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Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) II: porewater ammonia

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Abstract. The Asian clam (*Corbicula fluminea*) occurs in most of the southeastern US, often sharing habitat with native unionid mussels. Clam populations can reach high densities and, under conditions of low water flow and warm summer temperatures, may experience rapid die-offs. Clams are infaunal, so the interstitial zone may be subject to elevated levels of ammonia and reductions in dissolved oxygen (DO) that could affect organisms such as native mussels that also use this habitat. We conducted laboratory experiments to characterize concentrations of total ammonia and unionized ammonia (NH_3-N) produced in the sediment pore water and in overlying water as a result of clam die-offs. Sediment porewater NH_3-N concentrations ranged between 0.013 and 5.56 mg/L, levels that were consistently higher than NH_3-N concentrations in the overlying water. Levels of NH_3-N in both pore water and overlying water were positively correlated with temperature and density of clams involved in the die-offs. NH_3-N concentrations in chambers maintained at 28°C were 5.56 mg/L, ~20× levels in chambers maintained at 19°C. Increasing clam density from 200 to 1000 individuals/m^2 resulted in an increase in porewater NH_3-N from 0.17 to 0.55 mg/L. NH_3-N concentrations in some tests exceeded acutely toxic levels for some species of unionid mussels (0.022 to 5.56 mg/L). DO was always lower in pore water (2.01 to 6.74 mg/L) than in overlying water (5.02 to 8.67 mg/L) in chambers containing dead Asian clams, and low DO could have further exacerbated stress associated with exposure to NH_3-N. Overall, our results indicate that NH_3-N production and DO reductions associated with Asian clam die-offs could pose a risk to unionid mussels, particularly during warm low-flow summer months.

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

The exotic Asian clam, *Corbicula fluminea*, occurs in most drainages of the southeastern US, often sharing habitat with unionid mussels. In localities where the 2 groups co-occur, clams may contribute to the decline of unionids (e.g., Sickel 1973, Cherry et al. 1980, Williams et al. 1993, Sternberg and Bruenderman 1999, Barnes and Riggert 2000, Bruenderman et al. 2001). For example, Sickel (1973) noted a negative association between the presence of *Corbicula* and unionid populations in Georgia, and Bruenderman et al. (2001) observed increases in Asian clams and declines in unionid densities in the Little Black River Basin of Missouri. These field surveys may simply indicate that clams have a greater ability than unionids to proliferate in degraded habitat, but Clarke (1988) reported that losses of unionid populations coincided with increases of Asian clams populations in some undisturbed Atlantic coastal rivers. In one of the few studies that specifically examined the interaction between Asian clams and unionid mussels, Yeager et al. (2000) observed a positive correlation between juvenile mussel mortality and displacement from the sediments as densities of clams increased, and they observed ingestion of native-mussel glochidia by Asian clams. Other studies assessing the interaction of Asian clams with native mussels and their habitats have reported modest to dramatic declines in the food supply of native mussels (phytoplankton and seston) in locations heavily infested with Asian clams (Cohen et al. 1984, Lauritsen 1986, Leff et al. 1990).

Asian clam populations often are characterized by rapid die-offs, possibly from factors such as siltation from floods, and high summer temperatures with associated low dissolved oxygen (DO) levels (Sickel 1986, Phelps 1994). Such population die-offs have been observed during summer drought conditions in the New River (Giles County, Virginia; DSC, personal ob-

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servation), and in laboratory-held clams when the death of a few individuals led to mortality of the entire population (JRB, personal observation). The causative agents in the laboratory die-off were probably reduced DO concentrations and ammonia production from bacterial decomposition of the first dead individuals (Whiteman et al. 1996). Therefore, Asian clams also could impact unionid mussels through effects on water quality that occur during clam die-offs in the field.

Cherry et al. (2005) investigated this possibility by characterizing the sensitivity of juvenile and adult Asian clams and selected species of unionid mussel to unionized ammonia (NH_3-N) and by measuring NH_3-N production during induced die-offs of clams in laboratory artificial streams. They found that concentrations of NH_3-N occurring in overlying water during and after the clam die-off were well above concentrations that caused acute mortality of the bivalves. Their study underscored the possible effects of clam die-offs on unionid mussels, but it raised additional questions. Cherry et al. (2005) measured NH_3-N only in the water column, but both *Corbicula* and unionid mussels are infaunal (Ringwood and Keppler 2002), so NH_3-N and DO concentrations in pore water may have a greater influence on survival than levels in overlying water. Exposure to porewater NH_3-N may be a particular issue for juvenile mussels that live entirely within the interstitial zone (Buddensiek et al. 1993, Yeager et al. 1994). Juveniles may not be as capable as adult mussels of avoiding NH_3-N through prolonged valve closure, and both total and NH_3-N concentrations usually are higher in sediments than in overlying water (Chambers et al. 1992, Frazier et al. 1996, Augsperger et al. 2003). Elevated ammonia concentration in pore water was a major contributor to the decline of the fingernail clam (*Musculium transversum*) in the Illinois (Sparks and Sandusky 1981) and Mississippi (Wilson et al. 1995) rivers. These studies indicated that pore water may be an important source of ammonia exposure for benthic invertebrates such as native mussels (Newton et al. 2003).

Our study examined the accumulation of total ammonia and NH_3-N in pore water associated with an induced die-off of Asian clams under laboratory conditions. The objectives of our study were to: 1) characterize the relationships between the density of decaying Asian clams and temperature and concentrations of NH_3-N and DO in the water column of test chambers that contained only water and in the overlying water and pore water in chambers containing a standard gravel substrate, 2) determine the influence of flow, temperature, and time on porewater concentrations of NH_3-N and DO in a standard gravel substrate that contained dead clams, and 3) compare the results of tests conducted in chambers with a standard gravel substrate to chambers with a natural river sediment.

