GREAT LAKES FISHERY COMMISSION

2020 Project Completion Report[1]

Lampricide Residues in Sea Lamprey Larvae Carcasses recovered after 3-trifluoromethyl-4-nitrophenol (TFM) or TFM/Bayluscide Stream Treatments

by:

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Abstract

Lampricide concentrations in whole larval sea lamprey (*Petromyzon marinus*) carcasses collected after lampricide treatments were determined to support risk assessment for non-target organisms that may consume lampricide-laden carcasses. Dead larvae were collected by Sea Lamprey Control personnel following the Ford River (Delta County, Michigan) 4.1 $mg \cdot L^{-1}$ 3-trifluoromethyl-4-nitrophenol (TFM) treatment, Sturgeon River (Baraga County, Michigan) 0.64 $mg \cdot L^{-1}$ /7.1 $\mu g \cdot L^{-1}$ TFM/niclosamide treatment, and two treatments on the Chippewa River (Isabella County, Michigan). The upper reach of the Chippewa River was treated with 3.1 $mg \cdot L^{-1}$ /33 $\mu g \cdot L^{-1}$ TFM/Bayluscide and the lower reach was treated with 4.1 $mg \cdot L^{-1}$ TFM. Carcasses were removed from each stream via scap nets immediately after treatment completion. To assess instream degradation, half of the collected carcasses from both Chippewa River treatments were placed in cages and returned to the river for 2 days before they were analyzed for lampricide residues. The estimated mean and standard error of the mean (SEM) TFM concentration in the fresh carcasses (n = 80) collected from all the TFM and TFM/Bayluscide treated rivers was 4.6 $\mu g \cdot g^{-1}$ (SEM =1.1 $\mu g \cdot g^{-1}$). The mean concentration of niclosamide (the active ingredient in Bayluscide) in the fresh carcasses (n = 40) from the two rivers treated with TFM/ Bayluscide was 0.49 $\mu g \cdot g^{-1}$ (SEM = 0.21 $\mu g \cdot g^{-1}$). The mean 2-day postmortem carcasses from the Chippewa River TFM/Bayluscide treatment contained 0.14 $\mu g \cdot g^{-1}$ (3%) of the TFM and 0.41 $\mu g \cdot g^{-1}$ (64%) of the niclosamide found in the fresh-carcass group (4.4 $\mu g \cdot g^{-1}$ TFM and 0.64 $\mu g \cdot g^{-1}$ niclosamide). The mean 2-day postmortem carcasses from the Chippewa River TFM treatment contained 0.72 $\mu g \cdot g^{-1}$ (12%) compared to the 6.1 $\mu g \cdot g^{-1}$ of TFM found in the fresh-carcass group.

Introduction

Two active ingredients are used in lampricide formulations to control larval sea lamprey (*Petromyzon marinus*) populations in Great Lakes tributaries (Dawson, 2003; Hubert, 2003). The most commonly used lampricide formulations contain the active ingredient 3-trifluoromethyl-4-nitrophenol (TFM). The other active ingredient, 2',5-dichloro-4'-nitrosalicylanilide (niclosamide), is used in Bayluscide® formulations. TFM is applied alone or with $1\%_{(w/w)}$ Bayluscide. The addition of Bayluscide reduces the concentration of TFM required to kill larval sea lamprey (Dawson, 2003; Hubert 2003; Solomon, 2019). A granular formulation of Bayluscide (Bayluscide 3.2% Granular Sea

Lamprey Larvicide) is used to assess larval sea lamprey populations and to treat areas where the use of TFM would be cost prohibitive (Dawson, 2003).

Assessing the effects of the lampricides on non-target organisms is required to maintain the registration of the lampricides. The Sea Lamprey Control Program monitors, reports, and implements protocols to reduce unreasonable adverse non-target effects to fulfill section 6(a)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act, section 7 of the Endangered Species Act, and the annual National Pollutant Discharge Elimination System reports for the states of Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin (Solomon, 2019). Sea lamprey larvae that have succumbed to the lampricide and have exited their burrows are carried downstream during lampricide treatments. The lampricide-laden carcasses are susceptible to consumption by a variety of non-target fish and birds during or shortly after stream treatments (Cochran, 2009; Gilderhus, 1979).

