Acute toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) and a 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

Completion report prepared for
Lake Champlain Fish and Wildlife Management Cooperative
Vermont Department of Fish and Wildlife
111 West Street
Essex Junction, Vermont 05452

By
Michael A. Boogaard and Cynthia S. Kolar
U.S. Geological Survey
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

and
Diane L. Waller
Western Wisconsin Technical College
304 North Sixth Street
La Crosse, Wisconsin 54601

June 2004

U.S. Department of the Interior
U.S. Geological Survey

Suggested citation:

Boogaard, M. A., C. S. Kolar, and D. L. Waller. 2004. Acute toxicity of 3-trifluormethyl-4-nitrophenol (TFM) and a 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. Report submitted to the Lake Champlain Fish and Wildlife Management Cooperative, Essex Junction, Vermont, June 2004. 21 pp.

Contents

Introduction

The lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2'5-dichloro-4'-nitrosalicylanilide (niclosamide) have been used to control larval sea lamprey (*Petromyzon marinus*) in tributaries of the Great Lakes since the early 1960s. Niclosamide is presently used in combination with TFM to reduce the amount of TFM required to treat for sea lamprey, and as a granular bottom-release formulation to survey or control larval sea lamprey in lentic habitats. Application of the TFM:niclosamide combination is a cost-saving measure usually used where large quantities of TFM would normally be required to treat streams with high water flows. When used in combination, the TFM:niclosamide ratio ranges from 98.0:2.0 to 99.5:0.5 (Associate Committee on Scientific Criteria for Environmental Quality 1985). Niclosamide is also used outside the sea lamprey program as a molluscicide for aquatic snail control. Because of this, adverse effects of combination lampricide treatments with TFM and niclosamide on molluscs may be expected.

In recent years, there has been growing concern over the risk of lampricide applications to non-target fauna. The State of Vermont is especially concerned about aquatic species listed as threatened, endangered, or of special concern including several fishes, mussels, and amphibians native to tributary streams of Lake Champlain where lampricide treatments have been proposed. Because of these concerns, an interagency agreement was drafted between the Lake Champlain Fish and Wildlife Management Cooperative (LCFWMC) and the U.S. Geological Survey (USGS) to determine the acute effects of the lampricides to these native species. The Vermont Department of Fish and Wildlife, New York State Department of Environmental Conservation (NYSDEC), and the U.S. Fish and Wildlife Service (FWS) jointly manage the sea lamprey control effort in Lake Champlain under the framework of the LCFWMC (Fisheries Technical Committee 2001). The Great Lakes Fishery Commission, with interest in the entirety of the Great Lakes, has charged the USGS Upper Midwest Environmental Sciences Center (UMESC) with conducting acute toxicity tests with TFM and a TFM-1% niclosamide mixture on the giant floater (*Pyganodon grandis*), fragile papershell (*Leptodea fragilis*), and pink heelsplitter (*Potamilus alatus*) unionid mussels.

Freshwater mussels are an important part of the aquatic community in many waters, sometimes 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 (Turgeon et al. 1998). In the past century, the diversity and abundance of freshwater mussels have dramatically declined. About 70% of species are either 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).

The effects of TFM on several freshwater mussel 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). In general, these studies showed that many mussel species are resistant to TFM at concentrations typically applied to control sea lamprey larvae. In laboratory studies, for example, the threehorn wartyback (*Obliquaria reflexa)* was sensitive to TFM in the same concentration range as rainbow trout(*Oncorhynchus mykiss*) which has been shown to be unaffected by TFM concentrations up to 3.5 times the levels required to kill 100% of sea lamprey larvae. (Boogaard et al. 2003, Waller et al. 1993). In field trials using a TFM-1% niclosamide mixture (at two times the observed

minimum lethal concentration, or MLC, for sea lamprey larvae), Waller et al. (2003) found survival of Eastern elliptio (*Elliptio complanata*) and Eastern floater *(Pyganodon cataracta*) unaffected one year after exposure. Bills et al. (1992) showed that *Potamilus alatus* was sensitive to TFM, but 90% survived at concentrations typically encountered during stream treatments. Also, although 60% of mussels were initially narcotized (foot extended or shell gaped) at two times the TFM concentration required to kill sea lamprey, 50% of *P. alatus* recovered after the 14-day post-exposure observation period (Bills et al. 1992). Field and laboratory observations have since confirmed that TFM temporarily narcotizes unionids during exposure (Waller et al. 1998), but few quantitative data exist on the onset and duration of narcotization during treatment conditions. The concern is that narcotized mussels may be more vulnerable to predation and displacement current and that certain mussel species may be more sensitive to TFM.

