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28	Toxicity of ammonia to surf clam (Spisula solidissima) larvae in saltwater and
29	sediment elutriates
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51 Abstract

52 Ammonia is a natural component of sediments and has been identified as a common 53 contributor to toxicity in marine sediment, elutriate and porewater testing. In our study, 54 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 55 56 baseline sediment samples. Ammonia was then purged from aliquots of the baseline 57 sediment samples prior to elutriate preparation. Finally, ammonia was spiked into 58 aliquots of the purged elutriates to mimic ammonia concentrations measured in the 59 baseline elutriates. Toxicity was present in all of the baseline samples and was removed 60 in the ammonia purged samples. In most cases, toxicity was comparable in the ammonia 61 spiked samples to levels measured in the baseline samples. Water only toxicity tests 62 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 63 64 ammonia) and the EC50 for shell development was 2.35 mg/L total ammonia (0.12 mg/L 65 unionized ammonia). Toxicity endpoints calculated from the water only ammonia toxicity test were good predictors of effects observed in the sediment elutriate tests. 66 67 keywords: toxicity, embryos, larval bioassay, Spisula sp., ammonia, ocean disposal 68 69 70

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- 73

74 **1.0 INTRODUCTION**

75	Sediment toxicity tests have become routine for many environmental monitoring
76	programs (USEPA and USACE, 1998; Miller et al. 1990). Although a variety of test
77	methods exist, toxicological assessments of contaminated marine sediments generally
78	have emphasized acute end points with 10 d exposures using various species of
79	amphipods and polychaetes (USEPA 1994; ASTM 2007, 2006, 1992). There exists a
80	need to test organisms with different sensitivities and routes of exposures to provide
81	environmental managers and risk assessors more tools to evaluate the effects of
82	contaminants in the marine environment. This can be achieved though the use of
83	porewater and sediment elutriates.
84	
85	Embryonic and larval bivalves have been used as sensitive indicators for a variety of
86	pollutants (USEPA, 1989). Embryos and larvae are considered to be the most sensitive
87	life stages of bivalve mollusk species. Results of these acute survival and developmental
88	tests are often considered to be a good indicator of the acceptability of pollutant
89	concentrations to saltwater mollusk species in general (ASTM, 1994). Several bivalve
90	toxicity test methods are available for evaluating the toxicity of whole sediment,
91	porewater, and elutriates (ASTM, 1994; Hunt and Anderson 1993; USEPA and USACE
92	1991).
93	

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

97	during the years prior to World War II to one contributing over 70 percent of all clam
98	products used in the US by the 1970's (Ropes, 1982; Hurley and Walker, 1996). Within
99	the mid Atlantic region, the surf clam is a particularly important commercial clam species
100	because of its wide distribution (Ropes, 1982). The surf clam ranges from the Gulf of
101	Maine, Maine, USA, south to Cape Hatteras, North Carolina, USA (Fay et al., 1983), and
102	is found in shallow sub tidal zones to depths of 50 m (Weinberg and Helser, 1996).
103	Despite the economic and recreational importance of this species, very little toxicological
104	information could be found for Spisula solidissima. Nelson et al. (1988) tested 3 month
105	old juvenile S. solidissima with copper and Eyster and Morse (1984) exposed eggs to
106	silver prior to fertilization. Information related to ammonia toxicity to the surf clam was
107	limited in the literature. Ammonia has been found to be a principle toxicant in marine
108	sediments (Ho et al., 2002; Ankley, 1990). Ammonia is not a persistent or
109	bioaccumulative substance and is a naturally occurring compound in anoxic sediments
110	(Ankley et al., 1990). Ammonia in sediments may be naturally formed by microbial
111	degradation of natural organic matter, although it is likely augmented by the presence of
112	anthropogenic nitrogenous organic material. While there have been numerous studies
113	relating ammonia toxicity to marine organisms (Kohn et al., 1994; Moore et al., 1997;
114	Frazier et al., 1996; Ankle et al., 1990), species specific data relating to ammonia
115	sensitivity are needed due to the ubiquitous nature of ammonia in the environment and
116	for use in risk assessment and deriving water quality criteria for saltwater organisms
117	(ASTM, 1994). Bivalve larvae have been found to be one of the more sensitive life
118	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.

