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CHLORINE TOXICITY TO EARLY LIFE STAGES OF FRESHWATER MUSSELS (BIVALVIA: UNIONIDAE)

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Abstract—Chlorine (Cl) is a highly toxic, widely used halogen disinfectant that is present in point-source pollution discharges from wastewater treatment plants and industrial facilities. The U.S. Environmental Protection Agency freshwater criteria for Cl are 19 μg total residual Cl (TRC)/L as a maximum 1-h average concentration and 11 μg TRC/L as a maximum 4-d average; however, toxicological data for unionids were not used in these calculations. To address this void in the data, we conducted acute tests with glochidia from several species and 21-d bioassays with three-month-old *Epioblasma capsaeformis* and three-, six-, and 12-month-old *Villosa iris* juveniles. The 24-h lethal concentration 50 values for glochidia were between 70 and 220 μg TRC/L, which are 2.5 to 37 times higher than those reported in other studies for cladocerans. Significant declines in growth and survivorship were observed in the 21-d test with *E. capsaeformis* at 20 μg TRC/L. Lowest-observed-adverse-effects concentrations in bioassays with juvenile *V. iris* were higher (30–60 μg TRC/L) but showed a significant trend of declining toxicity with increased age. Although endpoints were above water quality criteria, the long life spans of unionids and potential implications of chronic exposure to endangered juvenile mussels still warrant concern.

Keywords—Chlorine   Freshwater mussels   Toxicity   Glochidia   Juveniles

INTRODUCTION

Chlorine (Cl) is a halogen disinfectant often used by wastewater treatment facilities to eliminate pathogenic organisms in discharges before their release into aquatic systems. It also has been used effectively as an agent to control biofouling by exotic bivalves in waterlines of industrial and electrical plants [1–5]. The high toxicity and relatively rapid dissipation rate of Cl from the water column make it an appealing chemical alternative [6]. The toxicity of Cl to aquatic life was first studied extensively during the late 1970s and early 1980s at both species and community levels so that researchers could assess environmental risk. In 1984, the U.S. Environmental Protection Agency (U.S. EPA) drafted water quality criteria (WQC) for Cl [7] and established acceptable levels that included a maximum 1-h concentration not to exceed 19 μg total residual chlorine (TRC)/L more than once every three years and a 4-d average concentration not to exceed 11 μg TRC/L.

At that time, the criteria were based on toxicological data available for 33 freshwater species; however, practicalities associated with regulation also were considered. When these experiments were conducted (1958–1982), TRC could be measured only at concentrations of 10 μg/L and above. Although chronic endpoints reported in the review of literature for the 1984 WQC for Cl suggested impairment at concentrations substantially lower (3.4 μg TRC/L), enforceable thresholds were constrained by limited technology and the credibility of data at that time. The Clean Water Act of 1977 (Section 304 a:1) required the U.S. EPA (http://www.epa.gov/owow/oceans/

regulatory/sec301tech/index.html) to publish WQC based on data that reflected the latest scientific knowledge. Since the initial drafting of WQC for Cl, advances in analytical methods have lowered TRC detection limits substantially. Consequently, more recent studies have reported adverse effects at concentrations well below acceptable criteria limits, as scientists have observed impairment of algae and periphyton communities at concentrations as low as 2 μg/L [8,9].

Freshwater mussels are the most rapidly declining faunal group in North America, and researchers are concerned for the continued existence of many species. Population studies report declines in the abundance and number of species and, perhaps more important, lack of recruitment at sites where diverse adult mussel assemblages are found [10,11]. Although in situ mussel surveys are useful for identifying impairment at specific sites, it is difficult for researchers to isolate variables and distinguish cause-and-effect relationships because of the complex anthropogenic inputs to river systems. Hence, it has been challenging for researchers to evaluate the severity of potential threats to water quality in rivers and their subsequent effects on recruitment of juveniles. To better understand potential toxicological effects of various contaminants on the life stages of freshwater mussels, researchers have developed approaches for conducting tests in the laboratory [12–14]. The results of recent laboratory studies have shown that early life stages are not only more sensitive than adults but also more susceptible to some contaminants than organisms used to derive safe water concentrations and/or assess environmental risk [12,14,15].

