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Ammonia Tolerance of the Bivalve *Mulinia lateralis* Sublethal Sediment Toxicity Test

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Several bivalve toxicity test methods are available for evaluating the toxicity of environmental samples including sediment interstitial waters and elutriates (Hunt and Anderson 1993). Only recently have whole sediment toxicity tests using bivalves been described in the literature (Burgess and Morrison 1994; Nipper and Roper 1995). The addition of these tests enhances our ability to determine adverse effects of contaminated sediments with a diversity of phyla and endpoints.

A standard methods manual for a ten-day juvenile *Mulinia lateralis* toxicity test was prepared recently for U.S. EPA Region VI as part of their dredged materials regulatory program (Pelletier et al. 1995). The method is based on the bivalve *M. lateralis* sublethal whole sediment toxicity test described by Burgess and Morrison (1994). As the manual was prepared, an additional aspect of the method was investigated: species ammonia tolerance. Water-only ammonia toxicity tests were conducted followed by ammonia-flushing studies to determine the residence times of various concentrations of naturally-occurring sediment ammonia in the method exposure chambers. Ammonia, which can be found at high environmental concentrations in sediments, is a concern in sediment testing because it has been shown to cause toxicity (e.g., Ankley et al. 1990).

MATERIALS AND METHODS

A stock solution of ammonia (10,000 mg/L) was prepared using reagent grade ammonium chloride at the beginning of each test. Treatment dilutions were made with appropriate amounts of the stock and l-µm filtered Narragansett Bay seawater (NBS). Nominal ammonia concentrations tested for the range finder were 0, 1, 10, 100, 1000 mg/L, while those used for the definitive test included 0, 3, 10, 30, 60, 100 mg/L. Test solutions were renewed daily with freshly prepared mixtures of the refrigerated original stock. Ammonia was measured using an Orion® ammonia ion-selective electrode (Boston, MA). Values reported below are measured.

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The test method experimental design is detailed in Burgess and Morrison (1994) and Pelletier et a1. (1995) and will be described briefly here. Test chambers consisted of 250-mL glass containers filled with 200 mL of the appropriate ammonia-spiked NBS mixture. Three replicate testing chambers were prepared per concentration. Each testing chamber contained 10 juvenile clams. Clams were sized by selecting those that passed through a 1.5-mm sieve and were retained on a 1.0-mm sieve. Tests were conducted at a temperature of 20 ± 2 °C and salinity of 32 ± 2 ‰. Water temperature, salinity, and pH were measured daily in every test chamber. Ammonia and dissolved oxygen were measured before renewals three times (days 0, 5, and 10) for the range finder test and four times (days 0, 3, 7, and 10) for the definitive test. After renewing the test solutions, test chambers were fed enough *Isochrysis galbana* and *Tetraselmus suecica* to result in final algal concentrations of 5 x 10^4 cells/mL. Exposure lights (2000-4000 lux) were kept on continuously to ensure that phytoplankton added as food did not contribute to a decline in water-column oxygen. At the initiation of each test, three random samples of 10 clams each were taken for initial dry weights. At the conclusion of each test, dry weights were measured and growth calculated for surviving clams. Growth measurements were based on the entire clam. Point estimates for survival and growth (i.e., LC_{50} and EC_{50}) were determined using the inhibition concentration (ICp) method (Klemm et al. 1994). Analysis of variance (ANOVA) was performed to determine the No Observed Effect Concentrations (NOECs).

To ensure residual ammonia had not been introduced into the overlying water during the set-up of tests, after 24 hr (Day 0) the water was removed (i.e., flushed) and replaced. Three marine sediments (Sites 2-4) exhibiting interstitial water ammonia concentrations ranging from approximately 14 to > 70 mg total ammonia/L were chosen to conduct ammonia flushing tests. Selected sediments showed acceptable survival (i.e., > 80%) in 10-day static test with the amphipod *Ampelisca abdita* and were considered non-toxic. In addition, a control sediment (Long Island Sound) having interstitial water concentrations of ~4 mg total ammonia/L was tested (Site 1). On Day -1, duplicate interstitial water samples were collected. Triplicate overlying water samples were collected on Days 0, 1 and 10.

Interstitial water ammonia was measured following centrifugation in teflone centrifuge tubes for one hour at 12,000 to 15,000xg at 4°C. Interstitial water salinity, pH and total ammonia were measured to calculate unionized ammonia (Hampson 1977). To initiate testing, chambers (250 mL) were filled with 50 mL of sediment and 150 mL of 1.0-µm filtered NBS and allowed to settle overnight. On Day 0, after overlying water samples were collected, all overlying water was decanted and each chamber refilled with fresh NBS. Once the sediment settled the organisms were added. Tests were static except for the removal of samples for ammonia analyses.

RESULTS AND DISCUSSION

The physical parameters pH, salinity and temperature averaged 7.79±0.20, 30±0.8 and 21.8±0.4, respectively, during the definitive ammonia test. The LC_{50} for the definitive test for total ammonia was 21.7 mg/L, while the LC_{50} for unionized ammonia was 0.6 mg/L (Table 1). The average weight of the clams

Table 1. Results of definitive ammonia toxicity tests with bivalve *M. lateralis* (pH 7.79, salinity 30 ‰ and temperature 21.8°C).