Given the concerns of the State of Vermont over the presence of threatened and endangered unionid species in areas proposed for lampricide applications, and the limited number of unionid species for which toxicity data to the lampricides has been determined, toxicity testing of additional unionid species is warranted. The State of Vermont has listed ten unionid species as being endangered or threatened, seven of which are found in the Lake Champlain tributary reaches that may be treated with lampricides (B. Chipman, personal communication, Vermont Department of Fish and Wildlife). The agreement with LCFWMC called for the UMESC to conduct lampricide risk assessments on three of these species: the giant floater, fragile papershell, and pink heelsplitter.

Materials and Methods

Test Articles

The isopropanol solution of the sodium salt formulation of TFM used in this study, with an average concentration of 38% active ingredient, was manufactured by Hoescht, Frankfurt, Germany under the commercial name Lamprecid® and was supplied by FWS. The niclosamide formulation used was Bayluscide® 70% Wettable Powder, the aminoethanol salt formulation of niclosamide, with an average niclosamide concentration of about 59%, and manufactured by ProServe, Inc., Memphis, Tennessee. The TFM technical material used for test concentration confirmation was purchased from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Niclosamide technical material was purchased from Sigma Chemical Company, St. Louis, Missouri.

Test Animals

Sea lamprey ammocoetes (90–110 mm) were collected from the Carp River, Marquette County, Michigan on 2–3 July 2001, from Harlow Creek, Marquette County, Michigan on 11–13 June 2002, and the Brule River, Douglas County, Wisconsin on 2 July 2003, using methods in Fodale et al. (1997). Sea lamprey larvae were transported to the UMESC in a 370-L hatchery tank and were held in 200-L fiberglass tanks supplied with 6 cm of washed sand to allow for burrowing. They were held a minimum of 14 days prior to testing to assess delayed handling mortality.

About 260 *Pyganodon grandis* (mean length 97.1 mm, range = 63–167 mm) were collected from the Bark River, Jefferson County, Wisconsin on 18 July 2001 and the Yellow River, Wood County, Wisconsin on 20 July 2001. Mussels were transported to the UMESC in

100-L coolers using methods in Newton et al. (2001) and were placed in 1.7x1.7x0.6 m cages in a backwater area of the Mississippi River near the UMESC. After 14 days delayed mortality from handling and transport was assessed. The surviving 179 *P. grandis* were measured and uniquely marked by attaching a plastic numbered tag with waterproof adhesive to each mussel. Mussels were transported to the UMESC in 100-L coolers and placed in 3.0x0.6x0.6 m fiberglass acclimation tanks at the same water temperature as was observed in the cages prior to transport (23.9 °C). Water temperature was slowly reduced over 4 days to the test temperature of 12 °C (ASTM 2000). Four additional mussels died during temperature acclimation, leaving 175 *P. grandis* for testing. Mussels were fed algae (concentrated from a UMESC culture pond using 10 µm mesh plankton nets) daily during the temperature acclimation and 14-day post-exposure periods. Mussels were not fed during the exposure period.

A total of 205 *L. fragilis* (mean length 112.5 mm, range = 50–145 mm) were collected from Pool 2 of the Upper Mississippi River near Hastings, Minnesota on 6–17 July 2002. A total of 223 *Potamilus alatus* (mean length 127.1 mm, range = 90–158 mm) were collected from the Wisconsin River near Prairie Du Sac, Wisconsin on 18–22 June 2003. Due to high mortality of *Pyganodon grandis* during the holding phase in the river in 2001, *L. fragilis* and *Potamilus alatus* were transported directly to the UMESC to be held at 12 °C for the 14-day acclimation period. Five *L. fragilis* and three *P. alatus* died before tagging leaving 200 and 220, respectively, for testing. Tagging and feeding procedures for *L. fragilis* and *P. alatus* followed those of *Pyganodon grandis*.