126

127 The presence of ammonia can complicate the interpretation of results of sediment 128 elutriate toxicity tests. High concentrations of ammonia in sediments could mask the 129 effects of other toxicants (Ankley et al., 1990). Depending on the study area and the 130 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 131 132 confirm ammonia as a toxicant (and also implicate other more hydrophobic, persistent 133 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 134 135 confounding factor and not a contaminant of concern in laboratory toxicity tests when 136 testing sediments proposed for ocean disposal. The objectives of our study were (1) to 137 determine the unionized ammonia (UIA) effect levels (i.e. EC20, EC25, and EC50) for 138 Spisula solidissima larval survival and shell development respectively; (2) to confirm and 139 define the role of ammonia as a causative toxicant to the survival and development of 140 Spisula solidissima larvae in marine elutriates samples; and (3) to determine if other 141 chemicals were contributing to elutriate toxicity.

143 2.0 MATERIALS AND METHODS

144

145 2.1 Definitive unionized ammonia toxicity test

- 146 Water only test solutions for the UIA toxicity test were prepared by spiking filtered, 0.45
- 147 Φm, saltwater with a 1000 mg/L TAN solution (Thermo Orion Applied Solution
- 148 Ammonia Standard, NH₄Cl). The nominal concentrations were 0, 1, 2, 4, 8 and 16 mg/L

149 TAN. The saltwater was collected from Manasquan Inlet, NJ, USA.

150

151 2.2 Test sediments

152 Samples for the sediment bioassays were collected from five locations within the Peconic

153 Estuary, NY, USA. Surficial samples were collected with a Van Veen grab sampler

154 modified to allow access to the top 2 cm of sediment. A reference sediment was obtained

155 from the Narrow River, a tributary of Narragansett Bay, RI, USA. The reference sample

156 has been previously tested and is a fine grained organically enriched sample that supports

157 high densities of the marine amphipod *Ampelisca abdita* but its suitability to support high

- 158 survival and hinge production with *Spisula solidissima* has not been previously
- 159 evaluated. All sediment samples were sieved through a 0.5 mm stainless steel sieve to

160 remove indigenous macrofauna, large debris, and any potential predators.

161

162 2.3 Ammonia purging of sediments

163 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
- 165 covered with 4500 mL of 0.45 micron filtered natural saltwater in a procedure developed

166	by Ferretti et al. (2000). The overlying water was aerated and the basins covered to
167	exclude dust and minimize evaporation. On the third and sixth day after setup, 90% of
168	the overlying saltwater was renewed. After seven days, the overlying water was
169	siphoned off and test elutriates were prepared from the purged sediments.
170	
171	2.4 Elutriate preparation
172	Each purged and unpurged sediment sample was homogenized for two minutes using a
173	mechanical stirrer. One part sediment and four parts filtered saltwater were then
174	combined and mixed on a rotary soil tumbler for 30 minutes and allowed to settle
175	overnight at 4 ^o C. Centrifugation or further filtration of the elutriates was not necessary.
176	A peristaltic pump was used to siphon the supernatants into 2 L containers. The elutriates
177	were tested within 24 h. A saltwater control was tested in parallel with the elutriates. A
178	1-L subsample from each purged elutriate sample was spiked with ammonia to match the
179	TAN measured in the corresponding unpurged elutriate sample.
180	
181	2.5 Test organisms
182	Adult Spisula solidissima were received from the Woods Hole Marine Biological
183	Laboratory, Woods Hole, MA, USA, in moist straw and held overnight at 4 $^{\circ}$ C. To
184	induce spawning, the chilled clams were placed in large basins with aerated saltwater at
185	20 $^{\circ}$ C. All specimens were separated into individual 2 L crystallization dishes with
186	seawater during release of gametes. Gametes from three males and three females were
187	prepared in accordance with ASTM methods (1994). Sperm and eggs were combined for
188	one hour and embryos were placed in test chambers within two hours of fertilization.

