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Sensitivity of freshwater molluscs to hydrilla-targeting herbicides: providing context for invasive aquatic weed control in diverse ecosystems

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Hydrilla (Hydrilla verticillata) is an invasive aquatic weed that has spread rapidly throughout the USA, especially in the southeast. A common control method is the application of aquatic herbicides, such as fluridone and endothall. However, there is limited documentation on the effects of herbicides commonly used to control hydrilla and other aquatic weeds on many non-target freshwater species and no published information exists on the toxicity of these herbicides to freshwater molluscs. We exposed juveniles (96 h) and glochidia (48 h) of the unionid mussel Lampsilis siliquoidea and adults (28 d) of Lampsilis fullerkati to a formulation of fluridone (Sonar – PR®) in laboratory toxicity tests. The early life stages of L. siliquoidea were also exposed to a formulation of the dipotassium salt of endothall (Aquathol – K®) in separate tests. Juveniles of the freshwater gastropod snail, Somatogyrus viriginicus (Lithoglyphidae), were exposed (96 h) to the Sonar – Genesis® fluridone formulation. Endpoints were survival (all species and life stages) as well as siphoning behavior and foot protrusion (adult mussels). Median lethal fluridone concentrations (LC50s) were 865 μg/L (95% CI, 729–1,026 μg/L) for glochidia (24 h), 511 μg/L (309–843 μg/L) for juvenile L. siliquoidea (96 h), and 500 μg/L (452–553 μg/L) for juvenile S. viriginicus (96 h). No mortality occurred in the 28-d exposure of adult L. fullerkati and we found no statistically significant effect of fluridone concentration on foot protrusion (p = 0.06) or siphoning behavior (p = 0.08). The 24-h LC50 for glochidia exposed to the dipotassium salt of endothall was 31.2 mg/L (30.3–32.2 mg/L) and the 96-h LC50 for juvenile mussels was 34.4 mg/L (29.3–40.5 mg/L). Freshwater molluscs were more sensitive to fluridone and endothall than most other species previously tested. Fluridone and endothall concentrations typically recommended for hydrilla treatment (5–15 μg/L and 1–5 mg/L, respectively) were not acutely toxic to the molluscs we tested and a 28-d exposure to fluridone was not lethal to adult mussels even at the highest concentration (300 μg/L), indicating minimal risk of short-term exposure effects.

Keywords: fluridone (Sonar); endothall (Aquathol); unionid mussels; snails; LC50; toxicity; invasive species

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Introduction

Freshwater systems are subject to many stressors, including point and non-point source pollution, extreme climatic events, habitat modification (e.g., dams), and invasive species (commonly anthropogenically introduced). Hydrilla (*Hydrilla verticillata*, Hydrocharitaceae) is a non-native aquatic invasive weed that was introduced into the United States in Florida in the early 1950s and has spread rapidly throughout the country, especially in the southeast (Gordon & Thomas 1997). Included on the Federal Noxious Weed List (USDA APHIS 2012), hydrilla can form vast monocultures, shade out native vegetation (FWC 2013), alter water quality parameters including dissolved oxygen (Pesacreta 1988), and can serve as a vector for a neurotoxic cyanobacteria that has been linked to avian vacuolar myelinopathy in several water birds and their predators (e.g., bald eagle *Haliaeetus leucocephalus* and great horned owl *Bubo virginianus*; Wiley et al. 2008; Williams et al. 2009).

Hydrilla produces numerous vegetative propagules (e.g., tubers, turions, and shoot fragments), and is frequently dispersed by humans via boat motors, trailers, and angling gear. Given the longevity of tubers in bottom sediments, eradication and/or long-term maintenance control is difficult (Langeland 1996). The most common control methods include application of aquatic herbicides, introduction of non-native grass carp (*Ctenopharyngodon idella*), and mechanical removal (Langeland 1996). Fluridone (Sonar®), a carotenoid synthesis inhibitor herbicide, is among the most commonly used aquatic herbicides for hydrilla management, and is typically prescribed for one to four months depending on the management objective and plant maturity. The dipotassium salt of endothall (Aquathol®) is also among the most commonly used aquatic herbicides for control of hydrilla and is typically prescribed two to three times during the growing season, each for a period of days. The impetus for this study was the recent introduction and persistence of hydrilla in two North Carolina, USA, ecosystems (Lake Waccamaw and the Eno River) with high biodiversity, high rates of endemism, and the presence of threatened and endangered species (Stager & Cahoon 1987; Smith et al. 2002; NCWRC 2005; LeGrand et al. 2013; NatureServe 2013). Here, the targeted use of herbicides has been recommended as the most effective hydrilla control method that is least likely to negatively affect native vegetation. However, increased information is needed on the potential effects of these herbicides on other non-target organisms.