Toxicity Tests

The limited number of individuals available did not allow us to replicate any of the treatment concentrations. Each test consisted of a 12-hour exposure period (to simulate a typical 12-hour lampricide treatment), during which test aquaria received one of nine chemical concentrations or untreated water (control), followed by a 14-day post-exposure recovery period. Exposures were conducted according to guidelines from the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), American Society for Testing and Materials (2000), and Standard Operating Procedures for Application of Lampricides in the Great Lakes Fishery Commission Integrated Management of Sea Lamprey Control Program (Klar and Schleen 2000) in a continuous-flow delivery system described by Garton (1980) with several modifications. Modifications were as follows. The dilution factor between chemical dilution cells was decreased from 50% to 20%. To minimize pH shifts, aeration was reduced by eliminating the float valve on the water supply, installing a large bore PVC (5.1 cm) 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). Duplicate dilution tubes were reduced to a single dilution tube, and plexiglass was replaced with double strength plate glass in the construction of the flow-through apparatus to eliminate the potential for adherence of niclosamide to the test chambers. Well water was used for all exposures. Water temperature, pH, and dissolved oxygen concentration were measured hourly in the test chambers throughout the 12-hour exposure period. Total alkalinity and total water hardness were determined in the control chamber at 0, 4, 8, and 12 hours according to methods described in the American Public Health Association (1989).

Stock solutions of the test material were prepared by diluting weighed portions of field grade TFM with deionized water or niclosamide with acetone. Test concentrations of TFM and niclosamide surrounded the expected MLC for sea lamprey based on the pH and total alkalinity of the test water (Klar and Schleen 2000). Water samples for analysis of TFM concentration were

removed from each test chamber hourly during the 12-hour exposure and were measured spectrophotometrically with a Beckman DU-640 spectrophotometer. Water samples for analysis of niclosamide concentration were removed every 2 hours and were measured with a Waters Millennium series 2010 LC Module I Plus high performance liquid chromatography (HPLC) 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.

Ten mussels were placed in each test chamber (containing 3–5 cm of washed sand) 24 hours before exposure. Because of the limited number of *P. grandis* and *L. fragilis*, mussels could not be added to all test chambers. Therefore, *P. grandis* was only added to the first six test chambers and the control chamber for the TFM and TFM-1% niclosamide exposures; while *L. fragilis* was added to the first seven test chambers and the control chamber for both exposures. Twenty five *P. grandis* and 40 *L. fragilis* remained in the holding tanks as controls (Table 1). Sufficient numbers of *Potamilus alatus* were available for all test and control chambers, with 20 remaining as controls (Table 1).

Table 1. Number of mussels available for testing, lampricide concentrations tested, and number of each species of mussels remaining in holding tanks as holding controls.

Species	Total individuals available for testing	Concentrations tested (mg/L)		Mussels remaining in holding tank
		TFM exposure	99% TFM–1% niclosamide exposure[a]	
Pyganodon grandis	175	6.3, 5.1, 4.1, 3.3, 2.6, 2.1 0.0	4.1, 3.3, 2.6, 2.1, 1.7, 1.3, 0.0	25
Leptodea fragilis	200	4.9, 4.1, 3.3, 2.7, 2.2, 1.8, 1.4, 0.0	3.9, 3.2, 2.6, 2.1, 1.7, 1.4, 1.1, 0.0	40
Potamilus alatus	220	3.8, 3.1, 2.6, 2.0, 1.7, 1.4, 1.0, 0.8, 0.6, 0.0	3.0, 2.4, 2.0, 1.6, 1.3, 1.0, 0.8, 0.6, 0.5, 0.0	20

[a]Concentrations reported in mg/L as TFM

Sea lamprey larvae were included in the exposures for direct toxicological comparison. For comparison, ten sea lamprey larvae were placed in wire cages and suspended above the sand substrate in each test and control chamber containing mussels. Sea lamprey mortalities were recorded hourly throughout the 12-hour exposure period and again at 12 hours post-exposure.

Observations of mussel narcotization behavior (which was defined as mussels with shells open, foot extension or loss of burrowing ability) were recorded hourly during the exposure period and at 36 hours post-exposure. Mussels were returned to the holding tanks at 36 hours post-exposure and final mortality was recorded at 14 days post-exposure. Because it was impossible to differentiate between mussels that were narcotized from those that were dead without extensive probing, which could increase stress and influence mortality, mussel mortalities were only recorded at 14 days post-exposure. Criteria for recovery from narcotization included re-closing of shell, active siphoning, and re-burrowing. Mussels that did not exhibit recovery after 14 days post-exposure were considered dead.

