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EFFECTS OF PORE-WATER AMMONIA ON IN SITU SURVIVAL AND GROWTH OF JUVENILE MUSSELS (*LAMPSILIS CARDIUM*) IN THE ST. CROIX RIVERWAY, WISCONSIN, USA

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Abstract—We conducted a series of in situ tests to evaluate the effects of pore-water ammonia on juvenile *Lampsilis cardium* in the St. Croix River (WI, USA). Threats to this river and its associated unionid fauna have accelerated in recent years because of its proximity to Minneapolis–St. Paul, Minnesota, USA. In 2000, caged juveniles were exposed to sediments and overlying water at 12 sites for 10 d. Survival and growth of juveniles was significantly different between sediment (mean, 47%) and water column (mean, 86%) exposures; however, these effects were unrelated to pore-water ammonia. During 2001, juveniles were exposed to sediments for 4, 10, and 28 d. Pore-water ammonia concentrations ranged from 0.3 to 62.0 μg NH_3-N/L in sediments and from 0.5 to 140.8 μg NH_3-N/L within exposure chambers. Survival (mean, 45, 28, and 41% at 4, 10, and 28 d, respectively) and growth (range, 3–45 μm/d) of juveniles were highly variable and generally unrelated to ammonia concentrations. Although laboratory studies have shown unionids to be quite sensitive to ammonia, further research is needed to identify the route(s) of ammonia exposure in unionids and to understand the factors that contribute to the spatial variability of ammonia in rivers.

Keywords—Unionids Ammonia In situ Pore water Juveniles

INTRODUCTION

Unionids have been used as in situ biomonitors of contaminant exposure [1–4]; however, comparatively less is known about their utility as indicators of contaminant effects [5,6]. In the past 10 years, adult unionids have been used to assess the effects of contaminants. For example, Beckvar et al. [3] found that growth of transplanted *Elliptio complanata* was negatively correlated with tissue concentrations of total mercury in the Sudbury River, Massachusetts, USA. Similarly, Gagné et al. [4] observed a reduction in growth in *E. complanata* exposed to a municipal effluent plume contaminated by estrogenic chemicals. To our knowledge, only three in situ studies have been conducted with juveniles, and these studies suggest that survival and growth are sensitive indicators of contaminant effects [7–9]. Although in situ testing with juveniles is relatively new, their sensitivity to contaminants and high growth rate, relative to adults, suggest that this life history stage has potential for further research. However, certain attributes of juveniles, such as their small size (~500 μm) and high natural mortality, create challenges in conducting in situ tests that are not currently addressed by existing guidelines [10].

Ammonia is one of the most common pollutants in aquatic systems and is toxic at relatively low concentrations. In addition to anthropogenic inputs, ammonia can be generated through natural processes, such as bacterial production through nitrogen fixation, ammonification, and dissimilatory reduction of nitrate. Unionids have been shown to be quite sensitive to ammonia, relative to other aquatic fauna [11], with laboratory median lethal concentration (LC50) values ranging from 40 to 284 μg NH_3-N/L [12–15]. Additionally, data on fingernail clams, another freshwater bivalve, were used to develop the ambient water quality criteria for ammonia [16]. Total ammonia nitrogen (TAN) exists in the environment as principally two forms in equilibrium: the ammonium ion (NH_4^+) and unionized ammonia (NH_3); however, toxicity of ammonia to aquatic organisms is generally attributed NH_3 [16].

The St. Croix River supports a diverse and abundant population of unionids, including two federally listed species. Although the upper reaches of the St. Croix River contain surface waters of high quality, threats to water and sediment quality have accelerated in recent years. Population expansion of the Minneapolis–St. Paul, Minnesota, USA, metropolitan area (~35 km west of the river) poses a significant threat to the river via increased inputs of nutrients, toxic contaminants, and suspended sediments from both point and non–point sources [17]. In particular, ammonia has been identified by the National Park Service as a potential threat to the riverine biota.

Our objectives were to determine if existing ammonia concentrations were adversely affecting survival and growth of juveniles in the St. Croix River and characterize TAN and NH_3 concentrations in pore water from the river during two consecutive years. We used juvenile *Lampsilis cardium* and pore-water ammonia in this assessment for several reasons. First, Buddensiek [18] suggests that juvenile unionids inhabit the interstitial zone of river beds, where they are restricted to areas with highly fluctuating conditions between overlying and pore waters. Also, laboratory data suggest that juvenile *Villosa iris*, which burrowed <1 cm into sediment, were not exposed to overlying water, but filtered pore water and fed on sediment-associated organic matter [19]. Second, toxicity of pore water has been suggested as a contributing factor in the decline of mollusks in the upper Mississippi River [20]. Third, concentrations of NH_3 in pore water were 6 to 30 times greater than

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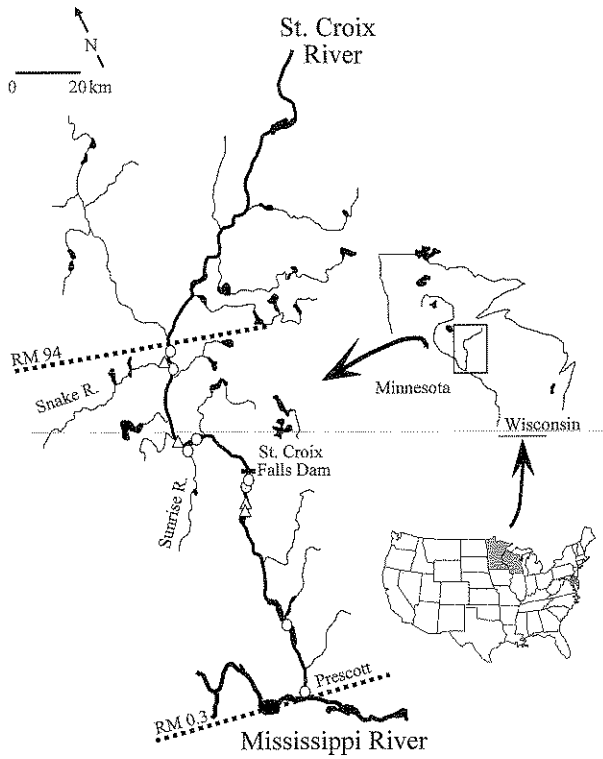

