Newton et al 2003

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Environmental Toxicology and Chemistry, Vol. 22, No. 11, pp. 2554–2560, 2003
Printed in the USA
0730-7268/04 $12.00 + .00

EFFECTS OF AMMONIA ON JUVENILE UNIONID MUSSELS (*LAMPSILIS CARDIUM*) IN LABORATORY SEDIMENT TOXICITY TESTS

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(*Received* 18 *July* 2002; *Accepted* 24 *January* 2003)

Abstract—Ammonia is a relatively toxic compound generated in water and sediments by heterotrophic bacteria and accumulates in sediments and pore water. Recent data suggest that unionid mussels are sensitive to un-ionized ammonia (NH_3) relative to other organisms. Existing sediment exposure systems are not suitable for ammonia toxicity studies with juvenile unionids; thus, we modified a system to expose juveniles to ammonia that was continuously infused into sediments. This system maintained consistent concentrations of ammonia in pore water up to 10 d. Juvenile *Lampsilis cardium* mussels were exposed to NH_3 in pore water in replicate 96-h and 10-d sediment toxicity tests. The 96-h median lethal concentrations (LC50s) were 127 and 165 μg NH_3-N/L, and the 10-d LC50s were 93 and 140 μg NH_3-N/L. The median effective concentrations (EC50s) (based on the proportion affected, including dead and inactive mussels) were 73 and 119 μg NH_3-N/L in the 96-h tests and 71 and 99 μg NH_3-N/L in the 10-d tests. Growth rate was substantially reduced at concentrations between 31 and 76 μg NH_3-N/L. The lethality results (when expressed as total ammonia) are about one-half the acute national water quality criteria for total ammonia, suggesting that existing criteria may not protect juvenile unionids.

Keywords—Unionids Ammonia Toxicity Juveniles Freshwater mussels

INTRODUCTION

Unionid mussels are the most rapidly declining faunal group in the United States. Freshwater mussels constitute the largest group of federally listed endangered or threatened invertebrates. Seventy-two percent of the 297 species and subspecies are listed as endangered, threatened, or of special concern [1]. Numerous factors have been hypothesized for these declines, including habitat loss, commercial exploitation, contaminants, and introduced species [2]; however, most of the studies that report unionid declines are based largely on anecdotal evidence of casual mechanisms. Because unionids are declining in rivers and lakes throughout much of the continental United States and some of these declines are occurring in relatively uncontaminated systems, it is unlikely that elevated (lethal) contaminant concentrations are responsible for these widespread declines. While localized chemical spills can result in mortality [3], the pervasive effects of chronic, low-level contamination are largely undocumented.

Recent data suggest that mollusks are quite sensitive to certain contaminants, especially ammonia, relative to other invertebrates and fishes [4–6]. Although many of the earlier studies were conducted with fingernail clams, substantial interest has arisen in standardizing laboratory toxicity tests with unionids. Conducting laboratory toxicity tests with unionids pose challenges that are not routinely encountered with more traditional test species. First, maintaining control survival above 80% is often difficult because unionids frequently die when held in the laboratory for extended periods. Second, exposure durations of days or even weeks are short relative to their long life spans (30–130 years [7]) that generally precludes the use of adults in toxicity assessments [8]. Third, although juveniles may be more sensitive than adults or glochidia [9,10], culturing juveniles is time consuming, and host fishes have been identified for only a fraction of mussel species.

Conducting water-only toxicity tests with unionids may not adequately address their route of exposure to contaminants. Adult unionids reside in bottom sediments and obtain food largely by filter feeding. However, recent evidence suggests that some juveniles (*Villosa iris*) may filter sediment pore water and feed on sediment-associated fine particulate organic matter [11]. This suggests that sediments may be a more realistic contaminant route of exposure for unionids. Sediments adsorb many contaminants, such as ammonia, that ultimately partition into pore water [12]. Toxicity of pore water has been suggested as a contributing factor in the decline of mollusks in the upper Mississippi River [13] and the Illinois River (USA) [14].