Results and Discussion

Water Quality

Mean values and ranges of water temperature, pH, total alkalinity, hardness, and dissolved oxygen for toxicity tests for all three mussel species are presented in Table 2.

Table 2. Mean water quality data (range) taken during toxicity tests of three species of unionids exposed to TFM or a TFM-1% niclosamide mixture.

Parameter	*Pyganodon grandis*	*Leptodea fragilis*	*Potamilus alatus*
Water temperature (°C)	13.1 (12.8–13.3)	12.9 (12.6–13.1)	12.8 (12.6–13.1)
pH	7.95 (7.84–8.07)	8.01 (7.94–8.15)	7.79 (7.73–7.85)
Total alkalinity (mg/L as $CaCO_3$)	114 (110–120)	116 (114–118)	131 (130–132)
Total hardness (mg/L as $CaCO_3$)	144 (140–150)	147 (144–150)	165 (164–168)
Dissolved oxygen (mg/L)	9.0 (8.4–10.3)	8.4 (7.9–8.7)	8.3 (7.7–8.7)

Behavioral Observations

We must note that because death and narcotization are indistinguishable, some of the mussels considered narcotized at 12-hour and 36-hour post-exposure may have been dead. Therefore, a quantitative assessment of mussel recovery from narcosis cannot be done. By comparing 12-hour and 36-hour post-exposure mortality/narcosis data with 14-day final mortality

data, we can determine if narcosis did occur. A decrease in mortality/narcosis from 12-hour and 36-hour post-exposure to 14-day post-exposure would indicate narcosis (Tables 3 – 5). It was clear that mussels that did not recover by 14 d post-exposure were dead.

After 6 hours, mortality/narcosis was evident among *Pyganodon grandis* in the TFM exposure and in the TFM-1% niclosamide mixture in concentrations at or greater than 2.0 times the observed MLC for sea lamprey larvae (5.1 mg/L for TFM and 2.6 mg/L for TFM-1% niclosamide; Tables 3 and 4). By 36 hours post-exposure, many *P. grandis* exposed either to TFM or to the TFM-1% niclosamide mixture that displayed some form of mortality/narcosis had recovered. In the TFM exposure, mortality/narcosis was observed 12-hour post-exposure in 20 of 40 mussels exposed to TFM concentrations ranging from 1.5 to 3.0 times the MLC for sea lamprey. Of those 20, eight had recovered by the 14 d post-exposure period. Similarly, in the TFM-1% niclosamide exposure, mortality/narcosis was evident 12 hours post-exposure in 18 of 30 mussels at concentrations from 2.0 to 3.2 times the MLC for sea lamprey. Of the 18, six had recovered by 14 days post-exposure. These results were similar to those observed by Bills et al. (1992), who noted 50% recovery of *Potamilus alatus* after exposure to TFM at concentrations 2.0 times the MLC for sea lamprey, and indicate that some narcotized mussels will recover through time. Narcosis, however, could increase mortality of mussels during chemical treatments of streams by increasing their vulnerability to predation or current displacement. Additional field studies would be necessary to determine if mortality related to narcosis is significant. Narcosis was not evident, however, at lampricide concentrations typically applied to control sea lampreys. Therefore, latent mussel mortality related to narcosis would likely not occur.

Neither *L. fragilis* nor *P. alatus* showed narcosis in response to TFM exposure even at the highest chemical concentrations (Table 3 and 4). Exposure of *P. alatus* to the TFM-1% niclosamide mixture, however, showed narcosis at the highest concentrations tested 36 hours post-exposure (Table 5), but these concentrations were more than twice the MLC for sea lamprey larvae and would not be encountered during treatment operations. The absence of narcosis among *L. fragilis* and *P. alatus* may benefit these species if narcosis-related mortality is a contributing factor to the overall mortality from lampricide exposure.