Fig. 1. Sampling sites used during in situ exposures of juvenile unionid mussels in the St. Croix River (WI, USA). Dashed lines delineate the study reach. Open circles were sites used in 2000 and 2001. Open triangles indicate sites used in 2000 only.

those in surface waters in the upper Mississippi River [21]. These data suggest that pore water may be an important exposure route of ammonia to juvenile unionids.

METHODS

Test organisms

Lampsilis cardium (Rafinesque 1820) was selected because it has a broad geographical distribution [22] (including the St. Croix River), is a congener of the federally endangered *L. higginsii*, and is relatively easy to culture in the laboratory. Juveniles were cultured using techniques described by Waller and Holland-Bartels [23] and were maintained in flow-through aquaria containing 22 ± 1°C well water for <6 d before being deployed into the river.

2000 field studies

The St. Croix River forms the northern border between Wisconsin and Minnesota, and terminates at the confluence of the Mississippi River, near Prescott, Wisconsin (Fig. 1). From August 28 to September 7, we conducted a 10-d preliminary in situ exposure at 12 sites in the river, including nine sites in the main channel, two sites on tributaries (Snake and Sunrise Rivers), and one embayment (Osceola Bay). The sites were chosen on the basis of the presence of depositional sediments, proximity to point-source discharges, or present and historical densities of unionids (Table 1). Four chambers were deployed at each site at a water depth of approximately 1 m; two were placed approximately 3 cm below the sediment–water interface, and two were placed approximately 5 cm above the sediment–water interface (Fig. 2). Chambers were placed in the water column to serve as a site control, address potential cage-related effects, and compare our results with those of Warren et al. [9]. The chambers were polyvinyl chloride cylinders (15.2 × 3.8-cm diameter), and each contained two 3.8-cm-diameter holes that were drilled approximately 1 cm from the chamber bottom. Each chamber was wrapped with 153 μm Nitex® (Sefar, Heiden, Switzerland) mesh, including the top and bottom, and was attached to a 107-cm plastic-coated garden stake with cable ties (Fig. 2). Twenty juveniles (5–6 d old) with visible foot or ciliary movement were allocated to each chamber. Chambers were held overnight in aerated well water until deployment. A subsample of ≥20 juveniles was preserved for later measurement of shell height (the greatest dorsal to ventral distance, perpendicular to the hinge).

At each site, pore water was sampled from sediments surrounding the chambers on days 0 and 10. At nine sites, the uppermost 10 cm of sediment were obtained from three 5.0-

Table 1. Mussel density (no./0.25 m²), species richness (no./0.25 m²), and sediment characteristics at 12 sites in the St. Croix River (WI, USA). Sites with the same river kilometer are designated with c = channel, t = tributary, and b = bay. Four sites were sampled during 2000 only (148t, 114c, 76c, and 76b)

	Mussel characteristics		Sediment characteristics (2000, 2001)							
River kilometer	Total density	Species richness	Bulk density (g/m³)	Water (%)	Volatile solids (%)	Gravel (%)	Coarse sand (%)	Medium sand (%)	Fine sand (%)	Silt and clay (%)
151	11.3[a]	2.8[a]	1.4, 1.6	21, 20	0.5, 0.5	<1, <1	56, 42	33, 49	11, 2	<1, 7
148t	0.0[a]	0.0[a]	1.7	17	0.8	56	40	4	<1	<1
148c	1.1[a]	1.0[a]	1.3, 1.5	22, 20	0.8, 0.6	20, 5	13, 39	52, 49	14, 6	1, 1
114c	16.3[b]	5.2[b]	1.1	29	2.0	42	15	27	12	5
114t	10.9[c]	2.8[c]	1.5, 1.4	19, 21	0.6, 0.3	1, <1	48, 5	45, 17	6, 21	<1, <1
101	7.7[b]	2.6[b]	1.1, 1.3	33, 28	3.8, 2.0	14, 2	43, 4	14, 17	23, 51	6, 26
81	7.3[b]	3.1[b]	1.3, 1.5	26, 19	1.5, 1.0	<1, 7	39, 15	46, 73	12, 20	4, 1
79	7.3[b]	3.1[b]	1.3, 1.8	26, 17	1.4, 0.6	24, 41	11, 37	59, 15	5, 5	2, 2
76c	2.3[d]	1.3[d]	1.3	23	0.9	<1	5	78	15	2
76b	0.2[a]	0.2[a]	1.1	30	2.5	1	35	31	23	11
30	4.7[d]	2.7[d]	1.7, 1.6	17, 16	0.6, 0.5	19, 15	53, 54	22, 23	6, 7	<1, <1
0.5	1.2[b]	1.2[b]	1.4, 1.5	23, 23	0.6, 0.4	<1, <1	10, 6	68, 58	21, 24	2, 12

[a] Dunn and Howard [36].
[b] D. Hornbach, (Malcalester College, St. Paul, MN, USA, personal communication).
[c] M. Davis, (Minnesota Department of Natural Resources, Lake City, MN, USA, personal communication).
[d] Holmberg et al. [17].