To evaluate species-specific risk resulting from a lampricide treatment, data on toxicity and chemical exposure are required. The acute toxicity from water-borne exposures to TFM or TFM plus Bayluscide have been reported in a variety of species (Applegate and King, 1962; Boogaard et al., 2003; Dawson et al., 1982, Gilderhus and Johnson, 1980). Oral toxicity data for fish, amphibians, and reptiles are lacking; however, some data on the lethal doses to cause death in 50% of test animals (LD50) on four species of birds exists (Hudson, 1979). Two of the species, mallards (*Anas platyrhynchos*) and ring-billed gulls (*Larus delawarensis*) have been observed feeding on sea lamprey larvae following lampricide treatments (Chiotti et al., 1987; Cochran, 2009). Acute single dose oral LD50 values of TFM to 1-year old drake mallards and immature ring-billed gulls are 108 $mg \cdot kg^{-1}$ and 87.5 $mg \cdot kg^{-1}$, respectively. The LD50 values for 14- to 17-weeks old mallard drakes and immature to adult ring-billed gulls from single oral exposure to field-grade TFM combined with Bayluscide (2% as niclosamide) were 165 mg TFM+niclosamide$\cdot kg^{-1}$ and 54 mg TFM+niclosamide$\cdot kg^{-1}$, respectively (Hudson 1979). Hudson (1979) did not include any data on the slopes of the toxicity curves for either lampricide. While the oral LD50 values exist for these birds, the chemical exposure data are sparse. Concern over the common tern (*Sterna hirundo*) dying from consuming sea lamprey larvae killed during Bayluscide treatments led to a laboratory study on the whole-body concentration of niclosamide in dead larvae (Hubert et al., 1999). Hubert et al. reported the mean whole-body burden of niclosamide was 3.53 $mg \cdot kg^{-1}$ (standard error of the mean (SEM) = 0.36 $mg \cdot kg^{-1}$) in larvae treated at the standard application rate with granular Bayluscide or

at 200 µg niclosamide·L^{-1} using the 70% Wettable Powder formulation. There are no data on the lampricide concentration in sea lamprey carcasses after TFM or combination TFM/Bayluscide treatments. Determining the missing concentration data will help to support risk assessments for non-target organisms that may consume lampricide-laden carcasses.

Objective

- The objective of this study was to determine the whole-body burden of TFM and niclosamide in larval sea lamprey killed during TFM or TFM/Bayluscide lampricide treatments. The whole-body burdens were evaluated immediately after death and 2 days after death. The objective of the study was met. The mean concentrations of TFM and nicolosamide in sea lamprey larvae carcasses ranged from 0.141 to 0.717 µg·g^{-1} and 0.343 µg·g^{-1} to 0.640 µg·g^{-1}, respectively. The body burden levels of TFM and niclosamide indicate that ring-billed gulls would need to consume in excess of 4,200 carcasses to achieve a body burden equivalent to the reported LD50.

Methods

Sample collection

Dead sea lamprey larvae were collected by Sea Lamprey Control personnel immediately after four lampricide treatments that were conducted using standard treatment protocols (Solomon, 2019). The Ford River was treated with TFM only (Figure 1). The Sturgeon River and the upper reach of the Chippewa River were treated with a combination of TFM and Bayluscide to achieve a niclosamide concentration equal to 1% of the TFM concentration. The lower Chippewa River was treated with TFM only, but it followed and was influenced by the TFM/ Bayluscide treatment in the upper reach of the river. Lampricide concentrations during each treatment varied and were based on ambient water quality and the treatment charts specified in the treatment protocols (Solomon, 2019). No additional water quality measurements were made beyond those collected by control agents to conduct the treatments. The two Chippewa River treatments included 2-day postmortem test groups (Table 1). Larvae carcasses were collected with scap nets and immediately placed in plastic bags on wet ice for up to 3 hours. Carcasses were temporarily stored at -20 °C, unless they were used for the 2-day postmortem assessment. Carcasses used for the 2-day postmortem assessments were

transferred to wire mesh cages and placed back in the river. Carcasses were removed from the cages 2 days later, placed on wet-ice for approximately 1 hour, then stored at -20 °C. Carcasses from the Ford and Sturgeon Rivers were transported to the U.S. Geological Survey Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin within 2 days of collection and stored at -80 °C until assayed for TFM and niclosamide. Carcasses from both Chippewa River treatments were held at -20 °C until after the final 2-day postmortem carcasses were removed from the river. All Chippewa River carcasses were transported within 6 days of their initial collection to UMESC where they were stored at -80 °C until assayed for TFM and niclosamide.