Prior to the drafting of the 1984 WQC for Cl, few studies had been conducted to determine the toxicity of Cl to fresh-

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water mussels. Even today, published data on the topic remain sparse. Thus, the intent of this study was to assess the level of risk that Cl toxicity poses to early life stages of unionids. To achieve this goal, a series of experiments were conducted with glochidia from various species of freshwater mussels to determine their tolerance to TRC. Glochidia are suitable as test organisms only for acute tests because substantial declines in survivorship occur during laboratory studies after only short periods, ranging from hours to days, depending on the species and water temperature [10,16–18]. Therefore, chronic tests were conducted using juvenile mussels for 21 d. We conducted bioassays with 3-, 6-, and 12-month-old juveniles of *Villosa iris* to examine the relationship between age and toxicity. We also conducted a 21-d test with juveniles of *Epioblasma capsaeformis*, a federally endangered species, to compare sensitivity between species.

MATERIALS AND METHODS

Test organisms

Glochidia. Gravid females of *V. iris* (rainbow mussel), *E. capsaeformis* (oyster mussel), *Epioblasma brevidens* (Cumberlandian combshell), and *Lampsilis fasciola* (wavyrayed lampmussel) were collected from the Clinch River, Virginia, USA. Gravid *Alasmidonta heterodon* (dwarf wedgemussel) were obtained from the Ashuilot River (NH, USA). *Epioblasma capsaeformis*, *E. brevidens*, and *A. heterodon* are federally endangered species. Specimens obtained from the Clinch River were transported back to the laboratory immediately after collection, while those from the Ashuilot River were mailed overnight in chilled coolers. Adults were acclimated to laboratory conditions in recirculating troughs maintained at 20 ± 2°C and fed a trialgal diet for at least 24 h prior to extraction of glochidia. Glochidia were extracted by gently prying open the shell of a gravid female and puncturing the marsupial gill with a 100-cc water-filled syringe that flushed out the glochidia. The process was repeated for each gill. Glochidia were then rinsed with clean water to remove excess gill material, and four samples of 25 to 50 glochidia were assessed for survivorship using a concentrated NaCl solution [10,13]. Glochidia were considered alive if the valves were open and responded to the addition of NaCl by closing or repeatedly closing and opening their valves. Glochidia that were closed prior to addition of the NaCl solution or open but exhibited no movement after exposure to the NaCl were recorded as dead. This assessment was based on the assumption that they would be unable to attach to host fish, which was previously described by Goudreau et al. [13]. Only glochidia from adults that had average viabilities of at least 90%, tested prior to the initiation of exposures, were used in experiments.

Juvenile mussels. Juvenile mussels were cultured at the Virginia Tech Freshwater Mollusk Conservation Center, Blacksburg, Montgomery County (VA, USA). The species of host fish used for *V. iris* was rock bass (*Ambloplites rupestris*), while banded sculpin (*Cottus carolinae*) was used for *E. capsaeformis*. Infestation of host fish followed the protocol of Zale and Neves [19]. After juveniles dropped from the host fish, they were maintained in recirculating aquaculture systems containing fine sediment and fed a daily diet of 30,000 cells/ml unicellular algae (*Neochloris oleoabundans*). Once juveniles reached their target age for testing, they were siphoned from the tanks, and their condition was assessed. Only mobile, pedal-feeding juveniles were used in bioassays. Two-month-

old juveniles were the youngest age class used during our tests based on results described in Valenti et al. [14].

Acute toxicity tests with glochidia

Treatments were 5, 10, 30, 60, 120, 250, and 500 µg TRC/L, plus a control. Calcium hypochlorite (high test hypochlorite [HTH]) was used as the toxicant, and moderately hard, reconstituted water was used as the diluent and control [20]. Test solutions of TRC were prepared and concentrations measured in 3-L plastic nalgene beakers. When needed, treatments were adjusted using stock solutions until the desired concentration was achieved. Concentrations were monitored three times per day and readjusted accordingly with stock solutions that were two times the treatment concentration to account for loss of chlorine.