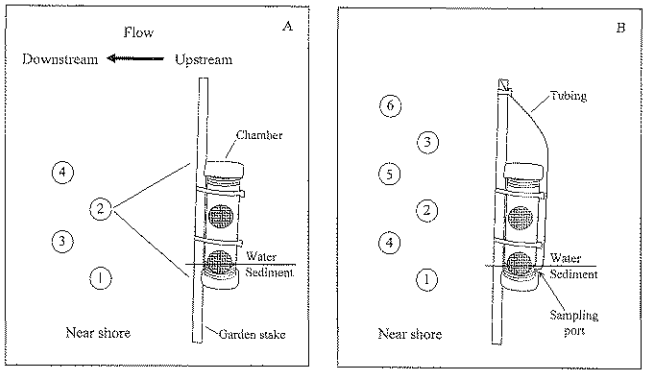

Fig. 2. Schematic of the experimental design and mussel exposure chambers at study sites located on the St. Croix River (WI, USA) during (**A**) 2000 and (**B**) 2001. (**A**) Mussels were deployed into chambers that were buried approximately 3 cm below the sediment–water interface (positions 1 and 2) and into chambers placed approximately 5 cm above the sediment–water interface (positions 3 and 4). (**B**) In 2001, all chambers were deployed approximately 3 cm below the sediment–water interface, and a sampling port was added to each chamber to obtain pore water.

cm-diameter cores. At the remaining sites, triplicate samples of the top 10 cm of sediment were obtained with a spatula or an Ekman dredge (GENEQ, Montreal, QC, Canada). Immediately on collection, sediment was measured for temperature and pH and stored on ice until centrifugation. All samples were centrifuged at 6,000 rpm for 20 min at 4°C. After centrifugation, pore water was filtered through a 0.7-μm glass-fiber filter and preserved to pH <2 with 3.6 N H_2SO_4. An additional sediment sample was obtained at each site for bulk density [24], water content [25], volatile solids [25], and particle size [26,27] analysis (Table 1). The particle size analysis was modified by omitting the H_2O_2 digestion, due to the low organic content (<4%), and was classified as gravel (>2 mm), coarse sand (0.5–2 mm), medium sand (250–500 μm), fine sand (63–250 μm), and silt and clay (<63 μm).

After 10 d, the chambers were retrieved and placed into coolers containing aerated river water. Juveniles from one chamber in the sediment and one in the water column at each site were counted and observed for mortality under ×40 magnification within 8 h of retrieval. Lack of movement, with an absence of ciliary action, and deterioration or absence of soft tissue indicated death. Live juveniles were fixed in 10% buffered formalin and preserved in 70% EtOH for later height measurements using an optical imaging system (Optimas, Bothell, WA, USA). We also retained and preserved indigenous organisms and qualitatively categorized the amount of algae within each chamber (low, medium, high).

2001 field studies

In 2001, we modified the experimental design in the following ways. First, several of the initial 12 sites were logistically inaccessible or we had difficulty obtaining intact sediments. Therefore, from July 20 to August 17, exposures were conducted at eight of the sites used in 2000, covering 151 km (Fig. 1). Second, we deployed six chambers approximately 3 cm below the sediment–water interface and randomly removed two chambers at each site after 4, 10, and 28 d of exposure (Fig. 2). We chose these test durations because they encompass the exposure periods used in other in situ studies with juveniles [7,9]. Third, the chambers were modified by the addition of a sampling port (0.36-cm diameter, located ~1 cm above the chamber bottom). A 1.2-m length of Tygon® (Saint-Gobain Performance Plastics, Akron, OH, USA) tubing (0.15-cm i.d.) was attached to the port with a nipple. The tubing was secured at the top of the stake with a cable tie (Fig. 2). This modification was made because we were concerned that measuring pore-water ammonia in the sediments surrounding the chambers may not reflect ammonia concentrations within the chamber. Also, the port allowed us to measure dissolved oxygen concentrations within the chamber (on days 10 and 28).

At each site, pore water was sampled from sediments surrounding the chambers and from within each chamber on days 4, 10, and 28. Triplicate sediment cores were obtained as previously described but were sectioned into 0- to 5-cm and 5- to 10-cm strata. Pore water was obtained within each chamber by attaching the tubing on the chamber port to a 5-ml polypropylene syringe. The first 2 ml of pore water were discarded to evacuate any surface water within the tubing; the remaining 3 ml were measured for temperature and pH, filtered through a 0.7-μm glass-fiber filter, and preserved to pH <2 with 3.6 N H_2SO_4. Current velocity was measured at each exposure duration at each site. Chlorophyll *a* samples were obtained in triplicate at each site on days 4, 10, and 28. The samples were obtained approximately 10 cm above the sediment–water interface with a 10-ml polypropylene syringe, transferred to a dark scintillation vial, and placed into a cooler containing ice. Samples were processed within 24 h by filtering a 5-ml sample through a 0.7-μm glass-fiber filter. The filters were frozen and later analyzed fluorometrically [25] using a Turner 10-AU (Sunnyvale, CA, USA).

All pore-water samples were analyzed for TAN with the automated phenate method on a Bran+Luebbe (Buffalo Grove, IL, USA) auto-analyzer [25]. Concentrations of NH_3 were calculated from site-, depth- (2001 only), and replicate-specific TAN, pH, and temperature measurements with the formula of Emerson et al. [28]. When characterizing ammonia in the river, we report both TAN and NH_3 concentrations for comparison with published data. However, our discussion focuses on NH_3 because it is the most biologically relevant form.

