



HAL
open science

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

James A. Ferretti, Diane F. Calesso

► To cite this version:

James A. Ferretti, Diane F. Calesso. Toxicity of ammonia to surf clam (*Spisula*) larvae in saltwater and sediment elutriates. *Marine Environmental Research*, 2011, 71 (3), pp.189. 10.1016/j.marenvres.2011.01.002 . hal-00673198

HAL Id: hal-00673198

<https://hal.science/hal-00673198>

Submitted on 23 Feb 2012

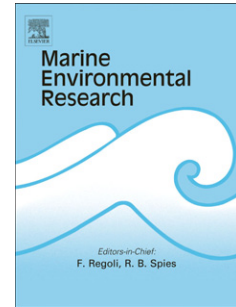
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

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

Authors: James A. Ferretti, Diane F. Calesso



PII: S0141-1136(11)00004-3

DOI: [10.1016/j.marenvres.2011.01.002](https://doi.org/10.1016/j.marenvres.2011.01.002)

Reference: MERE 3498

To appear in: *Marine Environmental Research*

Received Date: 25 August 2010

Revised Date: 13 January 2011

Accepted Date: 13 January 2011

Please cite this article as: Ferretti, J.A., Calesso, D.F. Toxicity of ammonia to surf clam (*Spisula solidissima*) larvae in saltwater and sediment elutriates, *Marine Environmental Research* (2011), doi: 10.1016/j.marenvres.2011.01.002

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

30

31

32 JAMES A. FERRETTI*^H, DIANE F. CALESSO ^H

33 H.U.S. Environmental Protection Agency, 2890 Woodbridge Avenue, Edison, NJ 08837,

34 USA

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49 *Corresponding Author's: 2890 Woodbridge Ave., Edison, N.J. 08837; Tel.:1 + 732-321-

50 6728; Fax: 1 + 732-906-6165; Email address: ferretti.jim@epa.gov

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
55 larvae of the surf clam, *Spisula solidissima*. Elutriates were prepared and tested using six
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
63 species. The LC50 for survival was 10.6 mg/L total ammonia (0.53 mg/L unionized
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
66 toxicity test were good predictors of effects observed in the sediment elutriate tests.

67

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

69

70

71

72

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 through 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

94 Scientific evaluation of bivalves are important because many species are commercially
95 and/or recreationally important. One of these species is the Surf Clam, *Spisula*
96 *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).
119

120 The American Society for Testing and Materials (1994) recommends an interim effect
121 concentration threshold for *Crassostrea. gigas* of 0.13 mg/L as unionized ammonia
122 (UIA) at a pH of 7.7 - 8.1. Geffard (2002) estimates the total ammonia nitrogen (TAN)
123 EC20 for *C. gigas* as 2.8 mg/L. Huber et al. (1997) found that 10 day juvenile *Mulinia*
124 *lateralis*, the dwarf surf clam, is one of the more ammonia sensitive species with an UIA
125 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.
131 For studies where ammonia is treated as a toxicity artifact, standard procedures to
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
134 sediment, elutriate or porewater studies. For example, ammonia may be considered a
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.

142

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, NH_4Cl). 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
164 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 °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 °C. To
184 induce spawning, the chilled clams were placed in large basins with aerated saltwater at
185 20 °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 °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.

210

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 °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
233 both the UIA definitive test and the sediment elutriate toxicity tests. For the saltwater
234 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, Tallahassee, 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 ± 0.06 , $19.7 \pm 0.4^{\circ}\text{C}$ and $31 \pm .5^{\circ}/_{\text{oo}}$ respectively. *Spisula solidissima* 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 ($\pm .01$). Normal hinge development
255 was the more sensitive endpoint. The 48 hour EC50 concentration for development was
256 0.53 mg/L TAN ($\pm .01$) and 0.12 mg/L UIA ($\pm .01$) (UIA EC25 for Development =
257 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.

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
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.