Approximately 25 to 50 glochidia were transferred with a fine-tip glass pipette to test chambers and were randomly assigned to the different treatments. Each test chamber was constructed of rigid plastic tubing (height = 14 cm, outside diameter = 2.5 cm) that had four 1-cm² openings removed from the base and covered with 50-micron Nitex mesh. Test chambers were placed in the 3-L beakers containing prepared treatment concentrations. Each treatment had four replicates ($n = 4$) of 25 to 50 glochidia per time interval (24 h). For example, a 48-h test would have eight test chambers, whereas a 72-h test would have 12. After each 24-h interval, four test chambers would be randomly selected, and the glochidia inside would be assessed for survivorship as previously described. Toxstat® Version 3.5 was used to calculate Spearman–Karber LC50 values [21]. A Wallace–Tiernan amperometric (Tonbridge, Kent, TN, USA) chlorine titrator was used to measure TRC concentrations.

21-d tests with juveniles

Toxicity tests were conducted for 21 d with six chlorine treatments that doubled in concentration, plus a control. Concentrations for bioassays with two-month-old *E. capsaeformis* and three-month-old *V. iris* were between 5 and 250 µg TRC/L, while those for bioassays with six- and 12-month-old *V. iris* were between 10 and 500 µg TRC/L. Treatments were prepared with HTH in 140-L recirculating aquaculture systems that were powered by 1.5-amp pumps. Each trough was filled with 120 L of a 50/50 (v/v) mix of dechlorinated tap water and reference water from Sinking Creek (Newport, VA, USA) and covered with Plexiglas to impede chlorine loss. Treatments were continuously spiked with stocks containing HTH at a rate of approximately 10 L/d, whereas the control received only the 50/50 (v/v) dechlorinated tap and river water mixture. Every 48 h, new stocks were created, and 20 L of water were removed from each trough. During all experiments, TRC concentrations in troughs were measured twice daily as described previously. Concentrations of free residual chlorine (FRC) and combined residual chlorine (CRC) were measured at the start and weekly thereafter.

Twenty juveniles were randomly allocated to each concentration. Each was held in test chambers similar to those described for the glochidia experiments. The only modifications were that a more porous Nitex mesh screen was used (200 microns), and each contained 2 ml of river sediment that were aerated, autoclaved, and aerated. Chambers were held upright with plastic test tube holders. Juveniles were randomly allocated into test chambers ($n = 140$) after shell lengths were measured using an ocular micrometer and dissecting micro-

scope. Test organisms were fed daily with 30,000 cells/ml *N. oleoabundans*. Temperature was maintained at 23 ± 1°C, and a 16:8-h light:dark photoperiod was established using an automatic timer.

After 21 d, juveniles were retrieved from test chambers by rinsing sediment onto a 200-micron sieve and then flushing with dechlorinated tap water. Sediment would pass through the sieve opening, leaving the juveniles. Final shell lengths were measured, and survivorship was determined. Individuals that did not move for 2 min were recorded as dead. Movement was defined as pedal feeding, active filtering, shell movement, or visceral mass movement observed through the translucent shell. Total growth was calculated by subtracting initial length from final length. No-observed-adverse-effect concentrations and lowest-observed-adverse-effect concentrations were determined for growth and survivorship based on the statistical approach described for *Pimephales promelas* in standard protocol [20] using Toxstat, Version 3.5 (α = 0.95).

RESULTS

Acute toxicity of TRC to glochidia

Average survivorship exceeded 90% for glochidia of all species after 24 h in control treatments. The three endangered species *Epioblasma brevidens*, *E. capsaeformis*, and *A. heterodon* were slightly more sensitive to chlorine than *L. fasciola* and far more sensitive than *V. iris* after 24 h of exposure (Table 1). At 250 μg TRC/L, average survivorship for these more sensitive species (<20%) was nearly half that of the respective value for *L. fasciola* (35%) and less than a third for *V. iris* (66%). In concentrations of 30 μg TRC/L and lower, survivorship remained greater than 90% for all species after 24 h, except *E. brevidens* (79–87%). All exposed glochidia died at 500 μg TRC/L.

