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Evaluation of Avoidance Behavior of Tadpole Madtoms (*Noturus gyrinus*) as a Surrogate for the Endangered Northern Madtom (*Noturus stigmosus*) in Response to Granular Bayluscide®

By Michael A. Boogaard, Richard A. Erickson, and Terrance D. Hubert

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
	Volume	
cubic meter (m^3)	6.290	barrel (petroleum, 1 barrel = 42 gal)
liter (L)	33.81402	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
cubic meter (m^3)	264.2	gallon (gal)
cubic decimeter (dm^3)	0.2642	gallon (gal)
cubic meter (m^3)	0.0002642	million gallons (Mgal)
cubic centimeter (cm^3)	0.06102	cubic inch (in^3)
cubic decimeter (dm^3)	61.02	cubic inch (in^3)
liter (L)	61.02	cubic inch (in^3)
cubic decimeter (dm^3)	0.03531	cubic foot (ft^3)
cubic meter (m^3)	35.31	cubic foot (ft^3)
cubic meter (m^3)	1.308	cubic yard (yd^3)
cubic kilometer (km^3)	0.2399	cubic mile (mi^3)
cubic meter (m^3)	0.0008107	acre-foot (acre-ft)
cubic hectometer (hm^3)	810.7	acre-foot (acre-ft)
	Flow rate	
cubic meter per second (m^3/s)	70.07	acre-foot per day (acre-ft/d)
	Density	

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as °C = (°F – 32) / 1.8.

Abbreviations

ASTM	American Society of Testing and Materials
CI	Confidence Interval
cm	centimeter
HEC	Huron-Erie Corridor
MS222	Tricaine methanesulfonate
SD	Standard Deviation
TFM	3-trifluoromethyl-4-nitrophenol
UMESC	Upper Midwest Environmental Sciences Center

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Introduction

The invasive sea lamprey (*Petromyzon marinus*) was first detected in Lake Erie in 1921 (Dymond, 1922) but was not considered a threat to the fish community until the late 1970s (Pearse and others, 1980). Efforts to control populations in the lake by selectively treating tributary streams with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) began in 1986. Suppression of sea lampreys was nearly immediate, as evidenced by declining larval, parasitic, and spawning abundance. Survival of lake trout (*Salvelinus namaycush*) markedly improved by the early 1990s (Sullivan and others, 2003). Despite continued control, however, the number of adult sea lampreys in Lake Erie was again approaching pre-control levels by 2005. In response to this increase, the Great Lakes Fishery Commission developed a large-scale sea lamprey control strategy for Lake Erie that involved treating all infested tributaries in the spring of 2008 and in the fall of 2009 (Neave and others, 2014). Traditionally, sea lamprey-producing streams are treated on a 3–4 year cycle depending on the size and age structure of the larvae present (Brege and others, 2003). Although the strategy reduced the number of adults in the lake, abundance in 2010 was still above pre-control and target levels (Sullivan and Adair, 2014), triggering a search for unknown sources of larval sea lampreys in hundreds of locations around Lake Erie.

The Huron-Erie Corridor (HEC) includes the St. Clair River, Lake St. Clair, and the Detroit River and connects Lake Huron with Lake Erie. Larval sea lampreys have been detected in small numbers in the HEC since 1975 but were not considered a threat because of poor water quality from contamination and intense predation inhibiting their downstream migration. Recent monitoring efforts have since confirmed some of these larvae are successfully migrating to Lake Erie (P. Hrodey, U.S. Fish and Wildlife Service, Marquette Biological Station, unpublished data, 2015). Because of an average discharge more than 5,000 cubic meters per second (Holtschlag and Koschik, 2002), the HEC cannot be treated effectively with TFM. Control agents are currently (2015) considering treatment strategies used on the St. Marys River, where a granular formulation of the lampricide Bayluscide®, the ethanolamine salt of the active ingredient niclosamide, is applied to areas with high larval densities. Bayluscide Granular Sea Lamprey Larvicide® has been used since the mid-1990s as a granular bottom-release formulation to survey or control larval sea lampreys in the St. Marys River and other areas where TFM applications are impractical. Extensive larval assessment efforts in the HEC from 2011 to 2013 identified a number of areas with high larval densities that could be effectively treated with the granular formulation (Neave and others, 2014). The granular formulation is typically applied at rates to achieve a Bayluscide® concentration of 11 milligrams per liter ([mg/L]; 9.3 mg/L active ingredient niclosamide) in the bottom 5 centimeters (cm) of the water column (Adair and Sullivan, 2009).

As with any application of pesticides in the environment, effects from granular Bayluscide® applications on nontarget organisms are a major concern. Because the granular formulation targets the bottom sediments where larval sea lampreys reside, other benthic organisms, such as native bottom-dwelling fishes, may be at risk. One species of particular concern identified as endangered by the Committee on the Status of Endangered Wildlife in Canada is the northern madtom (*Noturus stigmosus*). The northern madtom typically prefers large creeks and small rivers but has been found in deeper waters of the HEC.

