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Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) I: water-column ammonia levels and ammonia toxicity

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Abstract. The Asian clam (*Corbicula fluminea*) co-occurs with unionid mussels in many riverine ecosystems. Clam populations can reach high densities and may undergo rapid die-offs, particularly during the low flow and warm temperatures of summer drought. Our study objective was to determine whether ammonia produced by decaying clam tissues during die-offs could affect unionid mussels. We induced *C. fluminea* die-offs in artificial streams to simulate die-off effects in natural habitats, and measured total and unionized ammonia (NH_3-N) and dissolved oxygen (DO) in the overlying water. When water flow was stopped, reductions in DO preceded the onset of mortality in streams having the highest density of clams (~10,000 clams/m^2). NH_3-N concentrations in the overlying water increased in association with clam mortality and reached concentrations of up to 5.04 mg/L at 26 ± 2°C. Temperature significantly influenced the rate of DO reduction and NH_3-N production in the systems, while resumption of water flow led to rapid dissipation of NH_3-N from the water column. We also conducted laboratory experiments to determine median lethal concentrations (LC_{50}s) of total ammonia and NH_3-N for glochidia and juveniles of the unionid mussel *Villosa iris*, adults of the unionid *Pyganodon grandis*, and juveniles and adults of *C. fluminea*. The 96-h LC_{50}s (24-h for *V. iris* glochidia) for NH_3-N ranged from 0.11 mg/L for *V. iris* glochidia to 0.8 mg/L for adult *C. fluminea*, indicating that NH_3-N levels produced by Asian clam die-offs have the potential to exceed acute effects levels for at least some species of unionid mussels.

Key words: ammonia toxicity, Asian clam die-offs, unionids.

Native freshwater mussel populations have declined significantly in North America in recent decades, with 72% of species listed as extinct, endangered, threatened, or of special concern (Williams et al. 1993). Potential causes of this decline include habitat destruction, pollution, and introduced species including the Asian clam (*Corbicula fluminea*) and zebra mussel (*Dreissena polymorpha*) (Bogan 1993, Williams et al. 1993, Richter et al. 1997, Metcalfe-Smith et al. 1998).

Populations of the infaunal Asian clam often reach high densities (e.g., 269,105/m^2 in a newly colonized reach of the New River, Virginia; Cherry et al. 1986). Populations of this clam can undergo rapid die-offs initiated by factors such as increased silt loads during spring flooding, high and low temperature extremes, and low dissolved oxygen (DO) levels associated with decreased water flow (Sickel 1986, Strayer 1999). Elevated ammonia levels and decreased DO concentrations may occur in association with tissue decay during clam die-offs, but few studies have investigated how these factors change in association with a clam die-off or how they influence native unionid mussels that occur near dying Asian-clam populations.

In aquatic systems, ammonia occurs in both ionized (NH_4^+-N) and unionized (NH_3-N) forms, with pH and temperature determining the dominant species. NH_4^+-N is relatively nontoxic to aquatic organisms (Thurston et al. 1979).

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However, as pH increases, NH_3-N becomes the dominant species. NH_3-N is more lipid soluble than NH_4^+-N and readily passes across the gill membrane and causes toxic effects (Redner and Stickney 1979). In addition to mortality, NH_3-N may impair siphoning, impair byssal thread secretion, and deplete energy stores (Epifanio and Srna 1975, Reddy and Menon 1979, Chetty and Indira 1995).

We sought to further characterize the effects of ammonia on freshwater mussels by examining the potential for impacts arising from ammonia produced during an Asian clam die-off. Our 1st objective was to investigate the influence of *C. fluminea* population density on mortality rate, concentration of ammonia produced, and reductions in DO concentrations during clam die-offs induced in the laboratory. Our 2nd objective was to determine the acute sensitivity of 2 unionids (*Villosa iris* and *Pyganodon grandis*) and *C. fluminea* to unionized ammonia and compare acute sensitivity levels with ammonia levels produced from the clam die-off.

Methods

Artificial stream tests with Asian clams

We simulated natural die-offs of Asian clams collected from the Clinch River, Virginia, in miniature artificial streams consisting of lengths of vinyl gutter (47.7 cm long, 11.4 cm wide, and 6.4 cm deep). Each stream contained ~1 L of sediment and 2 L of dechlorinated municipal tap water. Masterflex® peristaltic pumps (Cole Parmer, Vernon Hills, Illinois) maintained flow-through at 30 mL/min. We obtained sediment from Sinking Creek, Virginia, then sieved (5 cm) and refrigerated (4°C) it for ≤2 wk prior to use.

