ACUTE TOXICITY OF TWO LAMPRICIDES, 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) AND A TFM:1% NICLOSAMIDE MIXTURE, TO SEA LAMPREY, THREE SPECIES OF UNIONIDS, HALIPLID WATER BEETLES, AND AMERICAN EEL

TECHNICAL REPORT 70

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May 2011

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Citation: Boogaard, M.A., and Rivera, J.E. 2011. Acute toxicity of two lampricides, 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture, to sea lamprey, three species of unionids, haliplid water beetles, and American eel. Great Lakes Fish. Comm. Tech. Rep. 70.

Great Lakes Fishery Commission
2100 Commonwealth Blvd., Suite 100
Ann Arbor, MI 48105-1563

May 2011

ISSN 0072-730X (print)
ISSN 2159-6352 (online)

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Printed on recycled paper.

TR70/5-2011/600

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ABSTRACT

We conducted a series of toxicological treatments with 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% 2′,5-dichloro-4′-nitrosalicylanilide (niclosamide) mixture, two compounds used to control larval sea lamprey (*Petromyzon marinus*) in Great Lakes tributaries, to evaluate the acute toxicity of the lampricides to a number of nontarget species of concern. Treatments were conducted with yellow stage American eel (*Anguilla rostrata*), adult and larval haliplid water beetles (*Haliplus* spp.), a surrogate for the endangered Hungerford's crawling water beetle (*Brychius hungerfordi*), and adults of three unionid species—giant floater (*Pyganadon grandis*), fragile papershell (*Leptodea fragilis*), and pink heelsplitter (*Potamilus alatus*). Treatments were conducted using a serial dilution system consisting of nine test concentrations and an untreated control with 20% dilution between concentrations. Narcosis was evident among giant floaters exposed to the TFM and the TFM:1% niclosamide mixture and among pink heelsplitters exposed to the TFM:1% niclosamide mixture only but mostly at concentrations greater than 2-fold that required to kill 100% of larval sea lamprey (minimum lethal concentration (MLC)). Tests with the haliplid beetle suggest the risks to the Hungerford's crawling water beetle associated with TFM applications are minimal. Concentrations over 2-fold the sea lamprey MLC did not kill adult or larval water beetles. Preliminary behavioral observations suggest water beetles may avoid treatment by crawling out of the water. Adult water beetles exposed to TFM at 3-fold the sea lamprey MLC were observed above the water line more often than controls. The lampricide TFM was not acutely toxic to American eel. Mortalities were rare among American eel exposed to TFM concentrations up to 7-fold the observed sea lamprey MLC. Similarly, for the TFM:1% niclosamide mixture, mortalities were rare among American eel exposed to nearly 5-fold the observed sea lamprey MLC. Overall, acute TFM toxicity

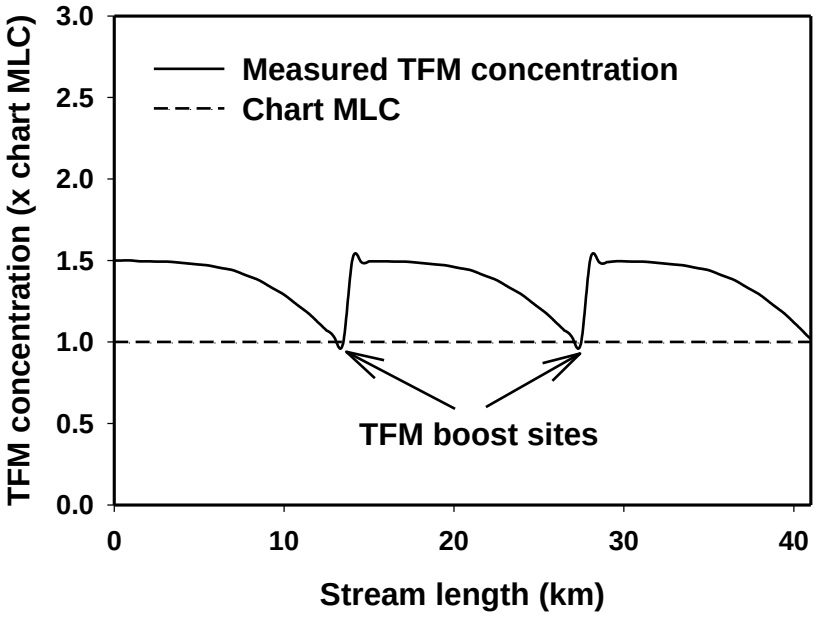
was not evident among any of the species examined in this study at concentrations targeted to control larval sea lamprey. Results for the adult unionids should be viewed with caution due to the lack of replication in the treatments.

INTRODUCTION

The lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2′,5-dichloro-4′-nitrosalicylanilide (niclosamide) are important components of the Great Lakes Fishery Commission's Integrated Management of Sea Lamprey Control Program. They are routinely used to kill larval sea lamprey (*Petromyzon marinus*) in tributaries to the Great Lakes. Sea lamprey control treatments (typically 12 h in duration) are conducted primarily with TFM, but niclosamide, toxic at much lower concentrations than TFM, may be used as an additive to reduce the amount of TFM and protect populations of burrowing mayflies (*Hexagenia* spp.) (Klar and Young 2004). Historical records of the U.S. Fish and Wildlife Service (USFWS) indicate that niclosamide has been used in about 15% of the streams treated over the last 23 yr (D. Lavis, USFWS, Ludington Biological Station, personal communication, 2008). Lampricide treatments target the vulnerable larval stage of the sea lamprey. After hatch, larval sea lamprey burrow into stream sediments where they remain for 3 to 15 yr before transforming into the parasitic stage that feeds on Great Lakes game fishes (Potter 1980; Youson 2003). The multi-year stream residence of larvae allows treatment of individual streams on a 3- to 4-yr rotating basis, thereby limiting the overall exposure of nontarget organisms to lampricides to 12 h over a span of 3 to 4 yr. Although the lampricides are selective to sea lamprey, some nontarget organisms are also vulnerable if lampricide levels unexpectedly exceed target concentrations (Boogaard et al. 2003). Lampricide concentrations up to 1.5 times the sea lamprey minimum lethal concentration (MLC), the lowest concentration that effectively kills 100% of larval sea lamprey, are applied initially to compensate for loss and dilution as the lampricide block moves downstream (Brege et al. 2003; Klar and Young 2004). In addition, supplemental lampricide applications are made at several points (boost sites) within a stream when lampricide levels approach the sea lamprey MLC. Fig.

1 shows a hypothetical profile of the TFM concentration in a stream during a typical lampricide treatment. Several areas of the stream are likely to receive lampricide concentrations up to 1.5 times the sea lamprey MLC, depending on the length of the treated stream and the number of supplemental boost sites.

Fig. 1. A hypthetical profile of 3-trifluoromethyl-4-nitrophenol (TFM) concentration in a stream during a typical treatment. Concentrations of TFM are shown as multiples of the predicted minimum lethal concentration (MLC) for sea lamprey. To maintain concentrations above the expected MLC as the lampricide block moves downstream, TFM is boosted at various sites.