Previous studies have shown that some fish species can detect and avoid Bayluscide®. Dawson and others (1998) noted that Eurasian ruffe (*Gymnocephalus cernuus*) significantly avoided the granular formulation of Bayluscide® at concentrations typically applied in the field to control larval sea lampreys. In a similar study, Boogaard and others (2008) showed that juvenile lake sturgeon also significantly avoided the granular formulation when applied at typical treatment rates.

Because of the northern madtom's endangered status and the likelihood that securing enough individuals for the study is low, the tadpole madtom (*Noturus gyrinus*) was identified by Fisheries and Oceans, Canada, Species at Risk officials as an acceptable surrogate for this study on the basis of similar life history characteristics and habitat requirements. The objective of this study was to document the vertical avoidance behavior of the tadpole madtom, as a surrogate for the northern madtom, in response to granular Bayluscide® when applied at typical treatment rates.

Methods

Tadpole madtoms with prior disease-free certification were obtained from Willocats Inc., Cochrane, Wisconsin, and held at 20 degrees Celsius (ºC) at the U.S. Geological Survey, Upper Midwest Environmental Sciences Center (UMESC), in well water for a minimum of 2 weeks prior to testing. One week before test initiation, madtoms were acclimated to the target test temperature of 14 ºC over a 2-day period (no more than 3 ºC/day), according to methods in the Annual Book of the American Society for Testing and Materials Standards (ASTM, 2014). Avoidance by tadpole madtoms of granular Bayluscide® was assessed in vertical columns according to methods in Dawson and others (1998) and Boogaard and others (2008). Fish were exposed in UMESC well water at 14 ºC. Dissolved oxygen and water temperature were measured and recorded for each column prior to the addition of the granules. Clear Plexiglas® columns measuring 107 cm in height and 30.5 cm in diameter were used to evaluate the potential for the normally bottom-dwelling madtom to move vertically in the water column to avoid niclosamide (the active ingredient in Bayluscide®) dissolving from the granules. One madtom was placed in each of six columns (three control columns and three treated columns randomly selected) and allowed to acclimate for 30 minutes. After the acclimation period, granular Bayluscide® was applied to the treated columns (1.17 grams) at a rate equivalent to 11 mg/L (9.3 mg/L niclosamide) in the bottom 5 cm of the water column. A similar amount of washed sand was concurrently added to the control columns. The behavior of madtoms was recorded with a video camera for 60 minutes to provide an adequate amount of time for dissolution of the Bayluscide® from the granules and for triggering a response. Vertical migration of madtoms to greater than 15 cm from the bottom of the column was considered avoidance. Avoidance observations for each fish were recorded at 30-second intervals throughout the 60-minute trial. Each set of trials was conducted 15 times to allow for statistical comparison (45 treated and 45 controls). Any mortalities were noted at the end of each trial. If a mortality occurred, the time of death or incapacitation was estimated from the video recording (cessation of any movement), and the avoidance evaluation for that fish was halted thereafter. Fish were used only once, and those that survived exposure were euthanized in a 200-mg/L solution of tricaine methanesulfonate (MS222). After each trial, exposure water from each column was passed through a carbon filter prior to being sent to the UMESC toxic waste line.

Vertical avoidance trials produced data consisting of the number of madtoms avoiding granular Bayluscide® at a given time. We were interested in determining whether the granular application caused madtoms to avoid the treatment and in predicting the proportion avoiding a given time after exposure. We initially planned to use the Gompertz model to demonstrate the nonlinear relation between the proportion of tadpole madtoms avoiding granular Bayluscide exposure and time (Bates and Watts, 1988). However, upon inspection of the data using a spline in the ggplot2 plotting package in R (fig. 1; Wickham, 2009), we determined that the response was nonmonotonic (fig. 1) and the Gompertz model would therefore be inappropriate. Instead, the model was fit using a generalized additive-mixed model (gamm; Zurr and others, 2009) using the gamm4 package in R (Wood and Scheipl, 2014; R Core Team, 2015). We modeled the probability of avoidance as a fixed effect of time (as a spline), treatment, and column. We modeled fish and trial as random effects, and a binomial error term was used within the model.

We assumed that avoidance between columns during the same trial and between trials was independent but that some covariance was likely within columns because repeated observations were made on the same population of fish. The experimental unit was the column (one fish per column) within a trial, and the model treated each column within and between trials independently. Ninety-five-percent confidence intervals were constructed for the proportion of madtoms avoiding at each point in time across treatment.

Results and Discussion

The mean water temperature for all avoidance trials was 13.8 °C (standard deviation, [SD] 0.3 °C) and ranged from 13.4 to 15.1 °C. Dissolved oxygen concentration averaged 7.8 mg/L (SD 0.4 mg/L) and ranged from 7.0 to 8.6 mg/L. Initial avoidance or the duration of time before vertical movement was first observed averaged 6.6 minutes (SD 4.6 minutes); avoidance was observed as early as 3.5 minutes and as late as 22.5 minutes after the addition of granular Bayluscide®. In addition, several madtoms maintained their position at the water surface throughout the 60-minute observation period. Overall mortality in the treated columns was high (67 percent) but not unexpected, given that fish were confined in the vertical columns and had no chance to swim horizontally away from the chemical. No mortalities were observed in the controls in any of the trials.

