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**Toxicity of ammonia to surf clam (*Spisula solidissima*) larvae in saltwater and
sediment elutriates**

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Abstract

Ammonia is a natural component of sediments and has been identified as a common contributor to toxicity in marine sediment, elutriate and porewater testing. In our study, the role of ammonia as a possible toxicant in sediment toxicity tests was evaluated using larvae of the surf clam, *Spisula solidissima*. Elutriates were prepared and tested using six baseline sediment samples. Ammonia was then purged from aliquots of the baseline sediment samples prior to elutriate preparation. Finally, ammonia was spiked into aliquots of the purged elutriates to mimic ammonia concentrations measured in the baseline elutriates. Toxicity was present in all of the baseline samples and was removed in the ammonia purged samples. In most cases, toxicity was comparable in the ammonia spiked samples to levels measured in the baseline samples. Water only toxicity tests revealed that larvae of the surf clam are one of the more ammonia sensitive marine species. The LC50 for survival was 10.6 mg/L total ammonia (0.53 mg/L unionized ammonia) and the EC50 for shell development was 2.35 mg/L total ammonia (0.12 mg/L unionized ammonia). Toxicity endpoints calculated from the water only ammonia toxicity test were good predictors of effects observed in the sediment elutriate tests.

keywords: toxicity, embryos, larval bioassay, *Spisula sp.*, ammonia, ocean disposal

1.0 INTRODUCTION

Sediment toxicity tests have become routine for many environmental monitoring programs (USEPA and USACE, 1998; Miller et al. 1990). Although a variety of test methods exist, toxicological assessments of contaminated marine sediments generally have emphasized acute end points with 10 d exposures using various species of amphipods and polychaetes (USEPA 1994; ASTM 2007, 2006, 1992). There exists a need to test organisms with different sensitivities and routes of exposures to provide environmental managers and risk assessors more tools to evaluate the effects of contaminants in the marine environment. This can be achieved through the use of porewater and sediment elutriates.

Embryonic and larval bivalves have been used as sensitive indicators for a variety of pollutants (USEPA, 1989). Embryos and larvae are considered to be the most sensitive life stages of bivalve mollusk species. Results of these acute survival and developmental tests are often considered to be a good indicator of the acceptability of pollutant concentrations to saltwater mollusk species in general (ASTM, 1994). Several bivalve toxicity test methods are available for evaluating the toxicity of whole sediment, porewater, and elutriates (ASTM, 1994; Hunt and Anderson 1993; USEPA and USACE 1991).

Scientific evaluation of bivalves are important because many species are commercially and/or recreationally important. One of these species is the Surf Clam, *Spisula solidissima*. The surf clam commercial fishery has grown from a small bait fishery

during the years prior to World War II to one contributing over 70 percent of all clam products used in the US by the 1970's (Ropes, 1982; Hurley and Walker, 1996). Within the mid Atlantic region, the surf clam is a particularly important commercial clam species because of its wide distribution (Ropes, 1982). The surf clam ranges from the Gulf of Maine, Maine, USA, south to Cape Hatteras, North Carolina, USA (Fay et al., 1983), and is found in shallow sub tidal zones to depths of 50 m (Weinberg and Helser, 1996). Despite the economic and recreational importance of this species, very little toxicological information could be found for *Spisula solidissima*. Nelson et al. (1988) tested 3 month old juvenile *S. solidissima* with copper and Eyster and Morse (1984) exposed eggs to silver prior to fertilization. Information related to ammonia toxicity to the surf clam was limited in the literature. Ammonia has been found to be a principle toxicant in marine sediments (Ho et al., 2002; Ankley, 1990). Ammonia is not a persistent or bioaccumulative substance and is a naturally occurring compound in anoxic sediments (Ankley et al., 1990). Ammonia in sediments may be naturally formed by microbial degradation of natural organic matter, although it is likely augmented by the presence of anthropogenic nitrogenous organic material. While there have been numerous studies relating ammonia toxicity to marine organisms (Kohn et al., 1994; Moore et al., 1997; Frazier et al., 1996; Ankley et al., 1990), species specific data relating to ammonia sensitivity are needed due to the ubiquitous nature of ammonia in the environment and for use in risk assessment and deriving water quality criteria for saltwater organisms (ASTM, 1994). Bivalve larvae have been found to be one of the more sensitive life stages to unionized ammonia (Geffard, 2002).