Methods

Collection and handling of C. fluminea

Corbicula fluminea were obtained from the Little Black River, Ripley County, Missouri. Clams were collected after being exposed by gently raking the top layer of substrate with a handheld garden rake. Individuals were placed in 40-L coolers containing fresh river water for transport back to Oklahoma State University. Water temperature in the coolers was maintained at ~21°C and aerated with portable bait pumps. Temperature of the river water at the time of collection was 25°C. At the University, the clams were placed in 75-L aerated aquaria receiving a flow-through supply of dechlorinated laboratory water that replaced the volume of the tanks every 4 h. Clams in the holding tanks were fed 200 mL of *Selenastrum capricornutum* (1.8×10^7 cells/mL) every 3 d.

Substrate and test chambers

Standard-gravel substrate.—Natural sediments often are very heterogeneous with respect to factors that could influence porewater NH_3-N concentrations; these factors include particle-size distribution, % organic matter, and presence of both micro- and macroorganisms. Therefore, a standard gravel substrate was chosen to minimize these confounding factors and allow more effective characterization of porewater NH_3-N derived from decaying clam tissues. The substrate was pea gravel (particle diameter = 5–10 mm) obtained from a local home-and-garden center. All gravel was rinsed ≥5× with dechlorinated laboratory water before being used for any tests.

Test chambers.—Test chambers for all experiments were prepared by placing 350 mL of standard gravel in 1-L polycarbonate jars. Each jar was filled with 650 mL of dechlorinated water. Of this water, ~150 mL was pore water. To facilitate collection of pore water, an air stone with a plastic airline attached was inserted ~3 cm into the sediment of each chamber. The desired volume of pore water was collected by gently applying suction to the airline with a 50-mL syringe. All test chambers were allowed to stabilize for 2 h before clams were added.

To obtain flow-through conditions, the 1-L test chambers were fitted with an inflow port 3.5 cm from the top of the chamber and 1 cm from the surface of the standard gravel. Each inflow port was connected to a length of 6-mm Tygon® tubing that ran through a Masterflex® L/S® peristaltic pump (Cole Parmer Instrument Company, Vernon Hills, Illinois) that drew dechlorinated laboratory water from a common reservoir. Water entering through the inflow port was allowed to flow out over the rim of the test chamber. Flow was maintained at 5 mL/min. Based on this flow rate, the overlying water in the test chambers was renewed at a rate of 1 exchange/h.

Clam density

Static, no substrate.—The effect of the number of decomposing clams (clam density) on concentrations of NH_3-N and DO in the water column was evaluated in glass beakers filled with laboratory water alone. The visceral masses of *C. fluminea* were collected by prying open the valves and removing the entire body. Zero, 1, 2, 4, or 8 visceral masses were placed in 100 mL of dechlorinated water in 200-mL glass beakers with 4 replicates/treatment. The beakers were covered with aluminum foil, placed in incubators, and maintained at either 17°C or 25°C for 4 d. Following incubation, water-column ammonia was measured as described below.

Static, standard gravel.—The effect of clam density on NH_3-N and DO concentrations in the pore water and overlying water was evaluated in test chambers containing standard gravel. The visceral masses of 0, 1, 2, 4, or 10 Asian clams were placed in the sediment of a test chamber with 4 replicates/treatment. The number of clams used yielded approximate densities of 100, 200, 400, or 1000/m^2 of substrate, respectively. The chambers were held at 22°C for 4 d without renewal of the overlying water. Following the incubation period, 10 mL of overlying and 10 mL of pore water were collected for NH_3-N analysis as described below.

Flow-through, standard gravel.—The effect of clam density (400/m^2 and 1000/m^2) on NH_3-N concentrations in the overlying water and pore water was also investigated under flow-through conditions for 10 d at 22°C with a flow rate of 5 mL/min (1 exchange/h) and 5 replicates/treatment. NH_3-N concentrations in the overlying water and pore water were measured on days 4, 7, and 10.

Other environmental factors

Three additional tests were conducted under flow-through conditions to evaluate the influence of temperature, flow rate, and substrate type on NH_3-N concentrations.

Temperature.—The effect of temperature (19°C, 22°C, and 28°C) on NH_3-N concentrations in the overlying water and pore water was investigated for 10 d with a flow rate of 5 mL/min (1 exchange/h), a clam density of 400 /m^2, and 5 replicates/treatment. NH_3-N concentrations in the overlying water and pore water were measured on days 4, 7, and 10 as described below.

Flow rate.—The effect of flow rate (5 mL/min and 25 mL/min) on NH_3-N concentrations in the overlying water and pore water was investigated for 10 d at 22°C with a clam density of 400/m^2 and 5 replicates/treatment. NH_3-N concentrations in the overlying water and pore water were measured on days 4, 7, and 10 as described below.

Substrate.—For the last component of the study, NH_3-N concentrations in overlying water and pore water in chambers with natural sediment from the Little Black River, Ripley County, Missouri, were compared with those in chambers with standard gravel. A shovel was used to remove ~40 kg of Little Black River sediment from a riffle/run zone (average depth of collection = ~10 cm and maximum depth = ~25 cm). The sediment was placed in polycarbonate buckets and transported back to the laboratory. Sediment was stored at 4°C until used in the tests. The effect of substrate type (standard-gravel or natural) and flow rate (static or 5 mL/min) on NH_3-N concentrations in the overlying and pore water was investigated for 4 d at 22°C with a clam density of 400 clams/m^2 and 5 replicates/treatment. NH_3-N concentrations in the overlying and pore water were measured on days 4, 7, and 10 as described below.

Water quality

Ammonia.—The concentration of total ammonia in overlying water and pore water was determined on days 0 and 4 for the 4-d tests and days 0, 4, 7, and 10 for the 10-d tests. Total ammonia was measured using a mini ammonia electrode (detection limit: 0.02 mg/L, Diamond General Development Corporation, Ann Arbor, Michigan) connected to an Accumet® portable AP63 pH/mV meter (Fisher Scientific, Pittsburgh, Pennsylvania). Total ammonia concentrations were determined from a standard curve prepared with NH_4Cl (Fisher Scientific). The actual analytical procedure followed instructions provided by the probe manufacturer. NH_3-N concentrations were estimated from the measured total values based on temperature and pH (Thurston et al. 1979). Both spiked samples and quality control standards were analyzed regularly to evaluate electrode performance.