Lake Waccamaw is a unique Carolina Bay Lake located in the southeastern coastal plain of North Carolina, USA, because it has a neutral pH, unlike other bay lakes and blackwater systems, which enable it to support high biodiversity (Stager & Cahoon 1987). It has been called a ‘notable center of endemism in the southeast’ (Smith et al. 2002), supporting several endemic and other rare species, including two endemic unionid mussels (state-listed threatened Waccamaw fatmucket *Lampsilis fullerkati* and state-listed endangered Waccamaw spike *Elliptio waccamawensis*) and two endemic freshwater snails (Waccamaw snail *Amnicola sp. 1* and Waccamaw silt snail *Cincinnatia sp. 1*; NCWRC 2005; LeGrand et al. 2013). The Eno River is located in the Piedmont region of North Carolina (USA), and supports a variety of rare species, including the Carolina madtom (*Noturus furiosus*, state-listed threatened), one state-threatened (*Lampsilis radiata*) and three state-endangered (*Fusconaia masoni, Lampsilis cariosa, Lasmigona subviridis*) freshwater mussels, and the only confirmed population of panhandle pebblesnail (*Somatogyrus viriginicus*) in the state (LeGrand et al. 2013).

Though toxicity data exist for some freshwater invertebrates and fishes (Crosby & Tucker 1966; Hamelink et al. 1986; Paul et al. 1994; Yi et al. 2011), to our knowledge, no information has been published on the toxicity of fluridone or endothall to freshwater...
Understanding the potential risks to this non-target faunal group is especially important because both freshwater mussels and snails are simultaneously highly imperilled and critically important to the functional ecology of freshwater systems (Lydeard et al. 2004; Downing et al. 2010; Allen et al. 2012; Johnson et al. 2013). The southeastern USA, where hydrilla is most prevalent, has the highest unionid mussel biodiversity and endemism compared to any other region on the planet and >71% of North America’s unionid species are endangered, threatened, or of special concern (Williams et al. 1993). Similarly, of the 703 freshwater gastropod species in USA and Canada, 278 (40%) are federally listed as endangered and >74% are considered imperilled (Johnson et al. 2013). Moreover, non-pulmonate snails and the early life stages of freshwater mussels are among the most sensitive aquatic organisms to several contaminants (e.g., atrazine, carbaryl (Conners & Black 2004); copper, ammonia (Besser et al. 2009)), and glyphosate-based chemicals which are among the most widely used herbicides (Bringolf et al. 2007). Potential risks of specific aquatic herbicides to freshwater molluscs should be assessed and balanced appropriately against the significant biological threat posed by invasive aquatic weeds like hydrilla. Further endangerment to these organisms may push some species to extinction and reduce common species to rare status.

Fluridone (market formulations tested: granular Sonar – PR® and liquid Sonar – Genesis®) and the dipotassium salt of endothall (hereafter, simply ‘endothall’; market formulation Aquathol – K®) applications are commonly prescribed for management of hydrilla and both were considered for management of hydrilla in Lake Waccamaw (NC DENR 2013) and the Eno River. Unlike many aquatic systems in the southeastern USA that hydrilla has invaded and which have relatively low biodiversity (e.g., reservoirs, canals, and ponds), the two aforementioned ecologically unique systems in North Carolina, as well as others requiring similar conservation management, dictate a more thorough assessment of potential hazards to non-target biota from herbicide treatment. Therefore, we chose species of direct relevance to these systems for toxicity testing in this study. The purpose of this study was to determine the sensitivity of freshwater mussels and snails to herbicides commonly used in control and management of hydrilla and other aquatic weeds and to consider those results in the context of typically proposed hydrilla treatments (e.g., Lake Waccamaw) and potential future treatment of other sensitive ecosystems (e.g., Eno River).