After 48 h of exposure, survivorship in chlorinated treatments differed only slightly for *V. iris* and *A. heterodon*, although it decreased substantially for *L. fasciola*. However, average survivorship of *L. fasciola* declined to less than 80% in the control after 48 h. Similar declines have been observed in past experiments, which may be attributable to *L. fasciola* having a shorter survivorship outside the marsupium. It is unclear whether the lower 48-h LC50 value for *L. fasciola* is attributable to increased Cl toxicity or to natural mortality. The 48-h LC50 values for *V. iris* and *A. heterodon* were 260 and 95 μg/L, respectively (Table 1). Control survivorship remained greater than 90% for *V. iris* after 72 h, yet the LC50 (180 μg/L) remained higher than the 24-h value for the other species tested.

21-d chlorine toxicity to V. iris

Significant declines in survivorship were recorded in experiments with three-and six-month-old *V. iris* juveniles (Fig. 1). Adverse effects were observed at lower concentrations in experiments with three-month-old juveniles as survivorship declined to 50% at 30 μg TRC/L. Survivorship for six-month-old juveniles remained ≥90% in concentrations as high as 120 μg/L and was significantly lower than the control only at concentrations ≥250 μg TRC/L. No concentration in our test caused significant declines in survivorship for 12-month-old juveniles, and survivorship remained 80% even at 500 μg TRC/L (Fig. 1).

All three age classes of juveniles mussels grew significantly less at concentrations ≥60 μg TRC/L ($p < 0.05$) than those

Table 1. Comparison of acute toxicological endpoints for common U.S. Environmental Protection Agency (U.S. EPA) test organisms and those for freshwater mussel glochidia generated in our study. Endpoints for glochidia bioassays are presented as lethal concentration 50 (LC50)

Species	Mean acute value (μg/L)
Common U.S. EPA test organisms	
Cladoceran (*Ceriodaphnia dubia*)	6–27[a]
	<80[b]
Cladoceran (*Daphnia magna*)	28[c]
	32[d]
Pugnose shiner (*Notropis arogenus*)	45[c]
Common shiner (*Notropis cornutus*)	51[c]
Lake trout (*Salvelinus namaycush*)	60[c]
Rainbow trout (*Oncorhynchus mykiss*)	62[c]
	59[d]
Copepod (*Epischura lacustris*)	63[c]
Amphipod (*Hyalella azteca*)	78[d]

Species	Time (h)	Mean LC50 value (μg/L)[e]
Freshwater mussel glochidia		
Rainbow mussel (*Villosa iris*)	24	220
	48	260
	72	180
Wavyrayed lampmussel (*Lampsilis fasciola*)	24	145
	48	80*
	72	90*
Oyster mussel (*Epioblasma capsaeformis*)	24	107
Cumberland combshell (*Epioblasma brevidens*)	24	70
Dwarf wedgemussel (*Alasmidonta heterodon*)	24	107
	48	95

[a] Taylor [22]. Values are for concentrations of free residual or combined forms of chlorine rather than total residual chlorine.
[b] Stewart et al. [6].
[c] Fisher et al. [23].
[d] 1984 chlorine water quality criteria [7].
[e] * = control survivorship below 80%.

in controls (Table 2). Average growth for individuals was reduced relative to controls by 37 to 80% in exposures with TRC concentration of 30 to 120 μg/L and by 90% in exposures of 250 μg/L and greater. On the basis of the results of our bioassays, we established lowest-observed-adverse-effect concentrations values of 30 μg/L for three-month-old *V. iris* and 60 μg/L for 6- and 12-month-old *V. iris* juveniles.

