Toxicity of Ammonia to Pacific Purple Sea Urchin (*Strongylocentrotus purpuratus*) Embryos

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Ammonia is a common constituent of aquatic and marine environments. It is present both as a natural breakdown product of nitrogenous organic matter and as a contaminant from wastewater discharges and river run-off. Ammonia occurs in both ionized (NH_4^+) and un-ionized (NH_3) forms. The prevalence of these two forms is dependent on the pH, temperature, and salinity of the water. For the conditions commonly encountered in the marine environment, more than 95% of the total ammonia is in the ionized form.

The toxicity of ammonia has been well established for many marine species (USEPA 1989). Early studies of ammonia toxicity indicated that the un-ionized form caused most of the toxicity (Willingham 1976). More recent studies have demonstrated that either toxicity from the ionized form is greater than expected or that toxicity results from an interaction between other parameters (e.g., pH) and the un-ionized form (Thurston and Russo 1981, Miller *et al*. 1990, Borgmann 1994).

Sediment samples were collected for toxicity testing as part of the Southern California Bight Pilot Project (SCBPP) (see other related articles in this annual report). Interstitial water extracted from sediments at most of these stations was found to be toxic to Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryos (see *Sediment Toxicity in the Southern California Bight*, in this annual report). Further, most of the toxic interstitial water samples had total ammonia concentrations greater than 4 mg/L, which previous work indicated might cause toxicity to sea urchin embryos (SCCWRP, unpublished data).

We conducted a series of experiments to investigate the source of the ammonia encountered and determine whether ammonia was the cause of the toxicity. The first was a sediment storage experiment to track interstitial water ammonia concentration over time. This experiment also examined the effect of sediment homogenization, a common sample preparation procedure, on interstitial water ammonia concentration. Additional experiments using seawater spiked with ammonia were conducted to determine the dose-response relationship between ammonia and toxicity to purple sea urchin embryos. Finally, an exposure of sea urchin embryos to spiked seawater at varying pH levels was conducted to determine the relative toxicities of ionized and un-ionized forms of ammonia. The results of this series of experiments are presented below.

MATERIALS AND METHODS

Sediment for the storage experiment was collected using a 0.1 m^2 Clamshell Box Core (MEC Analytical Systems, Inc., Carlsbad, CA). Sediment was collected from three stations (8C, 9C, 10C) of the County Sanitation Districts of Los Angeles County benthic sampling grid on the Palos Verdes Shelf that had characteristics (e.g., grain size and organic content) similar to those of SCBPP stations. Duplicate cores were taken from each station. The top 3-4 cm of sediment was taken from each core and transferred to each of two 0.5 L plastic jars. Aboard ship, 10-13 ml of interstitial water was collected from each replicate core, using a squeezing apparatus (Kalil and Goldhaber 1973) and transported to the laboratory on ice for pH and ammonia analysis.

Seven days after collection, the upper 2-3 cm of sediment from one replicate jar for each station was removed and centrifuged at 3,000 x g for 30 min. The supernatants were then analyzed for pH and ammonia. The sediment in the second replicate jar from each station

was homogenized and then split into two smaller plastic jars and stored at 4°C.

Subsamples of the undisturbed and homogenized sediments were analyzed for pH and ammonia on days 16, 21, and 41. A sediment sample was removed from each jar (care was taken to minimize additional mixing of the sediment) and the interstitial water was obtained by centrifugation as described previously. Interstitial water was also obtained on day 41 using the squeezing apparatus in order to examine the effect of differences in extraction methods on the results.

All pH measurements were made with an Orion model 290 A meter. Total ammonia measurements were conducted using an Orion model 95-12 ammonia electrode. Un-ionized ammonia concentrations were calculated using the methods of Hampson (1977). Grain-size analysis was performed on representative samples from each station using the methods of Plumb (1981). Total organic carbon (TOC) and total nitrogen (TN) analyses were performed using methods of SCCWRP (1992).