Freshwater mussels (Unionacea) are an important part of the aquatic community in many waters, historically comprising up to 90% of the total biomass of benthic communities (Ökland 1963). North America has the richest and most diverse unionacean fauna in the world, with more than 300 species and subspecies currently recognized in the family Unionidae (Turgeon et al. 1998). In the past century, the diversity and abundance of freshwater mussels have sharply declined. About 70% of unionid species are extinct, endangered, threatened, or listed as species of concern (Williams et al. 1993). Freshwater mussels are now recognized as one of the most imperiled faunal groups in North America (Master et al. 2000). Declines have been reported in the Great Lakes and, in particular, Lake St. Clair and western Lake Erie where reductions in unionid numbers have reached 99% (Metcalfe-Smith et al. 2002; U.S. Environmental Protection Agency and Environment Canada 2009). With the exception of western Lake Erie, the status and trends in abundance of Great Lakes unionids are not well documented. The U.S. Environmental Protection Agency and Environment Canada (2009), in their review of the overall state of Great Lakes mussel populations, noted that only a limited amount of data on mussel abundance is available.

The effects of TFM on the adults of several unionid species have previously been evaluated in laboratory and field toxicity tests and in post-treatment field observations (Smith 1967; Rye and King 1976; Bills et al. 1992; Waller et al. 1993; Waller et al. 1998; Waller et al. 2003). In general, these studies showed that the adults of many mussel species are resistant to TFM at concentrations typically applied to control sea lamprey larvae. Bills et al. (1992) showed that the pink heelsplitter (*Potamilus alatus*) was sensitive to TFM, but 90% survived at concentrations typically encountered during stream treatments. Also, although 60% of pink heelsplitters were initially narcotized at 2-fold the TFM concentration required to kill sea lamprey, 50% of the narcotized mussels recovered (Bills et al. 1992). Field and laboratory observations have since confirmed that TFM temporarily narcotizes some adult unionids during treatment (Waller et al. 1998), but few quantitative data exist on the onset of narcotization during treatment, the duration of narcotization following treatment, or the vulnerability of narcotized mussels to predation or current displacement. Information on the narcotizing effect and acute toxicity of lampricides was a specific need for three species of unionids—the giant floater (*Pyganadon grandis*), the fragile papershell (*Leptodea fragilis*), and the pink heelsplitter. All three are listed

by the state of Vermont as threatened or endangered, and they are present in small numbers in streams tributary to Lake Champlain that are routinely treated to control sea lamprey.

Another organism recently identified as potentially at risk from application of lampricide to control sea lamprey is the Hungerford's crawling water beetle (*Brychius hungerfordi*), which is classified as endangered by the U.S. Fish and Wildlife Service (1994). The species was listed as a federal Category 2 Candidate Species in 1984 (U.S. Fish and Wildlife Service 1984) and then as a Category 1 Endangered Species in 1994 (U.S. Fish and Wildlife Service 1994). The only stream known to have a population of the endangered beetle and to require regular lampricide treatments is the Carp Lake Outlet, a tributary to northeastern Lake Michigan located in Emmet County, Michigan. In compliance with the U.S. Endangered Species Act (1973), sea lamprey control managers must determine if lampricide treatment of the Carp Lake Outlet would adversely affect *B. hungerfordi*.

B. hungerfordi is a small (<7-mm long), yellowish-brown member of the Haliplidae family that prefers cool, fast-moving streams. Little is known of the biology of *B. hungerfordi*, but it is thought to be similar to that of other haliplids (Hickman 1929). Adult haliplids can live up to 1 yr and have been known to overwinter (Hilsenhoff and Brigham 1978; Grant et al. 2000). Eggs of haliplids are laid in spring and early summer, and the larvae may go through three instars before pupating in moist soil out of the water (Hickman 1929). Both adults and larvae are herbivorous, feeding mostly on filamentous algae (Matheson 1912). Adults must come to the water surface to breathe and trap air bubbles under their large, hind coxal plates for use as an oxygen supply when under water (Hickman 1930). It is not known if *B. hungerfordi* respires in the same manner. Recent research failed to confirm surface breathing among *B. hungerfordi* (Scholtens 2002), although Strand and Spangler (1994) observed continuous surfacing among individuals in the Maple River, Emmet County, Michigan, presumably to resupply their air stores. Given that adult haliplids do not absorb oxygen directly through their cuticle (Hickman 1930), untoward effects from exposure to lampricides during treatments may be minimal. Larvae, however, breathe through dorsal tracheal gills or microtrachial gills and absorb oxygen directly from the water, which may make them more vulnerable than adults to lampricides. Some adult haliplids are able to fly and leave the water (Hickman 1931), an ability that may limit their exposure to lampricides if they are able to detect

the chemicals. However, the ability to fly has not been documented among *B. hungerfordi*.

The rarity and protected status of *B. hungerfordi* makes collection of sufficient numbers for testing impossible. Consequently, tests to evaluate its sensitivity to the lampricide TFM must be conducted with a surrogate. To identify a suitable surrogate for *B. hungerfordi*, we queried several aquatic entomologists (Dr. David White, Murray State University, Murray, Kentucky; Dr. Robert Roughley, University of Manitoba, Winnipeg, Manitoba; and Dr. Roger (Mac) Strand, Northern Michigan University, Marquette, Michigan). They identified haliplid water beetles (*Haliplus* spp.) as water beetles with a life history similar to that of *Brychius* spp. and noted that they are common and easy to collect in large numbers.

American eel (*Anguilla rostrata*) are the only catadromous fish in North America. It has a complex life cycle with several discrete stages. In the Great Lakes, American eel (yellow stage) is found almost wholly in Lake Ontario where they may stay for 10 to 40 yr before maturing and returning to the sea as spawning adults referred to as silver eel (Facey and Van Den Avyle 1987; U.S. Fish and Wildlife Service 2005). Over recent decades, the number of young American eel entering the upper St. Lawrence River and Lake Ontario has been declining. Because of this decline, the province of Ontario, Canada, has proposed listing the American eel as a species at risk under the Ontario Endangered Species Act of 2007 (Ontario Ministry of Natural Resources 2009). Although the numbers of American eel entering dam bypass ladders on the upper St. Lawrence River have steadily increased since 2001, the annual totals are still <2% of those in the 1980s (Tremblay et al. 2006; Ontario Ministry of Natural Resources 2010). In the U.S., the American eel population has been in a severe decline since 1984 (Casselman 2003; Haro et al. 2000). Evidence of a declining population prompted a petition to list the American eel as endangered under the U.S. Endangered Species Act (Watts and Watts 2004; Federal Register 2005). After a thorough review of all available scientific and commercial information, the USFWS concluded that the American eel did not yet warrant listing as a threatened or endangered species but asked the public to continue submitting any new information on the status of, or threats to, the species (Federal Register 2007; Berger 2007). Federal and state wildlife agencies expressed concerns that yellow eel in Great Lakes tributaries may be at risk

during lampricide treatments as the toxicity of lampricides to American eel was not known.