We conducted 2 separate tests, with each test consisting of 3 phases: 1) phase 1 (days 0–14 for both tests) in which flow-through was maintained at 30 mL/min; 2) phase 2 in which flow was reduced to 0 mL/min to initiate die-offs (in both tests we terminated this phase and reinitiated flow after any observed spikes in stream ammonia concentrations began to decrease); and 3) phase 3 in which flow was restored to 30 mL/min until ammonia concentrations in streams that had a die-off returned to levels similar to those of controls and the test was terminated. The 2 tests differed in that we allowed water temperatures to fluctuate with incoming laboratory water in test 1, whereas we maintained water temperatures at 26 ± 3°C in test 2.

For each test, we placed Asian clams in streams at treatment densities of 0 (control), 2000, 5000, and 10,000 individuals/m² (2 replicates/density). We used digital calipers to determine clam lengths to the nearest 0.1 mm to ensure a balance of clam sizes (small: 5–10 mm, medium: 10.1–15 mm, and large: 15.1–25.0 mm) among the density treatments in each of the gutters. In test 1, the size distribution of clams was 47% small, 37% medium, and 16% large clams, whereas in test 2, the distribution was 27% small, 59% medium, and 14% large clams.

We measured temperature, pH, DO, conductivity, and mortality daily. We measured ammonia every other day until a clam die-off occurred and daily thereafter. We measured pH with an Accumet® (Fisher Scientific, Pittsburgh, Pennsylvania) pH meter with an Accumet® gel-filled combination electrode, DO with a Yellow Springs model 54A oxygen meter (RDP, Dayton, Ohio), and conductivity with a Hach® conductivity/TDS meter (Hach, Loveland, Colorado). We measured total ammonia with an ammonia/pH electrode (detection limit: 0.05 mg NH_3-N/L), connected to an Accumet® 1003 pH/mV meter (Fisher Scientific). Total ammonia concentrations were determined from a standard curve prepared from an NH_4Cl stock solution. The actual analytical procedure followed instructions provided by the probe manufacturer. We calculated NH_3-N concentrations from the measured total ammonia based on temperature and pH (Thurston et al. 1979). We analyzed spiked samples and quality-control standards regularly to evaluate electrode performance. We based the ammonia concentrations used to calculate LC_{50} values on the average of measured levels taken initially (before addition of organisms), during (every 24 h unless otherwise stated), and at the end of each bioassay.

During phase 2 of each test, we replaced water removed for ammonia analysis with ammonia-dosed water so that the ammonia concentration matched that of the stream from which water was taken. We counted the number of obviously gaping individuals or shells that were dislodged from the sediment to estimate daily mortality rates of clams. Most clams were at least partially visible in the sediment, so this approach provided the most reliable estimate of mortality without disturbing the test systems.

At test termination, we removed all clams and determined the number of living and dead individuals.

Water-column testing

Glochidia bioassays.—We obtained *V. iris* glochidia from gravid adults collected from the Clinch River at Pounding Mill, Tazewell County, Virginia. Upon collection, we placed adult mussels in 20-L coolers that we filled with river water for transport back to the laboratory. In the laboratory, we placed the mussels in a 240-L recirculating Living Stream unit (Frigid Units, Toledo, Ohio) maintained at 16°C and fed them a tri-algal mixture (*Scenedesmus quadricauda, Nannochloropsis oculata,* and *Selenastrum capricornutum* in equal cell densities; Beck 2001) supplemented with unfiltered water obtained from Sinking Creek, Newport, Giles County, Virginia. Prior to testing, we separated and maintained gravid adults in incubators in Sinking Creek water at 4°C to prevent premature release of glochidia. We obtained glochidia (which develop in a specialized gill chamber of the adult known as the marsupium) by gently prying the valves of the adult mussel open (2–3 mm), inserting a water-filled syringe into the engorged gill chamber, and flushing the chamber with water. We assessed viability (ability to close the valves in response to an external stimulus) of glochidia by exposing a sample (derived from ≥2 adult mussels) to a saturated solution of NaCl. If 95% of the individuals closed their valves within 1 min after addition of the salt solution, we considered them viable and used them for ammonia toxicity tests. We initiated toxicity tests within 2 h of glochidia release. The adult female mussels were returned to their point of collection.

We conducted glochidia tests in 12-well culture plates (Fisher Scientific) in an environmental chamber (25°C) under static conditions with a 16 h:8 h light:dark cycle. We tested 6 exposure concentrations (3.12, 6.25, 12.5, 25, 50, and 100 mg/L total ammonia prepared from an initial 1000 mg/L stock of NH_4Cl in distilled water) plus a control, with 3 replicates of 40 individuals/concentration. Each replicate well contained 5 mL of test solution. At the end of the 24-h exposure period, we determined glochidia viability using NaCl as described above. Glochidia that failed to close after 1 min in response to NaCl addition plus those that were closed prior to its addition were considered dead for the purpose of calculating a median lethal concentration (LC_{50}) (Jacobson 1990).