DO, pH, conductivity, alkalinity, and hardness were measured in each water sample (on days 0 and 4 for the 4-d tests and days 0, 4, 7, and 10 for the 10-d tests). DO was measured using a Model 50B Dissolved Oxygen Meter (YSI, Yellow Springs, Ohio), and pH was measured with an Accumet® portable AP62 pH/mV meter (Fisher Scientific). Conductivity was measured with a Hach® conductivity/TDS meter (Hach, Loveland, Colorado), and alkalinity and hardness were measured by titration (APHA 1995).

Statistics

All statistical analyses were done with SigmaStat statistical software (version 3.0, SPSS, Point Richmond, California). NH_3-N concentrations were evaluated for normality and heterogeneity of variance using Kolmogorov–Smirnov and Levene median tests, respectively. In cases where either of these assumptions was violated, the data were rank-transformed. Depending on the number of independent variables for a particular experiment (overlying water or pore water, clam density, flow rate, temperature, substrate type), a 1-, 2-, or 3-way analysis of variance (ANOVA) was conducted to determine if NH_3-N concentrations differed among treatments. Tukey's test was used to indicate which treatments were significantly different. All statistical tests were conducted at $\alpha = 0.05$. For the water-only exposures, linear regressions were used to assess the relationship between increasing clam density and temperature.

Results

Water-quality parameters

DO concentrations ranged from ~2 to ~8 mg/L across all experiments and were always lower in the pore water than the overlying water for any given treatment (Table 1). pH ranged from 6.61 to 7.99 across all experiments. At these pH values, 0.8 to 6% of total ammonia would have been NH_3-N. Conductivity ranged from 679 to 1177 μS/cm across all experiments. Alkalinity and hardness ranged from 36 to 53 and 116 to 130 mg/L (as $CaCO_3$), respectively.

Density

Static, no substrate.—Water-column concentrations of total ammonia were significantly related to the amount of clam visceral mass present in the water. Temperature significantly affected the concentration of total ammonia produced. The slope of the curve was more than an order of magnitude greater at 25°C than at 17°C. The regression equation for total ammonia vs visceral mass at 17°C was $y = 3.1x - 0.96$, whereas the equation at 27°C was $y = 35.9x - 23.6$ ($p < 0.001$ for both regressions).

Static, standard gravel.—NH_3-N concentrations in the pore water and overlying water of the static-test chambers increased significantly as clam density increased ($p \leq 0.018$; Fig. 1). After 4 d, NH_3-N concentrations in the overlying water ranged from 0.007 mg/L in the control (0 clams/m^2) to 0.271 mg/L in the high-density (1000 clams/m^2) treatment. Porewater NH_3-N concentrations ranged from 0.02 mg/L in the control to 0.550 mg/L in the high-density treatment. The average NH_3-N concentrations in pore water were always significantly greater than those in the overlying water regardless of clam density ($p \leq 0.004$).

Flow-through, standard gravel.—NH_3-N concentrations were significantly affected by the interaction of time, clam density, and whether the samples were from pore water or overlying water. Increasing clam density significantly affected NH_3-N concentrations, but concentrations differed among days, and the temporal

TABLE 1. Ranges of dissolved oxygen (DO) concentrations and pH in the pore water and overlying water, and maximum porewater concentrations of total ammonia and unionized ammonia (NH_3-N) observed in the test chambers in each of the experiments.

		DO (mg/L)		pH		Porewater ammonia	
Experiment	Treatment	Overlying water	Pore water	Overlying water	Pore water	NH_3-N (mg/L)	Total ammonia (mg N/L)
Density, static	0 clams/m^2	7.75–8.05	6.74–7.01	7.54–7.68	7.61–7.72	0.02	1.20
	200 clams/m^2	7.15–7.57	5.45–6.11	7.73–7.79	7.80–7.91	0.17	4.55
	400 clams/m^2	7.42–7.84	5.37–6.07	7.85–7.94	7.71–7.80	0.20	7.12
	1000 clams/m^2	5.62–6.43	3.60–4.41	7.23–7.67	6.77–6.87	0.55	788.3
Density, flow-through	400 clams/m^2	6.45–8.25	3.30–5.70	7.22–7.90	7.05–7.62	1.23	65.60
	1000 clams/m^2	5.17–6.62	2.01–4.41	7.22–7.67	6.77–7.35	4.80	472.2
Temperature, flow-through, 400 clams/m^2	19°C	6.40–8.67	4.18–6.54	7.03–7.81	7.08–7.42	0.23	39.84
	22°C	6.45–8.25	3.30–5.70	7.22–7.90	7.05–7.48	1.08	76.91
	28°C	4.60–6.58	2.72–3.80	7.14–7.70	6.61–7.27	5.56	652.1
Flow rate, 400 clams/m^2	5 mL/min	6.45–8.25	3.30–5.70	7.22–7.90	7.05–7.62	0.23	22.12
	25 mL/min	5.02–7.11	2.06–5.15	7.00–7.25	6.93–7.29	0.26	36.05
Substrate type, 400 clams/m^2	Standard-gravel, static	5.81–6.58	4.32–5.18	7.86–7.99	7.16–7.40	0.16	13.85
	Standard-gravel, flow-through	8.00–8.67	5.44–5.85	7.34–7.45	7.13–7.38	0.04	4.77
	Natural sediment, static	5.74–5.91	2.15–3.27	7.57–7.60	7.07–7.29	0.11	16.03
	Natural sediment, flow-through	5.49–5.87	2.68–3.58	6.87–6.95	6.85–6.92	0.11	27.59