	Ammonia (mg/L)	
Endpoint	**Total**	**Unionized**
LC_{50}	21.7	0.6
Upper CI*	27.7	0.7
Lower CI	19.0	0.5
EC_{50}	11.0	0.3
Lower CI	14.8	0.4
Upper CI	7.3	0.2
NOEC Survival	8.2	0.2
NOEC Growth	<2.3	<0.1

* Confidence Interval

increased by 4.3 fold in the controls. Survival of clams from the 30, 60 and 100-mg/L treatments were significantly different from control clams resulting in a NOEC of 8.2 mg/L for total ammonia and 0.2 mg/L for unionized ammonia (Table 1). The growth EC_{50} for total ammonia was 11.0 mg/L and 0.3 mg/L for unionized ammonia (Table 1). Dry weights of bivalves in the lowest tested concentration were significantly different from the controls and, therefore, the NOECs were < 2.3 mg/L and < 0.1 mg/L for total and unionized ammonia, respectively (Table 1).

Kohn et al. (1994) summarized the sensitivities of 11 marine species with unionized ammonia LC_{50}s ranging from 0.77 to 3.35 mg/L. In another study of the ammonia tolerances of four marine amphipods, *Rhepoxynius abronius* was the most sensitive with an unionized ammonia LC_{50} of 0.18 mg/L at pH 7.0 (C. *Schlekat, personal communication).* These data indicate *M. lateralis* is one of the more ammonia sensitive marine toxicity testing species.

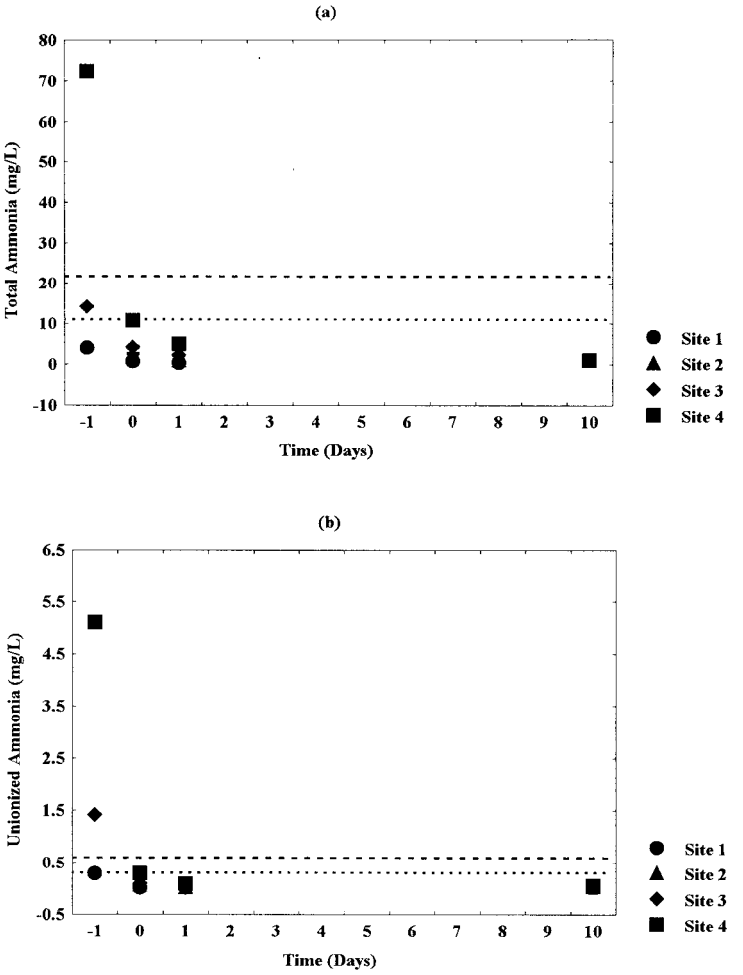

Figure 1. Decrease in total (a) and unionized (b) ammonia concentrations in overlying waters during flushing studies. Day 10 values are below detection limits (< 0.1 mg/L). Day -1 values are for sediment interstitial waters and Day 0, 1 and 10 values are for overlying waters. (-----) indicates the LC_{50} and (. . . . .) the EC_{50} effect levels (See Table 1).

In Figure 1, Day - 1 ammonia values show total ammonia interstitial water concentrations ranged from 2 mg/L to 72 mg/L. Site 2 was a heavy sand and insufficient interstitial water was isolated to perform ammonia measurements. By Day 1, overlying water ammonia levels decreased by an average of 55 **%** of Day 0 levels (Figure 1). Furthermore, by the conclusion of the test, overlying water concentrations were below detection (~0.1 mg/L), averaging a 95 % decrease from Day 0. Using the ammonia tolerance values from Table 1, by the first day of the exposure overlying water total and unionized ammonia concentrations were too low to adversely affect survival and growth (Figure 1a,b).

The sublethal sediment toxicity test using the marine bivalve *Mulinia lateralis* shows similar sensitivity to ammonia as other marine species. However, the flushing procedure performed at the initiation of each test was effective in removing nearly 100% of the ammonia, even at very high initial concentrations, from the exposure chamber overlying waters which is the primary exposure route to this species. Therefore, this method should not prove to be unduly sensitive to ammonia toxicity.

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