The objectives of this study were to determine:

1. The acute toxicity of TFM and a TFM:1% niclosamide mixture to adult giant floater, fragile papershell, and pink heelsplitter; adult and larval water beetles; and the yellow stage of the American eel
2. The TFM and TFM:1% niclosamide exposure-related narcosis effects among the three species of unionids
3. The TFM exposure-related avoidance response of adult water beetles

MATERIALS AND METHODS

Test Materials

The isopropanol solution of the sodium salt formulation of TFM used in this study, with an average concentration of 33% active ingredient by weight, was manufactured by Weylchem Frankfurt GmBH (Frankfurt, Germany) under the commercial name Lamprecid®*. The niclosamide formulation used was either Bayluscide 70% Wettable Powder or Bayluscide 20% Emulsifiable Concentrate, aminoethanol salt formulations of niclosamide, with an average niclosamide concentration of about 59% or 17%, respectively, manufactured by Coating Place, Inc. (Verona, Wisconsin). The niclosamide and TFM technical materials used to prepare analytical standards were purchased from Sigma (St. Louis, Missouri) and Aldrich Chemical Company (Milwaukee, Wisconsin), respectively.

*The use of brand name products does not imply endorsement by the U.S. Geological Survey.

Test Animals

Sea lamprey larvae (mean total length = 94 mm, range = 65-126 mm) were collected from tributaries in the Great Lakes basin using backpack electroshockers following methods in Weisser and Klar (1990). The sea lamprey were transported to the Upper Midwest Environmental Sciences Center (UMESC) and held in 200-L fiberglass tanks containing 6 cm of washed sand to allow burrowing. Flowing well water provided >1 tank-volume exchange•h^{-1} in the holding tanks. Because delayed mortality from collection and handling could compromise our test results, larvae were held a minimum of 14 d at 12 ºC prior to testing.

Giant floaters were collected from the Bark River, Jefferson County, and the Yellow River, Wood County, Wisconsin. Mussels were wrapped in wet burlap, placed in coolers, and transported to 1.7-m^3 cages in a backwater area of the Mississippi River near La Crosse, Wisconsin (Newton et al. 2001). To allow for any delayed mortality associated with collection and handling, mussels were held in the cages for 14 d prior to transport to the UMESC for testing. After 14 d, surviving giant floaters (mean total shell length = 97 mm, range = 63-167 mm) were measured (nearest mm) parallel to the hinge, marked with a numbered plastic tag, and transported to 1-m^3 acclimation tanks at 23.9 °C (the water temperature in the holding cages in the Mississippi River). Water temperature was reduced over 4 d to the test temperature (12 °C; American Society for Testing and Materials 2000). Fragile papershells were collected from Pool 2 of the Mississippi River near Hastings, Minnesota. Pink heelsplitters were collected from the Wisconsin River near Prairie Du Sac, Wisconsin. Fragile papershells and pink heelsplitters were transported directly to the UMESC; acclimated to the test temperature (12-13 °C) over a 3-d period (temperature changed no more than 3 ºC per day); and held in 320-L holding tanks, supplied with 5 cm of washed sand, for 14 d to allow for delayed mortality. After 14 d, surviving fragile papershells (mean total shell length = 112 mm, range = 50-145 mm) and pink heelsplitters (mean total shell length = 127 mm, range = 90-158 mm) were measured and tagged in the same manner as the giant floaters. Mussels were fed algae (concentrated from a UMESC culture pond using a 10-µm mesh plankton net) daily during laboratory holding.

Adult water beetles (>95% *Haliplus immaculicollis*, other species not identified) were collected from a small pond near Marquette, Michigan, and transported in aerated coolers to the UMESC for larval culture. The water beetles were maintained in a 320-L holding tank containing 8-10 cm of washed sand and 15-18 cm of well water at 21 °C. The water beetles were provided with filamentous algae, for food and egg deposition, collected from UMESC outdoor culture ponds. A mud bank was provided along one side of the holding tank to facilitate pupation. Third instar larval water beetles were recovered from the mud bank after 7 mo and used for larval treatments. For adult treatments, additional adult water beetles were collected from a backwater area of the Mississippi River near the UMESC.

American eel, yellow stage, was purchased from the Delaware Valley Fish Company, Inc., Norristown, Pennsylvania. American eel was placed in aerated bags and shipped to the UMESC. American eel was transferred to a 1,457-L, continuous-flow holding tank supplied with well water (4.4-7.0 °C flowing at about 9 $L \bullet min^{-1}$) and held a minimum of 14 d before acclimation (temperature changed no more than 3 $^{o}C \bullet day$) to the test temperature (12-13 °C). The mean weight of American eel was 67.2 g (range = 19.7-113.6 g) and the mean total length was 34.2 cm (range = 24.1-41.3 cm). The size of American eel tested was similar to that of eel migrating towards Lake Ontario via the bypass ladder at the Moses-Saunders Dam on the upper St. Lawrence River at Cornwall, Ontario. A subsample (N = 84) of American eel ascending the ladder in 2009 ranged in length from 22.5 to 43.3 cm and averaged 32.5 cm (Ontario Ministry of Natural Resources 2010).

Toxicity Tests

Treatments were conducted according to standard guidelines (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975; American Society for Testing and Materials 2000; Klar and Young 2004) in a continuous-flow delivery system described by Garton (1980) with several modifications. Modifications were as follows:

1. The dilution factor between chemical dilution cells was decreased from 50% to 20%
2. pH shifts were minimized as a result of reducing aeration by eliminating the float valve on the water supply, installing a large bore (5.1 cm) PVC water-supply tube that extended 6 cm below the head-box water line, and adding three mixing baffle plates in the head box (Bills and Johnson 1992)
3. Duplicate dilution tubes were reduced to a single dilution tube
4. Plexiglass was replaced with double-strength plate glass to eliminate the potential for adherence of niclosamide to the test chambers

Each diluter system consisted of a control and nine treatment concentrations (20% dilution between concentrations) delivered to 30-L treatment aquaria at 500 $ml \bullet min^{-1}$. Each treatment aquarium was equipped with a stand pipe positioned to allow 15 L of treatment water for an exchange rate of one aquarium volume every 30 min.

Each test consisted of a 12-h treatment period (to simulate a typical 12-h lampricide treatment), followed by a post-treatment recovery period (14 d for unionids and 1 d for water beetles and American eel) at the end of which final mortalities were recorded. Post-treatment recovery periods were longer for the unionids because narcotized mussels, which were difficult to differentiate from dead mussels (see below), could take longer than 1 d to recover, whereas narcosis does not occur among water beetles and American eel. Well water was used for all treatments. Water temperature, pH, and dissolved oxygen (DO) concentration were measured hourly in the treatment aquaria throughout the 12-h treatment period. Total alkalinity was determined in the control chamber at 0, 4, 8, and 12 h (American Public Health Association et al. 1989).