Mortality Observations

Mortality data show that TFM and 99% TFM-1% niclosamide were more toxic to sea lamprey larvae than to any of the unionid species (Tables 5 and 6). The observed MLC for sea lamprey larvae in the TFM exposures was between 2.0 and 2.7 mg/L (Table 5) and the no observed effect concentration (NOEC), or the greatest concentration at which no mortality was observed, for the unionid species was between 2.6 mg/L and 4.1 mg/L. The predicted MLCs for sea lamprey larvae exposed to TFM at the water qualities observed (pH 7.9 and alkalinity 114 mg/L as $CaCO_3$, for the *Pyganodon grandis* test, pH 8.0 and alkalinity 116 mg/L as $CaCO_3$ for the *L. fragilis* test, and pH 7.8 and alkalinity 131 mg/L as $CaCO_3$ for the *Potamilus alatus* test) were 2.3 mg/L, 2.7 mg/L, and 2.2 mg/L respectively (Klar and Schleen 2000).

Of the three mussel species tested, *P. alatus* was the least affected by exposure to TFM because no mortality 14 days post-exposure was observed (Table 5). The NOEC for *P. alatus* (3.8 mg/L) was 1.9 times greater than the observed sea lamprey MLC (2.0 mg/L; Table 5). *Leptodea fragilis* was intermediate in its toxicity to TFM. No mortality was observed among *L. fragilis* exposed to TFM at the observed or 1.5 times the observed MLCs for sea lamprey larvae (Table 5). However, 30% mortality of *L. fragilis* occurred among those exposed to 4.9 mg/L TFM (1.8 times the observed sea lamprey MLC).

Of the mussel species tested, TFM was the most toxic to *Pyganodon grandis*. Twenty percent mortality occurred at 2.1 mg/L TFM (the observed MLC for sea lamprey) and 60% mortality occurred at 6.3 mg/L TFM (3.0 times the observed MLC), although no mortality was observed at 2.6 mg/L (1.2 times the observed MLC). Mortality of all three unionid species was $\leq$ 10% in both the exposure and holding controls for the TFM exposures (Table 5).Mortality data of all three unionid species after exposure to the TFM-1% niclosamide mixture was < 10% at concentrations near the observed (Table 6) or the predicted sea lamprey MLC (can be found in Klar and Schleen 2000). At about two times the observed sea lamprey MLC, mortality was 10% in *Potamilus alatus*, 20% in *L. fragilis*, and 40% in *Pyganodon grandis*. At chemical concentrations about 2.5 times the observed MLC, mortality was 20% in *L. fragilis*, 40% in *Potamilus alatus*, and 50% in *Pyganodon grandis.* No unionid mortality was observed in the TFM-1% niclosamide exposure controls, and low mortality ($\leq$ 8%) was observed in the holding controls (Table 6).

The high *P. grandis* mortality (81 of 260 or 31%) before tagging and transport to the UMESC prompted a change from holding mussels in the Mississippi River prior to experimentation. Water temperatures in the area of the river exceeded 27 °C for several days during the period mussels were caged. The added stress from high water temperatures coupled with the stress of handling and transport may have been a factor in the high mortalities observed before transport to the UMESC. In addition, *P. grandis* populations commonly experience die-offs in mid to late summer (Diane Waller, personal communication) and numerous *P. grandis* shells were observed floating in the river during this time. Transporting *L. fragilis* and *Potamilus alatus* directly to the UMESC in holding tanks at 12 °C resulted in a significant reduction in mortality prior to tagging (5 of 205 for *L. fragilis* and 3 of 223 for *P. alatus*).

Table 3. Narcosis (shell gaped and/or foot extended) and mortality observations of giant floater (*Pyganodon grandis, n* = 10*)* after a 12-hour exposure to TFM and a 99%TFM-1%niclosamide mixture. Mortality (12 hour post-exposure) of sea lamprey (*Petromyzon marinus*, *n* = 10) larvae exposed to the lampricides concurrently with unionids is also reported.