Quality assurance

To evaluate the accuracy of TAN determinations, the following were analyzed with each batch of samples: quality control samples (Environmental Resource, WastWatR®, Arvada, CO, USA, lots 9938 and 8057, and Ultra Scientific, North Kingstown, RI, USA, ultracheck nutrient ampule 2, lot 73746), spiked samples, triplicate samples, and procedural blanks. Mean TAN concentrations in the three quality control samples were within certified ranges in all 27 samples. Recovery of TAN from nine triplicate spiked samples averaged 95%. Method precision (relative standard deviation) estimated from analysis of 17 triplicate samples averaged 1.8%. To reduce the variation in growth measurements, all juveniles were measured for shell height by one individual. Also, a 1,000-μm reference standard was measured five times daily prior to measuring juvenile height, and all values fell within two standard deviations. Precision of the height measures, estimated from analysis of 10 juveniles measured daily for 5 d, ranged from 0.8 to 6.1%. Sediment characterization included triplicate analysis of 25% of the samples. Method precision estimated from analysis of two triplicate subsamples averaged 1.6% for bulk density, 2.1% for water content, and 5.4% for volatile solids content. The relative standard deviation for particle size analysis of two triplicate subsamples averaged 7%.

Statistical analysis

In 2000, differences in arcsin-transformed survival of juveniles between water- and sediment-exposed chambers were analyzed with paired *t* tests. Regression analyses were used to examine the relation between survival (logistic model) or growth rate (linear model) of juveniles and TAN and NH_3 concentrations in pore water. We used Akaike's Information Criterion to judge the fit of the data to the models. In 2001, additional chamber-level (NH_3, pH, temperature, and dissolved oxygen) and site-level (current velocity and chlorophyll *a*) characteristics were included as independent variables. Growth rate (μm/d) of live mussels was calculated as the difference between the final and initial shell height divided by the days of exposure. For graphic purposes, we plotted NH_3 concentrations in the chambers against survival and growth of juveniles because all these were measured within the chamber.

Differences in pore-water NH_3 concentrations in 0- to 10-cm cores between 2000 and 2001 were analyzed with paired *t* tests. Regression analyses were used to examine the relation between the physical characteristics of sediment and TAN concentrations. Total ammonia nitrogen was chosen for regression analyses with sediment characteristics because TAN is produced in sediments by bacteria, whereas NH_3 concentrations are calculated from site-, depth- (2000 only), and replicate-specific TAN, pH, and temperature measurements [21]. In 2000, TAN concentrations were positively related with silt and clay content ($p = 0.05$) but were not related with any particle size fraction in 2001.

RESULTS

Recovery and survival

In 2000, we recovered all 48 chambers that were deployed into the river. We also recovered 89% of the mussels in the sediment-exposed chambers and 90% of the mussels in the water column–exposed chambers. Survival of juveniles was significantly lower in sediment-exposed chambers compared to those deployed in the water column ($p = 0.04$; Table 2). Across all sites, survival averaged 47% in the sediment and 86% in the water column. Survival was related to TAN concentrations ($p < 0.001$) but not with NH_3 concentrations ($p = 0.08$). Interestingly, the highest NH_3 concentration observed this year (187 μg NH_3-N/L) occurred at river kilometer 114t, which has a very dense and rich unionid community (Tables 1 and 2).

In 2001, we recovered 92% of the chambers from the river and 90, 86, and 71% of the mussels from the chambers during 4-, 10-, and 28-d exposures, respectively. Survival of juveniles averaged 45% at 4 d, 28% at 10 d, and 41% at 28 d (Fig. 3). At most sites, NH_3 concentrations fell within a narrow range (0–20 μg NH_3-N/L); however, within this range survival varied from 0 to 100%. The regression models based on survival at 28 d best fit our data, which is consistent with the 30-d minimum exposure duration for caged bivalves studies [10]. In these models, Akaike's Information Criterion was consistently lower (better fit to the data) than the models at 4 or 10 d (Table 3). The odds ratio (and its confidence limits) can be used as an additional measure of association between dependent and independent variables. This ratio provides a factor by which the odds of survival increase or decrease with each one-unit change in ammonia concentration. Ratios vary from 0 to ∞; a value of 1.0 indicates no relation between survival and ammonia. The only significant relations between NH_3 and sur-

Table 2. Survival (no. alive/no. recovered) and growth rate of juvenile *Lampsilis cardium* from one chamber deployed in the water column and another deployed in sediments from the St. Croix River (WI, USA) for 10 d during 2000. Un-ionized ammonia (NH_3) and total ammonia nitrogen (TAN) concentrations in pore water from sediments surrounding the chamber are listed for comparison. Sites with the same river kilometer are designated with c = channel, t = tributary, and b = bay

	Survival (%)		Growth rate (μm/d)		Ammonia species	
River kilometer	Water column	Sediment	Water column	Sediment	NH_3-N (μg/L)	TAN (mg/L)
151	69	100	8.2	6.0	26.5	1.65
148t	68	44	6.7	5.0	9.3	0.47
148c	94	16	5.3	5.2	10.2	0.89
114c	41	58	6.2	7.3	8.7	0.44
114t	100	13	5.4	5.4	186.9	4.31
101	82	47	7.3	5.6	20.1	5.86
81	89	0	7.9	—[a]	54.7	2.40
79	94	0	7.9	—[a]	10.1	1.05
76c	100	33	7.7	5.7	7.9	0.89
76b	94	100	8.6	6.2	45.8	12.73
30	95	100	8.7	7.7	22.5	1.78
0.5	100	50	10.5	8.4	148.1	5.86
Mean	86	47	7.5	6.3	—	—

[a] Growth rate was not calculated because mortality was 100%.