189	The sensitivity of a subset of the bivalve larvae was tested using potassium chloride as a
190	reference toxicant. Fertilized eggs develop into pyramid shaped, planktonic trochophore
191	larvae approximately 9 h after fertilization at 21.7 $^{\circ}$ C (Ropes, 1980) and reach veliger
192	larvae stage in 28 hours at 22.0 °C (Loosanoff and Davis, 1963). Although size was not a
193	measurement in this study, veliger larvae averaged 89 x 71 microns after 24 hours in a
194	study by Ropes (1980).
195	
196	2.6 Test conditions
197	Tests were conducted in accordance with ASTM E724. The test endpoints were survival
198	and shell development. The initial number of organisms per replicate was determined by
199	averaging the densities in ten dummy chambers which were inoculated at even intervals
200	among all of the test chambers. Spisula solidissima were exposed as embryos (60-120
201	minutes post fertilization) and exposed for 48 hours. Normally developed larvae (veliger
202	stage) were identified by their distinct "D" shaped shell at 125x magnification.
203	
204	2.7 Water quality
205	Three surrogate chambers were set up for each sediment elutriate treatment and the
206	saltwater control in the sediment bioassay tests for measurement of TAN, dissolved
207	oxygen and salinity at 0, 12, 24 and 48 h of testing. These surrogate chambers were
208	inoculated with embryos at the start of testing so that water quality parameters were
209	similar as possible to the actual test chambers.

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

212	ammonia spiked elutriate replicate was adjusted with 1.0 N and 0.1 N NaOH (0.05 - 0.2
213	mL), to match the pH of the corresponding unpurged sample. The pH of each spiked and
214	unpurged elutriate replicate was then measured every 12 hours of the 48 hour tests. Tests
215	were conducted in temperature controlled water baths (Lab Line Biotronnete) and
216	temperature was measured daily in each test chamber. Samples for TAN analysis were
217	preserved with concentrated sulfuric acid and stored at 4 $^{\rm O}$ C and held for less than 2
218	weeks prior to testing. Total ammonia measurements were performed using an Orion
219	Ionalyzer model 407A with an Orion model 95-12 ammonia sensitive electrode in
220	accordance with manufacturer's instructions and Standard Methods, Ammonia Selective
221	Electrode Method (APHA et al., 1999). Total ammonia nitrogen was converted to UIA
222	using the Free Ammonia-Nitrogen Calculator, December 1998, Purdue University, West
223	Lafayette, IN, USA and verified using the American Fisheries Society saltwater ammonia
224	calculator (http://www.fisheries.org/afs/hatchery.html).
225	
226	Dissolved oxygen, pH, and salinity were measured daily using a Yellow Springs
227	Instrument model 58 dissolved oxygen meter (Yellow Springs, OH, USA), a Hanna
228	Instruments pH meter model 9219 (Woonsocket, RI, USA), and a Reichert temperature

229 compensating refractometer (Depew, NY, USA), respectively.

230

231 2.8 Statistical analysis

232 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,

235	June 1994, U.S. EPA, Cincinnati, OH, USA) was used to calculate the UIA LC50 and
236	EC50 values for survival and development endpoints respectively. One way unpaired t-
237	tests ($p = 0.05$) were performed comparing sediment toxicity test elutriate treatments (i.e.
238	unpurged & purged, purged & spiked, unpurged & spiked) for each sample. A linear
239	regression was calculated between the transformed development endpoint and UIA using
240	Statistix Analytical Software, 1996, Tallahasse, FL, USA.
241	
242	3.0 RESULTS
243	
244	3.1 Definitive unionized ammonia toxicity test
245	The Manasquan saltwater control mean survival was 94.9% and normal development
246	89.7%. Both results meet the minimal acceptable limits of 70% and 60% for survival and
247	development, respectively (Table 1) (ASTM, 1994).
248	
249	The pH, temperature and salinity measured during the water only toxicity test averaged
250	8.14 \forall 0.06, 19.7 \forall 0.4 ^o C and 31 \forall .5 ^o / _{oo} respectively. <i>Spisula solidissima</i> survival and
251	development are listed in Table 1 along with measured TAN and calculated UIA values.
252	
253	The 48 hour LC50 concentration for survival in the water only exposures for ammonia
254	was 10.6 mg/L TAN (+/- 0.4) and 0.53 mg/L UIA (+/01). Normal hinge development
255	was the more sensitive endpoint. The 48 hour EC50 concentration for development was
256	0.53 mg/L TAN (+/01) and 0.12 mg/L UIA (+/01) (UIA EC25 for Development =
257	0.079 mg/L +006) (Table 1). There was a strong relationship established between