We used Sinking Creek water that had been filtered (30 μm) and aerated as the diluent for all tests. We measured DO, pH, temperature, ammonia, conductivity, alkalinity, and hardness in each of the treatment levels (combined sample derived from each replicate) before, during (every 24 h), and at the end of each bioassay. We analyzed DO, pH, temperature, ammonia, and conductivity as described above. We measured alkalinity and hardness by titration (APHA 1995).

Juvenile mussel and clam bioassays.—We obtained juvenile *Corbicula* from gravid adults we collected from the Clinch River, Virginia, using a funnel apparatus described by Doherty et al. (1987). We used juvenile *Corbicula* that were <48 h and 7 d old in the bioassays. We derived juvenile *V. iris* from larvae obtained as described above, and propagated them in the laboratory following methods described by Zale and Neves (1982). The glochidial larvae of unionid mussels attach to a vertebrate host, usually a fish (Keller and Ruessler 1997), to complete development to the juvenile stage. Host fish for *V. iris* were rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*). We used juvenile *V. iris* that were 5 d and 7 d old in the bioassays.

We conducted juvenile tests in 12-well culture plates in an environmental chamber (25°C) with a 16 h:8 h light:dark cycle. We tested 6 concentrations that ranged from 0.62 to 40 mg/L total ammonia-N for *V. iris* and 0.25 to 25 mg/L total ammonia-N for *C. fluminea,* with 4 replicate wells containing 5 juvenile bivalves for each concentration. Each replicate well contained 5 mL of test solution. We ran the tests for 96 h and recorded mortality every 24 h. We determined mortality by placing each test chamber under a dissecting microscope at 40× magnification, and viewing the internal anatomy of the juveniles through their transparent valves. We considered the organisms dead and removed them from the test chamber if no pedal locomotion, heartbeat, or ciliary movement was observed after 5 min.

Adult mussel and clam bioassays.—We obtained adult *C. fluminea* from the Clinch River, Virginia. We conducted 2 tests in 1-L glass beakers containing 750 mL of test solution, with 2 repli-

cates/concentration and 10 individuals/replicate. For the 1st test, we exposed the adult *C. fluminea* to concentrations ranging from 10 to 1000 mg/L total ammonia-N (0.3 serial dilution). For the 2nd test, we exposed the adults to concentrations ranging from 6.25 to 100 mg/L total ammonia-N (0.5 serial dilution). We obtained adult *Pyganodon grandis* mussels from Zetz's Fish Hatchery, Inwood, West Virginia, and exposed them in 16-L polycarbonate containers containing 10 L of test solution, with 2 replicates/concentration and 10 individuals/replicate. We used 5 concentrations ranging from 10 to 1000 mg/L total ammonia-N (0.3 serial dilution). We conducted the bioassays for both species at 25°C for 96 h, and we recorded mortality and removed dead individuals (gaping or no adductor response to light prying of valves) every 24 h.

Acute bioassays with standard test organisms.—We conducted bioassays with standard laboratory test organisms to determine how the response of these species compared to the bivalves that were tested. This issue is important because standard test species often are used to evaluate the quality of wastewater effluents that may be released into receiving systems containing populations of unionid mussels. Therefore, the sensitivities of standard test species to stressors such as NH_3-N should at least be comparable to those of mussels.

We obtained *Ceriodaphnia dubia* and *Pimephales promelas* from in-house cultures and conducted testing according to standard US Environmental Protection Agency (EPA) protocols (USEPA 1993). Our exposure concentrations ranged from 6.25 to 200 mg/L total ammonia-N for *C. dubia* (6 concentrations) and 1.56 to 25 mg/L for *P. promelas* (5 concentrations).

Statistical analyses

Artificial stream tests.—We used repeated measures analysis of variance (ANOVA) to determine whether NH_3-N concentration, mortality, and water chemistry values differed among density treatments, over time, among density treatments over time, and between temperatures. We judged significance at $\alpha = 0.05$. We analyzed data from the 1st, 2nd, and 3rd phases of each test separately. We used a Mann–Whitney Rank Sum test (Zar 1984) to compare the sizes of living and dead clams at the end of each test.

TABLE 1. Ranges of water-quality measurements for each phase of the 2 artificial stream tests.

	Temperature (°C)	pH	Conductivity (µS/cm)
Test 1			
Phase 1	16.3–22.1	7.39–8.04	180–390
Phase 2	17.2–24.1	7.54–8.57	280–2600
Phase 3	17.1–23.2	7.50–8.34	180–1600
Test 2			
Phase 1	23.6–27.1	7.40–7.98	240–330
Phase 2	25.1–26.3	7.47–8.25	260–1400
Phase 3	24.0–28.8	7.51–8.25	210–1400

Water-column testing.—We considered tests valid only if control mortality was ≤20%. All LC_{50}s were calculated using the Spearman–Karber method (Hamilton et al. 1977).