Existing protocols are not sufficient for conducting toxicity tests with ammonia and juvenile unionids. First, most toxicity tests have exposed juveniles to contaminants delivered via the water column [15,16], even though this may not adequately characterize their route of contaminant exposure. Second, traditional sediment toxicity tests expose organisms to a gradient of contaminated sediments from the field or a given contaminant is spiked into reference sediments in the laboratory. Neither of these systems is appropriate for use with ammonia because ammonia tends to diffuse from sediments into overlying water and can deplete concentrations of ammonia in pore water. Whiteman et al. [17] developed a system that reduced the diffusion of ammonia from sediment into water and maintained relatively constant pore-water ammonia concentrations for 10 d in tests with freshwater macroinvertebrates. Because

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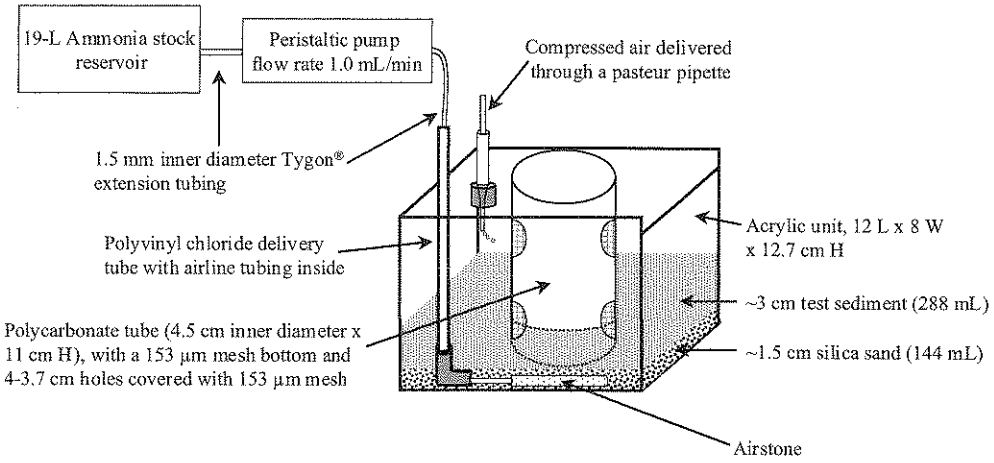

Fig. 1. Schematic diagram of the sediment dosing system used to deliver consistent concentrations of ammonia to juvenile unionids; Tygon® (Saint-Gobain Performance Plastics, Akron, OH, USA).

they worked with epibenthic species (*Hyallela azteca*), they intermittently renewed the overlying water to maintain ammonia concentrations below toxic levels.

Ammonia is ubiquitous in surface waters and is an integral part of the nitrogen cycle. Sources of ammonia to surface waters include precipitation, anthropogenic sources (industrial and sewage discharge and fertilizers), and natural processes, such as bacterial production through nitrogen fixation and ammonification. In anaerobic sediments, ammonia is generated by heterotrophic bacteria as a by-product of organic matter mineralization [18]. Total ammonia nitrogen (TAN) is primarily the sum of two forms in equilibrium: the ammonium ion (NH_4^+) and un-ionized ammonia (NH_3); although NH_3 is more toxic, it often represents only a small proportion of TAN, especially at low pH [19].

Our objectives were to modify the Whiteman et al. [17] system for use with unionids to determine if stable concentrations of ammonia could be maintained for 10 d and determine the lethal and sublethal effects of ammonia on juvenile *Lampsilis cardium* in laboratory sediment toxicity tests. We chose the freshwater *L. cardium* because it is common and widespread throughout much of the eastern United States [20], it is sexually dimorphic, its host fishes are known and easily maintained in the laboratory, and it is a congener of the federally endangered *L. higginsii*.

METHODS

System design

Each experimental unit (EU) consisted of an acrylic box, in which one of the narrower sides had a lower lip for overflow of treatment solutions (Fig. 1). In the bottom of each EU, we placed a 1.5-cm-deep layer of silica sand to act as a diffusion layer for the infused treatment solution. A piece of 153-μm Nitex® (Sefar, Heiden, Switzerland) mesh was placed on top of the sand to demarcate it from the test sediment. Then test sediment was layered 3.0 cm deep on top of the mesh. Ammonium chloride (NH_4Cl) solution was pumped into each EU via a 40-channel peristaltic pump, through Tygon® (Saint-Gobain Performance Plastics, Akron, OH, USA) and airline tubing, before connecting to an airstone buried in the sand layer. In theory, NH_4Cl gets distributed throughout the sand and then diffuses into the test sediment. Overlying water was not exchanged but overflowed into a recirculating water bath.

This system differed from that of Whiteman et al. [17] in several important ways. Because juvenile unionids are not epibenthic, we did not renew the overlying water. This simplified the system by decreasing the NH_4Cl gradient and minimizing diffusion between the sediment and overlying water. However, because we did not renew the overlying water, we had to make two other modifications. First, we aerated the overlying water in each EU with filtered, compressed air to maintain dissolved oxygen concentrations >70% of saturation without resuspending the test sediments. Second, our system employed discrete EUs, which did not share common overlying water and were individually aerated, in order not to violate the assumption of independence. Because juvenile unionids are small, a polycarbonate tube was inserted approximately 3 cm into the test sediments to facilitate recovery of juveniles. Mesh on the bottom and sides of this tube facilitated free exchange of the overlying water within the EU and allowed juveniles direct contact with sediment particles <153 μm (Fig. 1). Although juveniles were exposed to sediments within the tube, we analyzed pore water from all the sediment in the EU to obtain enough pore water for analyses. We assume that the pore-water ammonia concentrations inside and outside the tube are similar.