21-d chlorine toxicity to E. capsaeformis

Two-month-old juveniles of *E. capsaeformis* juveniles were more sensitive than any age class of *V. iris*. Growth was significantly reduced at concentrations of 20 μg TRC/L and higher, as exposed individuals grew less than 20% relative to those in the control (Fig. 2). Growth in the control and no-observable-adverse-effect concentrations exposure (10 μg/L) were 400 and 375 μm, respectively, differing by only 6%. The number of observed mortalities also was considerably high in the test, as 50% or more of the individuals died at concentrations of 30 μg/L and higher. All individuals in the 120-μg/L exposure died after 21 d of exposure, whereas those in the control and 5 μg/L had average survivorship of 80 and 100%, respectively.
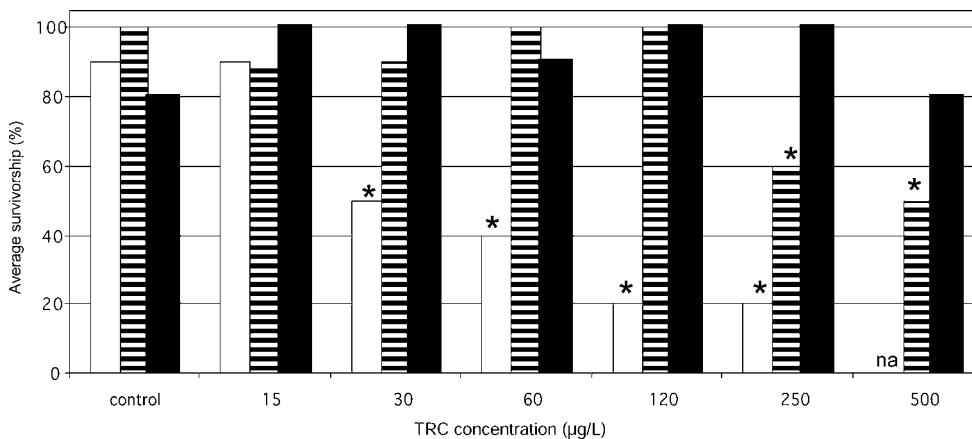
Fig. 1. Survivorship for 3-, 6-, and 12-month-old *Villosa iris* exposed to different concentrations of total residual chlorine (TRC). The asterisk (*) denotes significant differences from the control ($p = 0.05$). Three-month-old juveniles were not exposed to the 500-μg TRC/L treatment; this is depicted in the figure as na. □ = 3 month; ▤ = 6 month; ■ = 12 month.

Measured chlorine concentrations

The TRC concentrations were below detection limit in control treatments for all bioassays. The mean ± standard deviation for measured concentrations in the glochidia toxicity tests were 3.7 ± 0.8, 7.9 ± 1.9, 26.6 ± 4.1, 55.8 ± 6.3, 115.7 ± 6.0, 234.1 ± 15.3, and 482 ± 61.8 μg/L. Measured TRC concentrations in experiments with three-month-old *V. iris* were 5.2 ± 0.8, 13.9 ± 1.2, 28.1 ± 2.8, 63.3 ± 4.4, 115.9 ± 4.6, and 259 ± 11.7 μg/L. Measured TRC concentrations for the experiment with six-month-old *V. iris* were 17.3 ± 3.4, 30.9 ± 3.1, 56.4 ± 5.1, 123.3 ± 16.4, 242.9 ± 19.9, and 467.8 ± 56.8 μg/L. Measured TRC concentrations for the experiment with 12-month-old *V. iris* were 14.6 ± 2.8, 35.2 ± 5.3, 62.1 ± 4.9, 129.3 ± 11.8, 262.8 ± 24.1, and 524.4 ± 51.7 μg/L.

DISCUSSION

TRC concentrations

During glochidia and juvenile mussel bioassays, we were able to maintain TRC concentrations close to target levels by intermittently or continuously dosing test chambers with solutions of calcium hypochlorite. However, since no recognized uniform pattern was observed in the physicochemical interaction of Cl and water, it is difficult to accurately infer toxicity based solely on TRC concentrations [6]. Chlorine exists as one of several interim forms in water, depending on pH, temperature, and the presence of organic and nitrogenous compounds. When in water, Cl hydrolyzes to form FRC that may be either hypochlorous acid (HOCl) or hypochlorite salt (OCl^-). If ammonia is present, CRCs are formed that can be further grouped as monochloramine and dichloramine. These various forms of Cl have different stabilities in water and unique toxicities to aquatic life. During our study, we focused primarily on monitoring TRC because the U.S. EPA bases WQC solely on it; however, we also measured concentrations of FRC and CRC on several occasions during the 21-d tests and found approximately a 50:50 ratio.

