cost-effective and did not conform to best practices for integrated pest management. This led to the development and adoption of a quantitative larval assessment (quantitative assessment survey, QAS) methodology. The QAS methodology was combined with the adoption of the empiric stream treatment ranking system (ESTR) that ranks and selects streams for treatment based on cost, predicted effectiveness, and the projected number of larvae killed. The effectiveness of the proposed treatment is classified as high, medium, or low (99, 98, or 95% kill) and is based primarily on a qualitative judgment from observations of dead larvae during and after historical treatments. In some cases, the classification is based on direct measurements of pre- and post-treatment larval abundance of historical treatments (Christie *et al.* 2003).

### Characteristics of a Typical Treatment

A typical stream treatment consists of four components: larval assessment, collection of water chemistry and flow data, determination of MLC, and application of lampricide(s).

*Larval Assessment*

A survey of the stream (main branch and tributaries) is conducted to identify the upstream limit of larval sea lamprey infestation and to assess the size and age structure of the larval population. The size and age data are used to predict the numbers of larvae that have potential to metamorphose into the parasitic life stage in the coming year. This will determine whether and when to treat the river. The assessment data are also used to identify initial upstream application points on the main branch and all tributaries that require treatment.

*Collection of Water Chemistry and Flow Data*

This occurs prior to treatment and typically includes the following:

a) total alkalinity, pH, temperature, and dissolved oxygen in water from main branch and tributaries
b) total stream discharge in cubic meters per second
b) Stream hydrology as revealed by dye movements

*Determination of MLC*

This is done either with stream-side toxicity tests with larval sea lampreys captured from the river or is calculated using the pH-alkalinity model developed by Bills *et al.* (2003) using laboratory-derived data. These data, coupled with detailed records from previous treatments on the same stream, are then used by treatment managers to determine target lampricide concentrations, application rates, when to initiate the treatment, and where to locate booster application sites to counter lampricide loss as the chemical block moves downstream.

*Application of Lampricide*

Either TFM or TFM/niclosamide mixtures are then applied to the mainstream at the initial application point for 12 or more hours to achieve a 9-hour block of chemical above the target MLC (usually does not exceed 1.5 × MLC and does not exceed MLC when sensitive non-target species are present). Applications to tributaries are timed so that the arrival of lampricide at the convergence with the main branch coincides with the arrival of the main treatment block. During the treatment, water sampling typically continues with periodic measurements of pH, alkalinity and lampricide concentration.

## Example of a Large Treatment: Ford River, Michigan in 2000

The Ford River treatment took a total of 13 days; pre-treatment work was conducted over a 4–5 d period and a total of 176 km of stream was treated. Average stream discharge was 3.1 $m^3$/sec (historically very low) in the main branch of the river. Travel time for the lampricide block was 8 days from the initial application point to the river mouth. A total of 48 personnel and 3,400 staff hours were required to complete the treatment. Lampricide concentrations were targeted at 4.1 mg TFM/L in the upper reaches of the river and 3.1 mg TFM/L + 31 μg niclosamide/L in the lower portion. Total lampricide (based on active ingredient) applied to the river in 2000 was 2,114 kg of TFM, 22.7 kg of TFM Bars (a gradual release formulation of TFM), and 7.4 kg of niclosamide at a cost of $136,975.

## Effects of Water Chemistry on Lampricide Toxicity

Water pH and alkalinity both have substantial effects on the toxicity of TFM to sea lampreys but the effect is greatest for pH. TFM toxicity is about five times greater at pH 7 than at pH 8 when measured either as the 12 h LC50 (Fig. 2A) or as the 12 h LC99 (30 mg/L alkalinity, Fig. 2D). This difference in toxicity is thought to be largely due to the effect of pH on the ionization state of TFM (Fig. 2B), i.e., the relative proportions of the water-soluble phenolate anion and the lipid-soluble free phenol. Since the latter is much more permeable to the gills than the former (Hunn and Allen 1974, 1975), decreasing the pH will increase TFM uptake and therefore TFM toxicity. From pH 8.0 to 7.0 the free phenol fraction (Fig. 2B) increases from 2% to 20% of total TFM (Fig. 2C).

Increasing the alkalinity ($HCO_3^-$) reduces TFM toxicity separate from the effect of increasing pH (Fig. 2D). The mechanism for the protective effect of alkalinity is unknown but there are two possibilities: higher alkalinity may raise the pH of the gill micro-environment and thereby reduce the bioavailability of TFM (i.e., reduce the uptake of the free phenol) or it may compete with anion binding sites on the gills and thereby mitigate damage to the gill surface caused by the phenolate anion. Damage