Results

Artificial stream tests with Asian clams

Test 1 lasted 64 d; phase 2 occurred between days 14 and 42. Temperature (not controlled) fluctuated with the incoming laboratory water and ranged from 16.3 to 24.1°C (mean = 21.7°C) (Table 1). Test 2 lasted 34 d; phase 2 occurred between days 14 and 24. Temperature was constant at 26 ± 3°C.

Clam mortality.—At all densities, mortality gradually increased over the course of both tests, with significant ($p < 0.01$) density-dependent differences in mortality apparent in phases 2 and 3 (Fig. 1). In the high-density treatment, mortality increased sharply after day 34 and was complete by day 38 in test 1, and increased sharply after 17 d and was complete by day 21 in test 2. In the low- and medium-density treatments, average mortalities at test termination were 35.5% and 38.6%, respectively, in test 1 and 30.5% and 11.4%, respectively, in test 2. In test 1, mortality was significantly ($p < 0.0001$) affected by size in all density treatments. The median sizes of surviving clams ranged between 12.3 and 12.9 mm, whereas the median sizes of dead clams ranged between 8.2 and 8.5 mm. A similar size difference was not observed in test 2, in which the median sizes of surviving clams ranged between 12.2 and 12.5 mm and median sizes of dead clams ranged between 11.8 and 12.4 mm.

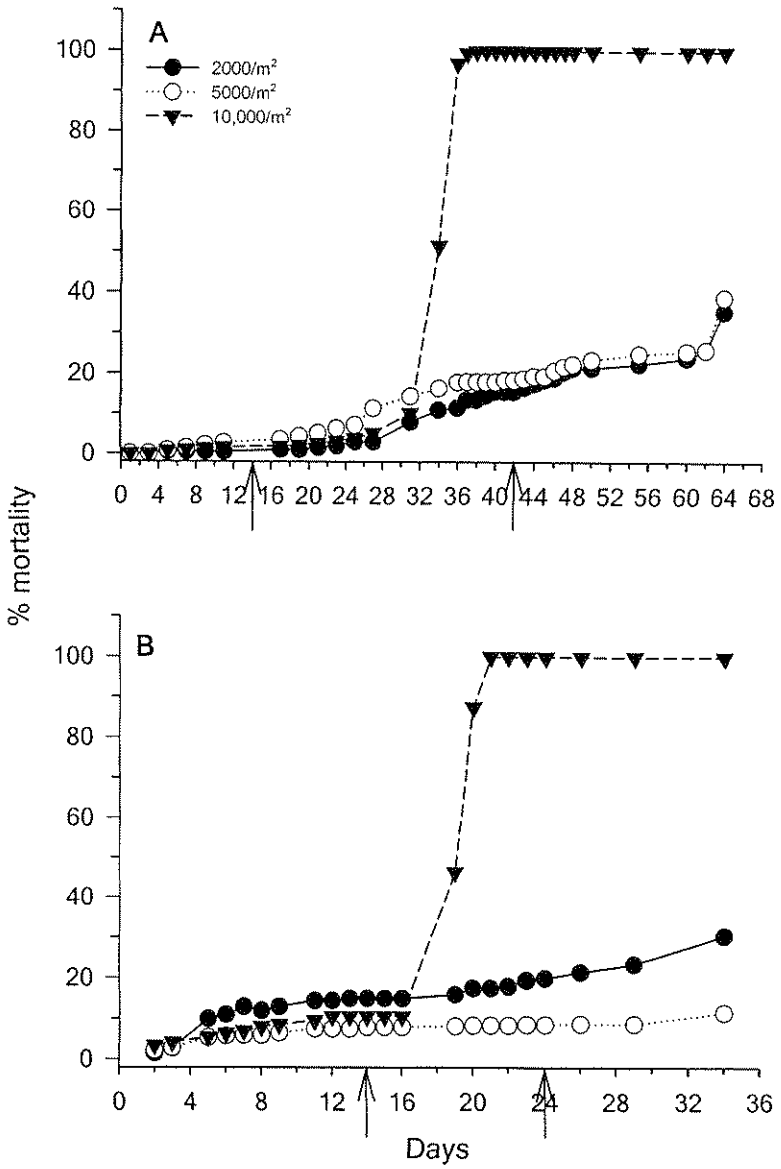

FIG. 1. Mortality of Asian clams for test 1 (A) and test 2 (B) in the artificial stream die-off experiments. Arrows indicate the beginning and end of phase 2, during which water flow to the streams was stopped.