Methods

Test organisms

Freshwater mussels are especially important non-target organisms for toxicity testing. They have been demonstrated as particularly susceptible to toxicants and other environmental stressors, in part because their larval life stage, glochidia, is an obligate parasite that requires encystment on a host fish to transform into the juvenile life stage (Cope et al. 2008). Therefore, juveniles and glochidia of the unionid mussel Lampsilis siliquoides (fatmucket) were used in fluridone (Sonar – PR®) and endothall acute toxicity tests; L. siliquoides is routinely used in toxicity testing due to its wide availability and ease of laboratory culture. Lampsilis siliquoides is a congener of the Lake Waccamaw endemic and state-listed as threatened Waccamaw fatmucket (Lampsilis fullerikati). Lampsilis siliquoides were supplied by the mussel culture laboratory at Missouri State University (Springfield, Missouri, USA). All glochidia were harvested from females <24 h before initiation of each test. All juveniles were propagated via host-fish infection, using
standard propagation and culture methods (Barnhart 2006), and ranged in age from 1 to 3 d, with an average shell length of 0.25 mm (± 0.14 mm, SD).

Adult *L. fullerkati* mussels were used in a 28-d chronic experiment. They were 33 months old at the time of the experiment, with an average shell length of 46.6 mm (± 3.3 mm; range 37.5–53.9 mm) and mean weight of 9.9 g (± 2.0 g; range 6.3–14.8 g). *Lampsilis fullerkati* were propagated at the Aquatic Epidemiology Conservation Laboratory, North Carolina State University College of Veterinary Medicine (Raleigh, North Carolina, USA), using a standard in vitro propagation protocol (Owen 2009).

The freshwater snail *Somatogyrus virginicus* was used in acute toxicity tests with the Sonar – Genesis® fluridone formulation; *S. virginicus* (Lithoglyphidae) is a rare, non-pulmonate snail with patchy distribution in Atlantic Slope streams of Virginia, North Carolina, and South Carolina (USA; NatureServe 2013). *Somatogyrus virginicus* is an annual species, in which most adults die soon after reproducing (Johnson et al. 2013). Juveniles were collected on 6 August 2013 from a viable population in the Eno River (near Hillsborough, North Carolina, USA) and were immediately transported to our laboratory at North Carolina State University for testing. Average shell length, as measured from the top down, perpendicular to the spiral, was 1.84 mm (± 0.37 mm). Based on earlier sampling in the Eno River on 2 May 2013, in which only adults and eggs were found, the juveniles tested were <3 months old.

**Experimental conditions**

We selected herbicide treatment concentrations based on recommended application rates for treatment of hydrilla, maximum application rates reported on the product label, and acute toxicity data reported for other taxa in peer-reviewed literature (Crosby & Tucker 1966; Sanders 1969; Hamelink et al. 1986; Paul et al. 1994; Yi et al. 2011) and on Material Data Safety Sheets (SePRO Corporation 2009, 2010, 2011, 2012; UPI 2011, 2012). An analytically verified 1304 µg/L (parts per billion) stock solution of Sonar – PR® (fluridone) formulation was prepared and provided by the SePRO Research and Technology Campus (Whitakers, North Carolina, USA). An analytically verified stock solution of Sonar – Genesis® formulation was prepared at 1383 µg/L. The fluridone formulations were shipped via overnight courier to our laboratory at North Carolina State University and immediately refrigerated until use in toxicity tests. Acute test concentrations of fluridone formulations ranged from 2.5 to 200 µg/L with an additional treatment at the stock solution concentrations (1304 µg/L for PR® and 1383 µg/L for Genesis®). Concentrations of Sonar – PR® in the chronic (28-d) experiment ranged from 5 to 300 µg/L. A concentrated formulation of endothall (Aquathol-K®), labeled as 4.23 lb/gal (~506,866 mg/L), was hand delivered by collaborating personnel in the Department of Crop Science, North Carolina State University, and subsequently diluted to a working stock of 1000 mg/L (parts per million). Test concentrations of endothall ranged from 0.5 to 1000 mg/L. Composite water samples (10 mL from each of three replicates, 30 mL total volume) were collected for herbicide concentration verification prior to placing organisms into the chambers, and again at 48 h; samples were stored at 4 ºC until they were shipped to SePRO analytical laboratory (fluridone quantified via HPLC) or the US Army Engineer Research and Development Center’s Environmental Laboratory (endothall quantified via immunoassay; Gainesville, Florida, USA).