TFM exposure					99%TFM-1% niclosamide exposure				
TFM concentration (mg/L)	Mussel narcosis or mortality			Sea lamprey percent mortality	TFM-niclosamide concentration (mg/L–ug/L)	Mussel narcosis or mortality			Sea lamprey percent mortality
	Percent mortality/narcosis		Percent mortality			Percent mortality/narcosis		Percent mortality	
	12 h	36 h	14 d			12 h	36 h	14 d	
6.3	100	60	60	100	4.1 – 41	70	60	30	100
5.1	50	40	30	100	3.3 – 33	70	50	50	100
4.1	30	30	20	100	2.6 – 26	40	40	40	100
3.3	20	10	10	100	2.1 – 21	0	0	0	100
2.6	0	0	0	100	1.7 – 17	20	20	20	100
2.1[a]	20	20	20	100	1.3 – 13[a]	10	10	10	100
1.7	---[b]	---[b]	---[b]	30	1.1 – 11	---[b]	---[b]	---[b]	40
1.4	---[b]	---[b]	---[b]	10	0.8 – 8	---[b]	---[b]	---[b]	0
1.1	---[b]	---[b]	---[b]	0	0.6 – 6	---[b]	---[b]	---[b]	0
Exposure control[c]	0	0	10	0	Exposure control[c]	0	0	0	0
Holding control[d]	0	0	8	NA	Holding control[d]	0	0	8	NA

[a]Observed minimum lethal concentration for sea lamprey
[b]Test concentrations not conducted because of insufficient numbers of organisms
[c]Exposure controls (*n* = 10) added to untreated exposure control aquarium
[d]Holding controls (*n* = 25) remained in holding tank from transport to the UMESC to 14 days post-exposure

Table 4. Narcosis (shell gaped and/or foot extended) and mortality observations of fragile papershell (*Leptodea fragilis, n*

TFM exposure					99%TFM-1% niclosamide exposure				
TFM concentration (mg/L)	Mussel narcosis or mortality			Sea lamprey percent mortality	TFM-niclosamide concentration (mg/L–ug/L)	Mussel narcosis or mortality			Sea lamprey percent mortality
	Percent mortality/narcosis		Percent mortality 14 d			Percent mortality/narcosis		Percent mortality 14 d	
	12 h	36 h				12 h	36 h		
4.9	20	30	30	100	3.9 – 41	20	20	30	100
4.1	0	0	0	100	3.2 – 36	10	20	20	100
3.3	10	10	10	100	2.6 – 28	0	0	20	100
2.7[a]	0	0	0	100	2.1 – 22	0	0	10	100
2.2	0	0	10	30	1.7 – 18	10	10	20	100
1.8	0	0	0	0	1.4 – 14[a]	0	0	0	100
1.4	0	0	0	0	1.1 – 11	0	0	0	10
1.1	---[b]	---[b]	---[b]	0	0.8 – 10	---[b]	---[b]	---[b]	0
0.9	---[b]	---[b]	---[b]	0	0.7 – 7	---[b]	---[b]	---[b]	0
Exposure control[c]	0	0	0	0	Exposure control[c]	0	0	0	0
Holding control[d]	0	0	7.5	NA	Holding control[d]	0	0	7.5	NA

= 10*)* after a 12-hour exposure to TFM and a 99%TFM-1% niclosamide mixture. Mortality (12 hour post-exposure) of sea lamprey (*Petromyzon marinus, n* = 10) larvae exposed to the lampricides concurrently with unionids is also reported.

[a]Observed minimum lethal concentration for sea lamprey

[b]Test concentrations not conducted because of insufficient numbers of organisms

[c]Exposure controls (n = 10) added to untreated exposure control aquarium

[d]Holding controls (n = 40) remained in holding tank from transport to the UMESC to 14 days post-exposure

Table 5. Narcosis (shell gaped and/or foot extended) and mortality observations of pink heelsplitter (*Potamilus alatus, n* = 10) after a 12-hour exposure to TFM and a 99%TFM-1% niclosamide mixture. Mortality (12 hour post-exposure) of sea lamprey (*Petromyzon marinus, n* = 10) larvae exposed to the lampricides concurrently with unionids is also reported.

TFM exposure					99%TFM-1% niclosamide exposure				
TFM concentration (mg/L)	Mussel narcosis or mortality			Sea lamprey percent mortality	TFM-niclosamide concentration (mg/L–ug/L)	Mussel narcosis or mortality			Sea lamprey percent mortality
	Percent mortality/narcosis		Percent mortality 14 d			Percent mortality/narcosis		Percent mortality 14 d	
	12 h	36 h				12 h	36 h		
3.8	0	0	0	100	3.0 – 42	10	80	60	100
3.1	0	0	0	100	2.4 – 34	0	50	40	100
2.6	0	0	0	100	2.0 – 268	0	40	10	100
2.0 [a]	0	0	0	100	1.6 – 22	0	0	0	100
1.7	0	0	0	30	1.3 – 17	0	0	0	100
1.4	0	0	0	0	1.0 – 15 [a]	0	0	0	100
1.0	0	0	0	0	0.8 – 10	0	0	0	20
0.8	0	0	0	0	0.6 – 9	0	0	0	0
0.6	0	0	0	0	0.5 – 7	0	0	0	0
Exposure control [b]	0	0	0	0	Exposure control [b]	0	0	0	0
Holding control [c]	0	0	5	NA	Holding control [c]	0	0	5	NA