Stock solutions of the treatment material were prepared by diluting weighed portions of field-grade TFM with deionized water or of niclosamide with methanol. Test concentrations of TFM and the TFM:1% niclosamide mixture were selected so as to bracket the expected MLC for sea lamprey based on the pH and total alkalinity of the test water (Klar and Young 2004). Water samples for analysis of TFM concentration were removed from each test chamber hourly during the 12-h treatment and were measured spectrophotometrically with a Beckman DU-640 spectrophotometer (Klar and Young 2004). Water samples for analysis of niclosamide concentration

were removed every 2 h and were measured with a Waters Millennium series 2010 LC Module I Plus high-performance liquid chromatography system using methods outlined by Dawson (1982). Concentrations of TFM and niclosamide were verified by comparison to analytical standards prepared by diluting weighed portions of the technical material with methanol and test water.

For the mussel treatments, ten mussels of each species were placed in the treatment aquaria (containing 3-5 cm of washed sand) 24 h before treatment. Because of the limited number of giant floaters and fragile papershells, mussels could not be added to all test chambers. Therefore, giant floaters were only added to treatment aquaria corresponding to the six highest concentrations and the control aquaria for the TFM and TFM:1% niclosamide treatments. Fragile papershells were added to treatment aquaria corresponding to the seven highest concentrations and the control aquaria. Twenty-five giant floaters and 20 fragile papershells were retained in the holding tanks as test controls. Sufficient numbers of pink heelsplitters were available for all treatment and control aquaria, including 20 for test controls. Ten sea lamprey larvae were placed in 5 cm x 15 cm x 25 cm wire-mesh cages and tested concurrently with each mussel treatment and control exposure to allow direct toxicological comparison. Giant floater tests were conducted in August 2002, whereas fragile papershells and pink heelsplitters were tested in July 2003 and July 2004, respectively.

After the initial 12-h treatment, mussels were returned to the holding tanks for the 14-d recovery period. Water temperatures in the holding tanks were slowly increased from 12 ºC to ambient temperature (21 ºC) over a 4-d period. Mussels were evaluated for narcosis and mortality daily during the 14-d recovery period, although it was not possible to reliably differentiate narcotized mussels from those that were dead. Both had a foot extended, some with a gaped shell, some not gaped. Narcotized mussels usually, but not always, moved their extended foot after gentle probing with a glass rod, whereas mussels that did not move their extended foot after probing were either narcotized or dead. However, mussels that did not respond to probing by 14 d post-treatment were considered dead. After 14 d, the number of mussels narcotized during the treatment was determined by subtracting the number of mussels categorized as dead from the number of mussels categorized as either narcotized or dead at 12 h post-treatment. For example, if one treatment aquarium had five mussels either narcotized or dead at 12 h

post-treatment and only three dead mussels at 14 d post-treatment, then the two mussels that recovered were considered narcotized.

Three sets of treatments were conducted in duplicate with water beetles: Treatment 1—larval water beetle treatments at TFM concentrations up to 2.0 times the predicted sea lamprey MLC, Treatment 2—larval water beetle treatments at TFM concentrations up to 3.8 times the sea lamprey MLC, and Treatment 3—adult water beetle treatments at TFM concentrations up to 3.0 times the sea lamprey MLC. During all three sets of exposures to TFM, water beetle larvae (N = 10 per replicate) and adults (N = 15 per replicate) were caged to ease mortality observation and to control access to air. Cages containing the larvae were completely submerged in the treatment aquaria. In Treatments 1 and 2, larval water beetles were placed in completely enclosed plastic-mesh cages (6 cm x 12 cm x 20 cm) and transferred to treatment aquaria the day before treatment. Larvae were added to the six highest treatment and control aquaria in Treatment 1, and to the four highest treatment and control aquaria in Treatment 2. In Treatment 3, adult water beetles were placed in cages positioned so that the tops of the cages were slightly above the water line (<0.5 cm) to allow adult water beetles to collect air while maintaining contact with the chemical. To determine if adult water beetles respond behaviorally to chemical treatment, preliminary data on avoidance were collected concurrent with the toxicity tests by placing ten caged adult water beetles per replicate in treatment aquaria corresponding to the highest (9.4 mg•L^{-1}) and middle (3.2 mg•L^{-1}) TFM concentrations and the control. Cages were positioned half out of the water column to allow avoidance by climbing up the side of the cage. We looked for effects of TFM concentration on the location of adult water beetles (above or below the water line), adjusted for time, using Generalized Estimating Equations based on Liang and Zeger (1986) in PROC GENMOD in SAS (Stokes et al. 1995). In Treatments 1 and 3, larval sea lamprey (N = 10 per replicate) were caged (5 cm x 15 cm x 25 cm wire-mesh cages) in all treatment and control aquaria for direct toxicological comparison. Larval sea lamprey were not included in Treatment 2 because all TFM concentrations tested exceeded lethal limits. Sea lamprey mortalities were recorded hourly throughout the treatment period and at 12 h post-treatment. Adult and larval water beetle mortalities were only recorded after 12 h post-treatment to minimize disturbance.

Three sets of treatments were conducted with the yellow stage of American eel. In Treatment 1 (duplicate TFM treatments) and Treatment 2 (duplicate TFM:1% niclosamide treatments), five American eels were placed in each treatment and control aquarium. Ten sea lamprey larvae were caged (5 cm x 15 cm x 25 cm wire-mesh cages) in all treatment and control aquaria to allow direct toxicological comparison. In Treatment 3, only single treatments were conducted with TFM and the TFM:1% niclosamide mixture due to a limited number of American eel. No sea lamprey larvae were tested in Treatment 3 because all TFM treatments exceeded lethal limits. Mortality results between each replicate in each of the three treatment sets were analyzed for significant differences by comparing potency ratios of the toxicity curves as defined in Litchfield and Wilcoxon (1949).

Data Analysis

Risk quotients (RQ) were calculated from data collected during the lampricide treatments to evaluate the likelihood of mortality for the five nontarget species. RQs are typically calculated as the predicted environmental concentration (PEC) of a chemical divided by the predicted no-effect concentration of the chemical (PNEC). For the PEC, we used the observed sea lamprey MLC (lowest lampricide concentration that produced 100% mortality among sea lamprey tested concurrently in the treatments) of lampricide for a given test multiplied by 1.5, and, for the PNEC, we used the no-observed-effect concentration (NOEC) (highest concentration with no mortality of nontarget species). For nontarget species, the risk of mortality during lampricide stream treatments increases as the RQ approaches one, values around one indicate a moderate risk of mortality, and values much greater than one indicate a high risk of mortality.

RESULTS

Test Water

The pH, alkalinity, temperature, and DO of the test water varied little during the 12-h treatments (Table 1). Coefficients of variation (CV) for pH, alkalinity, and temperature were all within 2% of the means, whereas CV for DO were all within 7% of the means.