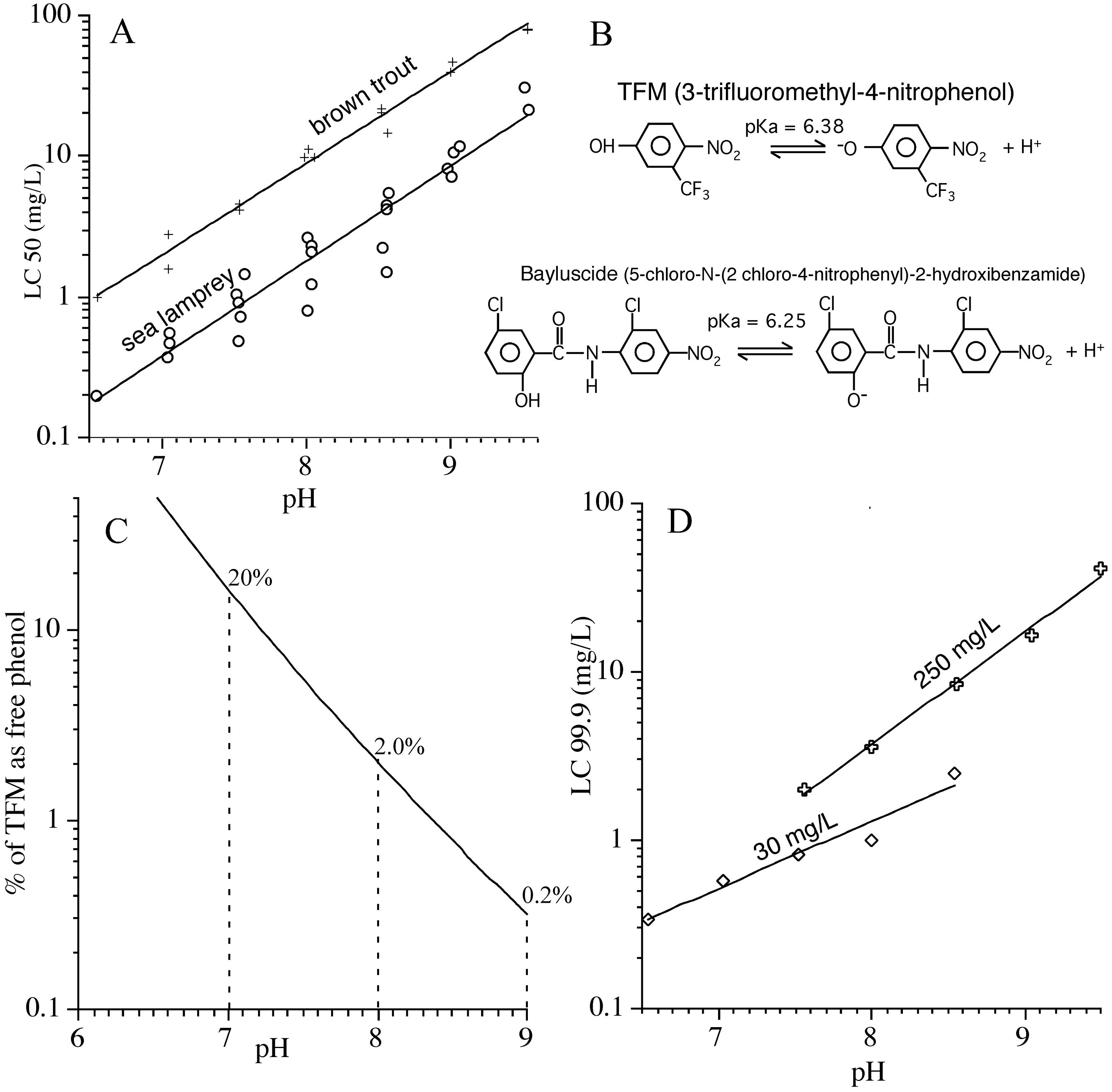

*FIG. 2. Effect of water chemistry on lampricide toxicity. A. Effect of pH on TFM toxicity expressed as LC50 (concentration that produces 50% mortality in 12 hours) to sea lamprey and brown trout. Graph drawn from data in Bills et al. 2003. B. Dissociation equilibria for TFM and Bayluscide. C. Fraction of total TFM present as TFM-OH (free phenol) in relation to pH. Curve calculated by rearrangement of the Henderson-Hasselbalch equation* $pH = pk_a + \log\left(\frac{TFM\text{-}O}{TFM\text{-}OH}\right)$ *and solving for [TFM-OH] using a pKa of 6.38. D. Effect of alkalinity (*$[HCO_3^-]$ *in mg/L) on TFM toxicity. Graph drawn from data in Bills* et al. *2003.*

to gill ion-uptake cells is one of the hallmarks of exposure of sea lampreys to TFM and is thought to contribute to their high susceptibility to lampricides (Mallatt *et al.* 1994).

The relative selectivity of TFM for sea lamprey is illustrated in Figure 2A. In this example, the toxicity of TFM to sea lamprey is about five fold greater than to brown trout (*Salmo trutta).* Note also that there is no apparent protective effect of alkalinity in brown trout (Fig. 2A).

### Present Status of Lamprey Populations

Lamprey populations are relatively stable in Lakes Erie and Ontario, declining in Lake Huron, but are showing an increasing trend in Lakes Superior and Michigan (Fig. 3). In Lake Superior adult sea lamprey abundance and wounding rates on lake trout have show an increasing trend since 1994. In Lake Michigan abundance also has shown a significant trend upward since 1994 and wounding rates above target since 1995. During the 1990s there were more sea lampreys in Lake Huron than in all the other Great Lakes combined (e.g., > 400,000 in 1993). However, increased control efforts (including lampricide treatment, trapping, and release of sterile males) on the St. Marys River, the major source of larvae, begun in 1997 have contributed substantially to the reduction of population to present levels. Nonetheless, lamprey abundance is still above target levels (dashed lines, Fig. 3) in all of the Great Lakes.