NH_3-N concentrations.—During Phase 1 of both experiments, NH_3-N concentrations did not differ between the control and density treatments. Concentrations ranged from non-detectable levels to 0.007 mg NH_3-N/L in test 1 (Fig. 2A) and from 0.002 to 0.044 mg NH_3-N/L in test 2 (Fig. 2B). A significant ($p < 0.01$) density-dependent difference in NH_3-N concentration was noted in phase 2 of both tests, with the highest concentrations measured in the high-density treatment. NH_3-N concentrations reached a maximum of 4.71 and 5.04 mg/L (tests 1 and 2, respectively) 2 to 3 d after 100% mortality had been reached in the high-density treatment. NH_3-N concentrations remained low in the low- and medium-density treatments of both tests (maximum = 0.015 mg/L and 0.022 mg/L in tests 1 and 2, respectively). NH_3-N

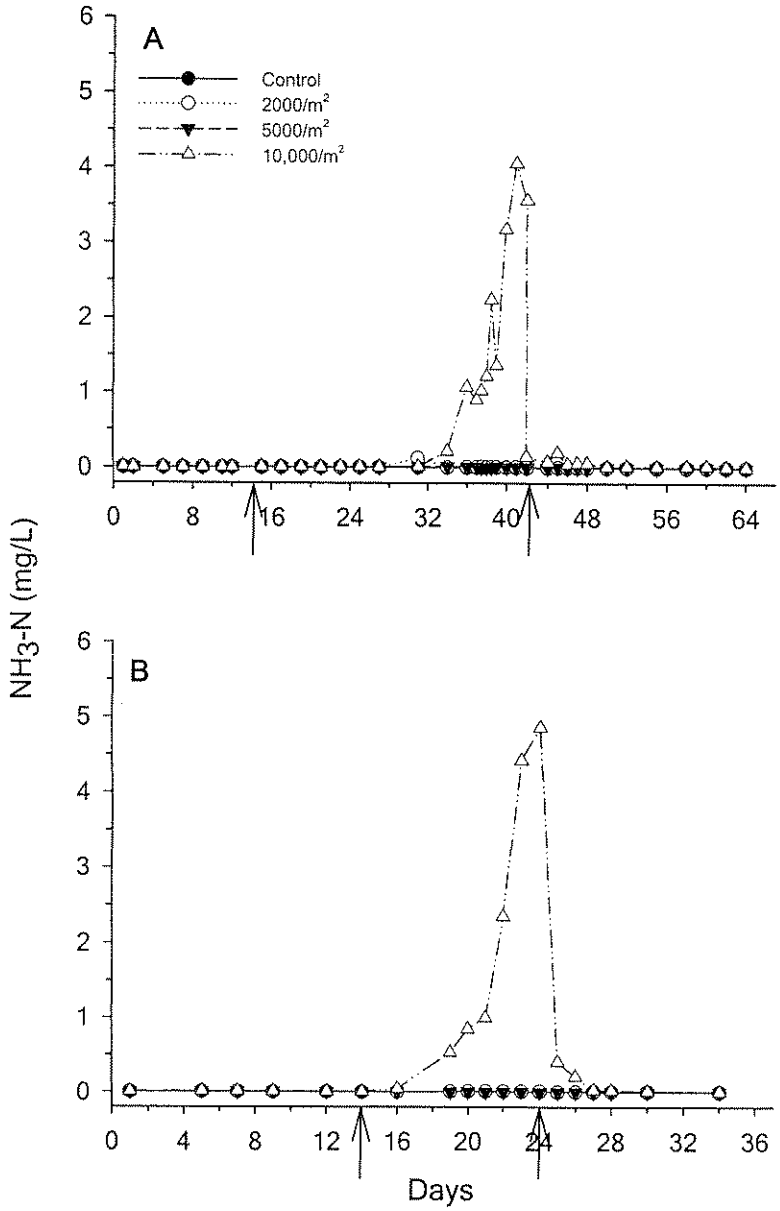

FIG. 2. Unionized ammonia (NH_3-N) concentrations in the water column for test 1 (A) and test 2 (B) in the artificial stream die-off experiments. Arrows indicate the beginning and end of phase 2 of each study, during which water flow to the streams was stopped.

concentrations dropped to 0.14 and 0.41 mg/L (tests 1 and 2, respectively) within 1 d of resuming water flow in the high-density treatments. NH_3-N concentrations dropped below levels found to be acutely toxic to any of the test species evaluated in the laboratory toxicity tests, including glochidia, within 4 d of resuming flow.

DO concentrations.—In the control and low- and medium-density treatments, DO concentrations generally remained >6 mg/L (Fig. 3A) and >4 mg/L (Fig. 3B) (tests 1 and 2, respectively) over the duration of both tests. In the high-density treatment, DO concentrations remained >6 mg/L during phase 1 of test 1 (Fig.