279

280

281 4.0 DISCUSSION

282 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 galloprovincialis* in an ammonia
289 reference toxicant test revealed a 48 hour EC50 of 0.036 mg/L UIA (McDonald, 2005).
290 *M. galloprovincialis* 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
338 contributor to toxicity in 69% of the samples tested. To support the hypothesis that UIA
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
361 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

368 **ACKNOWLEDGMENT**

369 This work was supported by the Division of Environmental Science and Assessment and
370 the Division of Environmental Policy and Protection of the U.S. EPA Region 2. We
371 thank Deborah Kay and Toshi Shintani for their analytical support, as well as Dennis
372 McChesney, U.S. EPA Region 2, and Robert Waters, New York Suffolk County

373 Department of Health Services, for sample collection.

374

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

376 Protection Agency

377

378

379 **6.0 REFERENCES**

380 Adams W.J., Berry W.J., Burton Jr. G.A., Ho K.T., Macdonald D., Scroggins R., Winger
381 P.V., 2001. Summary of the SETAC Workshop on Porewater Toxicity, March 18-22,
382 2000; Pensacola, FL. Society of Environmental Toxicology and Chemistry (SETAC).
383 Pensacola, FL, USA. 28p.

384

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

388

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

391

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

395

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

- 398 mollusks. ASTM E 724-94. ASTM. Philadelphia, PA.
399
- 400 American Society for Testing and Materials, 2006. Standard guide for conducting acute,
401 chronic and life cycle aquatic toxicity tests with polychaetous annelids. ASTM E
402 1562-00. ASTM. Philadelphia, PA.
403
- 404 American Society for Testing and Materials, 2007. Standard guide for conducting
405 sediment toxicity tests with polychaetous annelids. ASTM E 1611-00. ASTM.
406 Philadelphia, PA.
407
- 408 Becker D.S., Bilyard G.R., Ginn T.C., 1990. Comparisons between sediment toxicity
409 tests and alterations of benthic macroinvertebrate assemblages at a marine
410 superfund site: Commencement Bay, Washington. *Environmental Toxicology and*
411 *Chemistry* 9, 669-685.
412
- 413 Carr, R.S., and Chapman, D.C., 1992. Comparison of solid phase and porewater
414 approaches for assessing the quality of marine and estuarine sediments. *Journal of*
415 *Chemical Ecology* 7, 19-30.
416
- 417 Cesar, A., Marin-Guirao, L., Vita, R., 2002. Sensitivity of Mediterranean amphipods and
418 sea urchins to reference toxicants. *Ciencias Marinas* 28, 407-417.
419
- 420 Eyster, L.S. and Morse, M.P., 1984. Development of the Surf Clam (*Spisula solidissima*)
421 Following Exposure of Gametes, Embryos and Larvae to Silver. *Archives of*
422 *Environmental Contamination and Toxicology* 13, 641-646.
423

- 424 Fay, C. W., Neves, R. J., Pardue, G. B., 1983. Species Profiles: Life Histories and
425 Environmental Requirements of Coastal Fishes and Invertebrates (Mid-Atlantic),
426 Surf Clam. Department of Fisheries and Wildlife Sciences, Virginia Polytechnic
427 Institute and State University, Blacksburg, VA. Technical Report No. EL-82-4: 23
428 p.
- 429
- 430 Ferretti J.A., Calesso D.F., Hermon T.R., 2000. Evaluation of methods to remove
431 ammonia interference in marine sediment toxicity tests. *Environmental Toxicology*
432 *and Chemistry* 19, 1935-1941.
- 433
- 434 Frazier B.E., Naimo T.J., Sandheinrich M.B., 1996. Temporal and vertical distribution of
435 total ammonia nitrogen and unionized ammonia nitrogen in sediment porewater
436 from the upper Mississippi River. *Environmental Toxicology and Chemistry* 15, 92-
437 99.
- 438
- 439 Geffard O., Budzinski H., His E., Seaman M.N.L., Garrigues P., 2002. Relationships
440 between contaminant levels in marine sediments and their biological effects on
441 embryos of oysters, *Crassostrea gigas*. *Environmental Toxicology and Chemistry*
442 21, 2310-2318.
- 443
- 444 Geffard O., Budzinski H., Augagneur S., Seaman M.N., His E., 2001. Assessment of
445 sediment contamination by spermiotoxicity and embryotoxicity bioassays with sea
446 urchins (*Paracentrotus lividus*) and oysters (*Crassostrea gigas*). *Environmental*