## RESEARCH NEEDS

In April 2003, a workshop was held to transform the top ten research priorities (drafted in October 2002) of the Lampricide Control Task Force into this document. Participants decided that the two most important science issues related to the use of lampricides were *treatment effectiveness* and *non-target effects*. Other important issues included *mechanisms of toxicity and selectivity* and *new lampricide development.*

### Treatment Effectiveness

Research leading to improving treatment effectiveness is a top priority because lamprey populations in the Great Lakes have not been suppressed to target levels (Fig. 3). Even if populations were at prescribed targets and stable, improving treatment effectiveness would still be important for its potential cost savings and continued reduction of lampricide use as specified by the GLFC vision statement (GLFC 2001). Achieving improved treatment effectiveness is a challenge since it must balance maximizing ammocoete mortality and minimizing toxicity to non-target species. This constraint is greatest in those streams where sensitive and/or endangered non-target species are present.

The net effect of this constraint is to lower the margin of error in treatments (i.e., treatments are closer to the sub-lethal threshold). To mitigate this problem, extensive planning goes into every treatment, and detailed treatment records from past treatments for each stream are consulted to help treatment crews anticipate problems. Nonetheless, there are streams that are traditionally difficult to treat under the best of circumstances. Some variables, such as rainfall or unanticipated increases in stream pH, presence of beaver dams and landowner denying access, all which may be uncontrollable at the time of treatment, can lead to sublethal applications. Furthermore, treating at low discharge can amplify treatment problems. At low discharge the risk of dilution of lampricides by either groundwater or rainfall is greater and more pronounced fluctuations in water temperature and chemistry can be expected. Problems in filling backwaters and lagoon areas with TFM are also intensified at low flow.

In order to improvement treatment effectiveness, three major questions must be addressed:

1) *What are the major sources of parasitics*? Two major sources are possible: larvae that were not treated and larvae that survived treatment (treatment residuals). Untreated larvae are typically thought of as those that originate from untreated lentic areas and/or streams ranked too low for treatment. The latter group could also include larvae that escaped treatment by virtue of their location relative to the lampricide application. Treatment residuals are those larvae that were exposed to lampricide but survived. However, treatments that are "less than normal" effectiveness or "ineffective" appear to make up a minority of the total number of treatments (~15%; McDonald 2005), thus the major source of treatment residuals may be from treatments which are, by the standards used to judge treatments, regarded as effective. A decision tree of hypotheses for investigating the sources of parasitics is outlined in Figure 4.