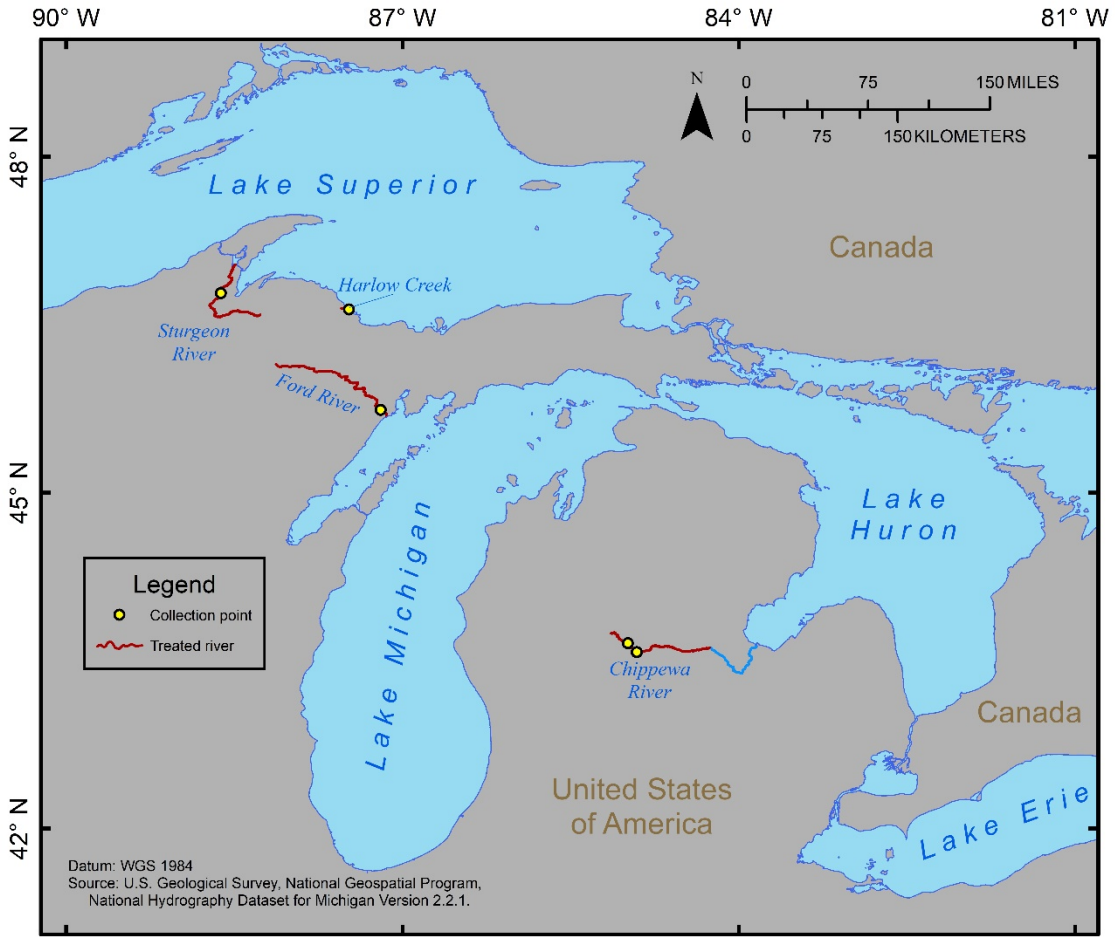

Figure 1. Locations in Michigan where sea lamprey carcasses were collected during TFM (Ford River near Schaffer and and lower Chippewa River near Will) and TFM/Bayluscide (Sturgeon River near Pelkie and upper Chippewa River near Lake Isabella) lampricide treatments and where sea lamprey were collected for analytical methods development (Harlow Creek).