We make this distinction because of observations regarding the toxicity of different forms of Cl to other freshwater organisms in prior studies. Taylor [22] observed that FRC was substantially more toxic to *Ceriodaphnia dubia* than CRC in
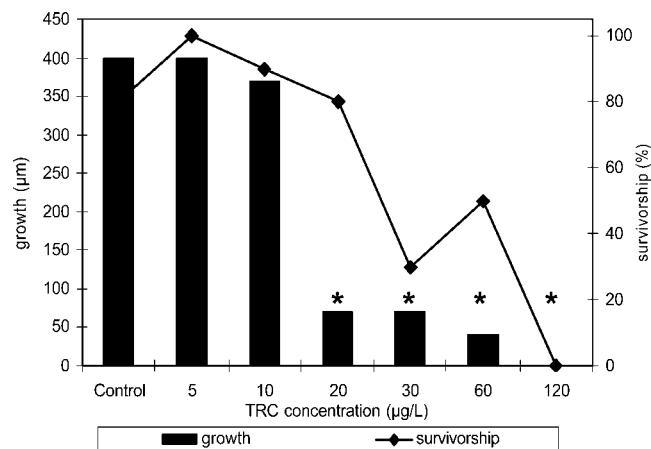
Table 2. Initial length and growth of *Villosa iris* juvenile mussels exposed to different concentrations of total residual chlorine (TRC) for 21 d

Age	Concn (μg TRC/L)	Initial length (μm)	Growth (μm)[a]
3-month	Control	1,290	680
	5	1,230	670
	15	1,300	550
	30	1,330	420
	60	1,340	20*
	120	1,390	10*
	250	1,190	70*
6-month	Control	1,900	680
	15	1,660	380
	30	1,840	140
	60	1,840	110*
	120	1,730	150*
	250	1,690	40*
	500	1,820	10*
12-month	Control	6,630	1,350
	15	6,550	1,240
	30	6,920	460
	60	6,580	110*
	120	6,870	240*
	250	6,330	10*
	500	7,230	50*

[a] * = significantly different that the control treatment ($p < 0.005$).

Fig. 2. Average survivorship and growth for two-month-old *Epioblasma capsaeformis* juveniles exposed for 21 d to different concentrations of total residual chlorine (TRC). TRC concentration in control was below detection limit. Measured concentrations and standard deviations in the treatments were 5 ± 1, 11 ± 2, 21 ± 3, 30 ± 4, 57 ± 4, and 126 ± 8 μg TRC/L, respectively. The asterisk (*) denotes significant differences from the control ($p = 0.05$).

continuous flow-through experiments, as LC50 values for HOCl and OCl⁻ were 6 and 5 μg/L, respectively, while those for monochloramine and dichloramine were 16 and 27 μg/L, respectively. The endpoints for FRC were seven to eight times lower in these experiments than in comparable experiments in which conditions were static and test solutions were not renewed. This disparity is likely attributable to the short half-life of FRC in water since Taylor [22] also observed that concentrations dropped below detection limit in <1 min during some experiments. Fisher et al. [23] reported similar trends pertaining to the tolerances of freshwater organisms during experiments comparing continuous versus intermittent exposures. In their study, respective LC50 values for the two test methods were 32 and 55 μg/L for *Daphnia magna*, 78 and 301 μg/L for *Hyalella azteca* (amphipod), 59 and 374 μg/L for *Oncorhynchus mykiss* (rainbow trout), and 304 and 572 μg/L for *Notemigonus crysoleucas* (golden shiner). We attribute the differences in the tolerances observed during these studies to the varying stability of constituents that make up TRC.

