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Influence of Potentially Confounding Factors on Sea Urchin Porewater Toxicity Tests

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Abstract. The influence of potentially confounding factors has been identified as a concern for interpreting sea urchin porewater toxicity test data. The results from >40 sediment-quality assessment surveys using early–life stages of the sea urchin *Arbacia punctulata* were compiled and examined to determine acceptable ranges of natural variables such as pH, ammonia, and dissolved organic carbon on the fertilization and embryological development endpoints. In addition, laboratory experiments were also conducted with *A. punctulata* and compared with information from the literature. Pore water with pH as low as 6.9 is an unlikely contributor to toxicity for the fertilization and embryological development tests with *A. punctulata*. Other species of sea urchin have narrower pH tolerance ranges. Ammonia is rarely a contributing factor in pore water toxicity tests using the fertilization endpoint, but the embryological development endpoint may be influenced by ammonia concentrations commonly found in porewater samples. Therefore, ammonia needs to be considered when interpreting results for the embryological development test. Humic acid does not affect sea urchin fertilization at saturation concentrations, but it could have an effect on the embryological development endpoint at near-saturation concentrations. There was no correlation between sediment total organic carbon concentrations and porewater dissolved organic carbon concentrations. Because of the potential for many varying substances to activate parthenogenesis in sea urchin eggs, it is recommended that a no-sperm control be included with every fertilization test treatment.

Porewater toxicity tests have been routinely employed to assess the bioavailability of sediment-associated contaminants since the late 1980s (Carr and Nipper 2003a). The potential influence of confounding factors, such as pH, ammonia, sulfides, and dissolved organic carbon (DOC), on interpretation of the results has contributed to the reluctance of regulators to adopt porewater testing (Scroggins *et al*. 2003; Nipper *et al*. 2003). A major recommendation from a recent workshop on porewater toxicity testing was the need to better understand the influence of these confounding factors on porewater toxicity tests (Carr & Nipper 2003b). Since the early 1990s, numerous large-scale comprehensive sediment-quality assessment studies have been conducted in our laboratory using samples from coastal areas of the United States, including Hawaii, Mexico, and the Philippines. This database comprises porewater toxicity tests with sea urchins that have been conducted using identical protocols for >40 different studies. It affords an opportunity to examine the influence of several potentially confounding factors on the test results. In addition, some parameters were tested in the laboratory at environmentally relevant concentrations to specifically evaluate the effects of these parameters on the different test endpoints. The results of the database analyses and these additional experiments are presented here to aid in the interpretation of porewater toxicity test results from past and for future studies. In addition, potentially confounding factors that could induce parthenogenesis, as well as the influence of naturally occurring haloaromatic compounds, are also discussed as they relate to the interpretation of pore water toxicity data.

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Materials and Methods

Porewater Collections

Pore water for these different studies was obtained by a variety of methods. For the majority of the samples, sediments were collected by Young or modified-Van Veen grab or with a box core. Usually only the top 2 cm was removed, and multiple grabs were composited and homogenized at a particular station (Carr *et al*. 1996a). In most of the studies conducted on the Texas coast, 10-cm i.d. cores of 8 to 10 cm depth were composited and homogenized and then subsampled for chemical analyses and toxicity testing (Carr *et al*. 1996b, 2000). In tropical areas with coarse sand sediments where it was not possible to collect samples with a grab, porewater samples were collected *in situ* by divers using a vacuum method described in Carr *et al*. (2003).

When composited sediment samples were collected, the sediments were placed in precleaned polyethylene bottles and shipped on blue ice to the Marine Ecotoxicology Research Station (MERS) in Corpus Christi, TX, where the pore water was extracted as soon as possible

after arrival (usually within 24 hours and none >48 hours). Porewater samples collected *in situ* were either centrifuged and then frozen or shipped to MERS on blue ice and then centrifuged and stored frozen as described later.

Porewater Extraction Procedure

Porewater was extracted from the sediments using a pneumatic extraction device (Carr 1998). This extractor is made of polyvinyl chloride, uses a 5-µm polyester filter, and has been used successfully in numerous sediment-quality assessment surveys (Carr and Chapman 1992, 1995; Carr *et al*. 1996a, 1996b). After extraction, the porewater samples were centrifuged in polycarbonate bottles at 1200 × *g* for 20 minutes to remove any suspended particulate material; the supernatant was collected and frozen at –20°C. The freezing–thawing procedure has been shown not to affect the toxicity of porewater samples compared with fresh samples as long as the samples are particle free before freezing (Carr and Chapman 1995).