Table 1. River, treatment date, location (watershed), collection condition and mean stream lampricide treatment concentration for each treatment where sea lamprey carcasses were collected for lampricide residue analyses

River (Date)	Location (Watershed)	Collection Condition	Mean Treatment Concentrations ($mg \cdot L^{-1}$ TFM / $\mu g \cdot L^{-1}$ niclosamide)
Ford (5/18/2010)	Delta County near Schaffer, Michigan (Lake Michigan)	Fresh	4.1 / -
Sturgeon (10/05/2010)	Baraga County near Pelkie, Michigan (Lake Superior)	Fresh	0.64 / 7.1
Chippewa (upper) (5/12/2012)	Isabella County upstream from Lake Isabella, Michigan (Lake Huron)	Fresh 2-Day Postmortem	3.1 / 33
Chippewa (lower) (5/13/2012)	Isabella County downstream from Lake Isabella near Winn, Michigan (Lake Huron)	Fresh 2-Day Postmortem	4.1 / -

Lampricide assay

An analytical method capable of assaying individual whole sea lamprey larvae carcasses for both TFM and niclosamide was developed and validated using larvae electrofished from Harlow Creek (Marquette Co., Michigan; 2012) and the Sturgeon River (Baraga County, Michigan; 2010) lampricide treatment. Twenty carcasses per group (120 total) were assayed to determine whole body burdens for TFM and niclosamide. Control (blank) water, control carcasses, fortified water, and fortified carcasses were analyzed with each sample set for quality assurance. Individual carcasses were weighed in 50-mL polypropylene centrifuge tubes (Corning Inc, Corning New York, USA) and digested with 100 µL of proteinase K (>600 $mAU \cdot mL^{-1}$) per gram of tissue (Qiagen GmbH, Hilden, Germany) for 5 hours at 54 ±2 °C. High performance liquid chromatography grade water (1.0 mL) and control carcasses were spiked with 500 µL of high performance liquid chromatography grade acetonitrile containing 3800 $ng \cdot mL^{-1}$ analytical grade TFM and 990 $ng \cdot mL^{-1}$ niclosamide (both from Sigma Aldrich Milwaukee, Wisconsin, USA). All other samples

received an equivalent aliquot of acetonitrile. All samples were diluted to 10.0 mL with acetonitrile then vortexed for 1-1.5 minutes. Magnesium sulfate (4 g; reagent grade; VWR, West Chester, Pennsylvania, USA) and sodium chloride (1 g; American Chemical Society grade; VWR, West Chester, Pennsylvania, USA) were added to each sample and vortexed again for 1-1.5 minutes. Samples were centrifuged for 10 minutes at 5000·relative centrifugal force at 4 °C. Supernatant (1 mL) was transferred to a 2-mL centrifuge tube (Part No. 5982-4921, Agilent Technologies, Agilent, Santa Clara, California, USA) and mixed with 150 mg of magnesium sulfate and 25 mg of C_{18}. Samples were vortexed for 1-1.5 minutes and centrifuged for 10 minutes at 10,000·relative centrifugal force and 20 °C. Approximately 0.5 mL of the supernatant was filtered through a 0.2 µm polytetrafluoroethylene syringe filter (Acrodisc; PALL Life Science, Westborough, Massachusetts, USA) into an amber vial (Part No. 186000847C; Waters, Milford, Massachusetts, USA) and then 5 µL injected into a liquid chromatograph triple quadrupole mass spectrometer system (Agilent Technologies, Santa Clara, California, USA) that consisted of: a model 1260 liquid chromatograph with a solvent degasser, a binary pumping system, an auto-sampler, a 45 ±0.8 °C column compartment containing a Kinetex XB-C_{18} analytical column (2.1 x 50 mm, 1.7 µm, 100 Å; Phenomenex, Torrance, California, USA), and a model 6460 mass detector equipped with an electrospray ionization Agilent Jet Stream source. The mobile phase was a mixture of A: 10 mM reagent grade ammonium formate (Sigma-Aldrich, Milwaukee, Wisconsin, USA) in E-pure water, mass spectrometry grade methanol, American Chemical Society grade formic acid (Sigma-Aldrich, Milwaukee, Wisconsin, USA) (800 mL + 200 mL + 0.1 mL) and B: 10 mM ammonium formate in E-pure water, mass spectrometry grade methanol, and mass spectrometry grade acetonitrile (100 mL + 400 mL + 500 mL). Retention times (TFM = 1.58 ± 0.02 minutes; niclosamide = 2.45 ± 0.02 minutes) were achieved using a 4.0-minute gradient starting at 0.4 mL·minute^{-1} flow and 20% mobile phase B (Table 2). Mass spectrometer acquisition was divided into three time segments. Flow for segment one (0-1.3 minutes) was diverted to waste. Segment two (1.3 – 2.2 minutes) was sent to the mass detector, and a multiple reaction monitoring scan with a delta electron multiplier voltage of 200 was conducted. The TFM precursor ion (206 mass·charge^{-1}) and product ion (176 mass·charge^{-1}) were set to unit resolution. The dwell (200 milliseconds), fragmentor (155 volts), collision energy (18 volts), and cell accelerator (7 volts) were optimized for negative polarity. The source parameters were optimized as follows: gas (350 °C and 9 mL·minute^{-1}), nebulizer (25 psi), sheath gas (400 °C and 11 L·minute^{-1}), capillary (3500 volts) and nozzle (500 volts). Segment three (2.2 – 4.0 minutes) consisted of a multiple reaction monitoring scan with a delta electron