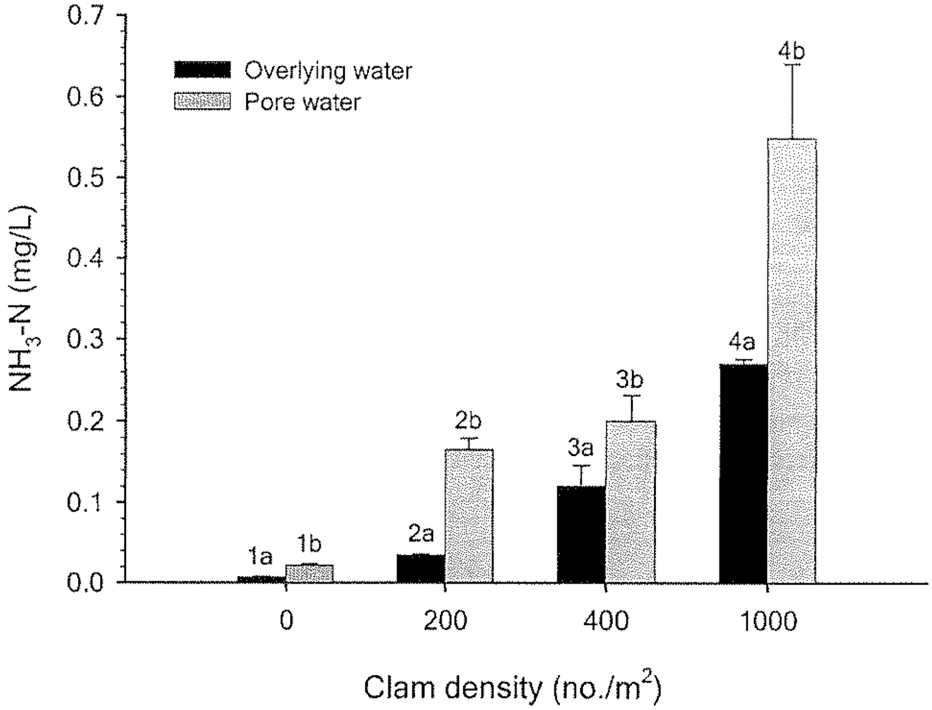

FIG. 1. Mean (+1 SD) unionized ammonia (NH_3-N) concentrations after 4 d in the overlying water and pore water of static-test chambers containing different densities of dead clams in standard-gravel substrate. Different numbers over bars indicate significant differences ($p < 0.05$) in NH_3-N concentrations among clam densities within a water type (overlying or pore). Different letters over bars indicate significant differences in NH_3-N concentrations between the overlying water and pore water within a given clam density.

patterns of changing concentrations differed between pore water and overlying water. On day 4, the mean NH_3-N concentrations in both pore water and overlying water were ≥2× higher in the high-density treatments than in the low-density treatments ($p = 0.003$) (Fig. 2A, B). Between days 4 and 7 in the low-density treatment, overlying-water and porewater NH_3-N concentrations increased significantly from 0.01 to 0.13 mg/L ($p < 0.001$), and from 0.27 mg/L to 1.23 mg/L, respectively (Fig. 2A). In the high-density treatment, over the same time period (days 4–7), overlying-water NH_3-N concentrations decreased from a high of 0.27 mg/L to a low of 0.03 mg/L, and porewater NH_3-N concentrations increased from 0.56 mg/L to 4.80 mg/L (Fig. 2B). On day 7, overlying-water NH_3-N concentrations were significantly higher in the low-density treatment than the high-density treatment ($p < 0.001$), but porewater NH_3-N concentration was ≥4× higher in the high-density treatment than in the low-density treatment ($p < 0.001$) (Fig. 2A, B). Between days 7 and 10, overlying-water NH_3-N concentrations did not change in the low-density treatment, but porewater concentrations decreased to 0.09 mg/L. However, between days 7 and 10 in the high-density treatment, overlying-water concentrations increased to 0.09 mg/L, and porewater concentrations remained high at 4.79 mg/L (Fig. 2A, B). By day 10, NH_3-N concentrations in the overlying water did not differ between low- and high-density treatments, and porewater NH_3-N concentrations were significantly lower in the low-density treatment than in the high-density treatment ($p < 0.001$) (Fig. 2A, B).

Temperature

Temperature significantly affected NH_3-N concentrations, but the effect varied with time. On day 4, NH_3-N concentrations were lowest at 19°C, intermediate at 22°C, and highest at 28°C