Before toxicity testing, the samples were thawed in a tepid (20°C) water bath. Sample salinity was measured with a Reichert refractometer (Cambridge Instruments, Inc., Buffalo, NY) and, if necessary, adjusted to 30 ± 1 ppt using purified deionized water or concentrated brine prepared by slow evaporation of seawater. It has been observed that a 10% addition of the reference pore water eliminates artifactual toxicity in the reconstituted brine reference sample, which we believe is caused by the loss or slow dissolution of essential trace elements from the concentrated brine. By adding this small amount of reference pore water to the reconstituted brine solution, these essential trace elements are restored and the toxicity eliminated. Temperature and dissolved oxygen (DO) were measured with YSI meters, and DO was adjusted by gently stirring the sample if the measured concentration was <80% saturation. Sulfide (as S^{-2}), total ammonia (expressed as nitrogen), and pH were measured with Orion meters (Orion Research, Inc., Boston, MA) and their respective probes. Unionized ammonia (expressed as nitrogen) concentrations were calculated for each sample based on the respective pH and total ammonia values and on test salinity (30 ppt) and temperature (20°C) (Bower and Bidwell 1978).

Porewater Toxicity Testing With Sea Urchins

Sediment toxicity was assessed using pore water in the sea urchin (*A. punctulata*) fertilization and embryological development tests according to the methodology described by Carr and Chapman (1992) and Carr *et al*. (1996a). The sea urchin fertilization test involved exposure of the sperm for 30 minutes, followed by the addition of a predetermined number of eggs. After an additional 30-minute incubation period, the test was terminated by the addition of 10% buffered formalin, and the percentage of fertilized eggs was determined. The sea urchin embryological development test was executed concurrently with the fertilization test. Eggs were prefertilized and then inserted into the exposure vials. The embryos were exposed to the test solutions for 48 hours, at which time the test was terminated by the addition of 10% buffered formalin. Aliquots from each of the five replicates were examined microscopically to determine the percentage of embryos that developed normally to the echinopluteus stage.

A. punctulata urchins used in this study were obtained from Gulf Specimen Company (Panacea, FL) or Marine Biologic Supplies (Woods Hole, MA). Each of the porewater samples was tested in a dilution series design at 100%, 50%, and 25% of the water quality–adjusted (salinity adjusted to 30 ± 1 ppt and DO >80% saturation, if necessary) sample with 5 replicates/treatment. Dilutions were made with 0.45 µm filtered seawater. A reference porewater sample collected from Redfish Bay, TX, which had been handled identically to the test samples, was included with each toxicity test as a negative control. This site is far removed from any known sources of contamination and has been used as a reference site in numerous studies (*e.g.*, Carr and Chapman 1992, 1995; Carr *et al.* 1996a, 1996b). A complete chemical and physical characterization of this reference sediment is provided elsewhere (Carr *et al*. 1998). In addition, a dilution series test with sodium dodecyl sulfate was included as a positive control.

Laboratory pH Experiments

A variety of methods were tried in an attempt to manipulate the pH of the test solutions, which either caused toxicity on their own or were impractical because of instability of the resulting pH. The first method tried, which was only partially successful, was the direct addition of 1 M or 0.1 M HCl to decrease pH or 1 M or 0.1 M NaOH to increase pH. Although it was found that the addition of NaOH to the solutions resulted in a stable pH of the test solution, this was the case only if the test vials were filled to near the top and only a small headspace was left; this modification was used for the pH unit treatments greater than ambient. Using our typical 5-ml sample size/replicate resulted in unacceptable pH stability (>0.5 pH unit change) during the test period, possibly resulting from the headspace air influencing the pH. However, successful pH stability could not be achieved with the acidified samples even with full vials, so a different approach using carbon dioxide was used for lower pHs.