In river systems, the proportion of the various constituents that make up TRC are determined by environmental conditions and thus specific for a given site. Particular sites on a reach of river may have a greater proportion existing as FRC, whereas other sites may have more CRC. It is important that future studies determine which constituents are most toxic to freshwater mussels so that environmental risk can be inferred accurately.

Glochidia

A substantial difference was observed in the sensitivity of glochidia from different species of mussels to TRC. Although few studies have examined their tolerance to Cl, other researchers have described similar variance in the sensitivities of glochidia to other toxicants. In experiments exposing glochidia from several species to mercury, Valenti et al. [24] reported acute endpoints that ranged between <8 and 43 μg/L. Cherry et al. [15] reported values as low as 37 μg/L for glochidia of *Lampsilis* and as high as 137 μg/L for those of *Pyganodon* in experiments testing the toxicity of copper. Additional studies also have reported substantial interspecific variability in the tolerances of glochidia from various species to other contaminants, such as malathion and ammonia [12,25]. It is unclear why some species have lower survivorship after being exposed to contaminants; however, it may be due to physiological differences. Although other factors affect the population size of endangered mussel species, such as the availability of fish host, reproductive timing, and brood type, toxicological effects also may impede recruitment. The results generated in this study add weight of evidence to the latter statement, as glochidia from the three species listed as federally endangered, *E. capsaeformis*, *E. brevidens*, and *A. heterodon*, were substantially more sensitive to TRC than either *V. iris* or *L. fasciola*.

Another study that has examined the toxicity of Cl to glochidia is Goudreau et al. [13]. The LC50 value reported in their study was 84 μg/L for *V. iris*, which is substantially lower than the comparable value calculated in our study (220 μg/L). Despite the large difference in endpoints, data generated in each study may reflect toxicological endpoints that are similar. First and foremost, the endpoint reported in their study is based on monochloramine rather than as TRC. When comparing the endpoint in relative terms, our endpoint may be

approximately 50% lower when related to the 50:50 FC-to-CRC ratio observed during our 21-d test. Furthermore, since pH was approximately 8, nearly all CRC in our test chambers would have existed as monochloramine rather than dichloramine. On the basis of this comparison and trends observed for other aquatic organisms, the LC50 values reported in our study for glochidia would be substantially lower if presented in terms of the constituents that make up TRC, such as FRC and CRC.

Although additional studies are warranted, it appears unlikely that Cl concentrations in the environment pose a substantial threat to glochidia of any species if instream concentrations meet current WQC. We support this position not only because our endpoints and those of other studies are higher than the acceptable 1-h maximum concentration (19 μg/L) but also because of ecological considerations. Successful attachment by most glochidia is likely to occur almost immediately after release from gravid females since this is when they are in the closest proximity to host fish. Therefore, the period of time in which glochidia are exposed to Cl while in the water column is rather brief. Furthermore, prior studies have suggested that attached glochidia or those brooded in females have a low risk of exposure to toxicants in the environment because they are afforded some level of physical protection by either the fish host or the marsupial gill [10]. Although glochidia that do not immediately attach to host fish may be found in stream drift where they may be exposed to toxicants for longer periods of times (>24 h), it is less likely that these individuals will come in contact with appropriate host fish [26].

Additional ecological considerations of interest include the presence of fish host species in areas affected by Cl pollution. Cherry et al. [27] reported avoidance behavior by fish species in areas receiving chlorinated effluents. Therefore, even if Cl concentrations are not at levels detrimental to the survival of glochidia, the absence of host fish would prevent attachment and recruitment. Additional studies have also reported that other species of freshwater organisms have substantially lower acute tolerances to Cl than those reported for glochidia in our study (Table 1). Therefore, freshwater organisms other than glochidia may be more appropriate as test organisms for assessing Cl pollution, especially since recent studies have reported some species with tolerances below current WQC [8,9,23].