The American Society for Testing and Materials (1994) recommends an interim effect concentration threshold for *Crassostrea. gigas* of 0.13 mg/L as unionized ammonia (UIA) at a pH of 7.7 - 8.1. Geffard (2002) estimates the total ammonia nitrogen (TAN) EC20 for *C. gigas* as 2.8 mg/L. Huber et al. (1997) found that 10 day juvenile *Mulinia lateralis*, the dwarf surf clam, is one of the more ammonia sensitive species with an UIA EC50 of 0.3 mg/L at pH 7.79.

The presence of ammonia can complicate the interpretation of results of sediment elutriate toxicity tests. High concentrations of ammonia in sediments could mask the effects of other toxicants (Ankley et al., 1990). Depending on the study area and the objectives of the experiments, ammonia may or may not be a contaminant of concern. For studies where ammonia is treated as a toxicity artifact, standard procedures to confirm ammonia as a toxicant (and also implicate other more hydrophobic, persistent compounds that are being masked by the ammonia toxicity) could be beneficial in some sediment, elutriate or porewater studies. For example, ammonia may be considered a confounding factor and not a contaminant of concern in laboratory toxicity tests when testing sediments proposed for ocean disposal. The objectives of our study were (1) to determine the unionized ammonia (UIA) effect levels (i.e. EC20, EC25, and EC50) for *Spisula solidissima* larval survival and shell development respectively; (2) to confirm and define the role of ammonia as a causative toxicant to the survival and development of *Spisula solidissima* larvae in marine elutriates samples; and (3) to determine if other chemicals were contributing to elutriate toxicity.

2.0 MATERIALS AND METHODS

2.1 Definitive unionized ammonia toxicity test

Water only test solutions for the UIA toxicity test were prepared by spiking filtered, 0.45 Φ m, saltwater with a 1000 mg/L TAN solution (Thermo Orion Applied Solution Ammonia Standard, NH_4Cl). The nominal concentrations were 0, 1, 2, 4, 8 and 16 mg/L TAN. The saltwater was collected from Manasquan Inlet, NJ, USA.

2.2 Test sediments

Samples for the sediment bioassays were collected from five locations within the Peconic Estuary, NY, USA. Surficial samples were collected with a Van Veen grab sampler modified to allow access to the top 2 cm of sediment. A reference sediment was obtained from the Narrow River, a tributary of Narragansett Bay, RI, USA. The reference sample has been previously tested and is a fine grained organically enriched sample that supports high densities of the marine amphipod *Ampelisca abdita* but its suitability to support high survival and hinge production with *Spisula solidissima* has not been previously evaluated. All sediment samples were sieved through a 0.5 mm stainless steel sieve to remove indigenous macrofauna, large debris, and any potential predators.

2.3 Ammonia purging of sediments

Sediments were homogenized for two minutes using a mechanical stirrer. A 1500 mL aliquot of each sediment sample was spread thinly in the bottom of a 15 L basin and covered with 4500 mL of 0.45 micron filtered natural saltwater in a procedure developed

by Ferretti et al. (2000). The overlying water was aerated and the basins covered to exclude dust and minimize evaporation. On the third and sixth day after setup, 90% of the overlying saltwater was renewed. After seven days, the overlying water was siphoned off and test elutriates were prepared from the purged sediments.

2.4 Elutriate preparation

Each purged and unpurged sediment sample was homogenized for two minutes using a mechanical stirrer. One part sediment and four parts filtered saltwater were then combined and mixed on a rotary soil tumbler for 30 minutes and allowed to settle overnight at 4 °C. Centrifugation or further filtration of the elutriates was not necessary. A peristaltic pump was used to siphon the supernatants into 2 L containers. The elutriates were tested within 24 h. A saltwater control was tested in parallel with the elutriates. A 1-L subsample from each purged elutriate sample was spiked with ammonia to match the TAN measured in the corresponding unpurged elutriate sample.

2.5 Test organisms

Adult *Spisula solidissima* were received from the Woods Hole Marine Biological Laboratory, Woods Hole, MA, USA, in moist straw and held overnight at 4 °C. To induce spawning, the chilled clams were placed in large basins with aerated saltwater at 20 °C. All specimens were separated into individual 2 L crystallization dishes with seawater during release of gametes. Gametes from three males and three females were prepared in accordance with ASTM methods (1994). Sperm and eggs were combined for one hour and embryos were placed in test chambers within two hours of fertilization.