The method successfully used to lower pH levels was based on methods described in the United States Environmental Protection Agency Marine Toxicity Identification Evaluation Phase I Guidance Document (1996). An atmospheric chamber measuring 70 × 48 × 38 cm was constructed of acrylic sheets with two gas entry ports for air and carbon dioxide and two glove ports for sample manipulation. By varying the levels of the two gasses, the pH of the solutions inside could be adjusted lower. The chamber was not airtight, but positive pressure from the gas influx prevented atmospheric gasses from entering. Typically the sample of seawater was placed in the chamber approximately 12 to 16 hours before testing and allowed to mix on a magnetic stir-plate until the stable test pH was achieved, whereupon the sample would be pipetted into sample vials also contained within the chamber. The entire acidification component of the fertilization test was performed within the chamber with ambient pH controls being tested outside the chamber at the same time. Sperm and egg dilutions were brought into the chamber through one of the glove ports. Only one pH level was tested at a time because the chamber can be adjusted only for a particular pH. Testing for the fertilization assay was done according to methods described earlier for standard porewater toxicity tests (5 ml/vial). Toxicity testing for the embryological development test was done according to similar pH adjustment procedures; however, vials were filled nearly to the top (approximately 23 ml) instead of the typical volume (approximately 5 ml). The vials, capped tightly after embryo introduction, were then removed from the atmospheric chamber and placed in an incubator at 20°C for 48 hours until the test was terminated by the addition of buffered formalin as described previously.

Three other methods were unsuccessfully tried to decrease pH in the test solutions, all with negative results. The first method was the addition of 1M boric acid as described by Novelli *et al*. (2003). Our testing found that boric acid appeared to be toxic to the sperm of *A. punctulata* and, therefore, was discarded as a possible adjustment buffer. A second method to decrease pH was tried based on the work of Smith and Clowes (1924). This method relied on the preparation of carbon dioxide–free water by acidifying seawater with 2 N HCl to a pH of approximately 3.0 and the addition of 10 N NaH_2PO_4 buffer to replace the lost buffering capacity. The resulting solution was brought back to test pH levels with 10 N NaOH. This method was found to be unworkable because the addition of any NaOH to the buffered carbon dioxide–free water resulted in heavy precipitation. Attempts to

remove the precipitates by centrifuging and decanting the supernatant resulted in unstable pH. In an attempt of our own design, it was noticed that the addition of the 10 N NaH_2PO_4 to seawater had an acidifying affect, which resulted in stable pHs. Unfortunately, NaH_2PO_4 was also toxic to sea urchin sperm, even at very low concentrations (unpublished data, 2004).

Porewater Dissolved Organic Carbon Measurement

DOC was measured in the porewater samples using an OI Analytic Model 1010 Wet Oxidation Total Organic Carbon Analyzer according to the model 1010 operator's manual (OI Analytic 1998). Previously filtered (0.45 μm cellulose nitrate) frozen samples were thawed in a tepid water bath on the day of analysis and run in duplicate. Samples were analyzed in total organic carbon (TOC) mode with 400 μl acid (5% phosphoric acid) and 4000 μl oxidant (200 g/L sodium persulfate). Total inorganic carbon react and detect times were 2:00 (minutes:seconds) and 1:35, respectively. Total organic carbon react and detect times were 8:30 and 2:00, respectively. At least one blank was run with each batch of samples. In addition, laboratory control samples and duplicates were run for every 10 to 15 samples. The analysis was repeated if the percent recovery of the laboratory control failed to meet the 90% to 110% level. Humic acid samples were also measured using this same method.

Statistical Analyses

Statistical comparisons among treatments for a particular study were made using analysis of variance (ANOVA) and Dunnett's one-tailed *t* test (which controls the experiment-wise error rate) on the arcsine square root–transformed data with the aid of SAS (SAS 1989). Before statistical analysis, the transformed data sets were screened for outliers (SAS 1992). Outliers were detected by comparing the Studentized residuals to a critical value from a *t*-distribution chosen using a Bonferroni-type adjustment. The adjustment is based on the number of observations, *n*, so that the overall probability of a type I error is at most 5%. The critical value (cv) is given by the following equation: $cv = t\ [df_{Error},\ 0.05/(2 \times n)]$. After omitting outliers but before further analysis, the transformed data sets were tested for normality and for homogeneity of variance using SAS/LAB software (SAS 1992).