The relation between the probability of avoidance and time was nonmonotonic because the control and treatment probabilities decreased after initially increasing (fig. 1). Time had a significant, nonlinear effect of the probability of avoidance ($z = -6.08$, $P < 0.001$). Treatments increased the odds of avoidance by a factor of 11.7 compared to controls (95-percent confidence interval 5.1–28.9, $z = 5.741$, $P < 0.001$). None of the columns affected the probability of avoidance (all *z*s < 1.4, all *P*s >0.160). The initial increase in avoidance observed in both treated and control columns was likely due to insufficient acclimation to the test system because the madtoms in control columns eventually settled back to the bottom (fig. 1). Also, the slight decrease in avoidance seen from madtoms in treated columns at the end of the exposure period could be due to overestimation of the time at which they became incapacitated and could no longer avoid the chemical.

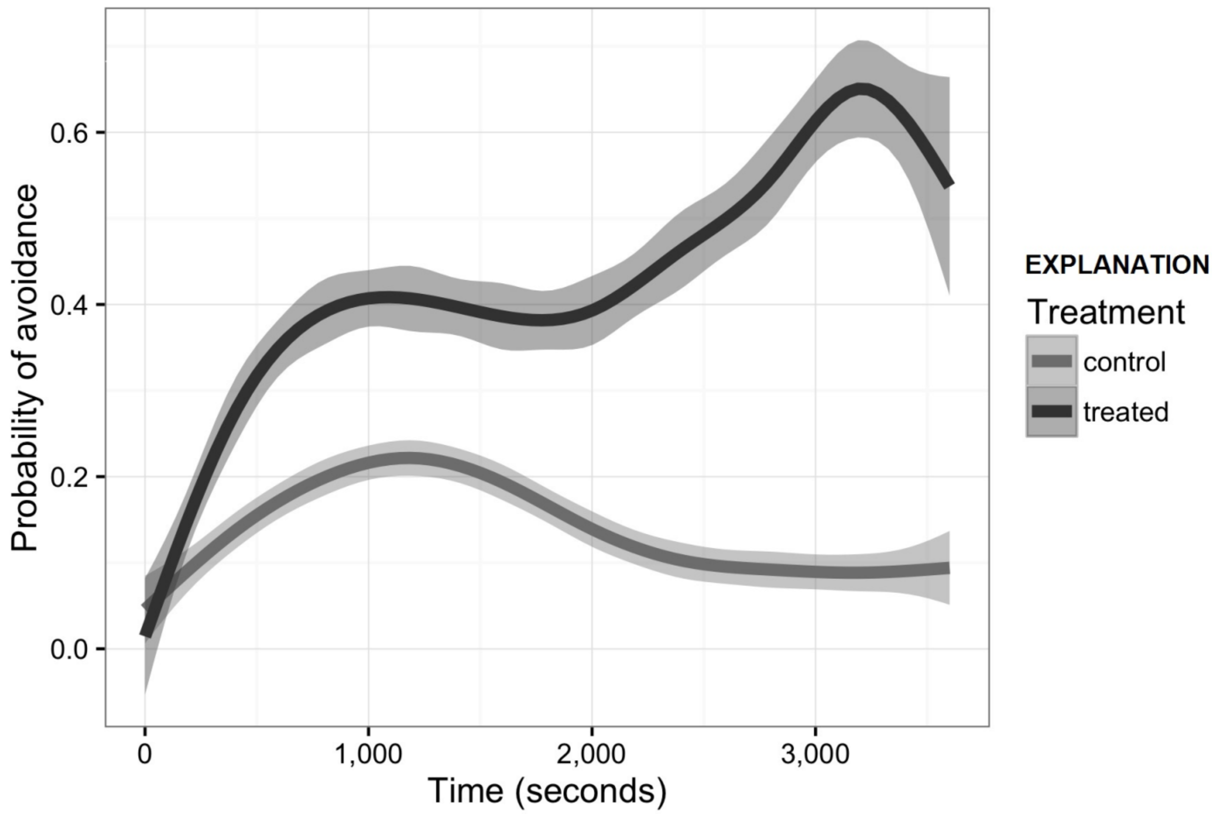

Figure 1. Relation between probability of avoidance and time of tadpole madtoms (*Noturus gyrinus*) exposed to Bayluscide® Granular Sea Lamprey Larvicide. The line is a spline, and the shaded region is the 95-percent confidence interval.

On the basis of the avoidance results from this study, it is likely that northern madtoms will be able to detect and avoid Bayluscide® from granular applications if their response is similar to that of the tadpole madtom and if they are provided an opportunity to escape. Current (2015) sea lamprey assessment/treatment protocols call for starting the application on the inshore side of the treatment plot and working outward, thereby providing an avenue for escape. However, if the application area is too large for escape, significant mortality may occur as evidenced by the mortalities observed in this study.

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