Three separate exposures were performed to determine the ammonia dose-response relationship. Exposure concentrations of ammonia were prepared from dilutions of a stock solution of NH_4Cl dissolved in 0.45 µm filtered laboratory seawater. Nominal total ammonia concentrations ranged from 1.0 to 5.6 mg/L. All ammonia solutions were prepared fresh for each exposure. Exposures were performed on Pacific purple sea urchin embryos for 72 h at 15°C, in 22 ml scintillation vials following the methods of USEPA (1995). All pH, ammonia, and total ammonia measurements and calculations were made as described above.

Embryo exposure and ammonia concentration preparation for the pH manipulation experiment used the same methods as the dose-response experiments. To manipulate pH, exposures were conducted inside three separate Plexiglas chambers. Testing was performed over a pH range of 7.7-8.7. A quantity of CO_2 equal to 1.2% of the chamber volume was added to lower the pH of test solutions to about 7.7. To achieve a pH of approximately 8.5, test solutions were adjusted to a pH of 8.5 using 0.1 M sodium carbonate and placed in a chamber containing a CO_2-free atmosphere (79% N_2/ 21%O_2). A test solution pH of about 8.7 was maintained by placing a 40 ml beaker containing a 5% KOH solution and a filter paper wick in a chamber containing regular air. Another set of samples was exposed at the ambient pH of about 8.1. For each pH, four replicates were exposed at each of five ammonia concentrations; the nominal values were 0, 2.5, 5.0, 10.0 and 20.0 mg/L total ammonia for pH 7.7 and 8.1, and 0, 1.0, 2.5, 5.0 and 10.0 mg/L total ammonia for pH 8.5 and 8.7. All exposures were conducted at 15°C for 72 h.

Either paired t-tests or Wilcoxon signed-rank tests (Jandel Scientific 1994) were used to compare sample types from the storage experiments. Median effective concentrations (EC50s) for all urchin exposures were calculated using the TOXIS software package (EcoAnalysis, Inc., Ojai, CA). Logit regressions of dose-response curves were calculated using SigmaPlot (Jandel Scientific 1994).

RESULTS

Storage Effects

The initial total ammonia concentration for all three stations was less than 2 mg/L. The values doubled after seven days of storage and by 41 days, all concentrations were 35 mg/L or greater (Figure 1). There was no significant difference between the homogenized and undisturbed samples at any single sampling time; however, when all times were pooled, the homogenized samples contained significantly more total ammonia than the undisturbed samples (Table 1). There was no significant difference in ammonia concentration between interstitial waters obtained by centrifugation and squeezing (Table 1). The pH of the interstitial water was fairly stable throughout the storage period, with the trend in most cases being slightly downward (Figure 2).

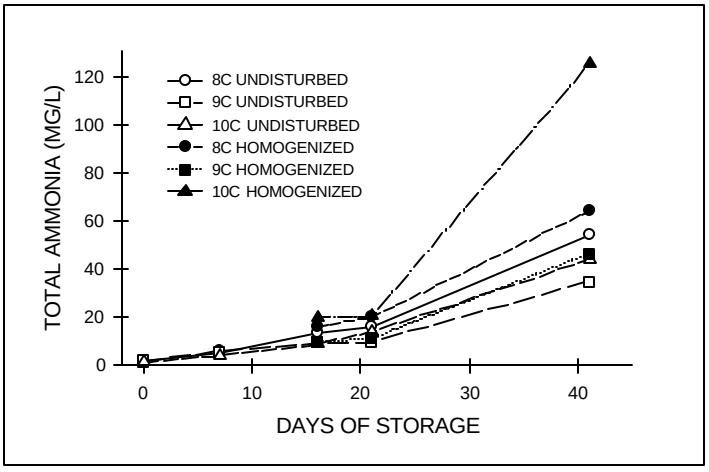

FIGURE 1. Changes in ammonia concentration of interstitial water from sediment storage experiment, plotted by duration of storage. (8C, 9C, and 10C are County Sanitation Districts of Los Angeles County benthic sampling stations.)