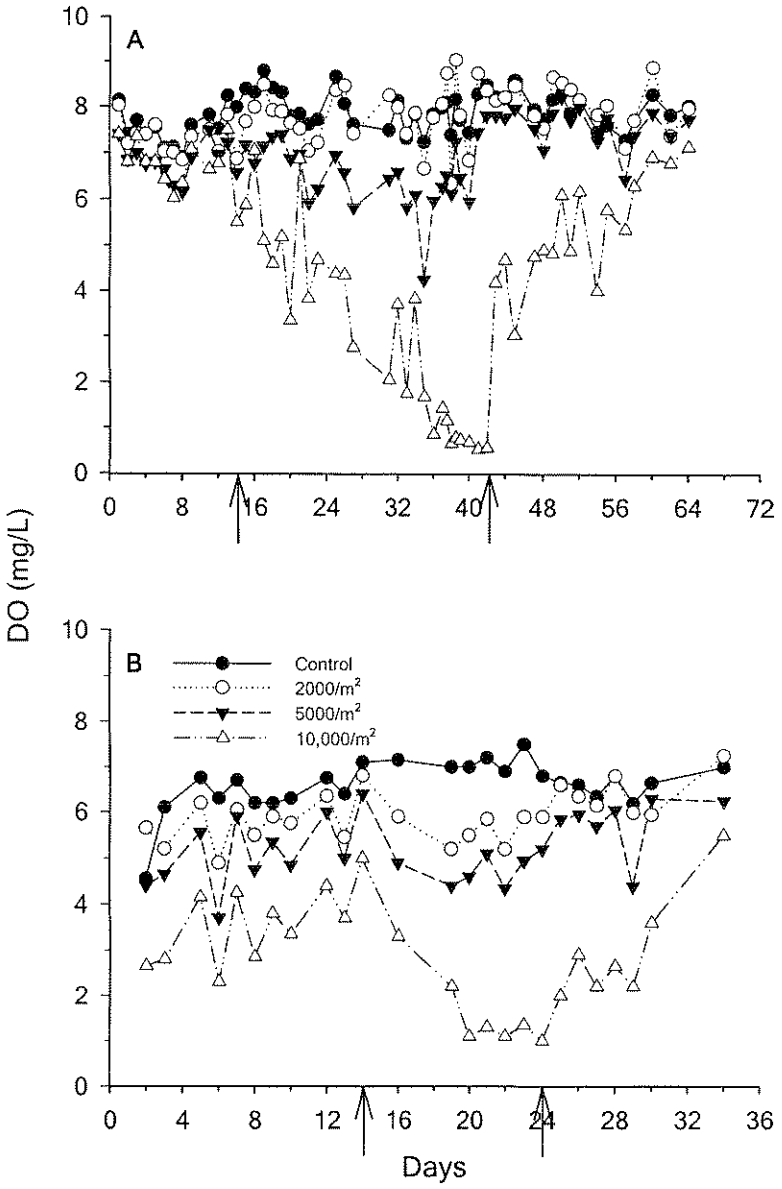

FIG. 3. Dissolved oxygen (DO) concentrations in the water column for test 1 (A) and test 2 (B) in the artificial stream die-off experiments. Arrows indicate the beginning and end of phase 2 of each study, during which water flow to the streams was stopped.

3A), but they dropped as low as 2.1 mg/L during phase 1 of test 2, even though water was flowing through the system (Fig. 3B). Once we stopped flow to the streams, DO concentrations declined steadily. In test 1, DO concentrations reached 2 mg/L by day 31 and were associated with a 40% increase in mortality of the clams. DO concentrations reached a low of 0.6 mg/L on day 42, 4 d after total mortality had occurred. In test 2, DO concentrations reached 3 mg/L by day 15 (1 d after flow was stopped) and dropped to 1 mg/L by day 20, just before total mortality occurred. Resuming flow to the streams increased DO concentrations to 4.2 to

TABLE 2. LC_{50} values for water-only exposure to NH_4Cl for 3 bivalve and 2 standard US Environmental Protection Agency (EPA) test species. Unless indicated, all values were generated from 96-h bioassays. CI = confidence interval.