The sensitivity of a subset of the bivalve larvae was tested using potassium chloride as a reference toxicant. Fertilized eggs develop into pyramid shaped, planktonic trochophore larvae approximately 9 h after fertilization at 21.7 °C (Ropes, 1980) and reach veliger larvae stage in 28 hours at 22.0 °C (Loosanoff and Davis, 1963). Although size was not a measurement in this study, veliger larvae averaged 89 x 71 microns after 24 hours in a study by Ropes (1980).

2.6 Test conditions

Tests were conducted in accordance with ASTM E724. The test endpoints were survival and shell development. The initial number of organisms per replicate was determined by averaging the densities in ten dummy chambers which were inoculated at even intervals among all of the test chambers. *Spisula solidissima* were exposed as embryos (60-120 minutes post fertilization) and exposed for 48 hours. Normally developed larvae (veliger stage) were identified by their distinct “D” shaped shell at 125x magnification.

2.7 Water quality

Three surrogate chambers were set up for each sediment elutriate treatment and the saltwater control in the sediment bioassay tests for measurement of TAN, dissolved oxygen and salinity at 0, 12, 24 and 48 h of testing. These surrogate chambers were inoculated with embryos at the start of testing so that water quality parameters were similar as possible to the actual test chambers.

Major factors affecting ammonia toxicity are temperature and pH. The pH of each

ammonia spiked elutriate replicate was adjusted with 1.0 N and 0.1 N NaOH (0.05 - 0.2 mL), to match the pH of the corresponding unpurged sample. The pH of each spiked and unpurged elutriate replicate was then measured every 12 hours of the 48 hour tests. Tests were conducted in temperature controlled water baths (Lab Line Biotronnete) and temperature was measured daily in each test chamber. Samples for TAN analysis were preserved with concentrated sulfuric acid and stored at 4 °C and held for less than 2 weeks prior to testing. Total ammonia measurements were performed using an Orion Ionalyzer model 407A with an Orion model 95-12 ammonia sensitive electrode in accordance with manufacturer's instructions and Standard Methods, *Ammonia Selective Electrode Method* (APHA et al., 1999). Total ammonia nitrogen was converted to UIA using the *Free Ammonia-Nitrogen Calculator*, December 1998, Purdue University, West Lafayette, IN, USA and verified using the American Fisheries Society saltwater ammonia calculator (<http://www.fisheries.org/afs/hatchery.html>).

Dissolved oxygen, pH, and salinity were measured daily using a Yellow Springs Instrument model 58 dissolved oxygen meter (Yellow Springs, OH, USA), a Hanna Instruments pH meter model 9219 (Woonsocket, RI, USA), and a Reichert temperature compensating refractometer (Depew, NY, USA), respectively.

2.8 Statistical analysis

Percent survival and percent normal shell development endpoints were calculated for both the UIA definitive test and the sediment elutriate toxicity tests. For the saltwater ammonia toxicity test, a linear interpolation method (*Toxicity Data Analysis Software*,

June 1994, U.S. EPA, Cincinnati, OH, USA) was used to calculate the UIA LC50 and EC50 values for survival and development endpoints respectively. One way unpaired t-tests ($p = 0.05$) were performed comparing sediment toxicity test elutriate treatments (i.e. unpurged & purged, purged & spiked, unpurged & spiked) for each sample. A linear regression was calculated between the transformed development endpoint and UIA using *Statistix Analytical Software*, 1996, Tallahassee, FL, USA.

3.0 RESULTS

3.1 Definitive unionized ammonia toxicity test

The Manasquan saltwater control mean survival was 94.9% and normal development 89.7%. Both results meet the minimal acceptable limits of 70% and 60% for survival and development, respectively (Table 1) (ASTM, 1994).

The pH, temperature and salinity measured during the water only toxicity test averaged 8.14 $\pm$ 0.06, 19.7 $\pm$ 0.4°C and 31 $\pm$.5‰ respectively. *Spisula solidissima* survival and development are listed in Table 1 along with measured TAN and calculated UIA values.