multiplier voltage of 200. The niclosamide precursor ion (325 mass·charge^{-1}) and product ion (289 mass·charge^{-1}) were set to unit resolution and the dwell (200 milliseconds), fragmentor (115 volts), collision energy (15 volts), cell accelerator (7 Volts) were optimized for negative polarity. The source remained unchanged from segment two. The system was tuned prior to use with six ions (112.92 to 1633.95 mass·charge^{-1}) bracketing the mass range. Ten calibration standards containing TFM (0.727 to 352 ng·mL^{-1}) and niclosamide (0.189 to 88.8 ng·mL^{-1}) bracketing the range of sample concentrations were injected at the beginning and end of each sample set. Quality control standards were injected after every tenth sample to verify the system was working properly throughout each sample set. MassHunter Acquisition and Quantitation software (Agilent Technologies, Santa Clara, California, USA) was used to control the system and quantify the resulting peaks. The mean TFM (n = 56) and niclosamide (n = 48) fortified control carcass recoveries were 99.3% (SEM =3.8) and 106.8% (SEM = 5.2) respectively.

Table 2. Liquid Chromatographic pump timetable set points.

Time (min)	Flow (mL·min^{-1})	Solvent A (%)	Solvent B (%)
0	0.4	30	70
2.0	0.4	25	75
2.2	-	5	95
3.0	0.6	-	-
3.2	-	5	95
3.4	-	80	20
3.8	0.6	-	-
4.0	0.4	80	20

Data analysis

Simple descriptive statistics were used to determine the mean and sample standard deviation (SD) of larvae masses, and lampricide concentrations for each test group. An analysis of variance was used to test if the variability between the group means was larger than the variability within each group. After determining homogeneity of variance, the Tukey's honestly significant difference test was used to test pairwise differences among the group means for significance with 95% confidence (R, version 3.6.1; R Core Team, 2017). Data collected for this study are available in Bernardy et al. (2020).

Results

The mean mass of fresh-carcass groups ranged from 0.85 g to 1.42 g and the mean mass of 2-day postmortem carcass groups ranged from 0.82 g to 0.95 g (Table 3). Differences in the mean TFM body burdens between groups were detected ($F_{(5/114)} = 114.1$, $P < 0.01$). The mean TFM whole body burdens in fresh-carcasses from all treatments ranged from 3.74 to 6.11 $\mu g \cdot g^{-1}$ and the 2-day postmortem body burdens ranged from 0.14 to 0.72 $\mu g \cdot g^{-1}$ (Table 3). The mean TFM body burden in lamprey carcasses collected immediately after the lower Chippewa River TFM application was greater than the TFM body burden in carcasses collected immediately after the other three treatments ($P < 0.01$; Table 3). Mean TFM body burden in carcasses held in situ for 2 days post treatment were similar across the treated streams ($P = 0.412$; Table 3) but were less than in carcasses collected immediately after treatment ($P < 0.01$; Table 3).