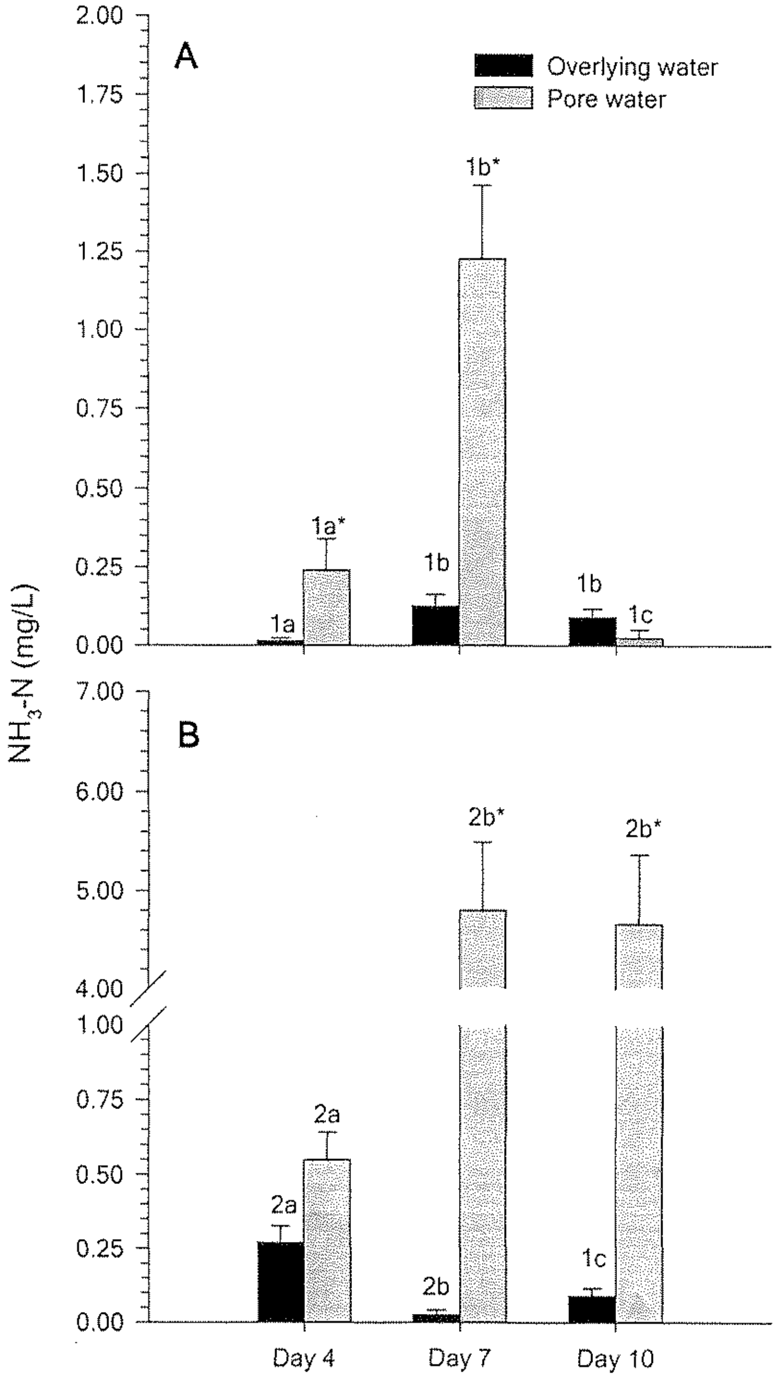

FIG. 2. Mean (+1 SD) unionized ammonia (NH_3-N) concentrations after 4, 7, or 10 d in the overlying water and pore water of flow-through chambers containing 400 (A) or 1000 (B) dead clams/m^2 in standard-gravel substrate. Different numbers over bars indicate significant differences ($p < 0.05$) in NH_3-N concentrations among clam densities within a given day and water type (overlying or pore). Different letters over bars indicate significant differences in NH_3-N concentrations between days but within a given clam density and water type. * indicates a significant difference in NH_3-N concentrations between the overlying water and pore water within a given clam density and day.

in overlying water and pore water ($p < 0.001$ for all comparisons; Fig. 3A, B, C). On days 7 and 10, overlying-water NH_3-N concentrations were significantly lower at 19°C and 28°C than at 22°C (Fig. 3A, B, C). On day 7, porewater NH_3-N concentrations followed the same pattern as on day 4. However, on day 10, porewater NH_3-N concentrations were lowest at 22°C (Fig. 3B), intermediate at 19°C (Fig. 2A), and highest at 28°C (Fig. 3C). At the 2 higher temperatures, porewater NH_3-N concentrations peaked on day 7 and declined by day 10 (Fig. 3B, C). NH_3-N concentrations were almost always significantly higher in the pore water than in overlying water regardless of temperature. The single exception occurred on day 10 in the 22°C treatment when NH_3-N concentrations in overlying water and pore water were the same (Fig. 3B).

Flow

NH_3-N concentrations in the overlying water of the 5- and 25-mL/min chambers remained low over the 10-d study, reaching peaks of ~0.02 mg/L on day 10 in both flow treatments (Fig. 4A, B). Porewater NH_3-N concentrations were significantly higher than overlying-water concentrations regardless of flow rate ($p < 0.001$). In the 5-mL/min treatment, porewater NH_3-N concentrations peaked on day 7 (Fig. 4A), whereas in the 25-mL/min treatment, porewater NH_3-N concentrations reached high levels by day 4 and remained elevated on day 7 (Fig. 4B). By day 7, porewater NH_3-N concentrations were similar between the 2 flow treatments (Fig. 4A, B). By day 10, porewater NH_3-N concentrations were lower in the 25-mL/min treatment than the 5-mL/min treatment, but this difference was not significant (Fig. 4A, B).

Substrate type

Overlying-water NH_3-N concentrations were significantly lower in flow-through than in static