[a]Observed minimum lethal concentration for sea lamprey

[b]Exposure controls (n = 10) added to untreated exposure control aquarium

[c]Holding controls (n = 40) remained in holding tank from transport to the UMESC to 14 days post-exposure

Summary

The results of this study should be applied cautiously as the chemical treatments could not be replicated due to insufficient numbers of individuals for testing. As stated earlier, *Pyganodon grandis* were the most sensitive of the unionids to the lampricides. A comparison of post-collection holding mortalities (prior to tagging) among the three species of unionids (81 of 260, or 31% for *P. grandis*; 5 of 205, or 2% for *L. fragilis*; and 3 of 223, or 1% for *Potamilus alatus*) brings into question the health of *Pyganodon grandis* prior to testing, however. The added stress of holding *P. grandis* in the river when water temperatures were high (>27 °C) and that the species often experiences a natural die-off in mid to late summer may have been a factor in the deaths observed during testing. Also, the atypical toxicological dose-response curve observed among *P. grandis* is further indicative of organisms of questionable health. In a normal dose-response curve, mortalities decrease with decreasing exposure concentrations. This was not the case with *P. grandis*, however. Final mortalities among *P. grandis* exposed to TFM were 0% at 2.6 mg/L yet 20% died at 2.1 mg/L, and in the 99% TFM-1% niclosamide exposure, mortalities were 0% at 2.1 mg/L (as TFM) but were 20% at 1.7 mg/L and 10% at 1.3 mg/L (as TFM; Tables 5 and 6).

Overall, the lampricide TFM and the 99% TFM-1% niclosamide mixture did not cause substantial narcosis or mortality among any of the three unionid mussel species tested at concentrations typically applied during stream applications to control sea lamprey larvae (Tables 3–6). Narcosis was evident among *P. grandis* exposed to TFM and the 99%TFM-1% niclosamide mixture and P. alatus (99% TFM-1% niclosamide mixture only) but only at concentrations far greater than what is typically applied to control sea lampreys. Lampricide concentrations up to 1.5 times the observed MLC for sea lamprey did not cause significant narcosis among the unionids tested (Tables 3–4). Occasionally, lampricide concentrations may be applied at 1.5 times the MLC for sea lamprey larvae at selected points within the stream to compensate for attenuation of the chemical bank (Klar and Schleen 2000). Even at this concentration, mortalities among *P. grandis*, *L. fragilis*, and *Potamilus alatus* would be minimal. These results are similar to those reported on the toxicity of the lampricides to *E. complanata* (0% at 1.8 times the sea lamprey MLC; Waller et al. 2003), *Pyganodon cataracta* (20% at 1.8 times the sea lamprey MLC; Waller et al. 2003), and *Potamilus alatus* (10% at 2.0 times the sea lamprey MLC, Bills et al. 1992).

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard methods for the examination of water and wastewater, 17th edition. APHA, Washington, D.C.

ASTM, American Society for Testing and Materials. 2000. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-96. In: 2000 Annual Book of ASTM Standards, American Society for Testing and Materials, West Conshohocken, PA. 11.05: 213–233.

Associate Committee on Scientific Criteria for Environmental Quality. 1985. TFM and Bayer 73: Lampricides in the Aquatic Environment. National Research Council Canada, Ottawa, Canada. NRCC No. 22488.

Bills, T. D. and D. A. Johnson. 1992. Effect of pH on the toxicity of TFM to sea lamprey and non-target species during a stream treatment. Great Lakes Fishery Commission Technical Report No. 57: 7–19.

Bills, T. D., J. J. Rach, L. L. Marking, and G. E. Howe. 1992. Effects of the lampricide 3-trifluoromethyl-4-nitrophenol on the pink heelsplitter. U.S. Fish and Wildlife Service, Resource Publication 183. 7 pp.