All experiments were static-renewal tests conducted in reconstituted soft water (ASTM 2007), with 90%–100% water renewal at 48 h in the 96-h acute non-aerated tests, and at 72-h intervals in the 28-d aerated experiment. Soft water was selected because it most
closely approximated the water quality parameters in most of the test organisms’ native ranges (e.g., Lake Waccamaw, Eno River). Quality assurance and control were ensured by conducting all tests according to the Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (ASTM 2006). No formalized guidelines exist for conducting experiments with freshwater snails or adult mussels, so the mussel guideline was used (ASTM 2006), as per protocol in other studies (Besser et al. 2009; Archambault et al. 2013). Organisms were acclimated from their culture water to the test water by placing them in a 50:50 solution of culture/reconstituted water for 2 h, then further diluting the culture water to a 25:75 ratio with reconstituted water, and held for an additional 2 h before being placed in 100% reconstituted water (ASTM 2006, 2007). Tests were conducted in light and temperature-controlled environmental chambers (Precision Model 818, Thermo Fisher Scientific, Marietta, Ohio, USA), held at 20 °C and LD 16:8. In the 24-h tests, ~150 glochidia were placed in each of three replicates per treatment. In the 96-h experiments, seven mussels or snails were placed in each of three replicates per treatment, with 10 organisms per replicate in controls (0 μg/L). Snails were transferred to untreated ASTM water at the conclusion of the test and held for 48 h for a post-exposure survival assessment to identify potential latent mortality effects (per J. Besser, 2013, e-mail to WGC; unreferenced). Mean water quality conditions among acute experiments were 27.8 mg CaCO₃/L alkalinity, 39.0 mg CaCO₃/L hardness, 220 μS/cm conductivity, 7.50 pH, and 8.47 mg/L dissolved oxygen (n = 4 for alkalinity and hardness, n = 36 for all other variables). In the 28-d experiment, five adult mussels were placed in each of three replicates per treatment. Mussels were fed a mixture of 1-mL Instant Algae® Shellfish Diet and 0.5-mL Nannochloropsis (Nanno 3600) concentrate diluted in 500 mL deionized water. Approximately, 6.25 mL of food mixture was added to each replicate (administered concentrations of 50,000 and 850,000 cells/mL solution, respectively; Reed Mariculture, Campbell, California, USA) every 72 h at least 2 h before each solution renewal (Mosher et al. 2012; Leonard 2013). Mean water quality conditions in the chronic experiment were 26.7 mg CaCO₃/L alkalinity, 44.9 mg CaCO₃/L hardness, 168 μS/cm conductivity, 7.49 pH, and 8.54 mg/L dissolved oxygen (n = 9 for alkalinity and hardness, n = 54 for pH, n = 60 for all other variables).

**Data collection and statistical analysis**

Viability was assessed at 24 h for a subsample of approximately 50 glochidia in each replicate. We assessed viability by exposing glochidia to a saturated NaCl solution and viewing them under a stereomicroscope; glochidia that exhibited a shell-closure response to salt were considered viable (ASTM 2006). At the end of each 96-h exposure, survival of juvenile mussels was assessed by viewing them under a stereomicroscope; juveniles that exhibited foot movement outside of the shell, foot movement inside the shell, or a detectable heartbeat within a five-minute observation period were considered alive (ASTM 2006). Snail survival was assessed similarly, by observing for righting or movement within five minutes. In the chronic experiment, survival of adult mussels was assessed visually every 72 h by observing for foot retraction or valve closure in response to dewatering during renewal in mussels with open shells. Because the shell of *L. fullerkiti* is thin and fragile, we made no attempt to check for resistance to opening, and mussels with tightly closed shells were assumed to be alive.

The effects of herbicide concentration on the survival of mussels and snails were analyzed by using survival data to generate median lethal concentrations (LC50s) and 95% confidence intervals (CIs) via the Trimmed Spearman-Karber method (Comprehensive
Environmental Toxicity Information Software (CETIS)™, v1.8.0.12, Tidepool Scientific, LLC, McKinleyville, California, USA). The LC50 was defined as the concentration that caused mortality in 50% of the individuals in the exposed sample, and the LC05 was defined as the concentration that caused mortality in 5% of the sample. LC values were considered significantly different when their 95% CIs did not overlap (i.e., \( \alpha = 0.05 \)).

In the 28-d experiment with adults, we made observations every 72 h of siphoning behavior and foot protrusion; mussels were given a binary designation of siphoning or not siphoning and assigned a binary score of foot protrusion or no foot protrusion (Leonard 2013). The effect of herbicide concentration on siphoning behavior and foot protrusion was analyzed using a repeated measures analysis of variance (PROC MIXED; SAS version 9.3; SAS Institute, Inc., Cary, North Carolina, USA). Significant effects (\( \alpha = 0.05 \)) of fluridone concentration were further analyzed using a Dunnett’s post hoc test.