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

261

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 duration of the test (data not presented). pH was measured between 7.8 and 8.3 for all 264 265 samples and generally did not vary by more than 0.10 pH unit within each unique sample. Survival in all samples was greater than 72.9% (data not presented). Since shell 266 267 development was the more sensitive endpoint, results and discussion will focus on this endpoint only. Shell development ranged from zero in four samples to over 92% in two 268 of the purged samples (Table 2). There was a significant increase in shell development in 269 270 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 samples (4 & 5) toxicity returned but the samples were not as toxic as the corresponding 274 275 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 was characterized with an r^2 value of 0.83. 278

279

4.0 DISCUSSION

4.1 Definitive unionized ammonia toxicity test

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

304	duration, salinity and other water quality related test conditions render direct comparisons
305	and relevance to other studies difficult. However, Spisula solidissima larvae appear to be
306	as or more sensitive to ammonia than values published for other commonly used
307	laboratory marine toxicity test organisms used for testing marine sediments, elutriates and
308	sediment porewaters. A summary of published ammonia threshold values for organisms
309	commonly tested in water only, sediment or sediment elutriate exposures are summarized
310	in Table 3.
311	
312	4.2 Sediment elutriate bioassays using bivalve larvae as a test organism
313	Bivalve larval tests have been commonly employed in ambient surveys (Becker et al.,
314	1990; His et al., 1999; Hunt and Anderson, 1993; Geffard et al., 2001) and have been
315	routinely used in testing of sediment elutriates of proposed dredged materials (USEPA
316	and USACE 1991). Our data show that Spisula solidissima are sensitive to relatively low
317	levels of ammonia and ammonia toxicity in elutriates prepared from surficial sediments
318	in our study was higher than expected. During testing of dredge material, the potential
319	for ammonia toxicity to test organisms is higher than surficial sediments because of the
320	general trend of increasing ammonia concentrations as sediment depth increases (Frazier
321	et al., 1996). Our water only ammonia toxicity tests predicted significant effects on
322	Spisula solidissima shell development and were confirmed by our tests on the six
323	sediment elutriates. All of the unpurged sediment elutriates had UIA similar or greater
324	than the calculated water only EC50 of 0.12 mg/L UIA and shell development was
325	significantly affected in all six unpurged samples (Table 2).
326	

327 To determine if UIA was causing the toxicity to *Spisula solidissima* in terms of shell 328 development, ammonia was removed from the baseline sediments by purging with 329 seawater. The UIA in all the purged samples was reduced below the EC50 of 0.12 mg/L. 330 Development increased significantly in all the purged samples when compared to the 331 corresponding unpurged sample (Figure 1). Shell development in sample 5 was 59.2% 332 after purging ammonia which is lower than the survivals obtained in the other five purged 333 elutriate samples (Table 2). This would indicate that other contaminants, in addition to 334 ammonia, are contributing to toxicity in Sample 5. Ho et al. (2002) found that during 335 toxicity identification evaluations of 13 sediment porewater samples that there was no 336 one predominant cause of toxicity. Rather, there were typically multiple causes including 337 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 338 339 is causing toxicity in the baseline sediments in our study, ammonia was added back into a 340 subsample from each purged sediment elutriate to mimic the TAN measured in the 341 unpurged elutriate (baseline) sample. Toxicity was measured in the six ammonia spiked 342 samples indicating that ammonia was responsible for some or all of the observed toxicity 343 in the unpurged sediment elutriates. Toxicity was not completely recovered in samples 4 344 and 5 of the ammonia spiked elutriates (Table 2). The non recovery of all of the observed 345 toxicity in the baseline samples could be due to variability of the ammonia measurements 346 or pH differences during the exposure. Other factors including changes in 347 oxidation/reduction potential or subsequent changes in contaminant availability (i.e. 348 sulfides) during the purging process may have contributed to the reduced toxicity 349 measured in these two ammonia spiked samples. However, from the lower than expected