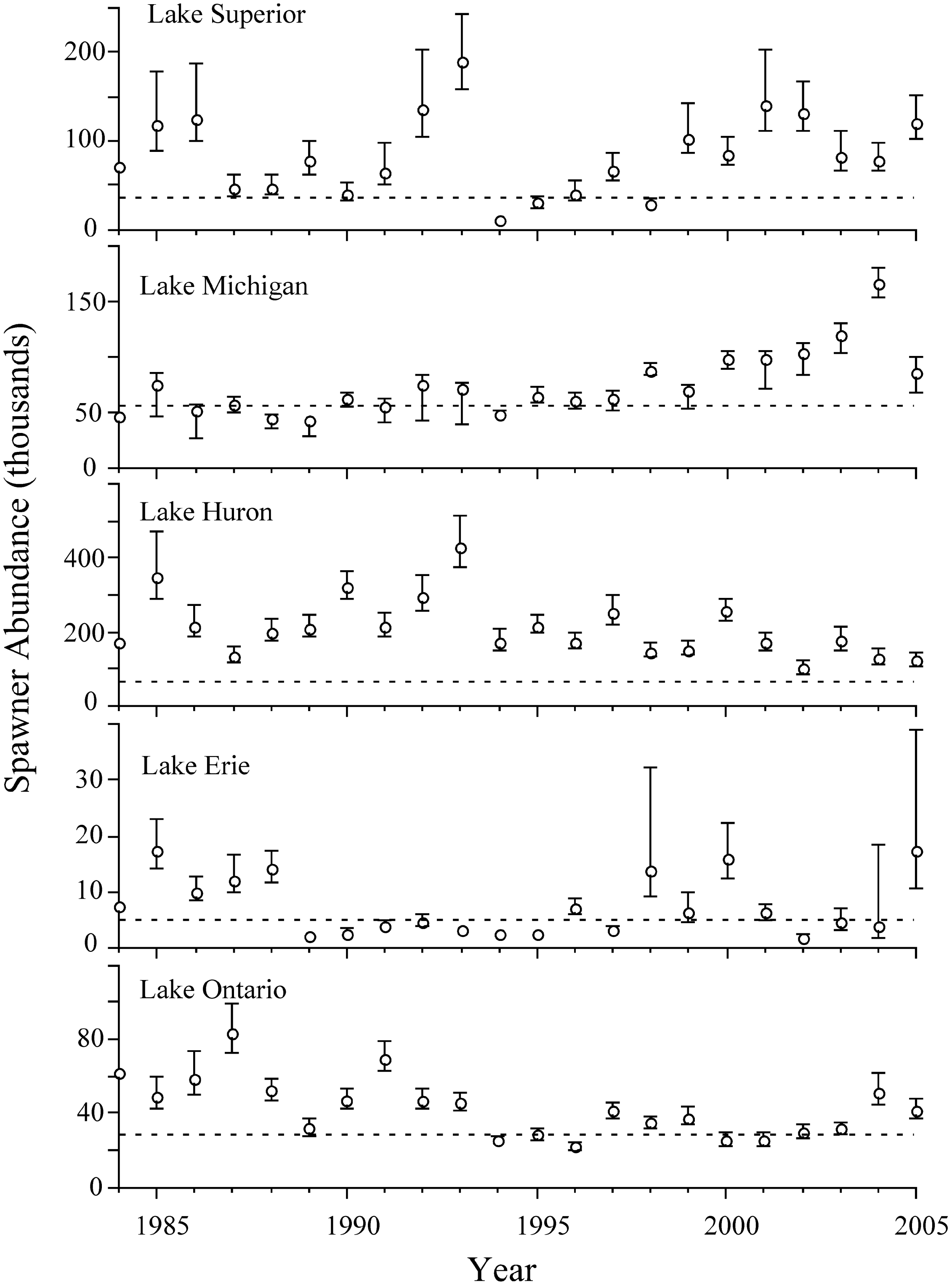

*FIG. 3. Annual lake-wide estimates of spawning-phase sea lamprey population size during 1984–2005 with 95% confidence intervals (vertical lines) and target level (dashed lines). Abundance is estimated by a combination of mark-recapture estimates of spawning phase migrants in streams with traps, and regression model-predicted numbers in streams without traps (Mullett* et al. *2003). Target values are set to meet Fish Community Objectives specific for each lake. Data from J. Adams, J. Richards and R. McDonald (2006). 2005 Lake-wide Spawner Estimates with Confidence Intervals. Unpublished GLFC technical report.*