The mean niclosamide body burdens in fresh-carcasses from the two TFM/Bayluscide treatments (Sturgeon and upper Chippewa Rivers) were 0.34 $\mu g \cdot g^{-1}$ (SD = 0.20 $\mu g \cdot g^{-1}$) and 0.64 $\mu g \cdot g^{-1}$ (SD = 0.19 $\mu g \cdot g^{-1}$), respectively (Table 3). The mean 2-day postmortem carcass concentration from the upper Chippewa River was 0.41 $\mu g \cdot g^{-1}$ (SD = 0.23 $\mu g \cdot g^{-1}$) (Table 3). Differences in the mean niclosamide carcass concentrations between groups were detected ($F_{(5,114)} = 42.78$, $P < 0.01$). The mean niclosamide concentration in fresh carcasses from the upper Chippewa River was greater than in fresh carcasses from the Sturgeon River and S-C-F and 2-day postmortem carcasses from the upper Chippewa River groups ($P < 0.01$; Table 3). Fresh and 2-day postmortem carcasses from the lower Chippewa River also contained niclosamide (mean 0.16 $\mu g \cdot g^{-1}$ (SD = 0.07 $\mu g \cdot g^{-1}$) and 0.18 $\mu g \cdot g^{-1}$ (SD = 0.11 $\mu g \cdot g^{-1}$)), at concentrations similar to each other ($P = 0.998$) and less than the fresh and 2-day postmortem carcasses from the upper Chippewa River groups ($P < 0.01$) and the Sturgeon River group ($P = 0.012$) (Table 3).

Table 3. Mean (standard deviation) mass, 3-trifluoromethyl-4-nitrophenol (TFM) concentration, and niclosamide concentration, of sea lamprey carcass groups collected immediately (fresh) or 2-days postmortem from the Ford, Sturgeon, and upper and lower reaches of the Chippewa Rivers.

				Whole-Body Burden	
River	River Treatment	Carcass Group	Larvae Mass (g)	TFM ($\mu g \cdot g^{-1}$)	Niclosamide ($\mu g \cdot g^{-1}$)
Ford	TFM	Fresh	0.85 (0.29)	4.04 (1.1)	<0.001 (0)
Chippewa (lower)	TFM[a]	Fresh	1.42 (0.50)	6.11 (1.2)	0.16 (0.07)
		2-day postmortem	0.82 (0.41)	0.72 (0.43)	0.18 (0.11)
Sturgeon	TFM/Bayluscide	Fresh	1.33 (0.81)	3.74 (1.5)	0.34 (0.20)
Chippewa (upper)	TFM/Bayluscide	Fresh	1.14 (0.44)	4.41 (0.74)	0.64 (0.19)
		2-day postmortem	0.95 (0.37)	0.14 (0.11)	0.41 (0.23)

[a] This treatment contained lampricide from the TFM/Bayluscide treatment on the upper Chippewa River.

Discussion

This study provides a dataset of lampricide concentrations found in sea lamprey larvae killed during two combined TFM/Bayluscide stream treatments, one TFM only treatment, and one TFM treatment that was influenced by a combined TFM/Bayluscide treatment conducted upstream. Our dataset is small and inclusion of data from additional treatments would clarify any patterns in the data.

The TFM concentrations in the fresh carcasses from all lampricide treatments were similar except the carcasses from the TFM only treatment in the lower Chippewa River had higher TFM concentrations. The fresh carcasses from the lower Chippewa River also contained some niclosamide. The TFM only treatment downstream from Lake Isabella on the lower Chippewa River was conducted 1 day after the TFM/Bayluscide treatment upstream from Lake Isabella. Animals dosed with a sub-lethal concentration of toxicant prior to receiving a lethal dose can accumulate a higher total body burden relative to animals that receive a more temporally compressed lethal dose (Conrad and Barton, 1978). It appears that the sea lamprey in the lower Chippewa River were exposed to lampricide from the upstream combination treatment. Lake Isabella likely diluted the lampricide as it passed through the lake. We have no data on the lampricide concentrations in or downstream from the lake from the treatment on the upper reach of the river.

While their TFM concentrations were similar, niclosamide concentrations in fresh carcasses from the Sturgeon River were lower than in those from the upper Chippewa River. The Sturgeon River was treated at a concentration almost 5x lower than the Chippewa River. The lampricide concentration in the water along with the water pH, alkalinity, hardness, temperature, exposure time, larval size, and condition factor all influence the whole-body burden (Muhametsafina et al., 2019; Siefkes, 2017).