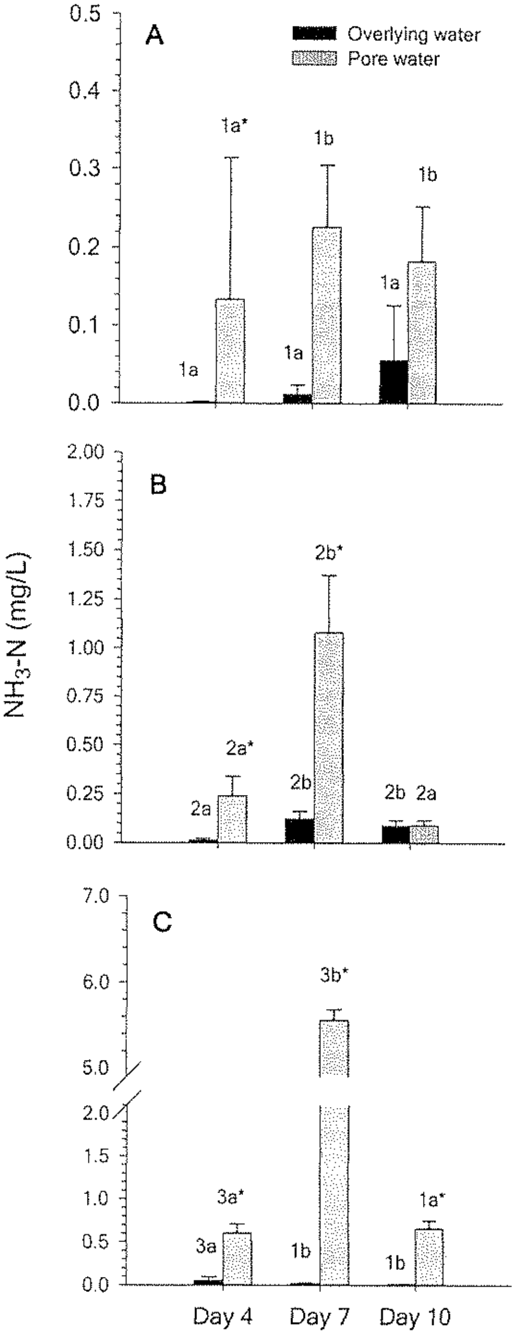

FIG. 3. Mean (+1 SD) unionized ammonia (NH_3-N) concentrations after 4, 7, or 10 d in the overlying water and pore water of flow-through chambers containing 400 dead clams/m^2 in standard-gravel substrate. Tests were conducted at 19°C (A), 22°C (B), or 28°C (C). Different numbers over bars indicate significant differences ($p < 0.05$) in NH_3-N concentrations among temperatures within a given day and water type (overlying or pore). Different letters over bars indicate significant differences in NH_3-N concentrations between days within a given temperature and water type. * indicates a significant difference in NH_3-N concentrations between the overlying water and pore water within a temperature and day.

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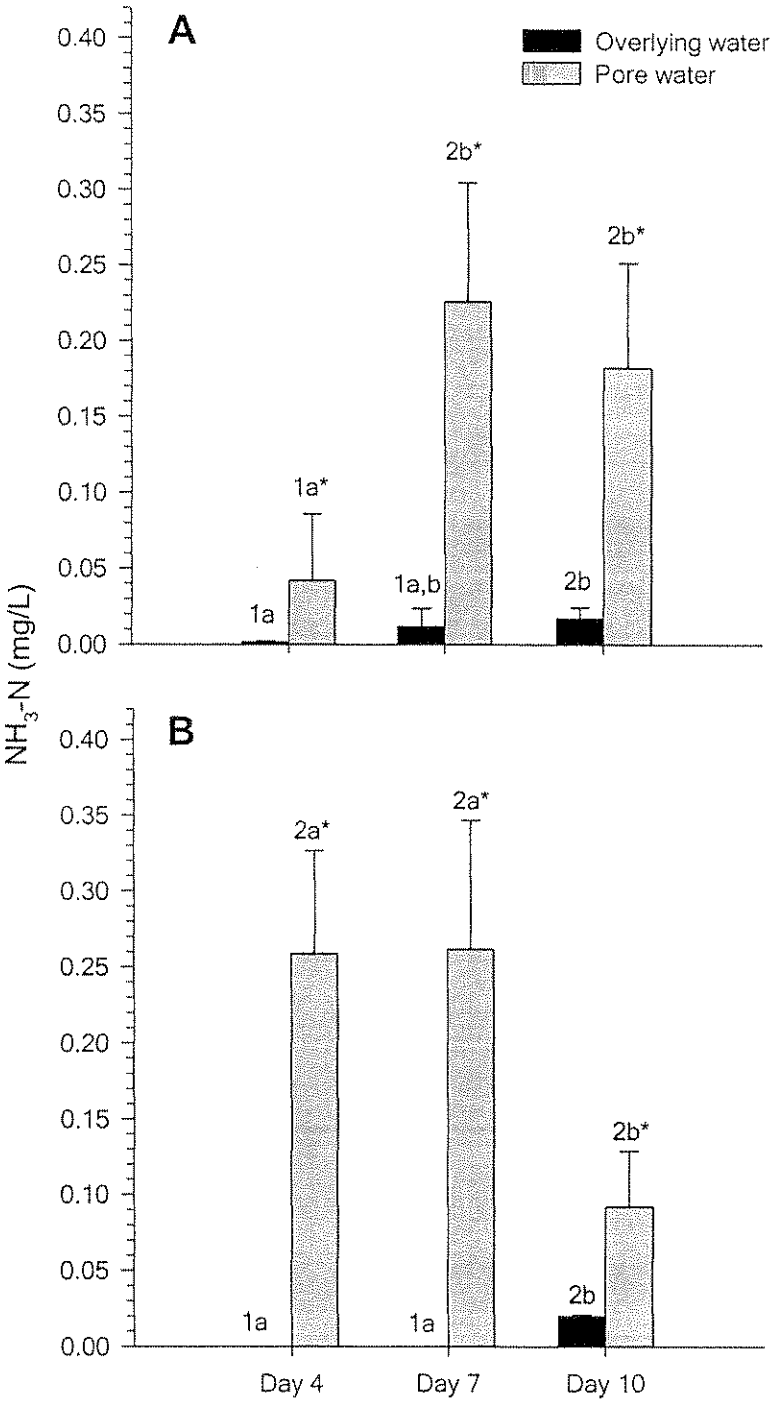

FIG. 4. Mean (+1 SD) unionized ammonia (NH_3-N) concentrations after 4, 7, or 10 d in the overlying water and pore water of flow-through chambers containing 400 dead clams/m^2 in standard-gravel substrate. Tests were conducted at 5 mL/min (A), or 25 mL/min (B). Different numbers over bars indicate significant differences ($p < 0.05$) in NH_3-N concentrations between flow rates within a day and water type (overlying or pore). Different letters over bars indicate significant differences in NH_3-N concentrations between days within a given flow rate and water type. * indicates a significant difference in NH_3-N concentrations between the overlying water and pore water within a given flow rate and day.