Assessment of temporal variation in ammonia

To determine if the system could maintain consistent TAN concentrations over 10 d, we chose concentrations of NH_4Cl that bracket the range for which acute toxicity has been observed in juvenile unionids. The low concentration was 5.7 mg TAN/L, and the high concentration was 57.4 mg TAN/L. These concentrations correspond to 100 and 1,000 μg NH_3-N/L based on a pH of 7.5 and a temperature of 21°C. Surficial sediments from a reference depositional area in the upper Mississippi River were taken with a Ponar grab (Wildco, Buffalo, NY, USA), homogenized, and stored at 4 ± 2°C for <7 d. This sediment has an organic content of 3% and a bulk density of 0.88 g/cm^3 and is 52% silt and clay (<63 μm), 24% fine sand (63–250 μm), 8% medium sand (250–500 μm), and 16% coarse sand (0.5–2 mm). Forty EUs were randomly positioned within a 21°C water bath, and 20 EUs were randomly assigned to each concentration. The photoperiod was 16:8 h light:dark. Stock solutions were renewed daily in 19-L carboys and adjusted to a pH of 7.5 ± 0.05 using 10% H_2SO_4 and a temperature of 21 ± 2°C. Flow rate (ml/min) of NH_4Cl into the sediment was maintained at 1.0 ml/min by randomly measuring 15% of the EUs each day and adjusting the pump speed

Table 1. Mean (±1 standard error) water quality characteristics in overlying water during a 10-d study of the temporal variation of ammonia in sediment pore water and during four sediment toxicity tests with juvenile *Lampsilis cardium*. Data are averaged over all ammonia concentrations

		Toxicity test			
Parameter	10-d temporal study	96-h test 1	96-h test 2	10-d test 1	10-d test 2
Temperature (°C)	20.8 ± 0.1	20.5 ± 0.1	21.2 ± 0.1	21.0 ± 0.1	21.3 ± 0.1
Dissolved oxygen (mg/L)	8.0 ± 0.1	8.6 ± 0.1	7.9 ± 0.1	7.7 ± 0.1	7.8 ± 0.1
pH	8.1 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	7.9 ± 0.1	8.1 ± 0.1
Alkalinity (mg $CaCO_3$/L)	139 ± 9	115 ± 1	90 ± 2	90 ± 4	75 ± 8
Hardness (mg $CaCO_3$/L)	190 ± 10	155 ± 1	124 ± 1	132 ± 1	123 ± 1
Conductivity (μS/cm)	NA[a]	3,819 ± 24	3,659 ± 46	3,502 ± 24	3,640 ± 41
Flow rate (ml/min)	0.99 ± 0.01	0.93 ± 0.01	0.95 ± 0.01	0.98 ± 0.01	0.96 ± 0.01

[a] NA = data not available.

accordingly. Dissolved oxygen, temperature, and pH of the overlying water (outside the tube) was measured daily in each EU. Alkalinity, hardness, and conductivity of the overlying water was measured in six randomly selected EUs on days 0, 4, and 10 (Table 1).

Culturing juveniles

Gravid *L. cardium* were obtained from the upper Mississippi River. For each test, glochidia from three females were used to infect approximately six largemouth bass (*Micropterus salmoides*, 8–13 cm long) as described in Waller and Holland-Bartels [21]. Encysted fish were held for 17 to 19 d in 38-L flow-through aquaria containing dechlorinated well water at 22°C. Encysted fish were fed fathead minnows, *Pimephales promelas*, until juveniles began to excyst. To determine postexcystment age, water from the aquaria bottoms was siphoned daily through a 153-μm sieve, and the contents were examined under a microscope. Juveniles from a given day were transferred into 4.4-cm-i.d. glass cylinders fitted with a 153-μm mesh bottom and suspended in a 38-L flow-through aquaria at 22°C until testing. For each test, we used juveniles from the day(s) of excystment for which we had the most juveniles. All tests were conducted with 3- to 5-d-old juveniles.

Toxicity tests with juveniles

We conducted replicate 96-h and 10-d toxicity tests exposing juveniles to increasing concentrations of ammonia delivered via sediment pore water. During the first 96-h and 10-d tests, we had six replicates of six NH_3 concentrations. During subsequent testing, we had difficulty obtaining females with viable glochidia, so we conducted the second 96-h and 10-d tests at the same time, with only three replicates of each concentration.