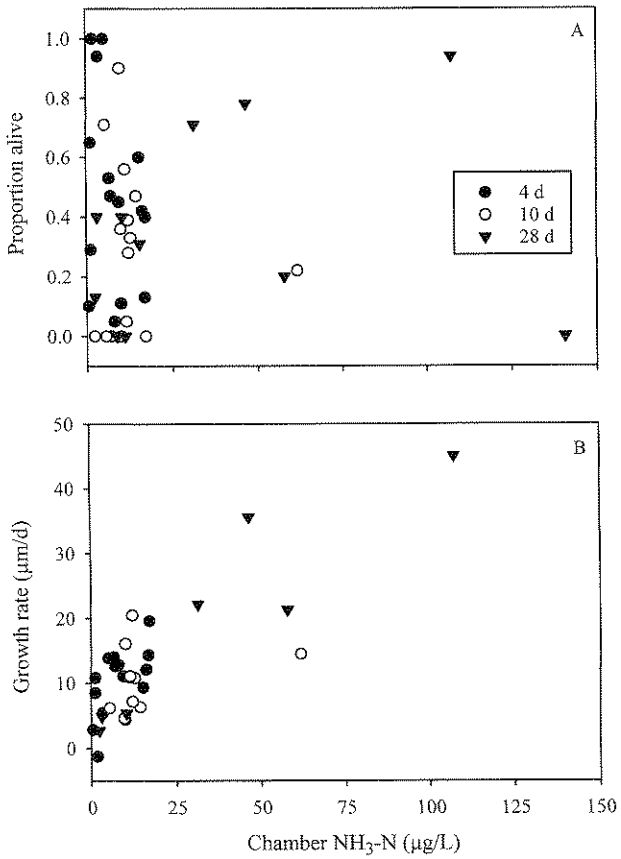

Fig. 3. (**A**) Proportion alive (no. alive/no. recovered) and (**B**) growth rate of juvenile *Lampsilis cardium* after 4, 10, and 28 d of exposure to un-ionized ammonia (NH_3) in pore water (measured from within chambers) over a 151-km reach of the St. Croix River (WI, USA) during 2001.

Table 3. Probability values, odds ratio (95% confidence limits), and Akaike's Information Criterion (AIC) from a logistic regression of survival (no. alive/no. recovered) of juvenile *Lampsilis cardium* exposed to un-ionized ammonia (NH_3) and total ammonia nitrogen (TAN) in pore water in the St. Croix River (WI, USA) during 2001. Pore water was sampled from 0- to 5-cm cores and pumped from within the chambers containing juveniles

Ammonia species	Sampling method	4-d exposure			10-d exposure			28-d exposure		
		p value	Odds ratio	AIC	*p* value	Odds ratio	AIC	*p* value	Odds ratio	AIC
NH_3	Core	0.002	0.98 (0.97–0.99)	397	<0.0001	1.47 (1.24-1.74)	229	<0.0001	0.53 (0.40–0.71)	167
	Chamber	0.001	0.94 (0.90–0.98)	396	0.56	0.99 (0.97–1.02)	318	0.09	1.01 (1.00–1.01)	200
TAN	Core	<0.0001	0.36 (0.22–0.57)	383	0.89	0.81 (0.05–13.59)	251	0.09	0.21 (0.03–1.31)	192
	Chamber	0.002	0.56 (0.38–0.81)	397	0.004	0.34 (0.17–0.71)	307	0.03	2.1 (1.1–4.2)	199

vival occurred when NH_3 was measured in the cores. This relation was positive (odds ratio >1) at 10 d and negative (odds ratio <1) at 28 d. In contrast, survival of juveniles was consistently related with TAN across all exposure durations and was negative in five of six cases (Table 3).

Other site- and chamber-specific variables may have influenced survival. Dissolved oxygen concentrations in the chambers were highly variable (range, 1.9–7.9 mg/L) but were positively related to juvenile survival ($p < 0.0001$) on days 10 and 28. In contrast, the temperature of the pore water within the chambers (range, 19.7–29.1°C) and chlorophyll *a* concentrations at the site (range, 2.3–32.1 mg/cm^3) negatively influenced survival on days 10 and 28 ($p < 0.0001$). Survival of juveniles was unrelated to current velocity (range, 0–0.45 m/s) at any exposure duration ($p > 0.05$).

Growth

In 2000, the growth rate of juveniles varied significantly between those deployed in the sediment and those deployed in the water column ($p = 0.01$; Table 2). Juveniles in the water column grew an average of 7.5 μm/d, while those deployed in the sediment grew an average of 6.3 μm/d. Interestingly, the highest growth rate of juveniles from both the water column and the sediment exposures occurred at river kilometer 0.5, which had one of the highest sediment NH_3 concentrations (148 μg NH_3-N/L). However, the growth rate of juveniles deployed in river sediments was unrelated to NH_3 concentrations ($p = 0.55$).

During the 2001 exposures, the growth rate of juveniles ranged from 2.9 to 19.6 μm/d at 4 d, from 4.4 to 20.5 μm/d at 10 d, and from 2.8 to 45.1 μm/d at 28 d (Fig. 3). Like survival, the growth rate of juveniles was highly variable (0–20 μm/d) over the 0- to 20-μg NH_3-N/L range. Concentrations of NH_3 in the chambers were positively related to growth at 4 and 28 d, and NH_3 concentrations in cores were negatively related to growth at 10 d. Total ammonia nitrogen concentrations were positively associated with growth at 10 d when sampled with cores and at 28 d when sampled from within the chambers (Table 4). Unlike survival, growth of juveniles was positively influenced by pore-water temperature at 4 and 10 d ($p = 0.03$ and 0.04, respectively) but not at 28 d ($p = 0.14$). Growth was negatively related to current velocity at 10 d ($p = 0.05$). Dissolved oxygen and chlorophyll *a* did not influence growth.