The lampricide body burden in sea lamprey carcasses decreased after being in the river for 2 days. On average, 2-day postmortem carcasses from the Chippewa River TFM/Bayluscide treatment contained 3% of the TFM and 64% of the niclosamide found in fresh carcasses. The mean 2-day postmortem carcasses from the Chippewa River TFM treatment contained 12% of the TFM present in the fresh carcass group. Non-target organisms that may consume 2-day old carcasses would receive a correspondingly smaller dose compared to those that consume carcasses immediately following a treatment.

In combination with good quality species-specific oral toxicity data our whole-body burden data may be used to evaluate the risk to nontargets associated with consuming dead larval sea lamprey. For example, estimates of the number of lamprey that a ring-billed gull might need to consume to achieve a certain dose (e.g., the reported LD50) can be estimated by applying relevant information into the following equation:

$$\text{Number of carcasses} = LD50 \times M_G \div (M_c \times B)$$

Where,

LD50 = 87.5 $mg \cdot kg^{-1}$ or 53.9 $mg \cdot kg^{-1}$ for TFM or TFM/ Bayluscide, respectively,

M_G = 0.450 kg (mean body mass of gulls, Warrington, 2001),

M_C = mean sea lamprey carcass mass in g (Table 3), and

B = mean lampricide (TFM + niclosamide) body burden in $mg \cdot g^{-1}$ (Table 3).

The number of carcasses that would have to be consumed to induce mortality in 50% of the ring-billed gulls is estimated to be 11,480 (9,758 g) in the Ford River, 2,726 (3,871 g) in the lower Chippewa River, 4,463 (5,936 g) in the Sturgeon River, and 4,212 (4,802 g) in the upper Chippewa River. Therefore, to reach a LD50 equivalent dose,

ring-billed gulls would need to consume 8.6 – 21.7 times their body weight in sea lamprey carcass. It appears unlikely that ring-billed gulls would consume enough sea lamprey carcasses to cause mortality; however, additional data, such as the slope of the dose-effect curve, are required to conduct an accurate risk assessment.

In this study we determined the concentration of TFM and niclosamide in the lamprey carcasses immediately after and 2 days after lampricide treatments induced mortality. Our data indicates that the TFM concentration in lamprey carcasses may be similar between TFM and TFM/Bayluscide treatments; however, niclosamide concentrations may be more variable among different treatments. Merging of dendritic stream treatments may increase residual lampricide concentrations in resident lamprey killed during treatments of downstream reaches, which may increase the risk to non-targets that consume them. We found that most of the TFM and a substantial amount of the niclosamide had dissipated from the carcasses 2 days after treatment. This whole-body burden data, in combination with appropriate toxicity data, may allow evaluation of the risk to non-target species that consume larval sea lamprey killed by TFM or TFM/Bayluscide treatments.

References

Applegate V.C., and Everett L. King E.L. 1962. Comparative toxicity of 3-trifluormethyl-4-nitrophenol (TFM) to larval lampreys and eleven species of fishes. Transactions of the American Fisheries Society. 91(4): 342-345. doi: 10.1577/1548-8659(1962)91[342:CTOTTT]2.0.CO;2.

Bernardy, J.A., Schloesser, N.A., and Schueller, J.R. 2020. Lamprey larvae carcasses following exposure to 3-trifluoromethyl-4-nitrophenol (TFM) or TFM plus 1% niclosamide. U.S. Geological Survey data release. https://doi.org/10.5066/F72Z13PG.

Boogaard, M.A., Bills, T.D., Johnson, D.A. 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. J. Gt. Lakes Res. 29: 529–541. doi:10.1016/S0380-1330(03)70514-0.

Chiotti, T. L., Engstrom-Heg, R., Hulbert, P., and Neuderfer, G. 1987. Summary report of TFM treatments in the Cayuga Lake system, September 1986. Bureau of Fisheries, New York State Department of Environmental Conservation Report. 66 pp.

Cochran, P.A. 2009. Predation on lampreys. *In* Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72. Edited by L.R. Brown, S.D. Chase, M.G. Mesa, R.J. Beamish, and P.B. Moyle American Fisheries Society. Bethesda, Maryland. pp. 139–152.