Table 1. Mean pH, alkalinity, temperature, and dissolved oxygen during 12-h lampricide treatments (3-trifluoromethyl-4-nitrophenol (TFM) and TFM:1% niclosamide) conducted on three species of unionid mussel, water beetles (*Haliplus* spp.), American eel, and sea lamprey. Means calculated from hourly recorded values.

Test species	Lampricide	pH	Alkalinity ($mg \bullet L^{-1}$ as $CaCO_3$)	Temperature (^oC)	Dissolved oxygen ($mg \bullet L^{-1}$)
Giant floater	TFM	7.94	114	13.1	8.9
	TFM:1% Nic	7.96	114	13.1	9.0
Fragile papershell	TFM	8.01	115	12.9	8.4
	TFM:1% Nic	8.03	116	12.9	8.4
Pink heelsplitter	TFM	7.78	131	12.8	8.4
	TFM:1% Nic	7.79	131	12.8	8.1
Larval *Haliplus* spp., treatment 1	TFM	7.90	118	12.5	9.1
Larval *Haliplus* spp., treatment 2	TFM	8.02	106	12.6	9.0
Adult *Haliplus* spp.	TFM	8.03	118	12.6	9.4
American eel and sea lamprey	TFM	7.79	132	13.2	9.8
	TFM:1% Nic	7.83	121	13.3	8.8
American eel	TFM	7.92	114	13.4	8.9
	TFM:1% Nic	7.92	115	13.4	8.8

Mussels

Narcosis Observations

Narcosis was evident among giant floaters after 6 h of exposure to TFM and the TFM:1% niclosamide mixture at concentrations ≥2.0 times (5.1 mg•L^{-1} for TFM and 2.6 mg•L^{-1} for TFM:1% niclosamide) the observed MLC for sea lamprey larvae. Narcosis was observed at 12 h post-treatment in 20 of 40 giant floaters exposed to TFM concentrations ranging from 1.5 to 3.0 times (3.3 mg•L^{-1} and 6.3 mg•L^{-1} TFM, respectively) the observed MLC for sea lamprey (Table 2). Of those 20, eight recovered by 14 d post-treatment (Table 2). Similarly, narcosis was evident at 12 h post-treatment in 18 of 30 giant floaters at TFM:1% niclosamide concentrations from 2.0 to 3.2 times (2.6 mg•L^{-1} and 4.1 mg•L^{-1} as TFM, respectively) the observed MLC for sea lamprey (Table 2). Of those 18, six recovered by 14 d post-treatment (Table 2).

Neither the fragile papershell nor the pink heelsplitter showed narcosis in response to the TFM treatment even at the highest chemical concentrations (Tables 3, 4). Although 20% narcosis (4.9 mg•L^{-1} TFM) and 10% narcosis (3.3 mg•L^{-1} TFM) among fragile papershells were recorded at 12 h post-treatment, these mussels did not recover after 14 d post-treatment and could have been dead at 12 h post-treatment (Table 3). Narcosis was observed among pink heelsplitters exposed to the TFM:1% niclosamide mixture at 14 d post-treatment (Table 4) but only at greater than 2 times the MLC for sea lamprey larvae.

Table 2. Narcosis and mortality of giant floaters (N = 10) and mortality of sea lamprey (N = 10) after 12-h unreplicated exposures to 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture. Also shown is the mortality of giant floaters 14 d after the 12-h treatments (mortality could not be distinguished from narcosis at the end of the 12-h treatments). Coefficients of variation for TFM concentrations were all within 2% of the means.

TFM exposures				TFM:1% niclosamide exposures			
	Giant floater (%)				Giant floater (%)		
TFM concentration ($mg \cdot L^{-1}$)	Narcosis/ mortality 12 h	Mortality 14 d	Sea lamprey mortality (%)	TFM:1% niclosamide concentration ($mg \cdot L^{-1}$ as TFM)	Narcosis/ mortality 12 h	Mortality 14 d	Sea lamprey mortality (%)
6.3	100	60	100	4.1	70	30	100
5.1	50	30	100	3.3	70	50	100
4.1	30	20	100	2.6	40	40	100
3.3	20	10	100	2.1	0	0	100
2.6	0	0	100	1.7	20	20	100
2.1[a]	20	20	100	1.3[a]	10	10	100
1.7	—[b]	—[b]	30	1.1	—[b]	—[b]	40
1.4	—[b]	—[b]	10	0.8	—[b]	—[b]	0
1.1	—[b]	—[b]	0	0.6	—[b]	—[b]	0
Treatment control	0	10	0	Treatment control	0	0	0
Test control[c]	0	8	NA	Test control[c]	0	8	NA

[a]Observed sea lamprey minimum lethal concentration as per Klar and Young (2004).
[b]Not evaluated because of insufficient numbers of giant floaters.
[c]Test controls (N = 25) observed to 14 d post-treatment.

Table 3. Narcosis and mortality of fragile papershells (N = 10) and mortality of sea lamprey (N = 10) after 12-h replicated exposures to 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture. Also shown is the mortality of fragile papershells 14 d after the 12-h treatments (mortality could not be distinguished from narcosis at the end of the 12-h treatments). Coefficients of variation for TFM concentrations were all within 3% of the means.

TFM exposures				TFM:1% niclosamide exposures			
	Fragile papershell (%)				Fragile papershell (%)		
TFM concentration ($mg \cdot L^{-1}$)	Narcosis/ mortality 12 h	Mortality 14 days	Sea lamprey mortality (%)	TFM:1% niclosamide concentration ($mg \cdot L^{-1}$ as TFM)	Narcosis/ mortality 12 h	Mortality 14 days	Sea lamprey mortality (%)
4.9	20	30	100	3.9	20	30	100
4.1	0	0	100	3.2	10	20	100
3.3	10	10	100	2.6	0	20	100
2.7[a]	0	0	100	2.1	0	10	100
2.2	0	10	30	1.7	10	20	100
1.8	0	0	0	1.4[a]	0	0	100
1.4	0	0	0	1.1	0	0	10
1.1	—[b]	—[b]	0	0.8	—[b]	—[b]	0
0.9	—[b]	—[b]	0	0.7	—[b]	—[b]	0
Treatment control	0	0	0	Treatment control	0	0	0
Test control[c]	0	7.5	NA	Test control[c]	0	7.5	NA

[a]Observed sea lamprey minimum lethal concentration as per Klar and Young (2004).
[b]Not evaluated because of insufficient numbers of fragile papershells.
[c]Test controls (N = 20) observed to 14 d post-treatment.

Table 4. Narcosis and mortality of the pink heelsplitters (N = 10) and mortality of sea lamprey (N = 10) after 12-h unreplicated exposures to 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture. Also shown is the mortality of pink heelsplitters 14 d after the 12-h treatments (mortality could not be distinguished from narcosis at the end of the12-h treatments). Coefficients of variation for TFM concentrations were all within 4% of the means.