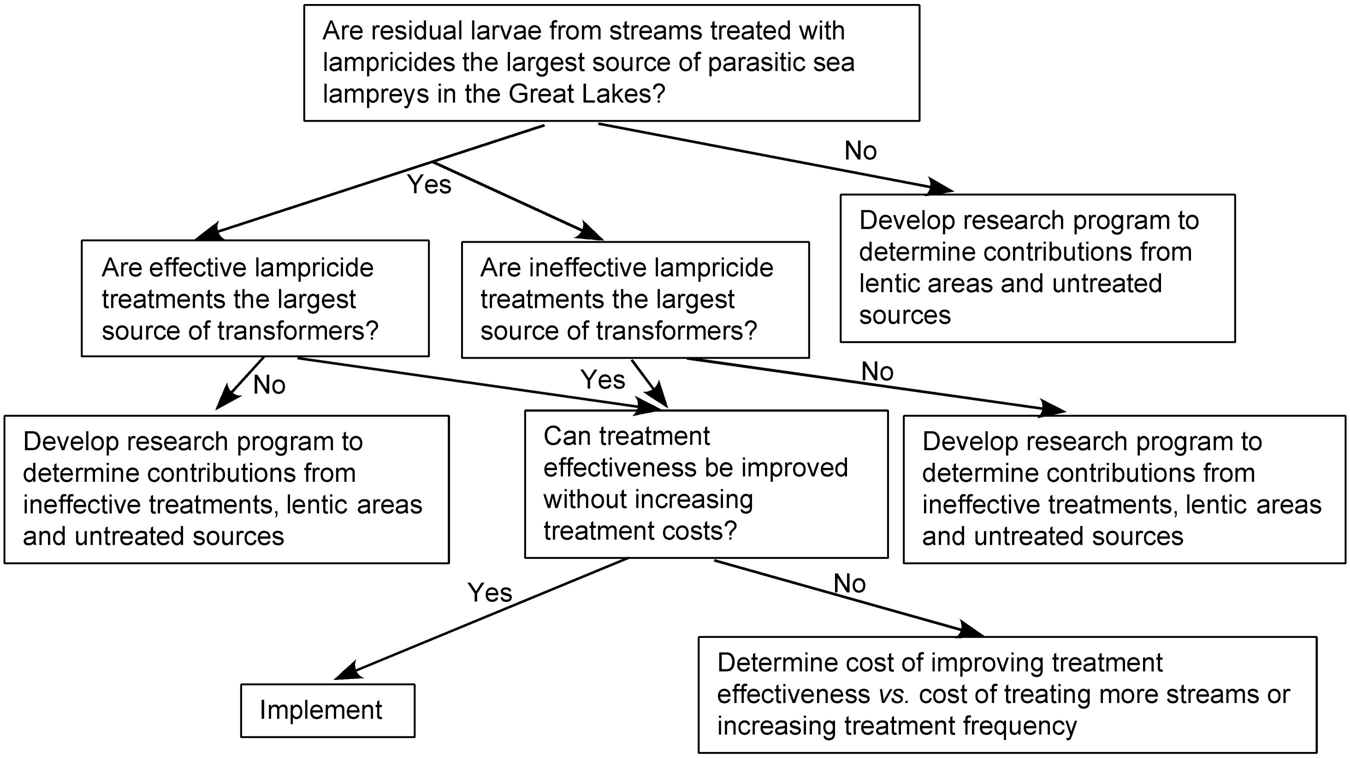

***FIG. 4. Decision tree of hypotheses concerning sources of parasitics.***

2) *What are the key factors producing treatment residuals*? Historical treatment records suggest that the primary factors affecting treatment effectiveness are stream discharge, beaver dams, rainfall, application of the sturgeon protocol, dilution of lampricide by ground water or at the stream mouth, and pH variation. What is their relative importance? What factors can we control? For those factors that are out of our control, how can we work around them, either modifying the treatment to account for them, or making the treatment more robust to changes in these factors?
3) *Does improving treatment effectiveness require additional resources and, if so, in what part of the program should they be applied?* Figure 5 considers two scenarios for increased investment in lampricide control: increasing personnel engaged in assessment/treatment and increasing lampricide usage.

## Non-target Effects of Lampricides

Although toxicity studies have not been completed on all fish and invertebrate species potentially susceptible to lampricides, a number of sensitive species have been identified over years of stream treatments and their relative sensitivity to lampricides tested in the laboratory (Boogaard *et al.* 2003, Dawson 2003, Hubert 2003). Extensive and controlled laboratory testing has shown a considerable variability in sensitivity to TFM amongst non-target species. Among the 15 teleosts examined by Boogaard *et al.* (2003), the most sensitive were members of the catfish family, Ictaluridae (black bullhead *Ictalurus melas*, channel catfish *Ictalurus punctatus,* tadpole madtom *Notorus gyrinus*). This group has a similar sensitivity to TFM as juvenile white sturgeon (Fig. 1) and are killed by TFM concentrations ranging from 1.3 to 1.7 × sea lamprey MLC. Adult mudpuppies (*Nectalurus maculosus*) are also in this category. Salmonids (rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, lake trout *Salvelinus namaycush*, Atlantic salmon *Salmo salar*) are less sensitive and can be killed at concentrations 3–5 × MLC. The least sensitive groups are the Centrarchidae (bluegill *Lepomis macrochirus*, green sunfish *Lepomis cyanellus*, smallmouth bass *Micropterus dolomieui*), and the Percidae (yellow perch *Perca flavescens*) which are killed at TFM