The 48 hour LC50 concentration for survival in the water only exposures for ammonia was 10.6 mg/L TAN ($\pm$ 0.4) and 0.53 mg/L UIA ($\pm$.01). Normal hinge development was the more sensitive endpoint. The 48 hour EC50 concentration for development was 0.53 mg/L TAN ($\pm$.01) and 0.12 mg/L UIA ($\pm$.01) (UIA EC25 for Development = 0.079 mg/L $\pm$.006) (Table 1). There was a strong relationship established between

258 UIA and Shell development with an r^2 value of 0.76. The regression equation using non
259 transformed survival data is $y = -238x + 78$, where 'x' is unionized ammonia in mg/L and
260 'y' is percent normal shell development.

262 3.2 Sediment elutriate toxicity tests (Nonpurged, Purged, and Purged+Ammonia Spiked)

263 Temperature, salinity and dissolved oxygen were within prescribed ranges for the
264 duration of the test (data not presented). pH was measured between 7.8 and 8.3 for all
265 samples and generally did not vary by more than 0.10 pH unit within each unique sample.
266 Survival in all samples was greater than 72.9% (data not presented). Since shell
267 development was the more sensitive endpoint, results and discussion will focus on this
268 endpoint only. Shell development ranged from zero in four samples to over 92% in two
269 of the purged samples (Table 2). There was a significant increase in shell development in
270 the elutriates which were purged of ammonia (Table 2; Figure 1). The survival in Sample
271 5 following purging of ammonia was not as high as measured in the other five samples
272 (Table 2). Toxicity was measured in the purged and spiked samples. Four of the six
273 samples exhibited toxicity similar to the unpurged (baseline elutriates). However in two
274 samples (4 & 5) toxicity returned but the samples were not as toxic as the corresponding
275 unpurged samples (Figure 1). Toxicity related to ammonia was evident in the reference
276 sample (REF) (Table 2). Ammonia was the primary causative toxicity agent in all
277 samples. Percent development in the unpurged (baseline) versus purged+spiked samples
278 was characterized with an r^2 value of 0.83.

4.0 DISCUSSION

4.1 Definitive unionized ammonia toxicity test

The sensitivity of *Spisula solidissima* larvae to ammonia in this study differed when compared to embryo/larvae of other bivalve species. Huber et al. (1997) measured ammonia sensitivity using juvenile *Mulinia lateralis*. The LC50 for *M. lateralis* survival was 0.6 mg/L UIA and the EC50 using growth as an endpoint was 0.3 mg/L UIA. A study of the pacific oyster, *Crassostrea gigas* (USEPA, 1993) revealed a 48 hour EC50 value of 0.13 mg/L. A recent study using *Mytilus galloprovincialis* in an ammonia reference toxicant test revealed a 48 hour EC50 of 0.036 mg/L UIA (McDonald, 2005). *M. galloprovincialis* embryos were almost an order of magnitude more sensitive to ammonia than *Spisula solidissima* that were tested in our study. Embryos are typically the most sensitive life stage (His et al., 1999) and mollusks are routinely more sensitive to contaminants than fish and echinoderms (Hunt and Anderson, 1993). Acute ammonia toxicity for 21 species of marine organisms was summarized by the U.S. EPA (1989). The most tolerant genera were adult mollusks with the Eastern Oyster, *Crassostrea virginica*, representing the most ammonia tolerant saltwater organism listed (19.1 mg/L UIA). However, due to the different life stages and the fact that most adult bivalves have the ability to stop siphoning during periods of stress, contaminant sensitivity comparisons to more sensitive bivalve larval stages are generally not valid. *Spisula solidissima* bivalve larvae typically use yolk material during its first 24 hours post fertilization. Also, very little information was found regarding ammonia sensitivity to bivalve larvae in general. Differences in pH, including within test variability, life stage of the organism tested, and endpoints measured (i.e. growth, shell development, viability, etc.), test

duration, salinity and other water quality related test conditions render direct comparisons and relevance to other studies difficult. However, *Spisula solidissima* larvae appear to be as or more sensitive to ammonia than values published for other commonly used laboratory marine toxicity test organisms used for testing marine sediments, elutriates and sediment porewaters. A summary of published ammonia threshold values for organisms commonly tested in water only, sediment or sediment elutriate exposures are summarized in Table 3.