TFM exposures				TFM:1% niclosamide exposures			
	Pink heelsplitter (%)				Pink heelsplitter (%)		
TFM concentration ($mg \cdot L^{-1}$)	Narcosis/ mortality 12 h	Mortality 14 days	Sea lamprey mortality (%)	TFM:1% niclosamide concentration ($mg \cdot L^{-1}$ as TFM)	Narcosis/ mortality 12 h	Mortality 14 days	Sea lamprey mortality (%)
3.8	0	0	100	3.0	10	60	100
3.1	0	0	100	2.4	0	40	100
2.6	0	0	100	2.0	0	10	100
2.0[a]	0	0	100	1.6	0	0	100
1.7	0	0	30	1.3	0	0	100
1.4	0	0	0	1.0[a]	0	0	100
1.0	0	0	0	0.8	0	0	20
0.8	0	0	0	0.6	0	0	0
0.6	0	0	0	0.5	0	0	0
Treatment control	0	0	0	Treatment control	0	0	0
Test control[b]	0	5	NA	Test control[b]	0	5	NA

[a]Observed sea lamprey minimum lethal concentration as per Klar and Young (2004).
[b]Test controls (N = 20) observed to 14 d post-treatment.

Mortality Observations

Mortality of all three unionid species tested was <10% in both the treatment and test controls for the TFM treatments (Tables 2-4). The lampricides TFM and TFM:1% niclosamide were more toxic to sea lamprey larvae than to any of the mussel species (Tables 2-4). Of the three mussel species tested, the pink heelsplitter was the least affected by exposure to TFM (Table 4). The NOEC for pink heelsplitter (≥3.8 mg•L^{-1}) was greater than 1.9 times the observed sea lamprey MLC (2.0 mg•L^{-1}) and the TFM-treatment RQ for the pink heelsplitter was ≤0.92. The fragile papershell was intermediate in its sensitivity to TFM with an RQ of 0.99. No mortality was observed among fragile papershells exposed to TFM at 1.0 times or 1.5 times the observed MLCs for sea lamprey larvae (Table 3). However, 30% mortality occurred among those exposed to 1.8 times (4.9 mg•L^{-1} TFM) the observed sea lamprey MLC. TFM was the most toxic to the giant floater with an RQ of 1.21, a value indicating a substantial risk of mortality during lampricide treatments. Although no mortality occurred at 1.2 times (2.6 mg•L^{-1}) the observed sea lamprey MLC, 20% mortality occurred at the observed MLC for sea lamprey (2.1 mg•L^{-1} TFM) and 60% mortality occurred at 3.0 times (6.3 mg•L^{-1} TFM) the observed MLC (Table 2). A comparison of post-collection holding mortalities (prior to testing) among the three unionid species (31% for the giant floater; 2% for the fragile papershell; 1% for the pink heelsplitter) suggests that the giant floater may not be a suitable test species or that we may not have had optimal holding conditions for this species. Prior to transport to the UMESC, giant floaters were held in the Mississippi River where water temperatures were >27 °C and stress from the warm water may have increased their susceptibility to the lampricides.

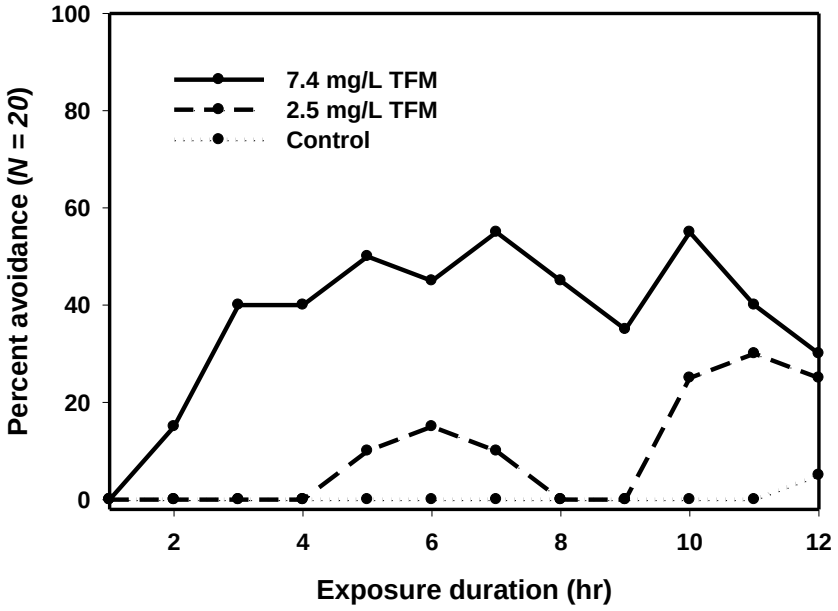
Unionid mortality was not observed in the TFM:1% niclosamide treatment controls, and mortality was low (<8%) in the test controls (Tables 2-4). Mortality of all three unionid species after exposure to the TFM:1% niclosamide mixture was <10% at concentrations 1.0-1.5 times the observed sea lamprey MLC but increased with increasing concentration (Tables 2-4). Calculated RQs for the three mussel species tested with the lampricide mixture were 0.93 for the giant floater, 1.50 for the fragile papershell, and 0.94 for the pink heelsplitter. The high RQ for the fragile papershell indicates a substantial risk of mortality from treatments using the TFM:1% niclosamide mixture.

Water Beetles

Behavioral Observations

Adult water beetles were not observed attempting to fly during the treatment period. They were observed crawling up the side of the plastic-mesh cage where they sometimes remained for the duration of the treatment. The water beetles began crawling out of the water after 2 h when exposed to 7.4 $mg \bullet L^{-1}$ TFM, 3 times the observed sea lamprey MLC (2.5 $mg \bullet L^{-1}$ TFM) and after 5 h when exposed to 1 time the observed sea lamprey MLC (Fig. 2). In the control treatments, water beetles rarely left the water. Water beetles in the 3 times sea lamprey MLC treatment were observed out of the water more often than those in the controls ($Z = 2.72$, $P = 0.01$). The location of adult water beetles in the 1 time sea lamprey MLC treatment was not different from that of adult water beetles in the controls ($Z = 1.66$, $P = 0.10$).

Fig. 2. The percentage of adult water beetles (*Haliplus* spp.) (*N* = 20) avoiding 2.5 and 7.4 mg•L^{-1} of 3-trifluoromethyl-4-nitrophenol (TFM) by crawling out of the water during 12-h exposures to the chemical. Also shown is the percentage of adult water beetles (*N* = 20) that were out of the water in the control aquarium.