The experimental design for the toxicity tests was similar to the temporal variation study with the following exceptions. Test sediment was added (by wt) on day −5, and well water (Na^+ and K^+ concentrations averaged 8.2 and 2.6 mg/L, respectively) was pumped into each EU on days −5 and −4. On day −3, we measured the pH and temperature of the test sediments in eight randomly selected EUs to estimate the fraction of NH_3 to expect under these conditions. These data were used to prepare the daily stock solutions of NH_4Cl that were temperature and pH adjusted as described earlier. On day 0, 20 juveniles were added to each EU. Juveniles were not fed during testing. An initial subsample of ≥30 juveniles was preserved for later size measurements. Alkalinity, hardness, and conductivity were measured on day 0 and day 4 (or 10) in two-thirds of the EUs at each concentration (Table 1).

On the last day of exposure, juveniles were assessed for survival. Juveniles were examined under ×10 magnification and classified as alive and active, alive but stressed, or dead. Alive and active juveniles were those whose foot could be seen moving either inside or outside the shell. Alive but stressed juveniles were those in which no foot movement was seen but ciliary activity was observed. Active and stressed mussels were measured for shell height using an optical imaging system (Optimas, Bothell, WA, USA). Growth rate (μm/d) was calculated as the difference between the mean juvenile height from a given EU and the mean juvenile height of the initial subsample divided by the days of exposure.

Ammonia analysis

For the temporal study, ammonia concentrations in pore water were determined for three randomly selected EUs from each concentration on days 1, 2, 4, 6, 8, and 10 and two EUs on day 3. During toxicity tests, ammonia concentrations in pore water were measured on the last day of exposure. At the end of each test, each EU was removed from the water bath, the overlying water was slowly decanted so that no visual resuspension of sediments occurred, and the sediment was homogenized in the EU using a plastic spoon. Fifty milliliters of sediment from each EU was centrifuged for 20 min at 6,000 rpm (5,210 *g*) and 4 ± 2°C. Pore water (~5 ml) was decanted, filtered through a 0.45-μm syringe filter, acidified to a pH <2 with 10% H_2SO_4, and stored at 4 ± 2°C for a maximum of 14 d. Samples were analyzed for TAN with the automated phenate method [22] on a Bran Luebbe (Buffalo Grove, IL, USA) auto-analyzer. Concentrations of NH_3 were calculated from EU-specific pH and temperature measurements with the formulas of Emerson et al. [23].

Quality assurance

To evaluate the accuracy of TAN determinations in pore water, the following were analyzed (in triplicate or quadruplicate) with each analytical batch of samples: one method blank, three experimental triplicates, three spiked samples, and three quality control samples (Environmental Resource Association WastWatR® [Arvada, CO, USA], lots 9938 and 8057, and UltraScientific [North Kingstown, RI, USA] ultracheck nutrient ampule 2, lot 73746). Measured concentrations of the quality control samples were within certified ranges in 58 of 60 individual measures. Across all tests, the recovery of TAN from analysis of 15 sets of spiked samples ranged from 84 to 112%. Method precision (relative standard deviation), estimated from analysis of 15 sets of triplicate samples, ranged from 0.8 to 44%. The calculated limit of detection was 0.02

mg TAN/L [22]. To assess the precision of the shell height measures, one individual measured 10 juveniles daily for 5 d, and the coefficient of variation among replicate measures of the same juvenile ranged from 0.8 to 6.1%.

Statistical analyses

To determine if TAN concentrations varied over time in our system, we fit a linear regression between TAN and time and used the likelihood ratio chi-squared test of significance of the effect of time [24]. If the effect of time was negligible, we fit a model for the overall mean by eliminating the effect of time and then used Wald 95% confidence limits around the measured TAN concentrations to determine where the nominal concentrations fell with respect to this interval. During toxicity tests, the median lethal concentration and the median effective concentration (based on the proportion affected) and their associated 95% confidence limits were calculated using probit analysis [25]. The proportion affected was calculated as the number of stressed juveniles plus the number of dead juveniles divided by the number recovered. Growth rate was analyzed using generalized linear models [24]. The NH_3 concentration equivalent to a 50% reduction in growth rate relative to controls (EC50 based on growth) was calculated by $[NH_3]_{100p} = (\ln p/b_1)$ where p is the predicted growth for some fraction of the growth rate of the controls (0.50) and b_1 is the slope of the regression line. Wald-type confidence intervals were calculated on the basis of a delta-method approximation to the variance of EC50.