4.2 Sediment elutriate bioassays using bivalve larvae as a test organism

Bivalve larval tests have been commonly employed in ambient surveys (Becker et al., 1990; His et al., 1999; Hunt and Anderson, 1993; Geffard et al., 2001) and have been routinely used in testing of sediment elutriates of proposed dredged materials (USEPA and USACE 1991). Our data show that *Spisula solidissima* are sensitive to relatively low levels of ammonia and ammonia toxicity in elutriates prepared from surficial sediments in our study was higher than expected. During testing of dredge material, the potential for ammonia toxicity to test organisms is higher than surficial sediments because of the general trend of increasing ammonia concentrations as sediment depth increases (Frazier et al., 1996). Our water only ammonia toxicity tests predicted significant effects on *Spisula solidissima* shell development and were confirmed by our tests on the six sediment elutriates. All of the unpurged sediment elutriates had UIA similar or greater than the calculated water only EC50 of 0.12 mg/L UIA and shell development was significantly affected in all six unpurged samples (Table 2).

To determine if UIA was causing the toxicity to *Spisula solidissima* in terms of shell development, ammonia was removed from the baseline sediments by purging with seawater. The UIA in all the purged samples was reduced below the EC50 of 0.12 mg/L. Development increased significantly in all the purged samples when compared to the corresponding unpurged sample (Figure 1). Shell development in sample 5 was 59.2% after purging ammonia which is lower than the survivals obtained in the other five purged elutriate samples (Table 2). This would indicate that other contaminants, in addition to ammonia, are contributing to toxicity in Sample 5. Ho et al. (2002) found that during toxicity identification evaluations of 13 sediment porewater samples that there was no one predominant cause of toxicity. Rather, there were typically multiple causes including ammonia, metals, and organics but ammonia was implicated as the sole or partial contributor to toxicity in 69% of the samples tested. To support the hypothesis that UIA is causing toxicity in the baseline sediments in our study, ammonia was added back into a subsample from each purged sediment elutriate to mimic the TAN measured in the unpurged elutriate (baseline) sample. Toxicity was measured in the six ammonia spiked samples indicating that ammonia was responsible for some or all of the observed toxicity in the unpurged sediment elutriates. Toxicity was not completely recovered in samples 4 and 5 of the ammonia spiked elutriates (Table 2). The non recovery of all of the observed toxicity in the baseline samples could be due to variability of the ammonia measurements or pH differences during the exposure. Other factors including changes in oxidation/reduction potential or subsequent changes in contaminant availability (i.e. sulfides) during the purging process may have contributed to the reduced toxicity measured in these two ammonia spiked samples. However, from the lower than expected

shell development in purged Sample 5, it is likely that there were other causative agents besides ammonia contributed to the observed toxicity in this sample.

5.0 CONCLUSIONS

Unionized ammonia has been shown to be toxic to marine test species in sediment, elutriate, and porewater samples. Ammonia can confound results when testing sediments. Water only toxicity tests indicate that larvae of the surf clam, *Spisula solidissima*, are one of the more ammonia sensitive marine species. The LC50 for survival was 10.58 mg/L TAN (0.53 mg/L UIA) and the EC50 for shell development was 2.35 mg/L TAN (0.12 mg/L UIA). Tests on elutriates prepared from surficial sediments indicate a potential for ammonia toxicity to the surf clam based on the water only ammonia toxicity data collected during this study. It is recommended that ammonia concentrations be measured on all elutriates prior to testing with embryos and larvae of marine bivalves and compared to published values. Lowering of ammonia to below toxicity thresholds in sediments prior to testing should be considered or choosing a more ammonia tolerant test species may be appropriate in other cases depending on the objective of the study.

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The use of trade names does not constitute endorsement by the U.S. Environmental Protection Agency

6.0 REFERENCES

Adams W.J., Berry W.J., Burton Jr. G.A., Ho K.T., Macdonald D., Scroggins R., Winger P.V., 2001. Summary of the SETAC Workshop on Porewater Toxicity, March 18-22, 2000; Pensacola, FL. Society of Environmental Toxicology and Chemistry (SETAC). Pensacola, FL, USA. 28p.

Ankley G.T., Katko A., Arthur J.W., 1990. Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin. Environmental Toxicology and Chemistry 9, 313-322.

APHA, AWWA, WEF, 1999. Standard methods for the examination of water and wastewater, 20th edition. Washington, DC, USA.

American Society for Testing and Materials, 1992. Standard guide for conducting 10 day static sediment toxicity tests with marine and estuarine amphipods. ASTM E 1367-92. ASTM. Philadelphia, PA.