- 447 Toxicology and Chemistry 20, 1605-1611.
- 448
- 449 Greenstein, D.J., Alzadjali, S., Bay, S.M., 1996. Toxicity of ammonia to purple sea
450 urchins. In: Southern California coastal Research Project Annual Report (1994-
451 1995), pp. 72-77.
- 452
- 453 His E., Beiras R., Seaman M.N.L., 1999. The assessment of marine pollution bioassays
454 with bivalve embryos and larvae. *Advances in Marine Biology*, Volume 37.
455 Southward AJ, Tyler PA, Young CM, Eds. Academic Press, New York, NY, USA.
456 pp. 1-178.
- 457
- 458 Ho K.T., Burgess R.M., Pelletier M.C., Serbst J.R., Ryba S.A., Cantwell M.G., Kuhn A.,
459 Raczelowski P., 2002. An overview of toxicant identification in sediments and
460 dredged materials. *Marine Pollution Bulletin* 44, 286-293.
- 461
- 462 Huber M., Pelletier M.C., Charles J.B., Burgess R.M., 1997. Ammonia tolerance of the
463 bivalve *Mulinia lateralis* sublethal sediment toxicity test. *Bulletin of*
464 *Environmental Contamination & Toxicology* 59, 292-291.
- 465
- 466 Hunt J.W., Anderson B.S., 1993. From Research to Routine: A review of toxicity testing
467 with marine mollusks. *Environmental Toxicology and Risk Assessment*. ASTM
468 STP 1179. Landis W.G., Hughes J.S., Lewis M.A., Eds., American Society for
469 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.

- 495
496 Nelson, D.A., Miller, J.E., and Calabrese, A., 1988. Effect of Heavy Metals on Bay
497 Scallops, Surf Clams, and Blue Mussels in Acute and Long Term Exposures.
498 Archives of Environmental Contamination and Toxicology 17, 595-600.
499
- 500 Ropes, J.W., 1982. The Atlantic coast surf clam fisher, 1965-1974. US National Marine
501 Fisheries. Services, Marine Fisheries Review 44(8), 1-14.
502
- 503 Ropes, J.W., 1980. Biological and fisheries data on the Atlantic surf clam, *Spisula*
504 *solidissima*. U.S. Dep. Comm., NOAA, NMFS, Northeast Fisheries Center Tech.
505 Ser. Rep No. 24, 91 p.
506
- 507 U.S. Environmental Protection Agency & U.S. Army Corps of Engineers. 1998.
508 Evaluation of dredged material proposed for discharge in waters of the U.S. -
509 testing manual. EPA/823/B-98-004. Washington, DC, USA.
510
- 511 U.S. Environmental Protection Agency. 1994. Methods for assessing the toxicity of
512 sediment associated contaminants with estuarine and marine amphipods.
513 EPA/600/R-94/025. Final Report. Office of Research and Development,
514 Washington, DC.
515
- 516 U.S. Environmental Protection Agency, 1993. Refinements of Current PSDDA
517 Bioassays: Final Report Summary. Water Division, Region 10, Seattle WA, EPA
518 910/R-9-93-014a.
519
- 520 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.

Research Highlights, MERE –D-10-00194

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	23.0
	∇ 8.1 ^{ab}						

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

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

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>Amplelisca 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

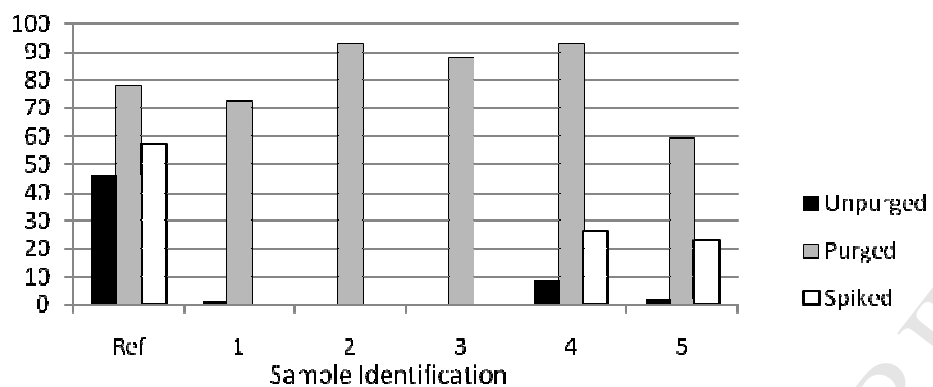


Figure 1. Comparison of percent normal shell development of bivalve larvae exposed to sediment elutriate samples which were tested as baseline, purged of ammonia and spiked to approximate baseline ammonia concentrations.