**Results**

**Herbicide concentration analysis**

Exposure accuracy (i.e., measured herbicide concentration compared to target concentration) was calculated as: exposure accuracy = \( \frac{P_m}{P_t} \times 100 \), where \( P_m \) is the measured herbicide concentration and \( P_t \) is the target concentration. The measured concentration of the fluridone stock solution used in tests with mussels (Sonar – PR) was 108.3% of the reported prepared concentration of 1304 \( \mu \)g/L, and the mean exposure accuracy in experiments was 119.9% (range 102%–176%) of target treatment concentrations. The verified concentration of the Sonar – Genesis formulation at the time of testing with juvenile snails was 87.0% of the initial reported concentration of 1383 \( \mu \)g/L, and had a mean exposure accuracy of 85.2% (range 80%–102%) in treatments prepared from the stock. The mean exposure accuracy in endothall (Aquathol-K®) experiments was 109.0% (range 100%–114%) of target treatment concentrations.

**Mussel toxicity**

Control viability at 24 and 48 h in glochidia tests was >90% of the initial viability that was assessed on arrival to the laboratory for all experiments, in accordance with testing guidelines (ASTM 2006). Control survival in experiments with juveniles was >90%, except in the endothall experiment at the 96-h time point. Even though control survival (73.3%) at 96 h in the endothall experiment was slightly below the 80% recommended in the standard guideline for toxicity tests with juvenile mussels (ASTM 2006), results are reported herein because survival was >90% in three of the low concentration treatments (1, 5, and 10 mg/L).

The 24-h LC50 for *L. siliquoidea* glochidia exposed to fluridone (Sonar – PR) was 865 \( \mu \)g/L (95% CI, 729–1026 \( \mu \)g/L) and the 48-h LC50 was 978 \( \mu \)g/L (787–1214 \( \mu \)g/L). The experiment with juveniles yielded a 48-h LC50 of 1197 \( \mu \)g/L (569–2522 \( \mu \)g/L) and a 96-h LC50 of 309–843 \( \mu \)g/L; (Table 1)). The 24-h LC05 for glochidia was 290 \( \mu \)g/L (0–598 \( \mu \)g/L); LC05s in the juvenile tests and at the 48-h time point of the glochidia test could not be determined due to the lack of two or more partial mortality responses among treatments. A chronic LC50 could not be determined for *L. fullerkati* at any time point during the 28-d test because no mortality occurred. Adult mussels exhibited only minor foot protrusion behavior during the experiment. Moreover, the degree of foot extension observed was minimal. Initially, foot extension was recorded using four
categories: (1) shell closed and/or foot not visible; (2) foot visible, but not extended beyond mantle margin; (3) foot extended beyond mantle; and (4) foot extended and swollen. Because observations \((n = 1050)\) were recorded as category 1 (67.5% of observations) or 2 (32.4%) in all but one case, foot protrusion data were analyzed as a binary function (i.e., foot extended or not) like the siphoning data. A category 3 observation was recorded only once, and category 4 was never observed. We found no statistically significant effect of fluridone concentration on foot protrusion \((p = 0.06)\) or siphoning behavior \((p = 0.08)\).

In endothall exposures, the glochidia 24-h LC50 was 31.2 mg/L (30.3–32.2 mg/L), and the 48-h LC50 was 27.6 mg/L (25.5–29.9 mg/L). The experiment with juvenile \(L.\ siliquoidea\) yielded a 48-h LC50 of 214 mg/L (134–342 mg/L) and a 96-h LC50 of 34.4 mg/L (29.3–40.5 mg/L; (Table 1)). The 48-h LC05 for juveniles was 34.6 mg/L (3.90–80.0 mg/L); other LC05s were not determined due to the lack of two or more partial mortality responses among treatments or poor fit.

Snail toxicity
The 96-h LC50 for \(S.\ virginicus\) exposed to fluridone (Sonar – Genesis\textsuperscript{®}) was 500 \(\mu g/L\) (452–553 \(\mu g/L\)) and the LC50 at 48-h post exposure was 409 \(\mu g/L\) (329–509 \(\mu g/L\); (Table 1)). The overlapping CIs between the two assessment time points indicate that there was no significant latent mortality in the exposed snails \((\alpha < 0.05)\). An LC50 at the 48-h time point and LC05s could not be determined due to the lack of two or more partial mortality responses among treatments.

