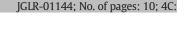
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In situ assessment of lampricide toxicity to age-0 lake sturgeon

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ABSTRACT

The lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2', 5-dichloro-4'-nitrosalicylanilide (niclosamide) are used to control sea lamprey (Petromyzon marinus), an invasive species in the Great Lakes. Age-0 lake sturgeon (Acipenser fulvescens), a species of conservation concern, share similar stream habitats with larval sea lampreys and these streams can be targeted for lampricide applications on a 3- to 5-year cycle. Previous laboratory research found that lake sturgeon smaller than 100 mm could be susceptible to lampricide treatments. We conducted stream-side toxicity (bioassay) and in situ studies in conjunction with 10 lampricide applications in nine Great Lakes tributaries to determine whether sea lamprey treatments could result in *in situ* age-0 lake sturgeon mortality, and developed a logistic model to help predict lake sturgeon survival during future treatments. In the bioassays the observed concentrations where no lake sturgeon mortality occurred (no observable effect concentration, NOEC) were at or greater than the observed sea lamprey minimum lethal concentration (MLC or LC99) in 7 of 10 tests. We found that the mean in situ survival of age-0 lake sturgeon during 10 lampricide applications was 80%, with a range of 45-100% survival within streams. Modeling indicated that in age-0 lake sturgeon survival was negatively correlated with absolute TFM concentration and stream alkalinity, and positively correlated with stream pH and temperature. Overall survival was higher than expected based on previous research, and we expect that these data will help managers with decisions on the trade-offs between sea lamprey control and the effect on stream-specific populations of age-0 lake sturgeon.

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1. Introduction

Rehabilitation activities aimed at protecting critical habitats and life stages of lake sturgeon (*Acipenser fulvescens*) have yet to realize significant increases in population abundance, in part due to the level of reduction in abundance coupled with the life history of this species (Auer, 1999; Welsh et al., 2008). Presently, the lake sturgeon is listed as endangered or threatened in the vast majority of their historic range (Auer, 1999; COSEWIC, 2006). Sea lamprey (*Petromyzon marinus*) are an invasive species that contributed to the decline in many fish stocks throughout the Great Lakes (Siefkes et al., 2013) and spawn in streams that are also suitable for lake sturgeon spawning, with the natal habitat of soft sediments and sand being shared by both species (Kempinger, 1996; Peake, 1999).

Of over 5000 tributaries to the Great Lakes, 57 are known to support at least some life stages of lake sturgeon and an additional 40 are thought to have historical evidence of lake sturgeon (Table 1). Of these 97 rivers, 72 are known to be currently infested or have had at

* Corresponding author. E-mail address: lisa.oconnor@dfo-mpo.gc.ca (L.M. O'Connor). least had one sea lamprey infestation since the beginning of sea lamprey surveys in the Great Lakes, and 46 of these receive lampricide applications on a regular (3-4) year cycle (Table 1). Sea lampreys are controlled in Great Lakes tributaries and estuaries by the application of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5dichloro-4'-nitrosalicylanilide (niclosamide) (Siefkes et al., 2013). Year- and stream-specific pH and alkalinity measures affect the toxicity of TFM to aquatic organisms (Bills et al., 2003). Alkalinity and pH data of a tributary are required to calculate the minimum amount of TFM required to kill 99.9% (LC99, or minimum lethal concentration, MLC) of sea lamprey larvae in the tributary. TFM application rates are typically 1.2-1.5 times the MLC to ensure that treatment efficacy is not affected by attenuation or dilution of lampricide as it moves downstream, with the goal of maintaining at least 9 h of exposure at or above the MLC throughout the length of infested stream. Niclosamide can be used in conjunction with TFM, typically at a rate of up to 1% by weight of active ingredient of the TFM applied, to reduce the target MLC for sea lampreys (Bills and Marking, 1976). Consequently, this reduces the amount and subsequent cost of TFM required to control larval sea lampreys, and is most often used in tributaries with pH > 7.0 or when the addition of niclosamide results in a substantial savings in cost (Gutreuter and Boogaard, 2007).

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Table 1

Summary of current and historical lake sturgeon streams in the Great Lakes with current or historical sea lamprey streams. Sea lamprey streams include those that have had at least one positive sea lamprey survey. Regular sea lamprey treatments are those that are conducted on a three to four year cycle, while irregular sea lamprey treatments are those ranging from a single treatment to those on a 5 to 10 year cycle.

Lake	Total streams	Current lake sturgeon streams	Historical lake sturgeon streams	Total lake sturgeon streams with sea lampreys	Lake sturgeon streams with regular sea lamprey treatments	Lake sturgeon streams with irregular sea lamprey treatments
Lake Superior	1566	16	6	21	10	11
Lake Huron	1761	16	17	25	18	7
Lake Michigan	511	17	10	18	15	3
Lake Erie	842	3	5	3	1	2
Lake Ontario	659	5	2	5	2	3
Total	5339	57	40	72	46	26

The effect of lampricides on non-target fishes has been a concern since the development of the lampricide application program in the 1960s (Applegate and King, 1962; Bills and Marking, 1976; Marking and Olson, 1975), and, more recently, specifically for lake sturgeon (Boogaard et al., 2003; Johnson et al., 1999). Steam pH was the primary factor in determining TFM toxicity to juvenile lake sturgeon 100 to 125 mm total length, but there was no significant mortality for this size group when lampricides were applied at a rate of $1.3 \times$ MLC or less (Johnson et al., 1999). Further, lake sturgeon sac fry and fingerlings < 100 mm were more susceptible than most teleosts when exposed to TFM at minimum lethal concentrations for sea lampreys (Boogaard et al., 2003).