Ammonia Toxicity

Results of the three ammonia dose-response exposures were combined for analysis. The percentage normal data plotted against the measured un-ionized ammonia concentration fitted well to a logit regression

(r^2=0.967; Figure 3). This dose-response curve was very steep, indicating that once the toxicity threshold concentration is achieved, small increases in ammonia concentration lead to large increases in toxicity. The mean EC50 for the three dose-response exposures was 3.8 mg/L total ammonia with a standard deviation of 0.2 mg/L. When expressed as un-ionized ammonia, the EC50 was 0.057 mg/L with a standard deviation of 0.005 mg/L.

TABLE 1. Paired t-test results for comparison of interstitial water sampling methods.

Comparison	Day	N	t	p	Significance
homogenized vs. undisturbed	16	3	1.47	0.278	ns
homogenized vs. undisturbed	21	3	2.90	0.100	ns
homogenized vs. undisturbed	41	3	1.45	0.280	ns
homogenized vs. undisturbed	16-41	9	-	0.009	*
centrifuged vs. squeezed	41	3	0.72	0.542	ns

[a]signed rank test performed instead of paired t-test.
ns=no significant difference.
s=significant differentes ($p \leq 0.05$).

pH/Ammonia Interactions

Dose-response curves based on total ammonia vary widely, indicating greater toxicity with increasing pH (Figure 4). When the data are expressed as un-ionized ammonia, the curves for each pH value overlap (Figure 5). For total ammonia concentration, small changes in pH led to large changes in both un-ionized ammonia concentration and toxicity. The EC50 values for total ammonia decreased (indicating greater toxicity) with increasing pH, while the EC50 values for un-ionized ammonia were very similar (Table 2).

DISCUSSION

The sediment storage experiment demonstrated that interstitial water ammonia concentration can change dramatically over time. Comparisons of the interstitial water concentrations with the dose-response curve for ammonia (Figure 3) indicates that the interstitial water ammonia increased from low levels at the time of collection to toxic levels after 41 days. The increases in ammonia are probably associated with microbial activity within the sediment.

While the ammonia concentration increased greatly over time, pH either remained the same throughout or decreased slightly. However, the pH decreases were sufficient to lower the un-ionized ammonia concentrations from about 1.5% to approximately 0.6% of the total ammonia concentration.

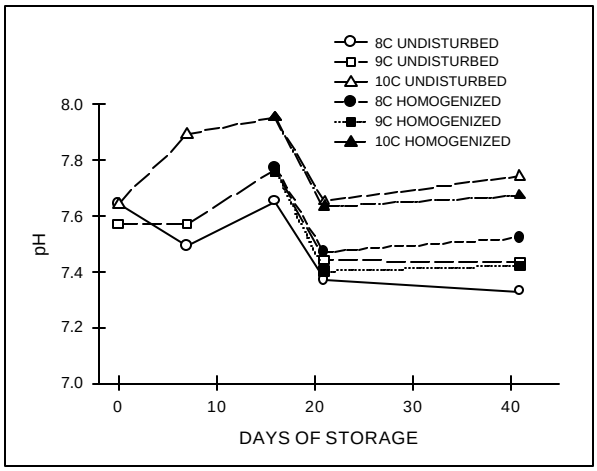

FIGURE 2. Changes in pH of interstitial water from sediment storage experiment, plotted by duration of storage. (8C, 9C, and 10C are County Sanitation Districts of Los Angeles County benthic sampling stations.)

Other studies have found changes in sediment toxicity with storage time. Dillon *et al*. (1994) found that sediment toxicity increased for the first four weeks of storage, but stabilized thereafter. Becker and Ginn (1995) tested the effects of sediment storage on three different organisms and found that toxicity to amphipods increased with time, whereas toxicity to polychaetes and bacteria was highly variable.

Homogenization of the test sediments tended to increase the ammonia content of the interstitial water; however, undisturbed samples also reached concentrations of ammonia that would be expected to cause toxicity. The increased ammonia level noted after homogenization may be due to increased bacterial activity associated with oxygenation of the sediment. Homogenization tended to increase interstitial water ammonia concentration for each sample, but the magnitude of change was sample specific. In this study, much larger changes were measured for sediment from station 10C after 41 days. Station-specific changes may have been the result of unintentional differences in sample handling during the experiment or to differences in the type of organic material present at each location.