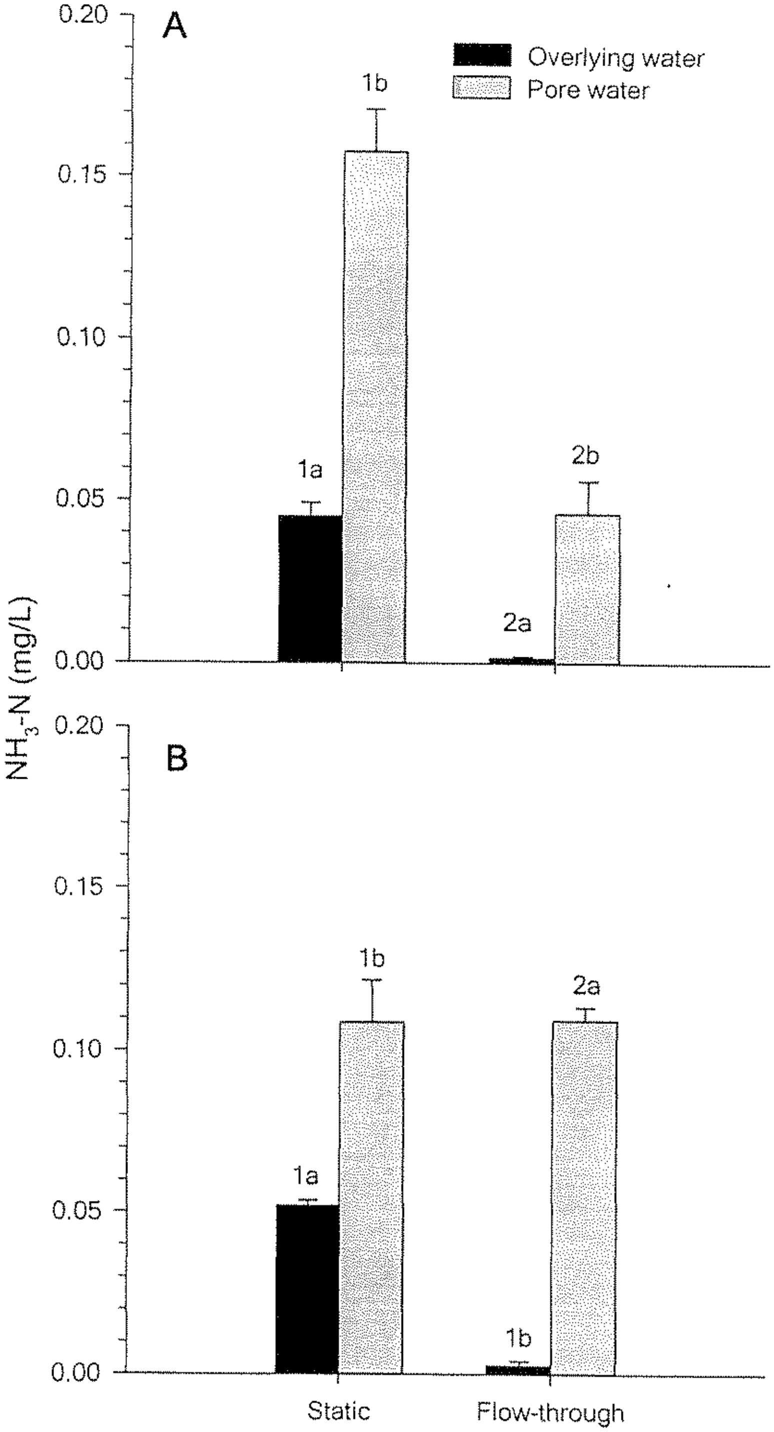

FIG. 5. Mean (+1 SD) unionized ammonia (NH_3-N) concentrations after 4 d in the overlying water and pore water of test chambers containing 400 dead clams/m^2 in either standard-gravel substrate (A) or natural substrate (Little Black River sediment) (B). Tests were conducted under static or flow-through (5 mL/min) conditions. Different numbers over bars indicate significant differences ($p < 0.05$) in NH_3-N concentrations between static and flow-through conditions within a given substrate type and water type (overlying or pore). Different

conditions regardless of the type of substrate ($p < 0.001$, Fig. 5A, B). Porewater NH_3-N concentrations were significantly lower in flow-through than in static conditions when standard-gravel substrate was used (Fig. 5A). However, porewater NH_3-N concentrations did not differ between flow-through and static conditions when natural substrate was used (Fig. 5B).

Discussion

Asian clam populations often are subject to rapid die-offs as a result of increased silt loads, temperature extremes, and low DO concentrations associated with decreased water flow (McMahon and Williams 1986, Sickel 1986). Strayer (1999) suggested that unionid mussels in the vicinity of these die-offs may be negatively affected as a result of elevated NH_3-N and reduced DO concentrations associated with decay of the clam tissues. A number of studies have investigated effects of NH_3-N and DO concentrations on unionids (Sparks and Strayer 1998, Bartsch et al. 2003, Mummert et al. 2003, Newton et al. 2003), but none have specifically evaluated how a *Corbicula* die-off could influence these variables.

Cherry et al. (2005) showed that NH_3-N accumulates in water overlying sediments that contain dead Asian clams, and our study clearly illustrates the potential for a clam die-off to result in the accumulation of much higher concentrations of NH_3-N in sediment pore water than in overlying water. For example, in flow-through chambers when densities of dead clams were $1000/m^2$, average total ammonia concentrations in pore water reached as high as 788 mg N/L after 7 d, whereas the corresponding overlying water had concentrations of 2.3 mg N/L. In the temperature experiment, NH_3-N concentrations in the pore water reached 5.56 mg/L after 7 d at 28°C. In contrast, corresponding concentrations in the overlying water averaged 0.80 and 0.03 mg/L for total ammonia and NH_3-N, respectively. Our results agree with those of other studies that have characterized porewater ammonia levels in natural systems (Ankley et al. 1990, Chambers et al. 1992, Frazier et al. 1996). Our results were expected given the lower water volume and lower exchange rates that characterize the sediment interstitial zone (Wetzel 1983, Frazier et al. 1996) and the extent to which ammonia may become associated with the sediments and partition into the pore water (Giesy et al. 1990).

Factors influencing NH_3-N concentrations in test chambers

Density.—Clam density in the sediment significantly influenced NH_3-N concentrations in both pore water and overlying water of the test chambers. In both static and flow-through tests, porewater NH_3-N concentrations were $>2\times$ as high in the highest-density treatment (1000 clams/m^2) as in the lower-density treatments (200 and 400 clams/m^2). These results were not surprising because more tissue mass (higher clam density) provided a greater amount of protein for degradation and ammonia production (Berner 1980, Wetzel 1983).