American Society for Testing and Materials, 1994. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve

mollusks. ASTM E 724-94. ASTM. Philadelphia, PA.

American Society for Testing and Materials, 2006. Standard guide for conducting acute, chronic and life cycle aquatic toxicity tests with polychaetous annelids. ASTM E 1562-00. ASTM. Philadelphia, PA.

American Society for Testing and Materials, 2007. Standard guide for conducting sediment toxicity tests with polychaetous annelids. ASTM E 1611-00. ASTM. Philadelphia, PA.

Becker D.S., Bilyard G.R., Ginn T.C., 1990. Comparisons between sediment toxicity tests and alterations of benthic macroinvertebrate assemblages at a marine superfund site: Commencement Bay, Washington. Environmental Toxicology and Chemistry 9, 669-685.

Carr, R.S., and Chapman, D.C., 1992. Comparison of solid phase and porewater approaches for assessing the quality of marine and estuarine sediments. Journal of Chemical Ecology 7, 19-30.

Cesar, A., Marin-Guirao, L., Vita, R., 2002. Sensitivity of Mediterranean amphipods and sea urchins to reference toxicants. Ciencias Marinas 28, 407-417.

Eyster, L.S. and Morse, M.P., 1984. Development of the Surf Clam (*Spisula solidissima*) Following Exposure of Gametes, Embryos and Larvae to Silver. Archives of Environmental contamination and Toxicology 13, 641-646.

- Fay, C. W., Neves, R. J., Pardue, G. B., 1983. Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (Mid-Atlantic), Surf Clam. Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA. Technical Report No. EL-82-4: 23 p.
- Ferretti J.A., Calesso D.F., Hermon T.R., 2000. Evaluation of methods to remove ammonia interference in marine sediment toxicity tests. *Environmental Toxicology and Chemistry* 19, 1935-1941.
- Frazier B.E., Naimo T.J., Sandheinrich M.B., 1996. Temporal and vertical distribution of total ammonia nitrogen and unionized ammonia nitrogen in sediment porewater from the upper Mississippi River. *Environmental Toxicology and Chemistry* 15, 92-99.
- Geffard O., Budzinski H., His E., Seaman M.N.L., Garrigues P., 2002. Relationships between contaminant levels in marine sediments and their biological effects on embryos of oysters, *Crassostrea gigas*. *Environmental Toxicology and Chemistry* 21, 2310-2318.
- Geffard O., Budzinski H., Augagneur S., Seaman M.N., His E., 2001. Assessment of sediment contamination by spermioxicity and embryotoxicity bioassays with sea urchins (*Paracentrotus lividus*) and oysters (*Crassostrea gigas*). *Environmental*

Toxicology and Chemistry 20, 1605-1611.

Greenstein, D.J., Alzadjali, S., Bay, S.M., 1996. Toxicity of ammonia to purple sea urchins. In: Southern California coastal Research Project Annual Report (1994-1995), pp. 72-77.

His E., Beiras R., Seaman M.N.L., 1999. The assessment of marine pollution bioassays with bivalve embryos and larvae. *Advances in Marine Biology*, Volume 37. Southward AJ, Tyler PA, Young CM, Eds. Academic Press, New York, NY, USA. pp. 1-178.

Ho K.T., Burgess R.M., Pelletier M.C., Serbst J.R., Ryba S.A., Cantwell M.G., Kuhn A., Raczelowski P., 2002. An overview of toxicant identification in sediments and dredged materials. *Marine Pollution Bulletin* 44, 286-293.

Huber M., Pelletier M.C., Charles J.B., Burgess R.M., 1997. Ammonia tolerance of the bivalve *Mulinia lateralis* sublethal sediment toxicity test. *Bulletin of Environmental Contamination & Toxicology* 59, 292-291.

Hunt J.W., Anderson B.S., 1993. From Research to Routine: A review of toxicity testing with marine mollusks. *Environmental Toxicology and Risk Assessment*. ASTM STP 1179. Landis W.G., Hughes J.S., Lewis M.A., Eds., American Society for Testing and Materials, Philadelphia, PA, USA. pp. 320-339.

470

471 Hurley D.H., Walker R.L., 1996. The effects of larval stocking density on growth,
472 survival, and development of laboratory reared *Spisula solidissima* (SAY, 1822).
473 Journal of Shellfish Research 15, 715-718.