Discussion
Our results indicate that the early life stages of \(L.\ siliquoidea\) are more acutely sensitive to fluridone than most other aquatic organisms that have been tested. In a multi-laboratory study evaluating the effects technical grade fluridone (i.e., active ingredient only) and a commercial formulation of Sonar\textsuperscript{®} on freshwater and marine invertebrates and fishes, Hamelink et al. (1986) reported a mean LC50 of 4.3 mg/L for invertebrates \((n = 15\) tests among six species) and a mean LC50 of 10.4 mg/L for fishes \((n = 28\) tests among five species) (Table 2). By comparison, at 24 h, \(L.\ siliquoidea\) glochidia were approximately five times more sensitive than invertebrates they tested and 12 times more sensitive than...
Table 2. Comparative acute toxicities of freshwater species exposed to technical or commercial formulations of fluridone and dipotassium salt of endothall.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Chemical grade</th>
<th>Time point</th>
<th>LC50 (mg/L)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrates</td>
<td></td>
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<tr>
<td><strong>FLURIDONE</strong></td>
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<tr>
<td>Arrenurus sp.</td>
<td>A water mite</td>
<td>Formulation</td>
<td>96 h</td>
<td>0.010</td>
<td>Yi et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technical</td>
<td>48 h</td>
<td>0.891</td>
<td>Yi et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technical</td>
<td>96 h</td>
<td>0.631</td>
<td>Yi et al. (2011)</td>
</tr>
<tr>
<td>Chironomus plumosus</td>
<td>A midge</td>
<td>Formulation</td>
<td>48 h</td>
<td>1.3</td>
<td>Hamelink et al. (1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technical</td>
<td>48 h</td>
<td>1.3</td>
<td>Hamelink et al. 1986</td>
</tr>
<tr>
<td>Gammarus pseudolimnaeus</td>
<td>An amphipod</td>
<td>Technical</td>
<td>96 h</td>
<td>2.1–4.1</td>
<td>Hamelink et al. 1986</td>
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<tr>
<td></td>
<td></td>
<td>Formulation</td>
<td>96 h</td>
<td>&gt;32</td>
<td>Hamelink et al. 1986</td>
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<td>48 h</td>
<td>3.6–3.9</td>
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<td></td>
<td></td>
<td>Technical</td>
<td>48 h</td>
<td>3.9–6.3</td>
<td>Hamelink et al. (1986)</td>
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<td>Fishes</td>
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<tr>
<td>Sander vitreus</td>
<td>Walleye</td>
<td>Formulation</td>
<td>96 h</td>
<td>1.8</td>
<td>Paul et al. (1994)</td>
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<td>Rainbow trout</td>
<td>Technical</td>
<td>96 h</td>
<td>4.2–11.7</td>
<td>Hamelink et al. 1986</td>
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<td></td>
<td></td>
<td>Formulation</td>
<td>96 h</td>
<td>7.1–8.1</td>
<td>Hamelink et al. (1986)</td>
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<td>Ictalurus punctatus</td>
<td>Channel catfish</td>
<td>Technical</td>
<td>96 h</td>
<td>8.2–15.0</td>
<td>Hamelink et al. 1986</td>
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<td>13.2</td>
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<td>12.1–13.0</td>
<td>Hamelink et al. (1986)</td>
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<td>Largemouth bass</td>
<td>Formulation</td>
<td>96 h</td>
<td>13</td>
<td>Paul et al. (1994)</td>
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<tr>
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<td>Fathead minnow</td>
<td>Technical</td>
<td>96 h</td>
<td>22</td>
<td>Hamelink et al. (1986)</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Chemical grade</th>
<th>Time point</th>
<th>LC50 (mg/L)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrates</td>
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<tr>
<td>Daphnia magna</td>
<td>A water flea</td>
<td>Technical</td>
<td>26 h (IC50)</td>
<td>46</td>
<td>Crosby and Tucker (1966)</td>
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<tr>
<td>Gammarus lacustris</td>
<td>An amphipod</td>
<td>Formulation</td>
<td>24 h</td>
<td>&gt; 100</td>
<td>Sanders (1969)</td>
</tr>
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<td>Fishes</td>
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<tr>
<td>Sander vitreus</td>
<td>Walleye (8–10 d)</td>
<td>Formulation</td>
<td>96 h</td>
<td>16</td>
<td>Paul et al. (1994)</td>
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<td>Walleye (41–43 d)</td>
<td>Formulation</td>
<td>96 h</td>
<td>54</td>
<td>Paul et al. (1994)</td>
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<td>Micropterus dolomieu</td>
<td>Smallmouth bass (&lt; 1 d)</td>
<td>Formulation</td>
<td>96 h</td>
<td>47</td>
<td>Paul et al. (1994)</td>
</tr>
<tr>
<td>Micropterus salmoides</td>
<td>Largemouth bass (9–13 d)</td>
<td>Formulation</td>
<td>96 h</td>
<td>130</td>
<td>Paul et al. (1994)</td>
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fishes, and juveniles at 96 h were approximately eight times more sensitive than other invertebrates and 20 times more sensitive than fishes. The closest relative to *L. siliquoidea* in their study was the eastern oyster (*Crassostrea virginica*); oyster embryos had a 48-h LC50 of 6.8 mg/L. *Lampsilis siliquoidea* was approximately 8 (24-h glochidia LC50) to 13 (96-h juvenile LC50) times more sensitive than oyster embryos. Another study determined the 96-h LC50s of fluridone for the early life stages of walleye (*Sander vitreus*), smallmouth bass (*Micropterus dolomieu*), and largemouth bass (*M. salmoides*) (Paul et al. 1994; Table 2), which were all more tolerant than the mussels tested here (Table 1). In a recent investigation of the toxicity of fluridone on male water mites (*Arrenurus* sp.), Yi et al. (2011) reported toxicities to technical grade fluridone similar to our Sonar—PR® commercial formulation results; however, they found water mites were 60 times more sensitive in tests with another commercial formulation (Sonar—AS®; Table 2).