A second criterion was also used to compare test means with reference means. Detectable significance criteria (DSC) were developed to determine the 95% confidence values based on power analysis of all similar tests performed by our laboratory (Carr and Biedenbach 1999). This value is the percent minimum significant difference from the reference necessary to detect a significant effect while minimizing type I errors. The DSC value for the sea urchin fertilization assay is 15.5% at $\alpha = 0.05$ and 19% at $\alpha = 0.01$. For the embryological development assay, the DSC value is 16.4% at $\alpha = 0.05$ and 20.6% at $\alpha = 0.01$. Only results that were significantly different from the reference sample and were below the DSC were considered significantly toxic.

The EC_{50} (median effective concentration) of the reference toxicants tests was calculated using the trimmed Spearman-Karber method (Hamilton *et al*. 1977) with Abbott's correction (Morgan 1992). Regression analyses were performed with the aid of SigmaPlot®) (SigmaPlot® 2000).

Results

The results of sea urchin fertilization tests with *A. punctulata* from 2424 samples regressed against the porewater unionized ammonia concentrations are shown in Figure 1. For the sea urchin fertilization test, only 0.6% of the 2424 samples tested had unionized ammonia concentration >800 μg/L, the lowest observed effect concentration (LOEC), and only 3.6% were greater than the no observed effect concentration (NOEC) of 400 μg/L. Comparable results for the embryological development test are shown in Figure 2. Of 1866 porewater samples, 18.6% of the undiluted samples had unionized ammonia >90 μg/L, the LOEC, whereas 25% were lower than the NOEC of 30 μg/L for the sea urchin embryological development endpoint. LOEC and NOEC values for both types of test were derived from laboratory experiments using filtered seawater spiked with NH_4Cl (Carr *et al*. 1996b).

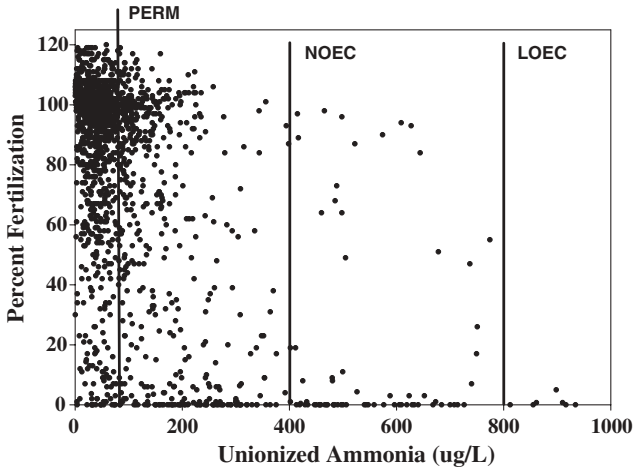

Fig. 1. Relationship between control-normalized fertilization test results with *A. punctulata* and the measured unionized ammonia concentration in field-collected porewater samples ($n = 2424$). Porewater effects range median (PERM) calculated as the 50th percentile of the unionized ammonia concentration in the toxic samples

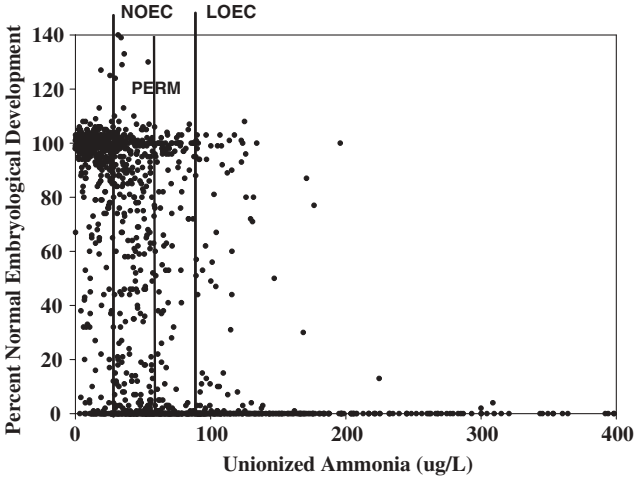

Fig. 2. Relationship between control-normalized normal embryological development with *A. punctulata* and the unionized-ammonia concentration in field-collected porewater samples ($n = 1866$). Porewater effects range median (PERM) was calculated as the 50th percentile of the unionized ammonia concentration in the toxic samples