RESULTS AND DISCUSSION

Existing methods for conducting sediment toxicity tests are not appropriate with ammonia and juvenile unionids largely because ammonia tends to dissociate from sediments into overlying water. Whiteman et al. [17] designed a system that overcame this issue; however, because their system was developed for use with epibenthic species, it required modification and testing prior to use with benthic juvenile unionids. In our system, total ammonia nitrogen concentrations averaged 5.5 mg TAN/L (range, 3.1–7.0) in the low concentration and 57.7 mg TAN/L (range, 53.0–60.6) in the high concentration. Nominal concentrations (5.7 and 57. 4 mg TAN/L) fell within the 95% confidence limits of the measured values (5.1–5.9 and 57.1–58.3 mg TAN/L, respectively). Total ammonia nitrogen concentrations did not vary significantly over time in either the low ($\chi^2 = 0.01$, $p = 0.94$) or the high ($\chi^2 = 1.05$, $p = 0.31$) concentration, suggesting that the system was capable of maintaining consistent concentrations of TAN in pore water over 10 d. Additionally, agreement of pore-water ammonia concentrations among replicate EUs was good. For example, the coefficient of variation among six sets of triplicate samples averaged 32% in the low concentration and 2% in the high concentration. The lowest concentration used in the present study (5.7 mg TAN/L or ~100 μg NH_3-N/L) is an environmentally realistic concentration. Concentrations of ammonia in pore water in the upper Mississippi River, for example, ranged from 0.07 to 10.0 mg TAN/L and from 1 to 175 μg NH_3-N/L during summer [26].

This system has utility as an exposure system for juvenile unionids and other small benthic organisms for ammonia and other volatile compounds. However, several issues warrant further research. The existing design allows juveniles contact with sediment particles <153 μm; this may limit its application in studies with larger-grained sediment. Second, although this system maintained temporally consistent TAN concentrations, horizontal and vertical variability in ammonia were not adequately addressed. We made a crude attempt at addressing the horizontal variation by measuring TAN concentrations in pore water from three locations in one EU over 4 d and found that the variability was negligible (coefficient of variation ranged from 2–6%). Additionally, the fact that measured TAN concentrations were within 88% of nominal argues against the existence of major cold spots that are not adequately infused. However, these issues need to be better evaluated prior to the application of this system in other studies.

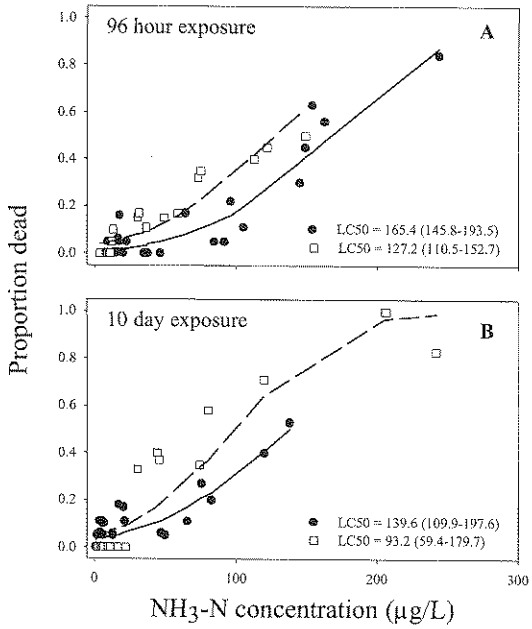

Fig. 2. Survival of juvenile *Lampsilis cardium* exposed to un-ionized ammonia (NH_3) in pore water during (**A**) 96-h and (**B**) 10-d exposures. Symbols are the actual data points (filled circles and open squares are replicate tests), and the lines are the model-predicted values. Values in parentheses are the 95% confidence limits around the median lethal concentration (LC50).

While adult unionids may be relatively insensitive to some pesticides, organic compounds, and wastewater effluents [15,27,28], their early life history stages are among the most sensitive aquatic fauna tested for metals [5,10], chlorine [29], and ammonia [29]. In the present study, *L. cardium* juveniles were quite sensitive to sedimentary ammonia (Fig. 2). Data on the effects of un-ionized ammonia (NH_3) on unionids for comparison are limited. Goudreau et al. [29] exposed *V. iris* glochidia to waterborne ammonia and calculated a 24-h LC50 of 284 μg NH_3/L. Wade [30] exposed 8-d-old *Anodonta imbecillis* to undiluted pore water from the Tennessee River (USA) and calculated a 9-d LC50 of 153 μg NH_3/L. Mummert et al. [31] exposed juvenile *V. iris* and *L. fasciola* to waterborne ammonia and reported 96-h LC50s of 120 and 280 μg NH_3/L, respectively. Myers-Kinzie [15] reported 96-h LC50s of 40 and 122 μg NH_3/L in <10-d-old *L. siliquoidea*. Although these data are from different species, test durations, and life history stages, they are similar to the LC50s observed in the present study (range, 93–165 μg NH_3-N/L; Fig. 2).