Mortality Observations

No significant differences in water beetle mortality were observed between the two replicates within each of the three treatment sets ($P \leq 0.05$). Therefore, mortality results for replicates were combined (Tables 5, 6). No larval water beetles died in Treatment 1 at TFM concentrations ranging from 1.6 to 4.6 mg•L^{-1}, 0.5 to 1.5 times the observed sea lamprey MLC (3.0 mg•L^{-1} TFM) (Table 5). Although one larva died in the control aquaria, we may have injured the animal while extracting it from the mesh cage after test

completion. Larval water beetles exposed to TFM concentrations ranging from 4.9 to 9.4 mg•L^{-1}, 1.6 to 3.1 times the observed sea lamprey MLC in Treatment 2, suffered only one mortality, which was at 1.6 times (Table 5). The calculated RQ for larval water beetles was 0.48. Mortality of adult water beetles in Treatment 3 was minimal with only 6.7% mortality at a TFM concentration of 5.9 mg•L^{-1}, 2.4 times the observed sea lamprey MLC (2.5 mg•L^{-1}) and 3.3% mortality at a TFM concentration of 2.0 mg•L^{-1}, 0.8 times the observed sea lamprey MLC (Table 6). No mortalities of adult water beetles occurred at the other treatment concentrations or in the controls. The calculated RQ for adult water beetles was 0.51. Results of larval and adult water beetle treatments suggest that the risk of death to *B. hungerfordi* from exposure to TFM is small during typical stream treatments.

Table 5. Mortality of larval water beetles (*Haliplus* spp.) (*N* = 20) and sea lamprey (*N* = 20) after 12-h replicated (*N* = 3) exposure to 3-trifluoromethyl-4-nitrophenol (TFM) in Upper Midwest Environmental Sciences Center well water. Coefficients of variation for TFM concentrations were all within 3% of the means.

TFM concentration ($mg \bullet L^{-1}$)	Exposure set	Mortality (%)	
		Larval water beetles	Sea lamprey
9.4	2	0	—[a]
7.6	2	0	—[a]
6.1	2	0	—[a]
4.9	2	5	—[a]
4.6	1	0	100
3.7	1	0	100
3.0[b]	1	0	100
2.5	1	0	95
2.0	1	0	60
1.6	1	0	10
1.3	1	—[a]	0
1.0	1	—[a]	0
0.8	1	—[a]	0
0.0	1	5	0

[a]Test animals not added to these aquaria.
[b]Observed sea lamprey minimum lethal concentration.

Table 6. Mortality of adult water beetles (*Haliplus* spp.) (*N* = 30) and sea lamprey (*N* = 20) after 12-h treatments replicated (*N* = 3) to 3-trifluoromethyl-4-nitrophenol (TFM). Coefficients of variation for TFM concentrations were all within 2% of the means.

TFM concentration ($mg \bullet L^{-1}$)	Mortality (%)	
	Adult water beetles	Larval sea lamprey
7.4	0	100
5.9	6.7	100
4.8	0	100
3.8	0	100
3.2	0	100
2.5[a]	0	100
2.0	3.3	5
1.6	0	0
1.3	0	0
0.0	0	0

[a]Observed sea lamprey minimum lethal concentration.

American Eel

Mortality Observations

No significant differences in mortality were observed between the two replicates within each of Treatment sets 1 and 2 ($P \leq 0.05$). Therefore, mortality results between replicates were combined, making for 10 American eels per replicate. Two American eels died during Treatment 1, one at the highest TFM concentration tested (7.8 mg•L^{-1}), 2.9 times the observed sea lamprey MLC (2.7 mg•L^{-1}) and the other at 1.7 mg•L^{-1}, 0.6 times the observed sea lamprey MLC (Table 7). The American eel that died at 0.6 times the MLC had a severe wound in the jaw area that was not noted when the animal was transferred to the treatment aquarium, and the injury could have contributed to its death. In Treatment 2, there was a single mortality among American eel at the highest concentration of the TFM:1% niclosamide mixture tested (5.0 mg•L^{-1} as TFM), 3.1 times the observed sea lamprey MLC (1.6 mg•L^{-1} as TFM) (Table 7). In Treatment 3, 60% mortality was observed among American eel at the highest TFM concentration tested (23.4 mg•L^{-1}), 8.7 times the observed sea lamprey MLC (2.7 mg•L^{-1}), but there were no mortalities at any other test concentration (Table 8). In the TFM:1% niclosamide treatment, there was 100% mortality at the highest TFM concentration tested (14.3 mg•L^{-1}), 8.9 times the observed sea lamprey MLC (1.6 mg•L^{-1}); 80% at the second highest TFM concentration tested (11.7 mg•L^{-1}), 7.3 times; and 60% at the third highest TFM concentration tested (9.5 mg•L^{-1}), 5.9 times; with no mortalities at any other test concentration (Table 8). The RQ for American eel was 0.21 for TFM and 0.31 for the TFM:1% niclosamide mixture. Results from the three treatments suggest that application of either formulation of lampricide to control larval sea lamprey poses little risk to American eel.

Table 7. Mortality of American eel (N = 10) and sea lamprey (N = 20) after 12-h replicated (N = 2) exposures to 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture. Coefficients of variation for TFM concentrations were all within 2% of the means. Tests were conducted in June 2006.

TFM			**TFM:1% niclosamide**		
	Mortality (%)			**Mortality (%)**	
Concentration (mg•L^{-1})	**American eel**	**Sea lamprey**	**Concentration (mg•L^{-1} as TFM)**	**American eel**	**Sea lamprey**
7.8	10	100	5.0	10	100
6.3	0	100	4.0	0	100
5.1	0	100	3.3	0	100
4.2	0	100	2.6	0	100
3.3	0	100	2.1	0	100
2.7[a]	0	100	1.6[a]	0	100
2.1	0	65	1.3	0	40
1.7	10[b]	10	1.1	0	5
1.4	0	0	0.8	0	0
Control	0	0	Control	0	0

[a]Observed sea lamprey minimum lethal concentration.
[b]Test animal had a severe wound in the jaw area.

Table 8. Mortality of American eel (N = 5) after 12-h unreplicated exposures to 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture. Coefficients of variation for TFM concentrations were all within 1% of the means. Tests were conducted in July 2006.

TFM concentration (mg•L^{-1})	**Mortality (%)**	**TFM:1% niclosamide concentration (mg•L^{-1} as TFM)**	**Mortality (%)**
23.4	60	14.3	100
19.0	0	11.7	80
15.2	0	9.5	60
12.4	0	7.6	0
9.7	0	6.1	0
7.8	0	4.8	0
6.1	0	3.8	0
5.0	0	3.0	0
3.9	0	2.4	0
Control	0	Control	0

DISCUSSION

Among adults of the three unionids tested, the giant floater was the most sensitive to lampricide exposure. The calculated TFM RQ for the giant floater (1.21) indicates that there is a substantial risk of mortality among giant floaters in areas of maximum TFM concentration (1.5 times the sea lamprey MLC) (Table 9). The calculated TFM RQs for the fragile papershell (0.99) and pink heelsplitter (0.92) were both below 1.0 suggesting substantial mortalities among adults of these mussel species should not occur during TFM treatments (Table 9). However, because the RQs for these species approach 1.0, there is potential for some mortality to occur among adults in areas where TFM concentrations approach 1.5 times the sea lamprey MLC. Our conclusions should be viewed with caution, however, due to the lack of replication in the mussel treatments. Additionally, at the time these tests were conducted, little was known about the culture and testing of earlier life stages of mussels that may be more sensitive to

lampricides. Calculated RQs among adult mussels exposed to the TFM:1% niclosamide mixture (giant floater 0.93, fragile papershell 1.50, and pink heelsplitter 0.94) indicate an increased risk of mortality among fragile papershells to the TFM:1% niclosamide mixture over TFM alone (Table 9). However, because niclosamide is also used in some states as a molluscicide to control mussels and snails in aquaculture production ponds and because niclosamide is highly toxic to unionids, it is unlikely the TFM:1% niclosamide mixture would be applied to streams with known populations of these mussels as verified by pre-treatment surveys.