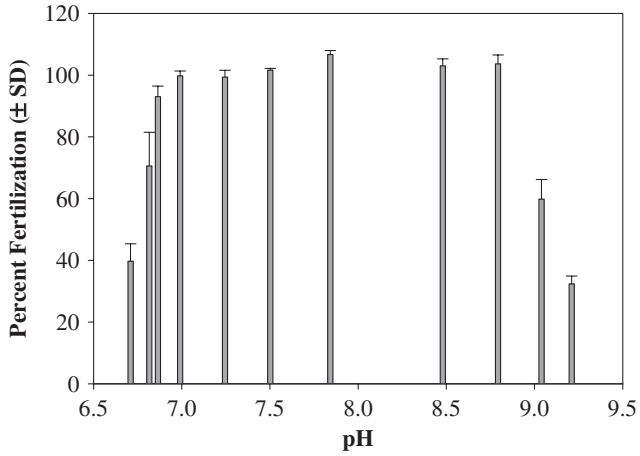

Fig. 3. Results of control-normalized percent fertilization with *A. punctulata* at different pH values

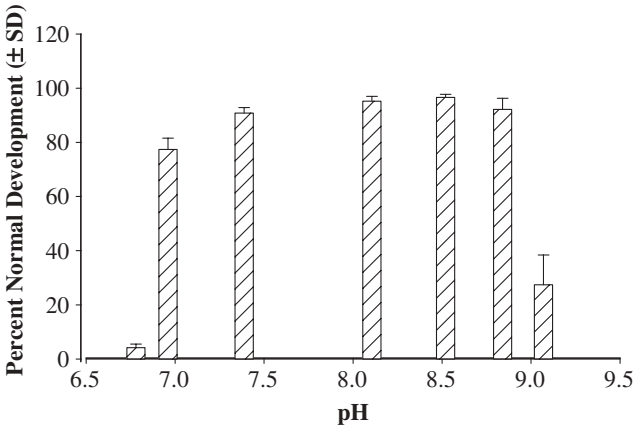

Fig. 5. Results of embryological development tests with *A. punctulata* at different pH values. Percent normal development values are control normalized

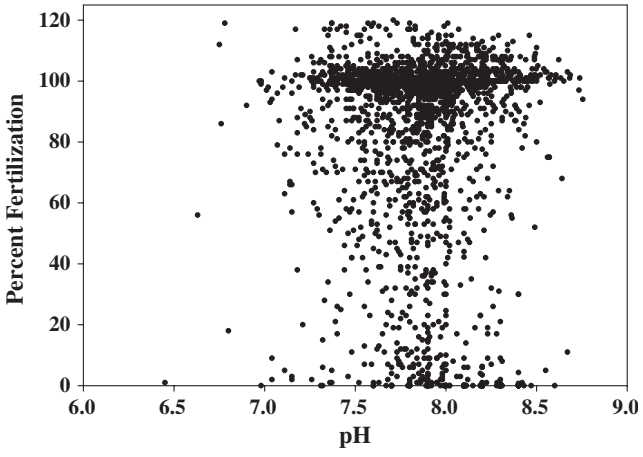

Fig. 4. Relationship between control-normalized fertilization test results with *A. punctulata* and pH of porewater samples from field-collected sediments (n = 2424)

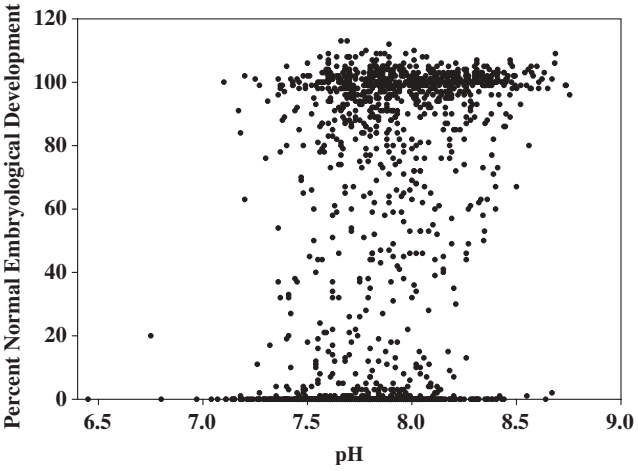

Fig. 6. Relationship between control-normalized embryological development with *A. punctulata* and pH of porewater samples from field-collected sediments (n = 1866)