- 350 shell development in purged Sample 5, it is likely that there were other causative agents
- 351 besides ammonia contributed to the observed toxicity in this sample.
- 352

353 **5.0 CONCLUSIONS**

- 354 Unionized ammonia has been shown to be toxic to marine test species in sediment,
- 355 elutriate, and porewater samples. Ammonia can confound results when testing
- 356 sediments. Water only toxicity tests indicate that larvae of the surf clam, Spisula
- 357 solidissima, are one of the more ammonia sensitive marine species. The LC50 for
- 358 survival was 10.58 mg/L TAN (0.53 mg/L UIA) and the EC50 for shell development was
- 359 2.35 mg/L TAN (0.12 mg/L UIA). Tests on elutriates prepared from surficial sediments
- 360 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
- 362 concentrations be measured on all elutriates prior to testing with embryos and larvae of
- 363 marine bivalves and compared to published values. Lowering of ammonia to below
- 364 toxicity thresholds in sediments prior to testing should be considered or choosing a more
- 365 ammonia tolerant test species may be appropriate in other cases depending on the

366 objective of the study.

367

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- 375 The use of trade names does not constitute endorsement by the U.S. Environmental
- 376 Protection Agency
- 377
- 378
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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.

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Table 1.Results of ammonia spiked seawater toxicity test with Spisula sp. larvae (pH 8.14 \forall .06, 19.7 \forall .4°C, 31 \forall 1 °/₀₀).

Targeted TAN (mg/L)	Measured TAN (mg./L)	Unionized Ammonia (mg/L)	Larval % Survival	Normal Larvae % Hinge Development
0	.09 ∀ .01	$.01 \forall 0$	94.9 ∀ 7.1	89.7 ∀ 6.7
1	.97∀ .02	.05 \(\no \)0	95.9 ∀ 13.1	85.5 ∀ 8.3
2	1.83 ∀ .06	.09 ∀ 0	95.8 ∀ 10.2	60.9 ∀ 6.7
4	3.71 ∀ .22	.18 ∀ .02	90.9 ∀ 8.1	2.2 ∀ .8
8	6.80 ∀ .12	.38 ∀ .09	79.9 ∀ 9.1	0
16	16.38 ∀ .14	.74 ∀ .05	2.2 ∀ 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.

Unpurged (Baseline)		Ammonia Purged		Ammonia Purged + Ammonia Spiked			
Sample ID	Ammonia Unionized (mg/L)	% Shell Development	Ammonia Unionized (mg/L)	% Shell Development	Ammonia Unionized (mg/L)	% Shell Development	
REF	.14 ∀ .03	$46.3 \forall 6.8^{a}$.03 ∀ .01	78.0 ∀ 7.3	.14 ∀ .01	56.9 ∀ .10.9ª	
1	.34 ∀ .07	$1.1 \forall 1.5^{a}$.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	$0.3 \; \forall \; 0.6^{\mathrm{a}}$.05 ∀ .01	87.6 ∀ 3.9	.32 ∀ .05	0 ^a	
4	.16 ∀ .07	$9.2 \forall 1.7^{a}$.05 ∀ .01	92.8 ∀ 9.2	.21 ∀ .02	26.1 \forall 10.9 ^{ab}	
5	.10 ∀ .01	$2.0 \forall 0.5^{a}$.01 \(\forall \.00	59.2 ∀ 14.8	.09 ∀ .02	23.0
∀ 8.1 ^{ab}							

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

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

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

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.