Table 9. Risk quotients (RQ) for selected nontarget species calculated from mortalities during 12-h exposures to 3-trifluoromethyl-4-nitrophenol (TFM) and a TFM:1% niclosamide mixture. The RQ is defined as 1.5 times the observed minimum lethal concentration (MLC) for sea lamprey divided by the no-observed-effect concentration (NOEC) for the nontarget species. The risk of mortality for the nontarget species during lampricide stream treatments increases as the RQ approaches 1, values around 1 indicate a moderate risk of mortality, and values much greater than 1 indicate a high risk of mortality. The RQs for adult unionids should be viewed with caution due to the lack of replication in the exposures.

Species and life stage	TFM (mg•L^{-1})			TFM:1% niclosamide (mg•L^{-1} as TFM)		
	Nontarget NOEC	Sea lamprey MLC	Sea lamprey MLC	Nontarget NOEC	Sea lamprey MLC	Sea lamprey MLC
Giant floater adult	2.6	2.1	1.21	2.1	1.3	0.93
Fragile papershell adult	4.1	2.7	0.99	1.4	1.4	1.50
Pink heelsplitter adult	≥3.8	2.0	≤0.92	1.6	1.0	0.94
Haliplus spp. larvae	9.4	3.0	0.48	___[a]	___[a]	___[a]
Haliplus spp. adult	7.4	2.5	0.51	___[a]	___[a]	___[a]
American eel yellow stage	19.0	2.7	0.21	7.6	1.6	0.31

[a]Not tested.

Overall, the two lampricides did not cause substantial narcosis among adults of the three unionid mussels tested at concentrations typically applied during stream applications to control sea lamprey larvae (Tables 2-4). Narcosis was evident among giant floaters exposed to TFM and the TFM:1% niclosamide mixture but rarely at concentrations ≤1.5 times the MLC for sea lamprey. Recovery from narcosis among giant floaters exposed to concentrations >1.5 times the MLC (37%) was similar to that observed by Bills et al. (1992), who noted 50% recovery of pink heelsplitters after exposure to TFM at concentrations 2.0 times the MLC for sea lamprey, indicating that some narcotized mussels will recover post-treatment. Narcosis was rarely evident, however, at lampricide concentrations typically applied to control sea lamprey. Among pink heelsplitters, narcosis was evident (TFM:1% niclosamide mixture only) but only at concentrations >2.0 times the sea lamprey MLC. Concentrations of this magnitude are unlikely during treatment operations where 1.5 times the sea lamprey MLC is the maximum

exposure level (Brege et al. 2003). Among fragile papershells, narcosis was not evident at any of the lampricide concentrations tested. Therefore, latent mortality of unionids related to narcosis should rarely occur during treatments. Again, because of the lack of replication, results should be viewed with caution.

Mortality was not evident among larval water beetles exposed to TFM concentrations up to 9.4 $mg \bullet L^{-1}$, 3.8 times the sea lamprey MLC, or among adults exposed to TFM concentrations up to 7.4 $mg \bullet L^{-1}$, 3.0 times the sea lamprey MLC. These lampricide levels are at least double the concentration typically encountered during treatments to control larval sea lamprey. The water beetle response to TFM suggests that the risk of mortality for larvae (RQ = 0.48, Table 9) and adults (RQ = 0.51, Table 9) of *B. hungerfordi* would be minimal during routine lampricide applications if their response to TFM is similar to that of the water beetles we tested.

Adult water beetles exposed to 7.4 $mg \bullet L^{-1}$, 3.0 times the observed sea lamprey MLC of TFM, limited their exposure to high TFM concentrations by leaving the water. Although we found no significant avoidance of TFM at concentrations similar to those maintained during lampricide treatments, the non-significant behavioral response may be an artifact of small sample size, and thus we cannot definitively predict whether adult water beetles would leave the water at treatment concentrations of TFM. Moreover, the potential avoidance behavior of *B. hungerfordi* is unknown as it is unclear if they have a dispersal flight similar to that of the water beetles we tested (Wilsmann and Strand 1990). Given our small sample size, (2 replicates), further testing will be required to fully evaluate whether adult water beetles avoid TFM concentrations similar to those used to control sea lamprey. If *B. hungerfordi* actively avoid treatment concentrations of TFM, they may be able to limit their exposure during treatment of the Carp Lake Outlet. Conversely, avoidance may result in the water beetles moving into areas of the stream unsuitable for their survival.

Results from the three treatments conducted on American eel indicate that TFM and TFM:1% niclosamide concentrations used for sea lamprey control in tributaries to the Great Lakes are not acutely toxic to them. In the two replicated treatments, excluding the death of one injured American eel, the lowest concentrations of TFM (7.8 $mg \bullet L^{-1}$ TFM) and the TFM:1% niclosamide mixture (5.0 $mg \bullet L^{-1}$ as TFM) with any mortality were still

about 3 times the observed sea lamprey MLC. In the unreplicated treatment, American eel mortality did not occur until lampricide concentrations approached 5 times the predicted sea lamprey MLC (23.4 mg•L^{-1} TFM and, for the TFM:1% niclosamide mixture, 9.5 mg•L^{-1} as TFM). Moreover, a check of historical treatment records revealed that no American eel mortalities were observed or reported during 455 lampricide applications to Lake Ontario tributaries since sea lamprey control began in that watershed in 1971 (W. Paul Sullivan, personal communication, 2010). In addition, since lampricides were first used to control sea lamprey in Great Lakes tributaries in 1958, over 3,000 treatments have been conducted in the U.S. and Canada, and no American eel mortalities have ever been reported to the U.S. Environmental Protection Agency or Canada's regulatory equivalent, Health Canada. The reporting of adverse incidents (e.g., nontarget mortalities) in the U.S. and Canada is required by pesticide regulations (Federal Register 1997; Department of Justice Canada 2006). Our tests (TFM RQ = 0.21, TFM:1% niclosamide RQ = 0.31; Table 9) and extensive field observations indicate that lampricide treatments pose little risk to American eel.

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