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Lethal and sub-lethal responses of native freshwater mussels exposed to granular Bayluscide®, a sea lamprey larvicide

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ABSTRACT

The invasive sea lamprey (*Petromyzon marinus*) poses a substantial threat to fish communities in the Great Lakes. Efforts to control sea lamprey populations typically involve treating tributary streams with lampricides on a recurring cycle. The presence of a substantial population of larval sea lampreys in the aquatic corridor between Lakes Huron and Erie prompted managers to propose a treatment using the granular formulation of Bayluscide® that targets larval sea lampreys that reside in sediments. However, these treatments could cause adverse effects on native freshwater mussels—imperiled animals that also reside in sediments. We estimated the risk of mortality and sub-lethal effects among eight species of adult and sub-adult mussels exposed to Bayluscide® for durations up to 8 h to mimic field applications. Mortality was appreciable in some species, especially in sub-adults (range, 23–51%). The lethal and sub-lethal effects were positively associated with the duration of exposure in most species and life stage combinations. Estimates of the median time of exposure that resulted in lethal and sub-lethal effects suggest that sub-adults were often affected by Bayluscide® earlier than adults. Siphoning activity and burrowing position of mussels during exposure may have moderated the uptake of Bayluscide® and may have influenced lethal and sub-lethal responses. Given that the various species and life stages were differentially affected, it will be difficult to predict the effects of Bayluscide® treatments on mussels.

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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 (Pearce et al., 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, while survival of lake trout (*Salvelinus namaycush*) markedly improved by the early 1990s (Sullivan et al., 2003). Despite continued control efforts, however, the number of adult sea lampreys in Lake Erie was again approaching pre-treatment levels by 2005 (Young and Klar, 2006). 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 (Adair and Young, 2009) and in the fall of 2009 (Sullivan and Adair, 2010). Sea lamprey producing streams are typically treated on a 3–4 year cycle depending on the size and age structure of the larvae present (Brege et al., 2003). A stream treatment is deemed effective if a minimum of 95% sea lamprey larval mortality is achieved based on pre and post population surveys (Lori Criger, U.S. Fish and Wildlife Service, Marquette Biological Station, personal communication, November 14, 2016).

The Huron Erie Corridor (HEC), comprised of the St. Clair River, Lake St. Clair, and the Detroit River, connects Lake Huron with Lake Erie. Larval sea lampreys have been detected in small numbers in the HEC since 1975, yet were not considered a threat due to poor water quality from pollution and intense predation during their downstream migration. Recent monitoring efforts have since confirmed some of these larvae are successfully migrating to Lake Erie (Adair and Sullivan, 2015). With an average discharge over 5000 m^3/s (Holtschlag and Koschik, 2002), the HEC cannot be treated effectively with TFM. Management agencies are currently considering treatment strategies used on the St. Marys River where a granular, encapsulated, formulation of the lampricide Bayluscide® (the ethanolamine salt of the active ingredient niclosamide) is applied to areas with high larval densities. Bayluscide® 3.2% Granular Sea Lamprey Larvicide® (hereafter referred to as Bayluscide®) has been used since the mid-1990s as a bottom-release formulation to survey or control larval sea lampreys in the St. Mary's River and other areas where TFM applications are impractical. Extensive monitoring in the HEC during 2011–2013 identified a number of areas with high larval densities that could be effectively treated with Bayluscide® (Adair and Sullivan, 2015). Bayluscide® is typically applied at rates to achieve a nominal peak concentration of 11 mg/L (9.3 mg/L active ingredient niclosamide) in the bottom 5 cm of the water column

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(Adair and Sullivan, 2009). A granular Bayluscide® treatment is deemed effective if a minimum of 75% sea lamprey mortality is achieved based on pre and post population surveys (Lori Criger, U.S. Fish and Wildlife Service, Marquette Biological Station, personal communication, November 16, 2016). Collectively, the use of TFM and Bayluscide® have successfully reduced sea lamprey numbers in the Great Lakes by over 90% since treatments were initiated in the 1960s (Crowe, 1975).

As with any application of pesticides into the environment, effects on non-target organisms are a concern. Because the granular formulation, Bayluscide®, targets bottom sediments where larval sea lampreys reside, other benthic organisms, such as native freshwater mussels (Unionoida, hereafter referred to as mussels), may be at risk. Considering that Bayluscide® was originally developed as a molluscicide to eliminate snails – the intermediate hosts of parasites causing schistosomiasis in humans (Gönnert, 1962) – mussels may be vulnerable to granular applications. At present, the effects of Bayluscide® on mussels are not known. In addition, the duration that Bayluscide® remains at toxic levels at the sediment-water interface is unknown, largely because methods to accurately quantify Bayluscide® concentrations in bottom sediments have never been developed.

Native freshwater mussels are critically imperiled on a global scale (Lydeard et al., 2004). In Canada, 61% of mussel species are considered to be endangered, threatened, or in decline (Canadian Endangered Species Conservation Council, 2011). To facilitate effective conservation, an understanding of the lethal and sub-lethal effects of pesticides on mussel assemblages is needed (Strayer, 2008). Resource managers identified 13 mussel species of interest due to potential overlap in distributions with larval sea lampreys in the HEC. Resident populations of these mussels could be adversely affected by proposed Bayluscide® treatments in the HEC. The objectives were to 1) estimate the risk of mortality as a function of exposure duration among adult and sub-adult mussel species exposed to environmentally-relevant concentrations of Bayluscide® and 2) evaluate the risk (probability and duration) of sub-lethal effects among adult and sub-adult mussel species exposed to environmentally-relevant concentrations of Bayluscide®.