Boogaard, M.A., T.D. Bills, and D.A. Johnson. 2003. Acute toxicity of TFM and a TFM/niclosamide mixture to selected species of fish, including lake sturgeon (*Acipenser fulvescens*) and mudpuppies (*Necturus maculosus*), in laboratory and field exposures. Journal of Great Lakes Research. 29, Supplement 1: 529-541.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. National Environmental Research Center, U.S. Environmental Protection Agency. EPA660/3-75-009. 61 p.

Dawson, V. K. 1982. A rapid high-performance liquid chromatography method for simultaneously determining the concentrations of TFM and Bayer 73 in water during lampricide treatments. Canadian Journal of Fisheries and Aquatic Sciences 39: 778–782.

Fisheries Technical Committee. 2001. Draft supplemental environmental impact statement - A long-term program of sea lamprey control in Lake Champlain. Lake Champlain Fish and Wildlife Management Cooperative. 356 pp.

Fodale, M., H. Quinlan, M. Towhey, G. Barner, D. Ollila, D. Cuddy, J. Weise, M. Stevens, J. Slade, S. Morkert, P. Sullivan, and A. Gonzalez. 1997. Larval assessment sampling protocol using the AbP-2 backpack shocker in Great lakes streams. 10pp.

Garton, R. R. 1980. A simple continuous-flow toxicant delivery system. Water Research 14: 227–230.

Klar, G. T. and L. P. Schleen. 2000. Standard operating procedures for application of lampricides in the Great Lakes Fishery Commission integrated management of sea lamprey (*Petromyzon marinus*) control program. U.S. Fish and Wildlife Service Technical Report. Special Report 92-001.3. USFWS, Marquette Biological Station, Marquette, MI. 912 pp.

Master, L. L., B. A. Stein, L. S. Kutner, and G. A. Hammerson. 2000. Vanishing assets: conservation status of U.S. species. In: Stein, B.A, L.S. Kutner, and J.S. Adams, editors. Precious heritage: The status of biodiversity in the United States. Oxford, UK: Oxford University Press 93–118.

Newton, T. J., E. M. Monroe, R. Kenyon, S. Gutreuter, K. I. Welke, and P. A. Thiel. 2001. Evaluation of relocation of unionid mussels into artificial ponds. Journal of the North American Benthological Society 20: 468–485.

Ökland, J. 1963. Notes of the population density, age distribution, growth, and habitat of *Anodonta piscinalis* Nilss. (Moll., Lammellibr.) in a eutrophic Norwegian lake. Nytt Magasin for Zoologi 11:19–43.

Rye, R. P., Jr., and King, E. L., Jr. 1976. Acute toxic effects of two lampricides to twenty-one freshwater invertebrates. Transactions of the American Fisheries Society 105: 322–326.

Smith, A. J. 1967. The effects of the lamprey larvicide, 3-trifluoromethyl-4-nitrophenol on selected aquatic invertebrates. Transactions of the American Fisheries Society 96: 410–413.

Turgeon, D. D., A. E. Bogan, E. V. Coan, W. K. Emerson, W. G. Lyons, W.L. Pratt, E. F .E. Roper, A. Scheltema, F. G. Thompson, and J. D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and CanadaBMollusks. Special Publication No. 26: Bethesda, Maryland, American Fisheries Society. 277p.

Waller, D. L., Rach, J. J., Marking, L. L., Cope, W. G., Fisher, S. W., and H. Dabrowska. 1993. Toxicity of candidate chemicals for zebra mussel control to selected non-target organisms. Journal of Great Lakes Research 19: 695–702.

Waller, D. L., Rach, J. J., Luoma, J. A. 1998. Acute toxicity and accumulation of the piscicide 3-trifluoromethyl-4-nitrophenol (TFM) in freshwater mussels (*Bivalvia: Unionidae*). Ecotoxicology 7: 113–121.

Waller, D. L., T. D. Bills, M. A. Boogaard, D. A. Johnson, and T. C. J. Doolittle. 2003. Effects of lampricide exposure on the survival, growth, and behavior on the unionid mussels *Elliptio complanata* and *Pyganodon cataracta*. Journal of Great Lakes Research xx: xxx-xxx Know these yet?.

Williams, J. D., M. L.Warren, Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries (Bethesda) 18: 6–22.