Pore-water ammonia concentrations in 2001

Concentrations of TAN and NH_3 in pore water varied substantially over the 151 km regardless of sampling method. Across all sites, TAN concentrations ranged from 0.02 to 2.2 mg TAN/L in the sediments and from 0.01 to 2.2 mg TAN/L in the chambers, and NH_3 concentrations ranged from 0.3 to 62.0 μg NH_3-N/L in the sediments and from 0.5 to 140.8 μg NH_3-N/L in the chambers (Fig. 4). The spatial variability in ammonia was substantial even among triplicate samples at a given site. For example, on day 4 at river kilometer 30, TAN concentrations in sediment varied threefold and ranged from 0.4 to 1.2 mg TAN/L (Fig. 4). Additionally, NH_3 concentrations among samples taken within 0.5 m of each other ranged from 28 to 74 μg NH_3-N/L. However, NH_3 concentrations in duplicate chambers at this site and time were similar (15 and 16 μg NH_3-N/L; 1.8 and 1.9 mg TAN/L). Ammonia concentrations also varied temporally over the 28-d exposure period. For example, NH_3 concentrations in pore water at river kilometer 114 averaged 1.8 μg NH_3-N/L at 0 d, 12.5 μg NH_3-N/L at 4 d, 5.9 μg NH_3-N/L at 10 d, and 4.2 μg NH_3-N/L at 28 d (Fig. 4). Similarly, NH_3 concentrations in pore water from the chambers at river kilometer 101 averaged 9.2 μg NH_3-N/L at 4 d, 12.0 μg NH_3-N/L at 10 d, and 39.0 μg NH_3-N/L at 28 d.

In general, pore-water NH_3 concentrations in the sediment

Table 4. Probability values, correlations coefficients (r^2), and Akaike's Information Criterion (AIC) from a linear regression of growth rate (μm/d) of juvenile *Lampsilis cardium* exposed to un-ionized ammonia (NH_3) and total ammonia nitrogen (TAN) in pore water the St. Croix River (WI, USA) during 2001. Pore water was sampled from 0- to 5-cm cores and pumped from within the chambers containing juveniles

Ammonia species	Sampling method	4-d exposure			10-d exposure			28-d exposure		
		p value	r^2	AIC	*p* value	r^2	AIC	*p* value	r^2	AIC
NH_3	Core	0.54	0.03	43.2	0.009	−0.65	35.0	0.31	−0.17	28.9
	Chamber	0.08	0.22	46.7	0.44	0.08	31.3	0.003	0.85	53.4
TAN	Core	0.29	0.09	45.6	0.002	0.78	19.3	0.43	0.11	47.4
	Chamber	0.49	0.04	46.3	0.22	0.18	33.8	0.01	0.74	32.6

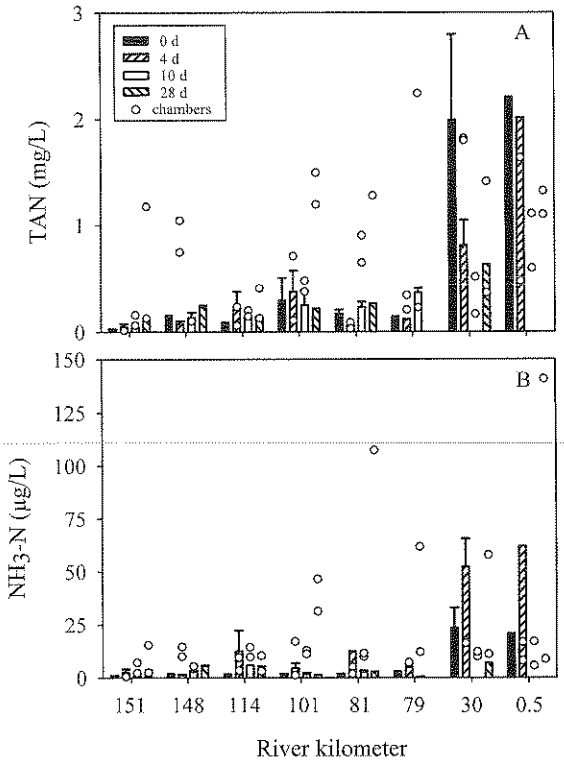

Fig. 4. Mean concentrations (±1 standard error) of (**A**) total ammonia nitrogen (TAN) and (**B**) un-ionized ammonia (NH_3) in pore water obtained from triplicate 0- to 5-cm cores (bars) after 0, 4, 10, and 28 d and in pore water obtained by pumping from within two chambers (open circles) after 4, 10, and 28 d over a 151-km reach of the St. Croix River (WI, USA) during 2001. Means without error bars have an $n \leq 2$.