Temperature.—Temperature clearly influenced ammonia production, with levels of NH_3-N in test chambers maintained at 28°C $\sim 20\times$ higher than levels in chambers maintained at 19°C. Temperature-related fluctuations in porewater NH_3-N with elevated concentrations occurring in the warm summer months have been reported in field studies (Sarda and Burton 1995, Frazier et al. 1996). Temperature effects on ammonia production ultimately are related to increases in both the density and metabolic rates of the microorganisms involved in decomposition (Kirchman and Rich 1997, Eiler et al. 2003). Brugger et al. (2001) and Fischer et al. (2002) determined that seasonal fluctuations in microbial density and production were a result of both temperature and quality of organic matter. In our study, initial concentrations of NH_3-N were higher in the warmer test chambers than in the cooler test chambers, but the subsequent decrease in NH_3-N levels in the warmer chambers was more rapid than in the cooler chambers, probably because the warm temperatures favored increased activity of nitrifying bacteria. Thus, high temperatures may cause a high initial pulse of ammonia in the sediment pore water, but a short overall exposure period to elevated ammonia concentrations.

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letters over bars indicate significant differences in NH_3-N concentrations between overlying water and pore water within a given substrate type and flow rate.

Flow—Increasing flow had 2 effects: NH_3-N concentrations in the overlying water and pore water of the test chambers were lower in higher-flow chambers, and NH_3-N dissipated more rapidly in higher-flow chambers. Increased interstitial DO levels associated with higher flow rates could have led to increased densities of aerobic nitrifying bacteria and enhanced losses of NH_3-N through its conversion to NO_3-N (Frazier et al. 1996). Chambers et al. (1992) found temporal changes in riverbed chemistry were related to flow, with high flows associated with decreased nutrients and increased DO concentrations.

Substrate type.—An important consideration in the present study was the extent to which N biogeochemistry in the gravel substrate matched that of natural sediment. Natural sediments contain both decomposing and nitrifying bacteria, so degradation of clam tissue and removal of ammonia should have happened faster in the natural substrate than in the standard-gravel substrate used in our chambers. In general, the NH_3-N concentrations in the 2 substrate types were comparable. Flow rate significantly affected NH_3-N concentrations in the water overlying both substrates, but the influence of flow rate on porewater concentrations in the Little Black Creek sediment was much less than its influence in the standard-gravel substrate. The mean particle size of Little Black Creek sediment (95% sand, 2.5% silt, and 2.5% clay) was smaller than that of the standard-gravel substrate, and Little Black Creek sediment probably was less permeable than the standard-gravel substrate. Lower permeability could have reduced NH_3-N exchange between overlying water and pore water in chambers with river sediment, thereby negating the effect of higher flow in those chambers. Thus, sediment characteristics such as particle size can influence the potential for NH_3-N to be flushed from the interstitial zone and, in turn, can influence the potential for NH_3-N effects on infaunal organisms. Previous studies also have observed higher NH_3-N and lower DO concentrations in sediments with high proportions of silt and fine particles than in sediments with large particle sizes (Chambers et al. 1992, Frazier et al. 1996).

Implications for native unionid mussels

Asian clams occur in most drainages of the southeastern US, and populations often reach densities of hundreds or thousands per m^2 (Cherry et al. 1986, Bruenderman et al. 2001, Hornbach 1992). In our study, NH_3-N concentrations in standard-gravel substrate reached 0.17 mg/L at the low density of 200 clams/m^2 and 4.8 mg/L at the maximum density of 1000/m^2 when experiments were conducted at 22°C. When clam density was 400/m^2 and the test was conducted at 28°C, NH_3-N concentrations reached 5.56 mg/L. These porewater NH_3-N concentrations would be sufficient to affect or kill unionid mussels. The 96-h median lethal concentration (LC_{50}) for NH_3-N was 0.78 mg/L and 0.59 mg/L for adult *C. fluminea* and *Pyganodon grandis*, respectively (Cherry et al. 2005). Earlier life stages were more sensitive, with 96-h LC_{50} values of 0.28 mg/L for juvenile *C. fluminea* and 0.38 mg/L for juvenile *Villosa iris*. Glochidia of *V. iris* were among the most sensitive of the organisms tested, with 24-h LC_{50} values of 0.11 mg/L. Other studies have found that NH_3-N concentrations below those produced from the decaying Asian clam tissues in our study could affect unionid mussels (Wade 1992, Mummert et al. 2003, Newton 2003).

The fact that the highest NH_3-N concentrations were found in the sediment pore water is important for juvenile unionids because juveniles live in the sediment interstitial zone (Yeager et al. 1994). Any stress on the mussels resulting from elevated levels of NH_3-N could be further exacerbated by the low levels of DO that tend to prevail in the interstitial zone and that would be further reduced during the decay of clam tissues. In contrast, the effects of elevated sediment NH_3-N and reduced DO may be irrelevant to glochidia because glochidia that come in contact with pore water have failed to encyst and, therefore, are not viable.

In conclusion, the interstitial zone of sediments may be affected by NH_3-N produced during an Asian clam die-off, with temperature and density of clams influencing the concentrations of NH_3-N that are produced. The extent to which flow may reduce NH_3-N concentrations in the sediments depends on sediment particle size and flow rate, although elevated NH_3-N levels may persist in pore water, even in flowing waters. The NH_3-N concentrations in pore water of our test chambers often were similar to concentrations reported as acutely toxic to unionid mussels. Our results indicate that Asian clam die-offs and the resultant impact on sediment quality could negatively affect unionid mussels. Moreover, because juvenile mussels often live in the interstitium and may have limited ability for behavioral avoidance, they may be particularly susceptible to NH_3-N. A controlled field study to further evaluate the influence of clam die-offs on sediment quality will be important to validate these laboratory results.

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