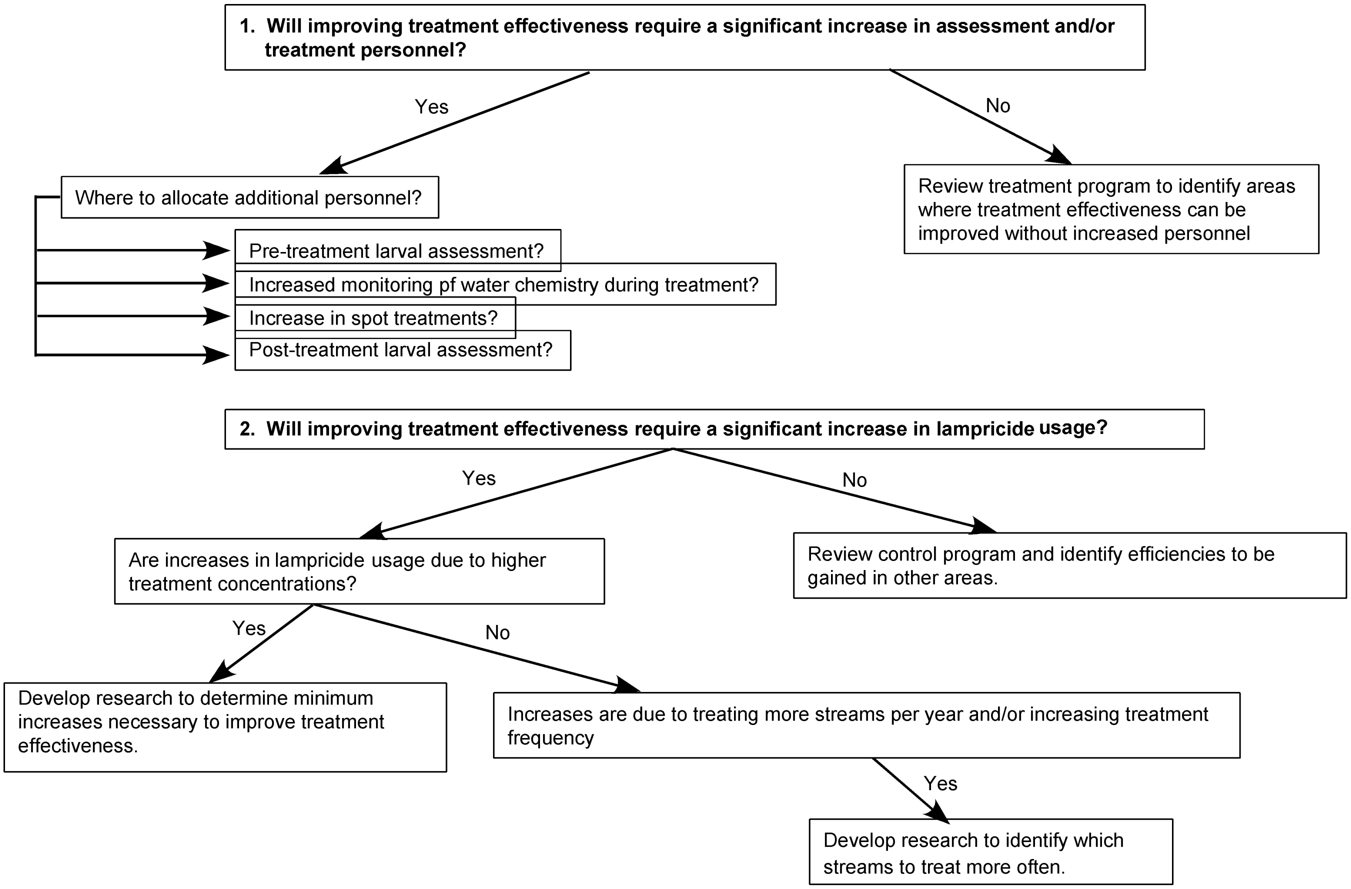

***FIG. 5. Decision tree of hypotheses concerning improving treatment effectiveness.***

concentrations 6–8 × MLC. In these studies all fish tested were young-of-the-year juveniles (1–2 g body mass) while the mudpuppies were adults. The authors noted that field observations suggest that juvenile mudpuppies are more sensitive to TFM than adults and are currently investigating TFM toxicity to juveniles. An earlier study on the toxicity of TFM to bullfrogs (*Rana catesbeiana;* Kane *et al.* 1993) showed that larvae are about 10 times more sensitive than adults. In this study the authors attributed their result to greater uptake of TFM from the water by larvae than adults. When each size class was exposed to TFM by intraperitoneal injection there was no difference in toxicity nor was there any difference in the efficiency of TFM detoxification between the two size classes.

Considerable progress has been made in identifying those non-target species that are at greatest risk of lethality from lampricide exposure (i.e., < 2 × MLC) and in minimizing that risk by developing species-specific treatment protocols (e.g., the sturgeon protocol, Klar and Scheen 2000). However, the focus of research to date has been mainly on non-target fish species. Future research should concentrate on the following questions:

1) Are there other vertebrate and invertebrate species that are sensitive to lampricide exposure? Particular attention should be paid to larval amphibians and mollusks and species that are endangered (e.g., Hungerford's crawling water beetle, *Brychius hungerfordi*).
2) What is the mechanistic basis for inter-specific differences in lampricide sensitivity? Are there inter-specific differences in rates of lampricide uptake, in sites of toxic action, or in efficiency of detoxification mechanisms? Progress in this area would assist with the task of identifying sensitive species and developing treatment protocols to minimize negative effects. Toxic mechanisms are addressed in more detail below.

3) Are there persistent sub-lethal effects of lampricide exposure, especially on long-lived sedentary species that may be exposed to more than one lampricide treatment (e.g., unionid mussels, Waller *et al.* 2003)? This research will lead to a better understanding of whether, and to what extent, there are long-term ecological effects of lampricide exposure on non-target species.

## Modes of Toxic Action

TFM acts at the cellular level by uncoupling oxidative phosphorylation causing cell death by metabolic arrest (Niblett and Ballantyne 1976, ACSCEQ 1985, Viant *et al.* 2001). Beyond the cellular level, however, there is no definitive explanation for the particular sensitivity of lampreys to TFM. In lampreys, relative to rainbow trout, TFM is taken up more readily (Lech and Statham 1975), causes more gill damage, particularly to iono-regulatory cells in the gills (Mallatt 1987, Mallatt *et al.* 1994) and is detoxified and excreted more slowly (Lech and Statham 1975, Kane *et al.* 1994) but the relative contribution of each to toxicity is unknown.

The mode of action of niclosamide is thought to be similar to that of TFM but the exact mechanism is unknown (ACSCEQ 1985). Niclosamide is about 43 times more toxic than TFM to larval lampreys but is not selective in toxicity, at least between rainbow trout and sea lamprey (Howell *et al.* 1964). However, more recent studies (Boogaard *et al.* 2003) suggest that the TFM/niclosamide mixture is slightly more selective than TFM alone in its toxicity to sea lampreys as compared to lake sturgeon at lengths > 10 cm (Fig. 1).