474

475 Kohn N.P., Word J.Q., Niyogi D.K., 1994. Acute toxicity of ammonia to four species of
476 marine amphipod. Marine Environmental Research 38, 1-15.

477

478 Kobayashi N., 1980. Comparative sensitivity of various developmental stages of sea
479 urchins to some chemicals. Marine Biology 58, 163-171.

480

481 Loosanoff V.L., Davis, H.C., 1963. Rearing of bivalve mollusks. Advances in Marine
482 Biology, Volume 1. Academic Press, NY. 410 p.

483

484 McDonald, B.G., 2005. Comparison of porewater and elutriate bivalve larval
485 development toxicity testing is a sediment quality triad framework. Ecotoxicology
486 and Environmental Safety 62, 383-390.

487

488 Miller D.C., Poucher S., Cardin J.A., Hansen D., 1990. The acute toxicity of ammonia to
489 marine fish and a mysid. Archives of Environmental Contamination and
490 Toxicology, 19, 40-48.

491

492 Moore D.W., Bridges T.S., Gray B.R., Duke B.M., 1997. Risk of ammonia toxicity
493 during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*.
494 Environmental Toxicology and Chemistry 16, 1020-1027.

- Nelson, D.A., Miller, J.E., and Calabrese, A., 1988. Effect of Heavy Metals on Bay Scallops, Surf Clams, and Blue Mussels in Acute and Long Term Exposures. Archives of Environmental Contamination and Toxicology 17, 595-600.
- Ropes, J.W., 1982. The Atlantic coast surf clam fisher, 1965-1974. US National Marine Fisheries. Services, Marine Fisheries Review 44(8), 1-14.
- Ropes, J.W., 1980. Biological and fisheries data on the Atlantic surf clam, *Spisula solidissima*. U.S. Dep. Comm., NOAA, NMFS, Northeast Fisheries Center Tech. Ser. Rep No. 24, 91 p.
- U.S. Environmental Protection Agency & U.S. Army Corps of Engineers. 1998. Evaluation of dredged material proposed for discharge in waters of the U.S. - testing manual. EPA/823/B-98-004. Washington, DC, USA.
- U.S. Environmental Protection Agency. 1994. Methods for assessing the toxicity of sediment associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Final Report. Office of Research and Development, Washington, DC.
- U.S. Environmental Protection Agency, 1993. Refinements of Current PSDDA Bioassays: Final Report Summary. Water Division, Region 10, Seattle WA, EPA 910/R-9-93-014a.
- USEPA and USACE, 1991. Evaluation of Dredged Material Proposed for Ocean

- 521 Disposal. Washington, DC. EPA-503/8-91/001.
522
- 523 U.S. Environmental Protection Agency. 1989. Ambient water quality criteria for
524 ammonia (saltwater), 1989. EPA/440/5-88-004. Office of Water, Washington, DC,
525 USA.
526
- 527 Weinberg J.R., Helser T.E., 1996. Growth of the Atlantic surf clam, *Spisula solidissima*,
528 from Georges Bank to the Delmarva Peninsula, USA. Marine Biology 126, 663-
529 674.

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Larvae of the Surf Clam, *Spisula solidissima* are one of the more sensitive marine species to ammonia.>
Reducing ammonia concentrations prior to testing with aquatic organisms should be considered. > The
purging and spiking test design delineated the role of ammonia in toxicity of marine sediment elutriates.

Table 1.

Results of ammonia spiked seawater toxicity test with *Spisula sp.* larvae (pH 8.14 $\pm$.06, 19.7 $\pm$.4°C, 31 $\pm$ 1 ‰).

Targeted TAN (mg/L)	Measured TAN (mg./L)	Unionized Ammonia (mg/L)	Larval % Survival	Normal Larvae % Hinge Development
0	.09 $\pm$.01	.01 $\pm$ 0	94.9 $\pm$ 7.1	89.7 $\pm$ 6.7
1	.97 $\pm$.02	.05 $\pm$ 0	95.9 $\pm$ 13.1	85.5 $\pm$ 8.3
2	1.83 $\pm$.06	.09 $\pm$ 0	95.8 $\pm$ 10.2	60.9 $\pm$ 6.7
4	3.71 $\pm$.22	.18 $\pm$.02	90.9 $\pm$ 8.1	2.2 $\pm$.8
8	6.80 $\pm$.12	.38 $\pm$.09	79.9 $\pm$ 9.1	0
16	16.38 $\pm$.14	.74 $\pm$.05	2.2 $\pm$ 1.5	0

TAN = Total Ammonia Nitrogen

Table 2.