We found no difference in ammonia content between the centrifugation and squeezing methods of interstitial water collection. However, ammonia was the only interstitial water constituent measured. Other investigators have found changes in interstitial water toxicity associated with collection technique (Carr and Chapman 1995).

Sensitivity of Pacific purple sea urchin embryos to ammonia was much greater than reported

for most other marine species. However, most of the ammonia toxicity data reported in the literature is for adults. USEPA (1989) provided acute ammonia lethal concentrations (LC50) for 21 saltwater species. Of these species, the most sensitive was the winter flounder *(Pleuronectes (=Pseudopleuronectes) americanus*) with a mean LC50 value of 0.49 mg/L un-ionized ammonia. The amphipod, *Ampelisca abdita* (used in whole sediment toxicity tests for the SCBPP), has a mean LC50 of 0.83 mg/L un-ionized ammonia (Kohn *et al*. 1994).

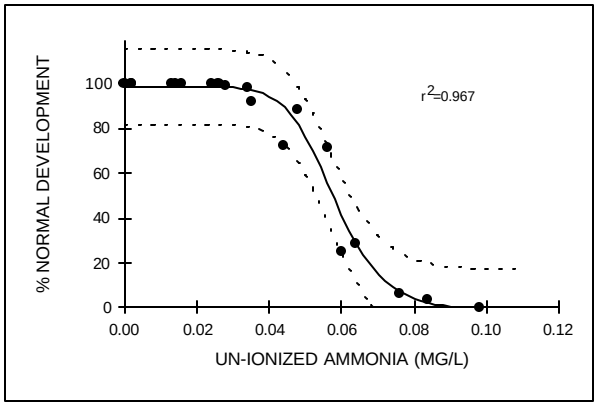

FIGURE 3. Changes in percent normally developed Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryos with the measured un-ionized ammonia concentration. Results are from three separate experiments. Solid line is logit regression. Dashed lines are 95% confidence intervals of the regression.

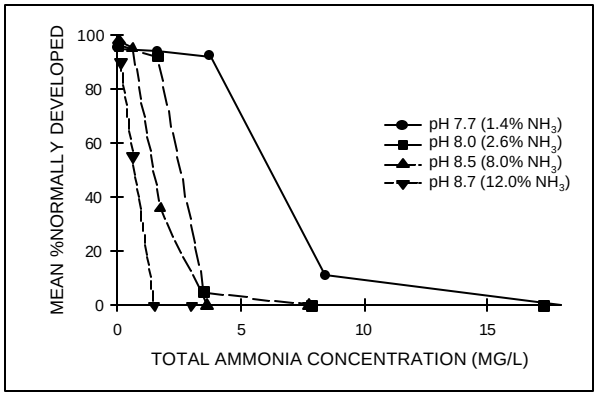

FIGURE 4. Dose response plots of percent normally developed Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryos versus total ammonia concentration at various pH levels.

Ammonia toxicity data for embryos of other invertebrate species are similar to our findings. Exposures of Pacific sand dollar (*Dendraster excentricus*) and Pacific oyster (*Crassostrea gigas*) larvae resulted in un-ionized ammonia LC50 values of 0.03 and 0.13 mg/L, respectively (USEPA 1993), while red abalone (*Haliotis rufescens*) embryos had an LC50 of 0.07 mg/L (Marine Pollution Studies Laboratory, California Dept. of Fish and Game, Granite Canyon, CA, unpublished data).

The increasing toxicity associated with elevated pH indicates that most of the toxicity from ammonia in our sea urchin embryo exposures was associated with the un-ionized form. This is consistent with what most previous studies have found (Willingham 1976, Schubauer-Berigan *et al*. 1995). However, a few researchers have found toxicity associated with the ionized form (Thurston and Russo 1981, Borgmann 1994). Although our spiking experiments were conducted in seawater, we expect that the speciation and toxicity of ammonia will be similar in interstitial water.