The mechanistic uncertainties outlined above suggest research on lampricides in at least three areas: uptake from the water, site of toxic action, and mechanism of detoxification. The specific questions should include the following:

1) *Uptake*. What is the rate of uptake from the water of each lampricide by sea lamprey and non-target species and how are the rates of uptake affected by pH and alkalinity?
2) *Site of toxic action.* Do differences exist in site of toxic action at the organ level among species? At what lampricide concentration is gill damage more prominent in sea lampreys than non-target species? Does the site of toxic action change in relation to exposure concentration? For example, are internal organs the primary target at lower concentrations? Do TFM and niclosamide have different sites of action?
3) *Mechanism of detoxification.* It is now fairly certain that all vertebrates use the same mechanism for detoxifying TFM and other phenolic compounds (Kane *et al.* 1993, 1994). The liver is the main site for detoxification and the process involves conjugating the phenolic compound with α-D-glucouronic acid, catalyzed by the glucuronyl transferase family of enzymes. The resulting conjugate is more polar and therefore more readily excreted in the bile. It is also clear that there are substantial differences amongst species in the efficiency of the glucuronyl transferase enzyme. In a four species comparison (sea lamprey, channel catfish, rainbow trout, and bluegill) a close correlation was found between enzyme efficiency and acute toxicity (Kane *et al.* 1994). The enzyme efficiency of the most tolerant species, the bluegill, was seven times and the 12h LC50 ten times that of sea lamprey. The other two teleosts were intermediate. What is less clear is whether this difference in detoxification efficiency is the sole explanation for inter-specific differences in tolerance to TFM, especially at MLC, the concentration used to kill 99.9% of lamprey larvae in a 9 h period. Under these conditions, in particular, the key question is how much damage is accumulated before detoxification begins, i.e., before TFM (and niclosamide) reach the liver to be detoxified?

## Lampricide Development

Findings from the above research will be key to two research initiatives: the exploration of new and more effective ways to apply existing lampricides, and the development of new lampricides.

1) *New application methods.* At least two possible directions should be considered:

a) Will longer exposures at lower concentrations (i.e., same or reduced amount of chemical) improve treatment effectiveness? If it is found, for example, that differences in detoxification capacity largely explain

***TABLE 1. Summary of research needs.***

TREATMENT EFFECTIVENESS

1. What are the major unknown populations and sources of treatment residuals that contribute to parasitic sea lamprey populations in the Great Lakes?
2. What are the key lampricide treatment variables that produce residual lampreys that survive treatments?
3. What additional personnel and lampricide will be required to improve treatment effectiveness, and in what part of the program should they be applied?

NON-TARGET EFFECTS

4. In addition to those species already identified as sensitive to lampricide exposure are there other vertebrate and invertebrate species that are also sensitive?
5. What is the mechanistic basis for inter-specific differences in lampricide sensitivity?
6. Are there sub-lethal effects of lampricides on non-target species?

MODES OF TOXIC ACTION

7. What is the rate of uptake from the water of each lampricide by lamprey and non-target species and how are the rates of uptake affected by pH and alkalinity?
8. Do differences exist in mode of toxic action among species?
9. At what lampricide concentration is gill damage more prominent in lampreys than non-target species?
10. Does the site of toxic action of lampricides change in relation to exposure concentration?
11. Do TFM and niclosamide have different sites of action?
12. How do larval or adult lamprey mechanisms for metabolizing and excreting lampricides compare those found in teleosts?

LAMPRICIDE DEVELOPMENT

13. Will longer exposures at lower concentrations improve treatment effectiveness?
14. Would lampricide pre-treatment at sub-lethal levels for several hours followed by a boost in concentration be more effective than current practices?
15. Will exposure to low levels of lampricide potentiate the toxicity of lampricide to sea lamprey? Would this approach increase the species selectivity?
16. Would pH reduction and buffering during lampricide treatment be cost-effective and lead to improved treatment effectiveness?
17. What are the synergistic toxic effects between existing lampricides and a new lampricide and can synergism be used to avoid non-target species' mortality?
18. What physiological, ecological, and biological characteristics of larval lamprey are different from other fish species and do these characteristics represent unique vulnerabilities to exploit in developing new lampricides.

the relative sensitivity of lamprey to lampricides then longer exposures should increase mortality in sea lamprey without similar effects on non-target species. Alternatively, would lampricide pre-treatment at sub-lethal levels for several hours followed by a boost in concentration be more effective? Will exposure to low levels of lampricide, well below non-target thresholds, potentiate the toxicity of lampricide to sea lamprey? Would this be a method for increasing the selectivity of lampricides?

b) Would pH reduction and buffering during lampricide treatment be cost-effective and lead to improved treatment effectiveness? TFM usage could be substantially reduced by controlling stream pH at a lower value (for each 1.0 unit reduction in pH there would be a ~10 fold reduction in TFM required, Fig. 2C). The important question is: Can pH reduction and control during stream treatments be accomplished cost effectively? The key hurdle with this approach would be in devising an effective method for reducing pH. One possible way would be by bubbling $CO_2$ as this would leave no residue and the pH would return to normal as the $CO_2$ escaped from solution. At non-bicarbonate buffer values typical of moderately hard fresh water a reduction of pH by one unit would require a $pCO_2$ (partial pressure of $CO_2$) of 15–20 mm Hg (atmospheric $pCO_2$ is 0.03 mm Hg). At worst, the direct effect on biota would be mild anaesthesia.