One of the inherent problems in conducting toxicity tests with unionids is they generally do poorly when held in the laboratory for extended time periods, perhaps as a result of inadequate or unknown food requirements. As a result, control survival in laboratory toxicity tests is often <80% (T.J. New-

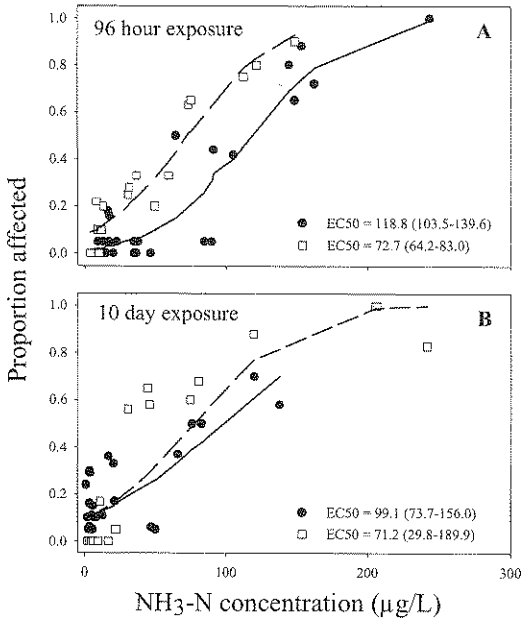

Fig. 3. Proportion of juvenile *Lampsilis cardium* that were affected (includes dead and inactive mussels) by exposure to un-ionized ammonia (NH_3) in pore water during (**A**) 96-h and (**B**) 10-d exposures. Symbols are the actual data points (filled circles and open squares are replicate tests), and the lines are the model-predicted values. Values in parentheses are the 95% confidence limits around the median effective concentration (EC50).

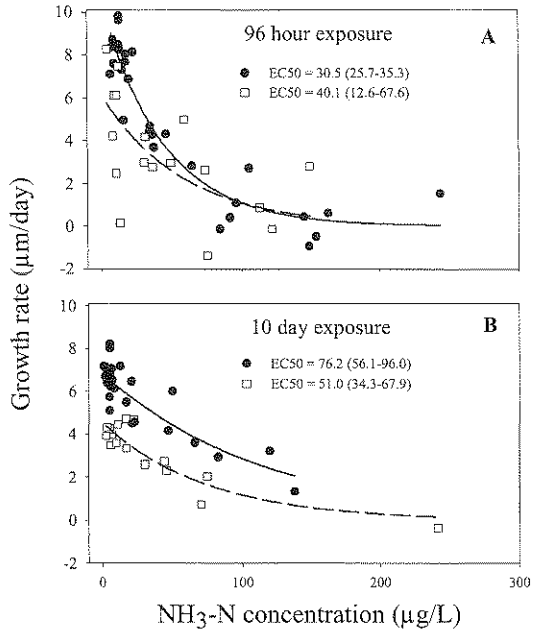

Fig. 4. Growth rate (based on shell height) of juvenile *Lampsilis cardium* exposed to un-ionized ammonia (NH_3) in pore water during (**A**) 96-h and (**B**) 10-d exposures. Symbols are the actual data points (filled circles and open squares are replicate tests), and the lines are the model-predicted values. Values in parentheses are the 95% confidence limits around the median effective concentration (EC50).

ton, unpublished data). In the present study, control survival ranged from 99 to 100% (Fig. 2). We attribute this, in part, to the presence of sediment in the EUs. Juvenile mussels may use pedal-sweep feeding, in which sediment is used as a substratum to collect food particles [11]; thus, the river sediments used in the present study may provide a food source for juveniles and ultimately enhance survival. The presence of sediment in toxicity tests with unionids may be critical for additional reasons. First, many contaminants, including ammonia, preferentially accumulate in sediments and pore water [26]. Second, because juveniles reside in sediments and may filter pore water [11], this could be an important route of contaminant exposure. However, more research is needed to identify the primary route(s) of contaminant exposure in unionids.