Species	Life stage	Age	Total ammonia (mg/L)		Unionized ammonia (mg NH_3-N/L)	
			LC_{50}	95% CI	LC_{50}	95% CI
Bivalves						
Villosa iris[a]	Glochidia	24 h	3.29	2.90–3.73	0.11	0.10–0.12
V. iris	Juvenile	5 d	7.81	7.00–8.70	0.62	0.57–0.67
V. iris	Juvenile	7 d	5.52	4.36–6.98	0.38	0.33–0.46
Pyganodon grandis	Adult		21.99[b] (18.8–25.1)	10.6–45.8	0.49[b] (0.44–0.54)	0.26–0.87
Corbicula fluminea	Juvenile	48 h	2.25	1.83–2.76	0.28	0.23–0.35
C. fluminea	Juvenile	7 d	1.00	0.82–1.24	0.09	0.07–0.11
C. fluminea	Juvenile	7 d	1.78	1.50–2.12	0.18	0.15–0.21
C. fluminea	Adult		13.96	11.98–16.26	0.88	0.78–0.99
Standard EPA test species						
Ceriodaphnia dubia[c]		24 h	14.52	11.3–18.7	0.07	0.05–0.11
Pimephales promelas[c]		24 h	8.96	8.15–9.87	1.18	1.08–1.27

[a] 24-h LC_{50}
[b] Mean LC_{50} of 2 tests. Values in parentheses are the range of the LC_{50}s for the 2 tests
[c] 48-h LC_{50}

7.1 mg/L in phase 3 of test 1, but DO concentrations remained <3 mg/L for almost 1 wk after flow was resumed in test 2.

Water quality.—pH, DO, conductivity, and temperature varied over each phase of the tests (Table 1). Significant ($p < 0.05$) increases in conductivity occurred in association with the onset of mortality in the high-density treatments during both tests.

Water-column testing

NH_3-N toxicity.—NH_3-N LC_{50} values ranged from 0.11 mg/L for *V. iris* glochidia (24-h exposure) to 0.62 mg/L for 5-d-old *V. iris* juveniles (96-h exposure). The mean 96-h LC_{50} from the 2 tests with *P. grandis* was 0.49 mg /L, whereas that for *C. fluminea* ranged from 0.14 mg/L (mean of 2 tests) for juveniles to 0.88 mg/L for adults (Table 2).

Comparisons with standard test organisms.—Based on the overlap of the 95% confidence intervals for LC_{50}s, *C. dubia* was more sensitive than *P. promelas* to NH_3-N (Table 2). *Ceriodaphnia dubia* had a lower LC_{50} than the bivalves, but the 95% confidence intervals overlapped with those of the *V. iris* glochidia and *C. fluminea* 7-d juveniles. In contrast, *P. promelas* was the least sensitive of all organisms tested (Table 2).

Discussion

Artificial stream tests with Asian clams

Die-offs of Asian clam populations in the field have been associated with extreme temperatures and low DO concentrations from decreased water flow (Sickel 1986). Cessation of water flow in the artificial streams in our study caused reductions in DO concentrations that varied in intensity with the density of clams in the streams. DO concentrations in the streams with the highest density of clams fell below 1 mg/L and preceded die-offs that eventually reached 100%, indicating that low DO concentrations may have been the primary cause for the die-off. Previous studies have reported that *C. fluminea* is relatively intolerant to hypoxia (Belanger et al. 1991, Johnson and McMahon 1998, Matthews and McMahon 1999), and the Asian clam and the zebra mussel, *D. polymorpha*, are among the least tolerant freshwater bivalves to low DO (Matthews and McMahon 1999). A number of natural and anthropogenic factors may initiate an Asian clam die-off, but it appears that low DO concentrations may be one of the most critical factors. Unionids could also have been negatively affected by the decreased DO concentrations observed in association with the clam mortality in

the artificial stream. Sparks and Strayer (1998) evaluated behavioral responses and survival of juvenile *Elliptio complanata* subjected to low DO concentrations in a laboratory flow-through study. They observed significant effects on behavior at a DO concentration of 2 mg/L, and significant mortality if the mussels were maintained at levels of 1 to 2 mg/L for ≥96 h.

Concentrations of NH_3-N detected in the overlying water during the clam die-off reached 4.71 and 5.04 mg/L (tests 1 and 2, respectively) in the high-density treatments. These concentrations are ~5× and ~10× higher than the LC_{50} values for *C. fluminea* and *P. grandis* adults, respectively, and >40× that for *V. iris* glochidia. The concentrations are also higher than the LC_{50} value for other native mussels including the glochidia of *Utterbackia imbecillis* and *Fusconaia masoni* (Augspurger et al. 2003), and juveniles of *Lasmigona subviridis* (M. C. Black, University of Georgia, Athens, Georgia, personal communication) and *Lampsilis siliquoidea* (Myers-Kinzie 1998). It is interesting that the NH_3-N concentrations (test 1: 0.013 mg/L, test 2: 0.044 mg/L) that were measured in the high-density treatment before the onset of clam mortality were lower than the 96-h LC_{50} concentrations for *C. fluminea* adults (0.88 mg/L). These results further support the idea that low DO concentrations may have been the primary factor initiating clam mortality, but the role of NH_3-N in the die-off cannot be discounted completely because NH_3-N concentrations may have been higher in the sediment pore water of the artificial streams than in the water column.