21-d toxicity to juveniles

A comparison of sensitivities for the three age classes of *V. iris* juveniles tested in our study revealed that younger mussels (three-month-olds) were more sensitive to TRC exposure than older juveniles (12-month-olds). The difference in sensitivities for the respective age-groups was more apparent when contrasting survivorship results. This observation is consistent with a trend often apparent for other freshwater species because early life stages of organisms are generally more sensitive to toxicant exposure than older, more developed individuals. Although chronic studies examining the toxicity of Cl to unionids are yet to be conducted, studies examining effects of Cl exposure to other species of bivalves are extensive because chlorination is often used as a biofouling control agent [2,3,28,29]. These studies also suggest that younger age classes of bivalves are more susceptible to Cl exposure than older classes. Researchers have commented that aquatic bivalves may be useful as surrogate test species for assessing environmental risk for Unionidae since they have similar physiological and ecological traits [30]. These similarities are useful for

interpreting toxicological impacts of exposure because most bivalves reside in the benthos, rely on suspension or deposit feeding, and have the affinity to accumulate trace metals from the water column, sediment, and interstitial water [30–32].

Of greater importance to our study are the behavioral similarities shared by bivalves, the most obvious one being their ability to temporally avoid toxicants by closing their valves for prolonged periods. When exposed to high concentrations of Cl, bivalves can avoid the uptake of toxicants by sealing their valves, reducing filtration, and relying on anaerobiosis, in some species for extended periods [2,3,28,29]. Consequently, because of this behavioral response, researchers often describe a time lag between the initiation of exposure and first observation of substantial mortality during laboratory studies. This time lag occurs even when exposure concentrations are extremely high and is typically 14 d or more for the bivalve species *Corbicula fluminea* and *Perna perna* [2,3,29]. The onset of mortality will not occur until energy resources are depleted or metabolic wastes reach toxic levels [29]. We attribute the greater susceptibility of earlier age classes of *V. iris* to TRC to the fact that younger mussels have less energy reserves and are unable to store as much wastes. The ability of the younger age class to avoid toxicant exposure may also be less because of thinner more permeable shells.

Exposure to Cl likely reduced the filtration rate of juveniles, thereby leading to less food being ingested. This would impair growth since juveniles would have less energy for assimilation into new body tissue. Although this reduction will not cause immediate death, it likely lowers an individual's fitness by reducing or prolonging development to sexual maturity. Slow growth rates could also result in latent mortality, as individuals may be unable to store adequate energy reserves to survive the winter.

Results of our 21-d tests provide additional evidence that further supports intraspecific variability in mussel tolerances to TRC, as endpoints for two-month-old *E. capsaeformis* were considerably lower than those for three-month-old *V. iris*. The growth and survivorship lowest-observed-adverse-effect concentrations for *E. capsaeformis* approached the current 4-d maximum WQC of 11 μg/L. If bioassays were conducted for durations longer than 21 d, it is likely that test endpoints would be substantially lower. Currently, the U.S. EPA defines chronic toxicity tests as exposures to individuals of a species that are equivalent to approximately one-tenth of their life span. Although bioassays this long in duration are not practical for unionids given their long life spans, which may exceed decades, it is important to note for conservation purposes. A subtle decline was observed in growth rates for individuals in the 10-μg/L treatment when compared to those in the control and 5-μg/L treatments. Impairment at lower concentrations would likely become more apparent over long exposure times and suggests that young juveniles may be at risk to Cl exposure at concentrations below current WQC.

CONCLUSION

Results of our bioassays suggest that glochidia are more tolerant of TRC than many aquatic species. In particular, researchers have reported toxicological endpoints for cladocerans that are substantially lower than those recorded in our study for glochidia. Relating the results of our study to the current WQC suggests that the environmental risk of Cl exposure to glochidia is fairly minimal.

Results of the 21-d tests with juvenile mussels do not cor-

respond with the acute toxicity assumption, as endpoints, especially those for younger age classes of *V. iris* and *E. capsaeformis*, were substantially lower. Given the long life spans of unionids, reasonable concern exists that current WQC are insufficient to protect the lengthy juvenile life stage of unionids. Although juvenile mussels may be able to survive high-dose acute exposures, the impact of long-term exposure to low doses may result in sublethal impairment that could lower their chances of surviving the multi-year, juvenile stage and being recruited to the reproducing population.

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