***TABLE 2. Internally funded research projects. Internal Research (R) and Technical Assistance (TA) related to lampricides recently completed (C), on-going (O) and proposed (P) by the U.S. Geological Survey's Hammond Bay Biological Station (HBBS) and the Upper Midwest Environmental Science Center (UMESC).***

| Title | R/TA | Status | Location | Contact |
|---|---|---|---|---|
| Prepare lampricide analytical standards for field use | TA | O | HBBS | 1 |
| Evaluate TFM samples for physical, chemical and toxicological properties | TA | O | HBBS | 1 |
| Do static and flow-through toxicity tests yield the same toxicity information? | R | P | HBBS | 1 |
| Effect of groundwater inflow on distribution of lampricide and on survival of sea lamprey larvae during a stream treatment | R | P | HBBS | 2 |
| Study of issues related to stream pH and lampricide treatments | R | O | UMESC | 3 |
| Lampricide toxicity to juvenile mudpuppies | TA | O | UMESC | 3 |
| Evaluation of an extended duration lampricide block as an alternative treatment strategy | R | P | UMESC | 3 |
| Avoidance of young-of-year lake sturgeon *(Acipenser fulvescens)* to Bayluscide 3.2% granular sea lamprey larvicide | R | P | UMESC | 3 |
| Re-evaluate accuracy of lower pH/lower alkalinity range of pH/alkalinity sea lamprey minimum lethal prediction model as a tool to define target treatment levels for effectively controlling larval sea lampreys with the lampricides TFM and niclosamide | TA | P | UMESC | 3 |
| Relative toxicity of the lampricides TFM and niclosamide to newly transformed and larval sea lamprey *(Petromyzon marinus)* | TA | O | UMESC | 3 |
| Residue levels of the lampricides TFM and niclosamide in moribund sea lamprey larvae following exposures to TFM and a TFM/1% niclosamide combination | R | P | UMESC | 4 |
| Dissipation of TFM and niclosamide following stream treatments | R | P | UMESC | 4 |
| Development of a glucuronyl transferase assay to assess the sensitivity of the lampricide TFM to nontarget species of concern | R | P | UMESC | 4 |
| Toxicity of the lampricides TFM and niclosamide to American eels *(Anguilla rostrata)* | TA | P | UMESC | 5 |
| Estimating treatment effectiveness using in-situ caged larval sea lampreys during lampricide control operations | TA | P | UMESC | 3 |
| Re-evaluation of the TFM pH/alkalinity sea lamprey minimum lethal prediction model using an improved statistical design | R | P | UMESC | 3 |
| Acute toxicity of TFM and a 99%TFM:1% niclosamide mixture to the northern brook lamprey (*Ichthyomyzon fossor*), American brook lamprey (*Lampetra appendix*), and sea lamprey (*Petromyzon marinus*) | TA | C | UMESC | 3 |
| Acute toxicity of TFM and a 99%TFM:1% niclosamide mixture to the giant floater (*Pyganodon grandis*), fragile papershell (*Leptodea fragilis*), and pink heelsplitter (*Potamilus alatus*) unionid mussels and sea lamprey (*Petromyzon marinus*) larvae | TA | C | UMESC | 3 |
| Relative toxicity of larval and adult *Haliplus sp.* to the lampricide TFM as a surrogate for the endangered Hungerford's crawling water beetle (*Brychius hungerfordi*) | TA | O | UMESC | 3 |

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2) *New lampricides*. The key to developing new lampricides will be a better understanding of the vulnerabilities that are unique to larval sea lamprey. Are their gills more permeable to organic toxicants? Are their branchial tissues or internal organs more sensitive to damage? What are the unique properties of their detoxification mechanisms? Recent evidence indicates that although sea lamprey possess detoxifying enzymes from the CYP1A family of mono-oxygenases (Whitfield *et al.* 2003) the enzymes are not inducible by ligands known to be potent inducers in teleosts (benzo(a)pyrene, Rotchell *et al.* 2000; or tetrachlorobiphenyl, Hahn *et al.* 1998). Does this mean that lampreys are particularly vulnerable to compounds that in other species induce mixed function oxygenases (MFOs)?

Although present evidence points to the conclusion that each factor may contribute to selective toxicity it will be important to know which is most important. Once that is known then a focused search for selective toxicants can be launched. Another factor to weigh is whether a new lampricide would be used alone or as a synergist with either TFM or niclosamide.

## CONCLUSIONS

Table 1 summarizes research needs related to the use of lampricides. For more information the reader is directed to Volume 29, Supplement 1 of the *Journal of Great Lakes Research,* Special Issue; “Sea Lamprey International Symposium (SLIS II), 2003. This volume consists of 60 papers developed from presentations made at the symposium. These papers cover the following areas: lamprey biology, ecology and assessment, alternative control, lampricide control, case studies, and symposium reports. Another useful resource, particularly to those individuals interested in pursuing studies of the mode of toxicity of lampricides, is the NRCC (National Research Council of Canada) report entitled “TFM and Bayer 73: lampricides in the aquatic environment” ACSCEQ (1985). The reader is also directed to the Sea Lamprey Research Completion Reports section of the GLFC web site (http://www.glfc.org/pubs_out/communi.php). This section contains 147 downloadable project completion reports from sea lamprey research projects completed between 1979 and present day. Of this total, 11 reports concern research in lampricide control. The reader should also be aware that the Commission funds internal research on lampricides as well. This research is mainly carried out at the Upper Midwest Environmental Science Center, and USGS Hammond Bay Biological Station. Table 2 summarizes recently completed, on-going and proposed research on lampricides at these facilities and provides contact information for the investigators.

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