Average un-ionized ammonia concentrations with average percentage *Spisula sp.* shell development in unpurged, purged and ammonia spiked sample elutriates.

Sample ID	Unpurged (Baseline)		Ammonia Purged		Ammonia Purged + Ammonia Spiked	
	Ammonia Unionized (mg/L)	% Shell Development	Ammonia Unionized (mg/L)	% Shell Development	Ammonia Unionized (mg/L)	% Shell Development
REF	.14 ∓ .03	46.3 ∓ 6.8 ^a	.03 ∓ .01	78.0 ∓ 7.3	.14 ∓ .01	56.9 ∓ 10.9 ^a
1	.34 ∓ .07	1.1 ∓ 1.5 ^a	.06 ∓ .01	72.4 ∓ 7.6	.32 ∓ .06	0 ^a
2	.37 ∓ .08	0 ^a	.04 ∓ .01	92.9 ∓ 8.4	.35 ∓ .06	0 ^a
3	.33 ∓ .06	0.3 ∓ 0.6 ^a	.05 ∓ .01	87.6 ∓ 3.9	.32 ∓ .05	0 ^a
4	.16 ∓ .07	9.2 ∓ 1.7 ^a	.05 ∓ .01	92.8 ∓ 9.2	.21 ∓ .02	26.1 ∓ 10.9 ^{ab}
5	.10 ∓ .01	2.0 ∓ 0.5 ^a	.01 ∓ .00		59.2 ∓ 14.8	.09 ∓ .02
	∓ 8.1 ^{ab}					23.0

^aSignificantly different than corresponding purged treatment, $p=0.05$; $df=4$.

^bSignificantly different than corresponding unpurged treatment, $p=0.05$, $df=4$.

Table 3. Unionized ammonia toxicity values (mg/L) reported for organisms commonly used in testing (water only exposures) of marine aqueous samples and elutriates

Organism	Type	Life Stage	Endpoint	Result	Reference
<i>Ampleliscia abdita</i>	Amphipod	Juveniles	LC50	0.83	Kohn, et al. (1994)
<i>Rhepoxynius abronius</i>	Amphipod	Juveniles	LC50	1.59	Kohn, et al. (1994)
<i>Eohaustorius estuarius</i>	Amphipod	Juveniles	LC50	2.49	Kohn, et al. (1994)
<i>Leptocheirus plumulosus</i>	Amphipod	Juveniles	LC50	0.70	Moore et al. (1997)
<i>Mysidopsis bahia</i>	Crustacean	Juveniles	EC25	0.23	Miller (1998)
<i>Menidia beryllina</i>	Fish	Larvae	EC25	0.06	Miller (1998)
<i>Dendraster excentricus</i>	Sand Dollar	Gametes	NOEC	0.04	USEPA (1993)
<i>Peronella japonica</i>	Sand Dollar	Gametes	NOEC	0.02	Kobayashi (1980)
<i>Arabcia punctulata</i>	Sea Urchin	Gametes	NOEC	0.09	Carr and Chapman (1992)
<i>Arabcia luxula</i>	Sea Urchin	Gametes	EC25	0.01	Cesar (2002)
<i>Strongylocentrotus purpuratus</i>	Sea Urchin	Gametes	NOEC	0.06	Grenstein et al. (1996)
<i>Mytillus galloprovincialis</i>	Mussel	Larvae	EC50	0.04	McDonald (2005)
<i>Crassostrea gigas</i>	Oyster	Larvae	NOEC	0.08	USEPA (1993)
			EC50	0.13	USEPA (1993)
<i>Spisula solidissima</i>	Clam	Larvae	EC25	0.08	This study
			EC50	0.12	This study

Amphipod, crustacean, and fish species tested using 96 hour exposures; Gamete and larval exposures ranged between 30 minute and 48 hour exposures

Figure 1. Comparison of percent normal shell development of bivalve larvae exposed to sediment elutriate samples which were tested as baseline, purged of ammonia and spiked to approximate baseline ammonia concentrations.