Methods

Test animals

Eight species of mussels were selected for study based on availability and the potential for overlap with larval sea lamprey distributions. Six adult and four sub-adult (defined as one or two growing seasons) species were obtained from hatchery and wild sources (two species were evaluated as both adults and sub-adults, Table 1). All mussels were shipped overnight express to, or collected by, personnel from the U.S. Fish and Wildlife Service, Genoa, WI. Within a few days, mussels were transported to the Upper Midwest Environmental Sciences Center (UMESC) in chilled coolers (7–12 °C) following methods in Newton et al. (2001). At UMESC, mussels were acclimated to 12 °C well water and held for 1–2 days in a 2.7 m × 0.7 m × 0.4 m fiberglass holding tank containing 10–15 cm of washed sand and 18–25 cm of well water to assess latent mortality from transport. Animal care and use procedures for mussels followed guidelines in ASTM (2013).

Granular Bayluscide® exposures

Each mussel was measured for shell length (Table 1) and uniquely marked by etching an identification number onto the shell. We also attached a 145 mm piece of buoyant fly fishing (adults) or 2 lb. test (sub-adults) line equipped with a small float secured at the top of the line with the same identification number. This was done to identify mussels that completely burrowed and were not visible during exposure to Bayluscide®. Mussels were exposed to Bayluscide® in one of five replicate glass aquaria (46 cm × 30 cm × 25 cm) containing ~10–15 cm of washed sand and ~20 L of 20 °C well water. Each species and life stage was tested separately. Twelve mussels were randomly assigned to each aquarium for all species except *Villosa iris* adults and *Pytochobranchus fasciolaris* sub-adults. Due to the limited number of individuals of these two species available for testing, there were only seven mussels per aquarium.

Table 1
Initial mean shell length (mm, 1 SEM) and source of native freshwater mussels used in laboratory toxicity tests with 3.2% granular Bayluscide®.

Species	Life stage	Mean length	Source
Lampsilis fasciola	Sub-adult	15.9 (0.3)	Hatchery (Clinch River, VA)
Ligumia nasuta	Adult	60.8 (0.3)	Hatchery (Nottoway River, VA)
Obovaria olivaria	Sub-adult	34.5 (0.3)	Hatchery (Iowa River, IA)
Obovaria olivaria	Adult	79.3 (1.0)	Wild (Chippewa River, WI)
Obovaria subrotunda	Adult	38.3 (1.0)	Wild (Duck River, TN)
Pleurobema sintoxia	Adult	63.5 (1.0)	Wild (Mississippi River, WI)
Pytochobranchus fasciolaris	Sub-adult	28.9 (0.4)	Hatchery (Powell River, VA)
Quadrula quadrula	Adult	61.4 (1.4)	Wild (Mississippi River, WI)
Villosa iris	Sub-adult	24.4 (0.8)	Hatchery (Clinch River, VA)
Villosa iris	Adult	46.5 (1.1)	Wild (Indian Creek, VA)

During the acclimation period, the temperature in each aquarium was increased incrementally (≤3 °C/day) from the transport temperature until it reached 20 °C (ASTM, 2000; Galbraith et al., 2012). Mussels were acclimated to this temperature for another 4–5 days (~1 week total acclimation) to allow mussels to burrow and to assess latent mortality from measuring, tagging, and transport. Most mussels had burrowed at least halfway into the sediments by day 2 of the acclimation period. During the acclimation period, water temperature and dissolved oxygen in each aquarium were recorded daily and flow into each aquarium was maintained at ~300 mL/min. Mussels were fed daily during the acclimation period by adding 450 mL of a stock solution containing 6 mL Nanno 360 and 12 mL Shellfish diet 1800 (Reed Mariculture, Campbell, CA) diluted in 11 L well water to each aquaria (Ganser et al., 2013). Prior to the addition of food, water from each aquarium was siphoned down to ~3–5 cm from the sediment surface (while still maintaining the 300 mL/min flow) to allow mussels time to feed.

Prior to applying Bayluscide® to each aquarium, one of three vertical positions (on the sediment surface, burrowed halfway, or totally burrowed) and status (dead or alive) of each mussel was recorded. Any mussels found dead prior to exposure were replaced, if available. No sub-adults were found dead prior to addition of Bayluscide®. Seven adults (two *Pleurobema sintoxia*, three *Obovaria olivaria*, and two *Obovaria subrotunda*) were found dead and were not replaced due to limited numbers available for testing. Water temperature and dissolved oxygen from each aquarium were recorded. At the start of each exposure (0 min), the flow into each aquarium was turned off and 2.23 ± 0.01 g of Bayluscide® was applied by a salt shaker over the water surface of each aquarium to achieve a nominal concentration of 9.3 mg/L niclosamide in the bottom 5 cm of the water column (the rate applied in field treatments).

Because Bayluscide® is only applied in field treatments at a nominal concentration of 9.3 mg/L niclosamide, a dose-response experiment was not appropriate. Rather, we conducted a duration-response experiment where duration was the number of minutes that each mussel was exposed to Bayluscide® and the responses were mortality and sub-lethal effects (see below). One mussel from each aquarium was randomly assigned to one of 12 exposure durations. Exposure durations were: control (0 min, no chemical exposure), 15, 30, 45, 60, 75, 90, 105, 120, 240, 360, and 480 min. An exception was that there were only seven exposure durations (0, 30, 60, 90, 120, 240, and 480 min) for *V. iris* adults and *P. fasciolaris* sub-adults due to a limited number of individuals available for testing. At each exposure duration, we estimated mortality, depth burrowed in substrate, and a suite of sub-lethal responses (i.e., siphoning activity, gaped valves, production of mucus, and rigid foot

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