The results of our experiments and the work of others lead to several recommendations regarding sample collection and handling. Sediment samples should be tested for toxicity as soon after collection as is feasible. If interstitial water is to be tested, it should be removed from sediment within a few days of collection. It may be best to freeze the interstitial water if testing cannot be done immediately. Carr and Chapman (1995) found that the toxicity of interstitial water collected by squeezing was unaffected by freezing and thawing; however, if interstitial water is collected by other methods, toxicity might increase after freezing.

If possible, the pH of interstitial water should not be adjusted before testing. An increase of only a couple of tenths of a pH unit can greatly increase the proportion of un-ionized ammonia in a sample, thus increasing toxicity. Interstitial water often has an initial pH lower than that of normal seawater. The pH often increases during the course of a toxicity test. If the initial pH is within the tolerance range of the organism being tested, it should not be adjusted.

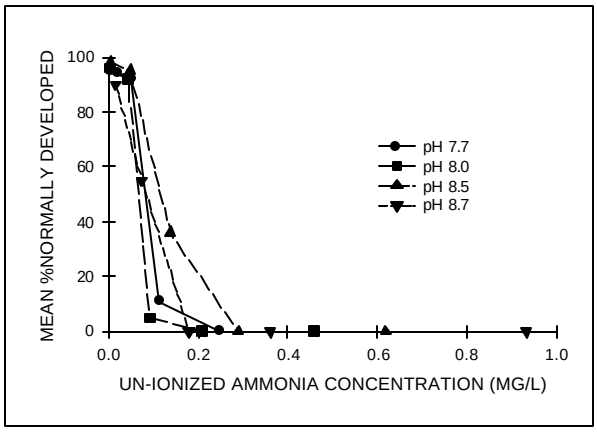

FIGURE 5. Dose response plots of percent normally developed Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryos versus un-ionized ammonia concentration at various pH levels.

TABLE 2. No observed effect concentration (NOEC) and median effective concentration (EC50) for Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryo exposures to ammonia at varying pH.

	Total Ammonia (mg/l)			Un-ionized Ammonia (mg/l)		
pH	NOEC	EC50	95% CI	NOEC	EC50	95% CL
7.7	4.50	7.20	6.16-7.88	0.060	0.096	0.082-0.106
8.1	2.00	2.98	2.65-3.26	0.066	0.098	0.087-0.108
8.4	0.61	1.38	1.25-1.52	0.038	0.088	0.079-0.097
8.7	<0.62	0.08[a]		<0.09	0.12[a]	

[a]EC50 was estimated from the dose response plot; therefore no confidence interval could be calculated.
95% CL=95% confidence intervals surrounding the EC50.

Caution should be exercised when interpreting sediment toxicity data obtained from test organisms that are highly sensitive to ammonia. Elevated ammonia concentrations may be present in whole sediment tests with animals such as amphipods, but concentrations attained in previous SCCWRP experiments have not been high enough to influence the results. Ammonia may be a significant interference in whole sediment toxicity tests of other sediment types. The high sensitivity of sea urchin embryos is likely to result in interferences when testing interstitial water samples. Results for most of the interstitial water samples tested during the SCBPP were affected by ammonia toxicity (see related article in this annual report) and the data presented in this article aided in identifying this interference. Previous SCCWRP toxicity tests of interstitial water used test methods (e.g., sea urchin fertilization test, Microtox) that are much less sensitive to ammonia; results from these studies do not reflect ammonia toxicity interferences.

Work at SCCWRP is continuing on issues regarding sediment sampling, handling, and storage. Future studies will involve spiking interstitial water with ammonia and testing various means of storing samples.

CONCLUSIONS

Ammonia levels of sediments stored at 4°C can increase greatly with time due to microbial activity and thus increase the toxicity of interstitial water samples. Sediment and interstitial water tests should be conducted as rapidly after sediment collection as possible.

Un-ionized ammonia is more toxic to Pacific purple sea urchin embryos than the ionized form. Since pH plays a large role in determining the relative abundance of the two forms of ammonia, the pH of interstitial water samples should not be adjusted.

Knowledge of the un-ionized ammonia concentration and sensitivity of the test organism is essential for evaluating sediment toxicity results.

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