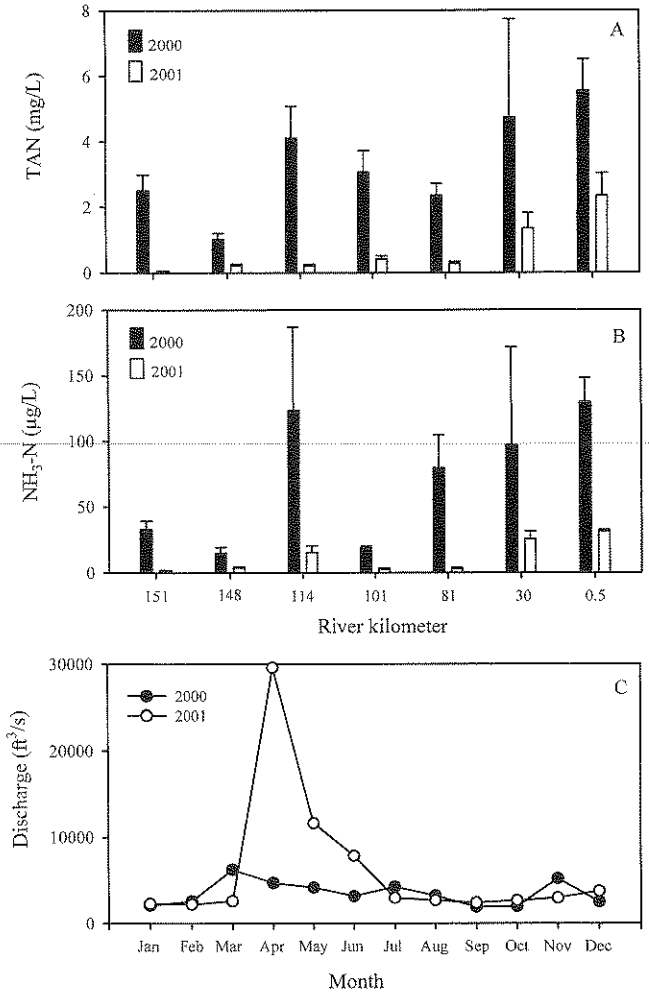

Fig. 5. Mean concentration (±1 standard error) of (**A**) total ammonia nitrogen (TAN) and (**B**) un-ionized ammonia (NH_3) in pore water obtained from 0- to 10-cm cores in the St. Croix River (WI, USA) during 2000 (averaged over 0 and 10 d) and 2001 (averaged over 0, 4, 10, and 28 d). (**C**) The discharge of the St. Croix River at St. Croix Falls during the study period.

and within the chambers varied with river kilometer. In the middle reaches (river kilometers 101, 81, and 79), chamber NH_3 concentrations were generally higher than those in the sediments. However, in the upper (river kilometers 151, 148, and 114) and lower reaches (river kilometers 30 and 0.5), pore-water NH_3 concentrations were similar in the sediment and within the chambers (Fig. 4).

Comparison of ammonia concentrations between 2000 and 2001

Concentrations of TAN and NH_3 in sediment pore water varied significantly ($p < 0.001$ and $p = 0.008$, respectively) between 2000 and 2001 over a 151-km reach of the St. Croix River (Fig. 5). Total ammonia nitrogen concentrations, averaged over all exposure durations, ranged from 1.0 to 5.6 mg TAN/L in 2000 and from 0.05 to 2.3 mg TAN/L in 2001, a fivefold decline when averaged across all sites. Similarly, NH_3 concentrations declined sixfold, ranging from 14.7 to 129.7 µg/L in 2000 and from 1.6 to 31.4 µg/L in 2001. The reduction in ammonia between years may be related to a flood event that occurred on the river in the spring of 2001 (Fig. 5).

DISCUSSION

In the present study, we were unable to consistently predict the survival of juvenile *L. cardium* on the basis of pore-water ammonia concentrations. However, we observed significant differences in survival of juveniles between sediment-exposed (47%) and water column–exposed chambers (86%). Our data compare favorably with a field bioassay in which six-week-old juvenile *Utterbackia imbecillis*, exposed to sediments that received agricultural runoff in an impoundment of the Tennessee River (USA), had lower mean survival than those exposed to overlying water (26 and 72%, respectively) [9]. Likewise, in a laboratory study, fingernail clams had significantly higher survival when placed 1 to 2 cm above the sediment–water interface (89%) compared to those that were allowed to burrow into sediments (77%) [29]. These data are consistent with the fact that many contaminants, including ammonia, concentrate in sediments and associated pore water [21,30] and suggest that certain conditions associated with river sediments (contaminants) or chamber-related artifacts (dissolved oxygen) may have contributed to the reduced survival.

Similar to survival, growth rate of juvenile *L. cardium* was not consistently predicted by pore-water ammonia concentrations. Few published in situ studies exist on growth rates of juveniles for comparison. Buddensiek [8] reported the maximum growth rate and highest survival of juvenile *Margaritifera margaritifera* when deployed in a slightly polluted river, relative to those deployed in three less polluted rivers, presumably because the more polluted river provided a better food source. Juvenile *M. margaritifera* grew approximately 4 µm/

d (shell length data inferred from graphic) in the polluted river and approximately 1 μm/d in the other three rivers [8]. However, growth and survival were negatively correlated with overlying ammonia concentrations (range, 0.16–0.34 mg TAN/L). In a laboratory study, the average growth rate of juvenile *L. cardium* was 4 μm/d over an ammonia gradient of 0 to 200 μg NH_3-N/L [15]. In contrast, in this study the average growth rate of juvenile *L. cardium* was 11 μm/d over an ammonia gradient of <1 to 62 μg NH_3-N/L.