Conrad, M.E., and Barton, J. 1978. Factors affecting the absorption and excretion of lead in the rat. Gastroenterology. **74**: 731-740.

Dawson, V.K. 2003. Environmental fate and effects of the lampricide Bayluscide: a review. J. Gt. Lakes Res. **29**: 475–492. doi:10.1016/S0380-1330(03)70509-7.

Dawson, V.K., Sills, J.B., and Luhning, C.W. 1982. Accumulation and loss of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73) by fish: laboratory studies. Investig. Fish Control 90: 1–5.

Gilderhus, P.A. 1979. Effects of granular 2',5-dichloro -4'-nitrosalicylanilide (Bayer 73) on benthic macroinvertebrates in a lake environment. Technical Report No. 34. Great Lakes Fishery Commission, Ann Arbor, Michigan.

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

Hubert, T.D. 2003. Environmental fate and effects of the lampricide TFM: a review. J. Gt. Lakes Res. 29: 456–474. doi:10.1016/S0380-1330(03)70508-5.

Hubert, T.D., Boogaard, M.A., Bills, T.D., Johnson, D.A., and Schleen, L. 1999. Determination of niclosamide concentrations in sea lamprey larvae following exposure to two formulations of Bayluscide Project Completion Repot. Great Lakes Fishery Commission, Ann Arbor, Michigan.

Hudson, R. 1979. Toxicities of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73) to four bird species. Investig. Fish Control 89.

Muhametsafina, A., Birceanu, O., Hlina, B.L., Tessier, L.R., and Wilkie, M.P. 2019. Warmer waters increase the larval sea lamprey's (*Petromyzon marinus*) tolerance to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). J. Gt. Lakes Res. 45: 921–933

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Siefkes MJ (2017) Use of physiological knowledge to control the invasive sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes. Conserv Physiol 5(1): cox031; doi:10.1093/conphys/cox031.

Solomon, B. (Editor). 2019. Standard operating procedures for the application of lampricides in the Great Lakes Fishery Commission Integrated Management of Sea Lamprey (*Petromyzon marinus*) Control Program (No. SCL 04-001.10). Marquette Biological Station, U.S. Fish and Wildlife Service, Marquette, Michigan.

Warrington, P.D. 2001. Animal weights and their food and water requirements, Resource Document. Water Quality, Water Management Branch, Environment and Resource Division, Ministry of Environment, Lands and Parks. https://www2.gov.bc.ca/assets/gov/environment/air-land-water/water/waterquality/water-quality-reference-documents/animal_weights_and_their_food_and_water_requirements.pdf.

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Deliverables

Reports

Bernardy, J.A. and Schloesser, N.A., 2020. Lampricide residues in sea lamprey larvae carcasses recovered after 3-trifluoromethyl-4-nitrophenol (TFM) or TFM/Bayluscide stream treatments. 16 pp.

Data and Analysis Code

Bernardy, J.A., Schloesser, N.A., and Schueller, J.R. 2020. Lamprey larvae carcasses following exposure to 3-trifluoromethyl-4-nitrophenol (TFM) or TFM plus 1% Niclosamide. U.S. Geological Survey data release. https://doi.org/10.5066/F72Z13PG.

Research highlights

- The whole-body burdens of TFM and/or niclosamide were determined in dead larval sea lamprey collected immediately after or 2 days after either a TFM or a combined TFM/Bayluscide lampricide treatment.
- The mean TFM whole body burden in fresh-dead sea lamprey carcasses from all treatments ranged from 3.74 to 6.11 $\mu g \cdot g^{-1}$ and 2-day postmortem body burden ranged from 0.14 to 0.72 $\mu g \cdot g^{-1}$.
- The mean niclosamide body burden in fresh-dead sea lamprey carcasses ranged from 0.34 $\mu g \cdot g^{-1}$ to 0.64 $\mu g \cdot g^{-1}$. The mean 2-day postmortem niclosamide body burden was 0.41 $\mu g \cdot g^{-1}$.
- The estimated number of carcasses required to induce mortality in 50% of ring-billed gulls are: 11,480 (9,758 g) in the Ford River, 2,726 (3,871 g) in the lower Chippewa River, 4,463 (5,936 g) in the Sturgeon River, and 4,212 (4,802 g) in the upper Chippewa River.