Due to concerns about mortality of age-0 lake sturgeon < 100 mm in total length, a protocol for lampricide applications in U.S. tributaries with known lake sturgeon populations was developed to 1) restrict the amount of TFM applied to $1.0 \times$ MLC and TFM/niclosamide to $1.2 \times$ MLC, and 2) ensure that treatments of these streams occurred after August 1, when the majority of lake sturgeon are expected to be >100 mm in length (Adair and Sullivan, 2009). However, application of lampricides late in the year and at reduced concentrations has raised concerns among sea lamprey control managers for several reasons. First, prior to adopting the revised treatment protocol, field personnel had observed only 10 dead lake sturgeon in over 16,000 posttreatment collections following 1800 lampricide stream treatments that occurred from 1959 to 2000 (Johnson et al., 1999). Between 2001 and 2012, an additional 982 lampricide treatments have been conducted and only 3 dead lake sturgeon were observed during this time (unpublished USFWS and DFO treatment reports). During the supplemental lampricide applications that are conducted during lampricide treatments (Adair and Sullivan, 2009), survey crews cover the entire length of sea lamprey infested portion of the river, looking for sea lamprey escapement areas. During these surveys, crews look for both sea lamprey and any non-target species mortalities, paying special attention to any lake sturgeon mortality (Adair and Sullivan, 2009). Because larval sea lamprey and lake sturgeon lack swim bladders, both sink to the bottom when dead; thus crews used to surveying stream bottom for sea lamprey mortality are skilled at looking for affected lake sturgeon. Nevertheless, extensive effort by the U.S. Fish and Wildlife Service, Little River Band of Ottawa Indians, and the Michigan Department of Natural Resources found 31 dead age-0 lake sturgeon during the 2014 lampricide treatment of the Muskegon River, (S. Nowicki, USFWS, 2015, personal communication); more than all other stream treatments combined. This indicated that lampricideinduced mortality of age-0 lake sturgeon could be greater than previously observed and that discovery of dead age-0 lake sturgeon may require a concerted effort. Second, since the revised treatment protocol was adopted on lake sturgeon producing streams, wounding rates among native fishes and population estimates of larval and spawning sea lampreys in the upper Great Lakes have increased (Slade, 2012; Sullivan et al., 2013). Lastly, survival of sea lamprey larvae in lake sturgeon producing streams was greater following treatments conducted in late September and beyond when the revised protocol was followed compared to earlier in the season (Scholefield et al., 2008). As a result, treatments were required more frequently on some large rivers, in some cases every one or two years, compared to their normal treatment cycle of once every three to four years, due to the number of these residual sea lampreys (Boogaard et al., 2011). When all these points are combined, the concern was that management actions taken to protect lake sturgeon may have resulted in increased sea lamprey production, increased treatment frequency of large rivers, and subsequently increased the frequency of exposure of age-0 lake sturgeon to TFM in these rivers.

The adherence to the restricted lampricide application protocols may not result in the expected benefits to lake sturgeon survival because observations of lake sturgeon mortality in laboratory tests do not correspond to the in-stream observations during and immediately following lampricide application. Instead, reduced lampricide application rate may increase the production of sea lampreys to the Great Lakes thereby increasing the likelihood of sea lamprey induced mortality on older lake sturgeon (Patrick et al., 2009), and more frequently expose cohorts of age-0 lake sturgeon to lampricide applications. During 2010 and 2011, we conducted a study to better understand the disparity between laboratory and field observations, and provide in situ observations of lake sturgeon exposed to lampricide application. Our specific research objectives were to 1) compare the calculated toxicity of TFM or TFM/niclosamide based on pH and alkalinity measures with observed mortality of age-0 lake sturgeon and sea lampreys in controlled, pretreatment bioassays; 2) evaluate in situ mortality of age-0 lake sturgeon held in cages during TFM and/or TFM/niclosamide treatments; and 3) develop a predictive model of lampricide-induced, age-0 lake sturgeon mortality based on stream-specific lampricide applications to kill sea lamprey larvae. Based on the most recent bioassay studies (Boogaard et al., 2003; Johnson et al., 1999), we expected moderate to high lake sturgeon mortality in situ and in the bioassays when TFM and TFM/niclosamide concentrations exceeded the MLC for sea lampreys.

2. Methods

2.1. Study sites

Site selection was based on the criteria that the streams: 1) were scheduled to be treated with TFM or TFM/niclosamide during 2010 or 2011; 2) represented a range of discharge, pH and alkalinity values typically encountered during lampricide applications (Table 2); and 3) where possible, were used by lake sturgeon for spawning. Streams that met this criteria were: the Kaministiquia River and its independently treated tributary, the Whitefish River, and the Batchawana and Two-Hearted rivers (Lake Superior); the Mississagi, Rifle and Pigeon rivers (Lake Huron); and the Millecoquins, and Sturgeon rivers (Lake Michigan; Fig. 1). The Rifle River was treated in two separate parts; the upper section was treated exclusively with TFM and the lower section with TFM/niclosamide. The two sections were treated as independent observations and were assessed separately, resulting in 10 treatments to evaluate *in situ* lampricide-induced lake sturgeon mortality.

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