Guidelines for conducting in situ studies with caged bivalves recommend measuring temperature, dissolved oxygen, and chlorophyll *a* because these variables may influence the survival and growth of bivalves [10]. These variables likely influenced survival and growth of *L. cardium* in the St. Croix River. The temperature of pore water in the chambers in the present study was negatively related to survival and positively related to growth. A similar relation was observed by Buddensiek [8] when juvenile *M. margaritifera* were caged in four rivers in northern Germany. Dissolved oxygen concentrations in the chambers positively influenced survival. Sparks and Strayer [31] found that survival of juvenile *E. complanata* was significantly reduced at dissolved oxygen concentrations of 1.3 mg/L and behavior was affected at 2 to 4 mg/L. During the 10- and 28-d exposures in our study, dissolved oxygen concentrations dropped below 4 mg/L at six of the eight sites. Chlorophyll *a* concentrations were negatively related to survival of *L. cardium* but were unrelated to growth. If chlorophyll *a* concentrations were high enough to result in an accumulation of algae in or on the chambers, water flow through the chambers may have been reduced. This could ultimately affect survival. The presence of indigenous organisms within the chambers may also influence survival [8,10]. However, given that we recovered most of the juveniles within the chambers and found a wide variety of organisms (copepoda, ostracoda, chironomidae) among sites, including potential predators, it seems unlikely that predation had a major influence on survival.

The method of deploying the chambers and their design may have influenced the survival and growth of juveniles. We did not follow the cage design in the American Society for Testing and Materials guideline, which recommends that adults are deployed in individual compartments [10]. Given that the juveniles we deployed were approximately 300 μm, they could not be individually compartmentalized. Rather, our chambers were similar in design to other in situ studies with caged juveniles [7,9]. Because some of our survival estimates were as high as 100%, it seems unlikely that the design of the chamber was problematic. However, we cannot rule out the confounding effects associated with chamber deployment. Our chambers were deployed approximately 3 cm below the sediment–water interface; however, because of the shifting nature of river sediments, we are uncertain that the chambers were maintained at this depth. If the chambers became too deeply buried or biofouled, survival may have decreased, perhaps as a result of low dissolved oxygen concentrations or as a result of a buildup of waste products. Interestingly, Buddensiek [8] found that the in situ survival rates of juvenile *M. margaritifera* were significantly greater in the presence of chironomids, a fact that he attributed to the role of fecal pellets in providing nutrition.

Because juvenile unionids reside in sediments and feed on sediment-associated particles, it is possible that sediments and associated pore water are a likely exposure route for ammonia and presumably other contaminants. We are not certain that the water we removed from the chambers was pore water, although we have some evidence that suggests it was not surface water. The pH of the water removed from the chamber averaged 0.4 pH units less than the pH of the surrounding surface water, which is consistent with this being pore water [21]. Even if the water in the chamber was not pore water, it was still the water that the juveniles were exposed to.

The spatial and temporal variation in pore-water ammonia concentration in the St. Croix River may have contributed to the variation in survival and growth of juveniles. Ammonia is known to be more stable in anoxic conditions because it undergoes less microbial degradation [32]; perhaps the sandy, well-oxygenated sediments in the St. Croix River contributed to the variability in ammonia. Additionally, heterogeneity in ammonia concentrations is also influenced by a host of microbially mediated processes, such as nitrification and denitrification. Sarda and Burton [33] found significant differences in pore-water TAN concentrations at distances of 30 to 60 cm. Similarly, TAN concentrations varied 10-fold when measured monthly (for 10 months) in the upper Mississippi River [21].

Ammonia concentrations in the St. Croix River decreased at least fivefold between 2000 and 2001, and thus a narrow range existed in the river during the bulk of our study (2001). We attribute the substantial decrease in pore-water ammonia concentrations to the high flows associated with a record flood event that occurred in April 2001 (Fig. 5). These high flows presumably allowed a greater exchange between surface water and sediments and decreased NH_3 concentrations in surficial sediments. Chambers et al. [34] suggest that interannual changes in discharge act as set points in defining the composition and chemistry of bottom sediments. Furthermore, concentrations of contaminants associated with bed sediments in the upper Mississippi River declined precipitously following a major flood event, perhaps as a result of dilution by coarser and relatively less polluted sediments [35].

In conjunction with our field study, we conducted a series of laboratory toxicity tests to assess the effects of NH_3 in pore water on survival and growth of juvenile *L. cardium*. In this study, Newton et al. [15] observed a consistent dose–response relation and reported LC50 values ranging from 93 to 165 μg NH_3-N/L and median effective concentration values (based on growth rate) ranging from 31 to 76 μg NH_3-N/L, concentrations that were observed in the St. Croix River, especially in the lower reaches. The different responses observed between our field and laboratory studies was not unexpected given the multitude of factors that likely influence survival and growth in the field. We offer three hypotheses regarding these discrepancies. First, NH_3 concentrations in the river are highly dynamic and may fluctuate substantially because of numerous environmental conditions, whereas in the laboratory, juveniles were exposed to relatively consistent NH_3 concentrations under controlled conditions [15]. Second, although the laboratory study was conducted with river sediment, the substantial differences in growth rates suggests that perhaps some unknown requirement was not provided in the laboratory environment. Third, the range in pore-water NH_3 concentrations in the St. Croix River may not be wide enough to see an effect on these endpoints at this time.

In conclusion, existing concentrations of NH_3 in pore water in the St. Croix River do not appear to be adversely affecting juvenile *L. cardium*. However, the literature strongly demonstrates that unionids are sensitive to relatively low concentrations of NH_3. We hypothesize that rivers may have episodes

of toxicity, such that when certain environmental conditions are present, they create a toxic environment for riverine biota. For ammonia, these conditions include high sediment temperature and high sediment pH, conditions under which a large percentage of TAN is in the more toxic NH_3 form. Although we sampled during the late summer, given the dynamic nature of ammonia, our sampling interval may not have encompassed the maximum range of NH_3 in the river. Further research is needed to identify the route(s) of exposure in unionids and the processes that control ammonia dynamics in rivers prior to understanding the effects of ammonia on this imperiled faunal group.

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