Density of clams and water temperature clearly were important determinants underlying the potential effects that a clam die-off may have on a local habitat. In our study, significant effects of die-offs on DO and NH_3-N were largely restricted to streams having a clam density of 10,000/m^2. This density, while not unheard of (e.g., Cherry et al. 1986), may represent the high end of the range of densities in natural populations. Comparison of the DO profiles in test 1 (with an average temperature of 21.7°C) and test 2 (in which temperature was maintained at 26°C) indicates that warm temperatures can significantly exacerbate the extent to which a clam die-off influences DO, both with respect to the rate at which O_2 is depleted and the ambient levels that may precede mortality.

Water-column testing

Too few laboratory bioassays were conducted with each test organism to allow statistical comparison of the LC_{50}s, but some generalizations and comparisons with data from the literature are possible. The glochidia of *V. iris* and juvenile *C. fluminea* appeared to be the most sensitive to NH_3-N. This result was expected because previous studies have shown that early life-stage bivalves are among the most sensitive aquatic organisms to metals (Keller and Zam 1991, Jacobson et al. 1993), some organic compounds (Weinstein 2001), and chlorine and ammonia (Goudreau et al. 1993, Augspurger et al. 2003, Mummert et al. 2003, Newton et al. 2003). Newton et al. (2003) reported 96-h LC_{50}s for juvenile *Lampsilis cardium* that ranged from 0.127 to 0.165 mg NH_3-N/L, a result similar to the results of Mummert et al. (2003) for juvenile *L. fasciola* (0.28 mg/L) and juvenile *V. iris* (0.12 mg/L), the results of Goudreau et al. (1993) for *V. iris* glochidia (24-h LC_{50} = 0.284 mg/L), and those presented in our study.

Interspecific comparisons

Early life stages of mussels are more sensitive to NH_3-N than adults, and they also may be more sensitive than other invertebrate and fish species to NH_3-N (Arthur et al. 1987, Mummert et al. 2003, Newton et al. 2003). This greater sensitivity of immature mussels to NH_3-N is important because debate has arisen over whether current US federal water-quality criteria for ammonia (USEPA 1999) protect unionids. Mummert et al. (2003) stated that the USEPA water-quality criteria for total ammonia (critical maximum concentration [CMC] = 5.62 mg/L when salmonids are present) were protective of the 2 unionid species (juvenile *V. iris* and juvenile *L. fasciola*) evaluated in their study. However, Augsperger et al. (2003) concluded that the criteria may not be protective, and said that the CMC was 1.75 mg/L total ammonia if toxicity data for a number of mussel species were included in its derivation. This recalculated CMC value would have been protective for *V. iris* glochidia in our study, but the current USEPA values would not.

An additional point of concern regarding species-specific differences in sensitivity to ammonia lies in the differences between some species

of unionids and other organisms used for routine evaluation of whole effluent toxicity (WET). In our study, fathead minnows were significantly less sensitive to NH_3-N than any of the bivalve species tested. Similarly, Mummert et al. (2003) observed that *Daphnia magna* was less sensitive to NH_3-N than the freshwater mussel species they examined. In contrast, both Mummert et al. (2003) and our study indicated that *C. dubia* had similar or higher sensitivity to NH_3-N than unionids. Considering that some state pollution discharge and elimination permits require only acute toxicity tests with species such as fathead minnows and *D. pulex* (JRB, personal observation), routine WET biomonitoring may also fail to protect the unionid fauna in some receiving systems.

In conclusion, studies investigating the potential impacts of Asian clams on native unionid mussels have largely focused on competitive interactions (e.g., Yeager et al. 1999), although the strength of the interaction may vary on a case-by-case basis (see Strayer 1999 for a review). Effects of clam die-offs on water quality (production of NH_3-N and associated biological oxygen demand) also has been identified as a mechanism by which clams can affect native mussels (Strayer 1999, Bruenderman et al. 2001), but no previous work has specifically evaluated this issue. Our study indicates that the NH_3-N concentrations produced by clam die-offs have the potential to exceed acute effect levels for juvenile mussels, and that this effect is dependent on factors such as clam density, temperature, and water flow. This effect of NH_3-N could be further exacerbated by the reductions in DO levels that occur in association with clam mortality. It is important to note that we measured water quality and NH_3-N in the water column. Juvenile unionids largely dwell in the interstitial zone of the sediments (Sparks and Strayer 1998), so conditions in the pore water may provide a better indication of potential effects on native mussels, particularly because water exchange is reduced and water-quality impacts may be magnified in the interstitial zone (Cooper et al. 2005).

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