In the context of typical treatment prescriptions for hydrilla, all of the mussel toxicity data generated in tests with Sonar® PR, including those generally reported for regulatory purposes (24 h for glochidia, 96 h for juveniles), are two or more orders of magnitude greater than the water column treatment maximum target concentration for Lake Waccamaw (5 μg/L), and are more than three times higher than the maximum label application rate of 150 μg/L (SePRO Corporation 2012).

As with fluridone, freshwater mussels were also more acutely sensitive to endothall than most other tested organisms. Median effective concentrations (EC50s) and LC50s for 11 species range from >100 to 1071 mg/L; channel catfish (*Ictalurus punctatus*) and coho salmon (*Oncorhynchus kisutch*) were the most sensitive in the group, and the bluegill sunfish (*Lepomis macrochirus*) was the most tolerant (UPI 2012). The nearest relative to unionid mussels included in the ecotoxicity data was the eastern oyster, which had a 96-h sublethal EC50 (shell deposition) of 335 mg/L (UPI 2012), approximately 10 times greater than our 24-h glochidia and 96-h juvenile LC50s for *L. siliquoidea*. Acute values reported for some species in other studies showed sensitivities more similar to those of unionid mussels, including early life stage smallmouth bass (aged <1 d) and walleye fry (aged 41–43 d; Paul et al. 1994), and *Daphnia magna* (26-h median immobilization concentration (IC50); Crosby & Tucker 1966) (Table 2). Walleye 8–10 days old (96-h LC50 = 16 mg/L; Paul et al. 1994) were approximately twice as sensitive as the *L. siliquoidea* tested here (Tables 1 and 2). There was good agreement in our data among the LC50s for glochidia and the 96-h juvenile LC50, suggesting a defined threshold of tolerance; most mussels survived at concentrations ≤10 mg/L and experienced complete mortality at concentrations ≥100 mg/L. Despite being among the most sensitive species tested to date, the toxicity data for *L. siliquoidea* are 6–34 times higher than the recommended application rate of endothall for treatment of hydrilla (1–5 mg/L; UPI 2011). The 24-h glochidia and 96-h juvenile LC50s are approximately one order of magnitude greater than the application rate, indicating a smaller margin of error in applying endothall compared to fluridone. It should be stressed that an LC50 is not protective of a population (i.e., only 50% are expected to survive at the LC50 concentration).

