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Growth and behavioral effects of the lampricide TFM on non-target fish species

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ABSTRACT

Although the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been used for over 60 years to control sea lamprey *Petromyzon marinus* in the Laurentian Great Lakes, its potential non-lethal impacts on non-target species have not been fully evaluated. We exposed juveniles of two species of fish (lake sturgeon *Acipenser fulvescens* and rainbow trout *Oncorhynchus mykiss*) and one adult fish species (fathead minnows *Pimephales promelas*) to various concentrations of TFM (0.25–7.5 mg/L) in three sets of experiments examining TFM effects on growth, avoidance of TFM treated water, and predation susceptibility. Lake sturgeon and rainbow trout were monitored for two weeks after a 12 hour exposure to TFM to observe differences in instantaneous growth among four treatment levels (0, 2.5, 5, and 7.5 mg/L TFM). Growth rates did not differ significantly among control and treated fish of either species. Next, potential avoidance of TFM by rainbow trout was evaluated in a test tank where half the water was contaminated with TFM (0, 0.25, or 2.5 mg/L). No avoidance behavior was observed as rainbow trout spent equal amounts of time in TFM and control water. Finally, fathead minnows were exposed at three different concentrations of TFM (0, 2.5, and 7.5 mg/L) and placed in mesocosms with a non-exposed largemouth bass *Micropterus salmoides* predator. Two separate trials were performed, both with no significant differences due to treatments. In summary, results indicate that for the conditions tested, TFM has no detectable sub-lethal effects on growth, avoidance behavior, or predation mortality on the fish species tested.

Introduction

The sea lamprey *Petromyzon marinus* invaded the upper Laurentian Great Lakes in the early 20th century through the construction of shipping canals (Smith and Tibbles, 1980). Due to its parasitism of large-bodied fishes, sea lamprey contributed towards the precipitous decline of several Great Lakes fish populations starting in the 1940s (Smith and Tibbles, 1980). To minimize the spread and deleterious impacts of lamprey, active control programs to limit sea lamprey were developed and implemented throughout the Great Lakes. In particular, the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been widely used to control larval sea lampreys in tributaries of the Great Lakes. While several alternative control strategies have been developed and applied, lamprey control programs remain reliant on this toxicant.

Although effective at controlling sea lamprey, the effects of lampricides on non-target fish species are not well understood. From acute toxicity data, it is known that there is a wide range of sensitivities to TFM among and within fish families, with up to 7-fold or more changes in LC_{50} values (concentration at which the fish experiences 50% mortality; Boogaard et al., 2003). Laboratory studies have shown that early life stages of lake sturgeon *Acipenser fulvescens* are relatively sensitive to TFM and TFM/1% niclosamide compared to other fish species (Boogaard et al., 2003). In a series of laboratory experiments, these authors reported that, larvae and small (<100 mm in total length) age-0 juvenile lake sturgeon experienced LC_{50} at or less than the LC_{50} for larval sea lamprey. This finding has led to the development of a protocol which stipulates that in streams with larval sturgeon, lampricide concentrations must not exceed the Maximum Lethal Concentration (MLC, defined as the lampricide concentration that produces 99.9% sea lamprey mortality during a 9 hour exposure); however, this protocol is not always followed (Klar and Schleen, 2000). Other fishes such as ictalurids are also sensitive to TFM with toxicity ratios (calculated as the non-target LC_{50} divided by the predicted sea lamprey MLC) of 1.5 or less compared to about 1.8 in lake sturgeon (Boogaard et al., 2003). Rainbow trout *Oncorhynchus mykiss* and other salmonids in general are moderately sensitive (toxicity ratios of 3.1–4.6), while several other fish species, such as centrarchids, appear to be relatively tolerant (toxicity ratios of 6.3–8.8) (Boogaard et al., 2003).

With some exceptions, most past studies suggest that concentrations of TFM experienced by fish in streams will not lead to direct mortality for most non-target species (Boogaard et al., 2003). However, a significant limitation from previous studies with lake sturgeon and other non-target fish species is that only lethal effects were measured

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and there may be a suite of sub-lethal effects on non-target species (e.g., growth, development, predator avoidance). Studies on a variety of systems and toxicants suggest that such sub-lethal effects may alter ecological interactions and ultimately lead to increased mortality rates, i.e., toxicants indirectly causing lethality (e.g., Relyea and Diecks, 2008). In fact, by altering ecological interactions, sensitivity to environmental factors, and susceptibility to other toxicants, indirect effects may ultimately have greater population-level effects than would a moderate rate of direct toxicant mortality (e.g., Peacor and Werner, 2004; Relyea and Hoverman, 2006).

Data collected over the past 30 years on the toxicity and environmental fate of TFM indicate that it breaks down quickly in the environment, does not bioaccumulate, and is quickly detoxified in non-target fish species. These findings have led to the conclusion that TFM-based lampricides should pose minimal risk to aquatic organisms (Dawson, 2003; Hubert, 2003). However, because almost all of the lampricide toxicity studies performed to date have evaluated lethality as the only endpoint, more studies are needed that examine sub-lethal effects, particularly ecologically-relevant sub-lethal effects which may cascade to have population-level consequences such as changes in growth, behavior, and susceptibility to predation. There is a critical need for this understanding considering that currently over 170 Great Lakes tributaries are treated with lampricides on average once every 4 years (Christie and Goddard, 2003).

Our objective was to evaluate sub-lethal effects of TFM on non-target fish species. We explored how representative species, specifically lake sturgeon, rainbow trout, and fathead minnow *Pimephales promelas*, respond to TFM exposure by tracking growth, behavior (avoidance of TFM treated water) and susceptibility to predation. We hypothesized that TFM would lead to short-term reductions in growth (De Boeck et al., 1997), avoidance behavior (Beitinger, 1990) and increased susceptibility to predation (Little et al., 1990).