Most laboratory tests with unionids use lethality as the test endpoint. The small mass of juveniles often precludes the use of more traditional sublethal endpoints (growth and reproduction) and may preclude biochemical analyses. We used the proportion affected as a sublethal indicator because inactive juveniles may be more prone to predation in the field. Although we observed a dose–response relation between this endpoint and NH_3 (<10% were affected in the controls, and 71–97% were affected in the highest NH_3 treatment), this endpoint was highly variable and subjective (Fig. 3). Additionally, the EC50s (based on the proportion affected) were 57 to 76% of the LC50s, suggesting that this endpoint was not sensitive to NH_3 in this species. This endpoint has been used in prior studies with similar results. Warren [32] exposed 1- to 3-d-old *Utterbackia imbecillis* to Cu and Cd and found that the 96-h EC50s were 59 and 84% of the LC50s, respectively. Lasee [9] exposed 7-d-old juvenile *L. cardium* to Cd and determined that the 7-d EC50 was 64% of the LC50. More sensitive sublethal endpoints need to be found in this taxonomic group.

Recent advances in computer-aided optical imaging systems allows growth of juveniles to be measured with a high degree of precision. In this study, growth was a sensitive indicator of ammonia exposure; EC50s (based on growth) ranged from 31 to 76 µg NH_3-N/L (Fig. 4). After 96 h, the growth rate of juvenile *L. cardium* in the highest NH_3 treatment was reduced by 65 and 83% relative to the controls (Fig. 4). After 10 d, the growth rate was reduced by 57 and 100% relative to the controls. However, juveniles in the control treatment grew well, adding an average of 28 µm of new shell material after 96 h and 46 µm after 10 d. Mean growth rates of juveniles in the control treatment in the present study (6.5, 7.2, 5.6, and 4.2 µm/d) were similar to growth rates observed by Lasee [9]. In the Lasee [9] study, juvenile *L. cardium* grew an average of 6.6 µm/d in the control treatment but grew only 3.6 µm/d when exposed to 50 µg Cd/L for 7 d.

A critical question in toxicity tests with unionids is the appropriate test duration. For acute tests with juveniles, most investigators have used 48- or 96-h exposures [5,15,28]. In the present study, although no substantial differences were observed in any of the responses (survival, proportion affected, growth rate) between the 96-h and 10-d exposures, substantially less variation occurred in the 10-d exposures, especially with growth rate (Fig. 4). For example, the residual variance from the statistical models for growth rate were 1.08 and 1.99 in the 96-h test and 0.76 and 0.61 in the 10-d tests—an average reduction in variance of 45%. This reduction in variance translates into better predictive capabilities, which is critical if these data are used for regulatory purposes. Longer exposure durations should be considered in acute tests with these long-lived organisms. McKinney and Wade [16] also suggest a minimum exposure duration of 5 to 9 d to estimate an acute endpoint in toxicity tests with juveniles.

Chronic data on the effects of contaminants on unionids are limited, but exposure durations of 7 and 9 d have been used [16,27,30]. We chose a 10-d exposure duration for two

Table 2. Median lethal (LC50) and effective concentrations ([EC50] based on the proportion affected and growth rate) of un-ionized ammonia (NH_3), total ammonia nitrogen (TAN), and TAN normalized to pH 8 during four sediment toxicity tests with juvenile *Lampsilis cardium* (95% confidence limits in parentheses). Un-ionized ammonia data are in μg NH_3-N/L, and TAN data are in mg TAN/L

	Toxicity test			
Ammonia speciation, endpoint	96-h test 1	96-h test 2	10-d test 1	10-d test 2
NH_3, LC50	165.4 (145.8–193.5)	127.2 (110.5–152.7)	139.6 (109.9–197.6)	93.2 (59.4–179.7)
NH_3, EC50 (proportion affected)	118.8 (103.5–139.6)	72.7 (64.2–83.0)	99.1 (73.7–156.0)	71.2 (29.8–189.9)
NH_3, EC50 (growth)	30.5 (25.7–35.3)	40.1 (12.6–67.6)	76.2 (56.1–96.0)	51.0 (34.3–67.9)
TAN, LC50	23.7 (20.9–27.6)	23.5 (20.3–28.5)	13.4 (10.5–19.8)	12.0 (8.1–20.5)
TAN, EC50 (proportion affected)	17.7 (15.7–20.3)	13.4 (11.2–16.4)	9.7 (7.3–15.2)	9.1 (4.9–22.1)
TAN, EC50 (growth)	4.5 (3.8–5.2)	7.5 (1.9–13.2)	7.5 (5.9–9.1)	6.2 (4.5–7.9)
TAN at pH 8, LC50	6.7	6.0	3.8	3.4
TAN at pH 8, EC50 (proportion affected)	8.0	5.8	4.4	4.1
TAN at pH 8, EC50 (growth)	2.0	3.2	3.4	2.8

reasons. First, we wanted the test durations to be comparable to standardized tests and published data on other invertebrates. Second, based on preliminary culture data for this species in our laboratory, mortality of juvenile *L. cardium* substantially increased after approximately 30 d in the laboratory. A test duration of 10 d is obviously a small fraction of a unionid's life span, but this must be balanced against the ability to maintain test organisms in a healthy and viable condition during testing. Until the basic biological requirements for this taxonomic group are better understood, chronic test durations may be shorter than is ecologically relevant.