We did not find any significant effects of fluridone on lethal or sublethal endpoints in tests with *L. fullerkati*, suggesting that adult mussels were tolerant to the range of concentrations used over 28 d, and may be tolerant to seasonal exposures at 5 μg/L during treatment of hydrilla infestations. However, many other endpoints could be explored, and some may provide more insight into effects from chronic exposure. Relevant toxicological endpoints in sublethal studies of freshwater mussel sensitivity to other contaminants that may be applied in future fluridone and endothall studies include growth (in juveniles, Bringolf et al. 2007; Wang et al. 2007, 2011, 2013), glochidial metamorphosis success...
(Hazelton et al. 2013), female mantle lure display (Bringolf et al. 2010; Hazelton et al. 2013; Leonard 2013), hemolymph and tissue analysis (Archambault et al. 2013; Leonard 2013), movement and burrowing (Flynn & Spellman 2009; Archambault et al. 2013; Hazelton et al. 2014), and metabolomics (Leonard 2013). We attempted to evaluate female mantle lure display in our experiment, but we had few females per replicate, thus there was insufficient statistical power to make sound inferences. We did note, however, that mussels in all treatments except for the highest concentration (300 μg/L) were periodically observed displaying mantle lures (stage 3 or higher, as per Bringolf et al. 2010). Our observations suggest that fluridone applied at a typically prescribed rate of 5 μg/L may not affect unionid mantle lure display. However, more statistically robust experimentation is needed to confirm a lack of effect, and to elucidate any other potential reproductive effects of fluridone.

The freshwater snail, *S. viriginicus*, was equally sensitive to the fluridone formulation *Sonar – Genesis®* as juvenile mussels were to the *Sonar – PR®* formulation (Table 1), and much more sensitive than other animals previously tested in commercial formulations of *Sonar®* (Hamelink et al. 1986; Paul et al. 1994), except for water mites (Yi et al. 2011) (Table 2). The reported acute values for *Sonar – Genesis®* were 1.8 mg/L (96-h LC50) for walleye and 3.6 mg/L (48-h EC50) for *Daphnia* (SePRO Corporation 2011), which are values 3.6 to 7.2-fold higher than the snail 96-h LC50. In experiments with *S. viriginicus*, both the 96 and 48-h post exposure LC50s were approximately two orders of magnitude higher than typical treatment concentrations recommended for hydriilla, and more than three times higher than the maximum label rate of application (SePRO Corporation 2010). Moreover, adult snails suffered no mortality in previous tests in our laboratory that had a maximum treatment concentration of 500 μg/L ((96-h LC50 > 500 μg/L), Archambault, Bergeron, and Cope, unpublished data). However, caution should be used in interpreting acute duration data, because slow-release or slow-acting herbicides like fluridone typically require extended exposure when treating hydriilla. Further, whole life cycle studies are especially important for *S. viriginicus* and other species that have an annual reproductive ecology, where the timing of hydriilla and other weed growth — and therefore herbicide treatment — coincides with egg laying, juvenile hatching and growth, and adult senescence. Because *S. viriginicus* adults die after reproduction (Johnson et al. 2013), negative effects to one cohort could result in further species decline.

In summary, we found that the fluridone and endothall concentrations typically recommended for hydriilla treatment were not acutely toxic to the freshwater molluscs tested in this study, and a 28-d exposure to fluridone was not lethal to adult mussels even at the highest concentration, indicating minimal risk of short-term effects to non-target species, including several protected and rare species. We also found that freshwater molluscs were more sensitive to fluridone and endothall than most other species previously tested. The mussels and snails studied here represent hundreds of highly imperilled freshwater gastropods and unionoids, and our findings may signal their greater sensitivity to herbicides than other species commonly studied in aquatic toxicity testing (e.g., *Daphnia* spp., *Hyalella* spp., fathead minnows (*Pimephales promelas*)). Furthermore, chemicals like fluridone and endothall are sometimes used in combination to increase effectiveness against aquatic weeds, and are rarely the only chemicals present in surface waters (i.e., aquatic contaminants). They also are typically applied over a longer duration than our test exposures. Though fluridone and endothall have been used for aquatic weed management for decades, more research is needed to elucidate any potential risk to less-studied non-target taxa, including molluscs, especially given hydriilla’s encroachment into
more systems across the country with high native biodiversity and endemism (e.g., Lake Waccamaw, Eno River). By providing a more thorough picture of the potential ecological risk associated with applying such herbicides for control of invasive aquatic weeds, resource managers can more confidently evaluate them as an option among other management choices (e.g., no treatment, grass carp control, and mechanical removal) and their associated risks. Topics warranting future study include acute exposures of endothall to snails; chronic exposures of juvenile mussels and snails to fluridone and endothall; evaluation of short- and long-term sublethal effects to juvenile and adult molluscs (e.g., reproduction, transformation success, growth, and biomarkers); indirect effects (e.g., effects on diet/food availability); whole life cycle exposures; and multi-stressor studies.

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References