Methods

Growth trials

We conducted a series of growth trials using age-0 rainbow trout and age-0 lake sturgeon. In August 2011, we obtained fish from the Genoa National Fish Hatchery in Genoa, WI, and transported them to Purdue University's Baker Aquatic Research Laboratory in West Lafayette, IN where we conducted all experiments. Lake sturgeon and rainbow trout were housed in 150 and 38 L flow-through aquariums, respectively, at a constant temperature between 13 and 14 °C prior to use in experiments. Prior to experiments, we fed rainbow trout Rangen 11–45 grower pellet feed once daily and we fed lake sturgeon a diet of chironomid larvae daily.

We set up twenty four 78 L experimental tanks in a flow-through system. Incoming well water was maintained at a flow of 7–15 mL/s, a constant temperature between 13 and 14 °C, and a 14 hour photoperiod. Throughout all experiments, we checked water quality parameters daily and alkalinity (~288 mg/L $CaCO_3$) and pH (~8.5) did not vary significantly within and between experiments. Based on our median pH and alkalinity values, the $LC_{99.9}$ for sea lamprey is ~9.0 mg/L (Bills et al., 2003). We conducted two exposures with slightly different methods, one using lake sturgeon and one using rainbow trout. In both exposures, we exposed 18 fish in six experimental tanks (72 total fish) to one of four TFM concentrations: control (0 mg/L), low (2.5 mg/L), medium (5 mg/L), or high (7.5 mg/L). We chose these TFM concentrations as they represent a range of dosages that may be experienced in natural systems where treatments should not exceed $MLC_{99.9}$ for sea lamprey (Boogaard et al., 2003). During exposures, we gradually increased TFM concentrations over a 2 hour period and then maintained concentrations for 8 h. At this point, we slowly reduced concentrations to zero for a 12 hour exposure replicating concentrations and length of treatment during an actual stream dosing.

We used a portable HACH 2400 (Loveland, CO) spectrometer to measure TFM absorption at a wavelength of 400 nm following a previously described protocol (Klar and Schleen, 1999). We derived TFM concentrations from absorption values through standard curve comparison, and we generated standard slopes from serial dilutions with 99.9% pure TFM from Sigma-Aldrich (Product# N27802, St. Louis, MO, USA) and Milli-Q water. We buffered standards and water samples to a pH of 9 before absorption readings by adding 2 drops of a concentrated sodium tetraborate solution per cuvette. We then determined residual TFM absorption by adding 2 drops of a 10% sulfuric acid per cuvette. We chose a random set of four of the six tanks per concentration and measured TFM every hour during the 12 hour exposure period.

Prior to and following exposure, we measured total length and mass of all fish and used these measurements to calculate instantaneous growth rates. To control for pseudo-replication, we used tank means as elementary units for all subsequent analyses. We compared differences in instantaneous growth among treatments using repeated measures ANOVA ($\alpha = 0.05$).

Lake sturgeon exposure

We added three lake sturgeon to each experimental tank (mean total length = 146.1 mm ± 17.2 mm) eight days before exposure and clipped pelvic fins (left, right, or middle) in order to differentiate among individuals in each tank. We measured (to 1 mm total length) and weighed (to 0.01 g) each fish when placing them in tanks and again one day prior to exposure. On the day of exposure, we shut off inflow water and slowly dripped TFM into the exposure tanks. After 3 h, target concentrations were reached at low, medium, and high levels. We took water samples every hour for the first 3 h and again at hours 10–12 to record TFM concentrations in exposure tanks (Fig. 1A). We recorded dissolved oxygen (DO) and temperature at regular intervals through the exposure. At

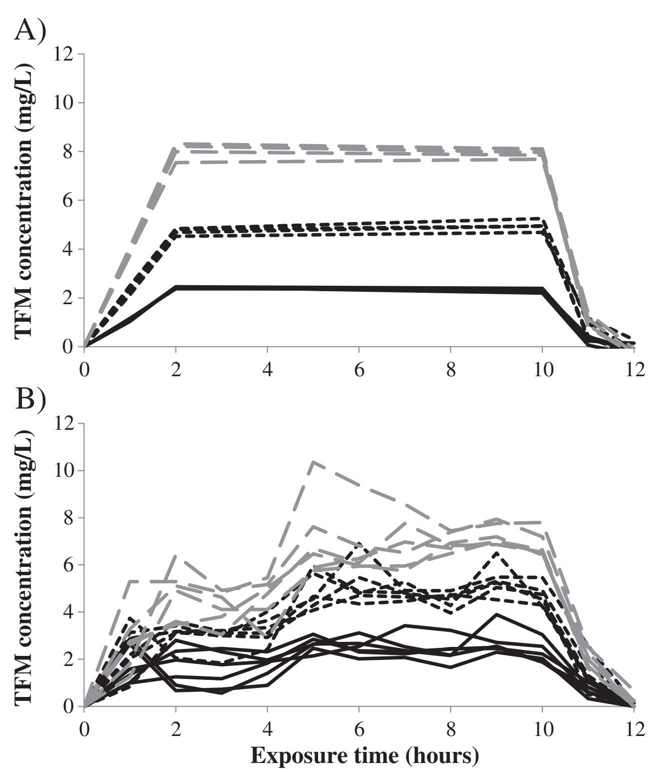

Fig. 1. Representative concentrations of TFM during experimental dosing of lake sturgeon (A) and rainbow trout (B). Each line represents concentrations in an individual tank and different line styles depict separate target concentrations (low—solid line, medium—dashed line, and high—hatched line). Control concentrations are not shown, but were always 0.00 mg/L.