Water quality criteria for ammonia may not protect juvenile unionids [15,33]. The acute criterion (criteria maximum concentration) for TAN when salmonids are absent is 8.4 mg TAN/L at pH 8 [19]. Because this criterion is based on TAN, we recalculated our LC50s and EC50s on the basis of TAN and then normalized these to pH 8 using formulas in U.S. Environmental Protection Agency [19]. The acute criterion is about double the concentrations we report for lethality in juvenile *L. cardium* (Table 2). The chronic criterion (criteria continuous concentration) when fish early life history stages are present is 1.7 mg TAN/L at pH 8 and 20°C [19]. This criterion is lower than the EC50s based on the proportion affected but similar to the EC50s based on growth (Table 2). Furthermore, both the acute and the chronic calculations were made without any additional margins of safety used to protect threatened or endangered species. Thus, existing water quality criteria for ammonia may not be protective of early life history stages in these imperiled organisms.

This study was done in conjunction with a field study where juvenile *L. cardium* were exposed in situ for up to 28 d to a gradient of NH_3 concentrations in pore water in the St. Croix River [34]. This river is relatively uncontaminated and contains a high density and diversity of unionids but is faced with the threat of eutrophication from urbanization (the metropolitan area of Minneapolis–St. Paul, Minnesota, USA, lies ~35 km west of the river). In the river, mean NH_3 concentrations in pore water ranged from 0.1 to 107 μg NH_3-N/L—concentrations that approach and often exceed those adversely affecting survival and growth in the laboratory. However, in the field, survival and growth of juveniles was generally unrelated to NH_3 [34].

To our knowledge, no comparable studies have attempted to field validate the results of laboratory toxicity data in unionids. In field assessments, unionids have been used largely as indicators of exposure to contaminants and not as indicators of effects of contaminants, as was done in the present study. We hypothesize that the lack of agreement between the laboratory and field exposures is the result of several factors. First, in the laboratory, juveniles were exposed to relatively constant concentrations of NH_3, whereas in the field, juveniles were more likely exposed to a range of NH_3 concentrations as a result of storm events and seasonal changes in pH and temperature. Second, NH_3 concentrations in pore water in the river were highly variable, even over the small spatial and temporal scales studied. This variability, coupled with the inherent variability in individual survival and growth estimates, likely masked any potential effect of NH_3 on juveniles. These data suggest that more research is needed to identify additional sublethal endpoints in juveniles and to understand the processes that control ammonia dynamics in rivers at various temporal and spatial scales.

CONCLUSION

The modified sediment infusion system provides temporally consistent ammonia concentrations and has application to studies with other small benthic organisms and other volatile compounds. Additionally, the system was relatively inexpensive to construct (total cost of materials is ~$5,000, of which ~$3,500 was the peristaltic pump), and it was easy to calibrate, run, and maintain. However, the maintenance of temporally consistent ammonia concentrations, while necessary for reproducible laboratory tests, may not be representative of the temporal variation in ammonia in pore water in the field.

The toxicity tests documented the sensitivity of one species of juvenile mussel to NH_3. Mortality was observed at concentrations as low as 93 μg NH_3-N/L, and significant reductions in growth occurred at concentrations as low as 31 μg NH_3-N/L. These concentrations are at or below the acute national water quality criteria, suggesting that existing criteria may not protect juvenile unionids. Although no substantial differences were observed in test responses between the 96-h and 10-d test durations, considerably less variation was associated with the 10-d exposures. Test durations of approximately 10 d should be considered as acute exposures in these long-lived organisms. Comparison of these data to a companion field study were not realistic because of differences in the frequency of exposure to ammonia and the high variability in NH_3 in pore water in the field. Clearly, more research on the sensitivity of early life history stages of unionids to contaminants needs to be evaluated to determine if pervasive, low-

level contamination is contributing to widespread declines in this imperiled faunal group.

Acknowledgement—This research was done in collaboration with staff from the National Park Service, St. Croix National Scenic Riverway, who provided substantial guidance on river-related issues. The authors are grateful to Lori Vingum and Xiaoli Yuan for analytical support and to Steve Gutreuter for statistical guidance. LeeAnne Thorson provided assistance in maintaining unionids and conducting water quality measures during the tests. Two anonymous reviewers made helpful comments on the manuscript.

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