The acceptable pH range for the fertilization endpoint in filtered seawater exposures with *A. punctulata*, with the pH adjusted using carbon dioxide or NaOH, was 6.9 to 8.8 (Fig. 3). Porewater samples from field studies with pHs as low as 6.8 were not toxic to sea urchin fertilization (Fig. 4). For the embryological development endpoint, the acceptable pH range was also 6.9 to 8.8 (Fig. 5). This compares well with the data for the embryological development test from the field studies (Fig. 6). Less than 4% of the porewater samples collected in field studies by the methods described had sulfide concentration higher than the detection limit of 10 μg/L. Of the porewater samples with measurable concentrations of sulfide, 88% and 99% were toxic in the fertilization and embryological development tests, respectively (data not shown).

No decrease in fertilization was observed in laboratory tests with sea urchins exposed to humic acid dissolved in filtered seawater at saturation concentrations (43 mg/L), but an EC_{50} of 32.4 mg/L (NOEC = 23 mg/L, LOEC = 43 mg/L) was calculated for the embryological development endpoint. There was no clear correlation between measured DOC in porewater samples and toxicity to sea urchin fertilization (Fig. 7) or embryological development (Fig. 8). Less than 3% of the 544 porewater samples measured for DOC in our laboratory have been observed to be higher than the embryological development EC_{50}. There was no correlation between the whole-sediment TOC levels and the associated porewater DOC concentrations (Fig. 9).

Discussion

The fertilization endpoint with *A. punctulata* is relatively insensitive to ammonia. Only a small percentage of field-collected porewater samples exhibited concentrations greater than the water-only NOEC of 400 μg/L unionized ammonia. Using the approach of Long *et al*. (1995), the porewater effects range median concentration (PERM; 50th percentile unionized ammonia value of the significantly toxic samples) was calculated to be 85 μg/L. It can be inferred from the data shown in Figure 1 that ammonia is not primarily responsible for the

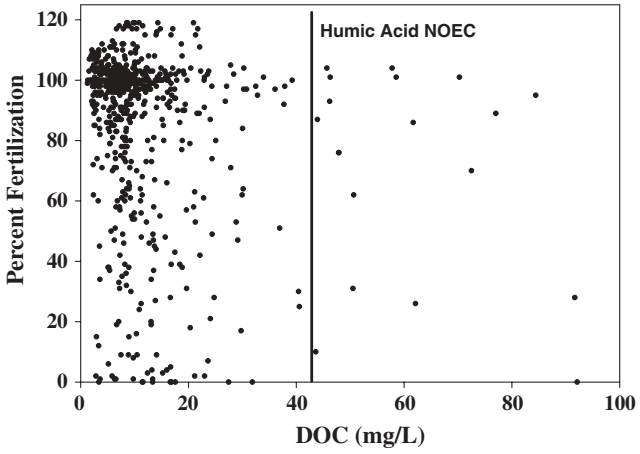

Fig. 7. Relationship between control-normalized fertilization test results with *A. punctulata* and the measured DOC in field-collected porewater samples (n = 678). The NOEC value for humic acid derived from water-only laboratory experiments was the saturation concentration in seawater

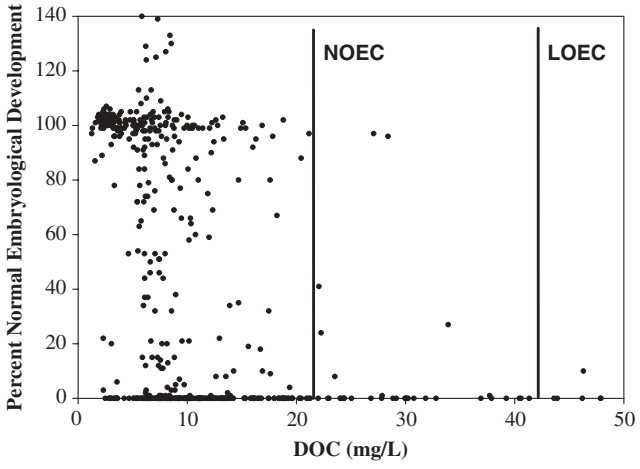

Fig. 8. Relationship between control-normalized embryological development test results with *A. punctulata* and the measured DOC in field-collected porewater samples (n = 544). NOEC and LOEC values for humic acid were derived from water-only laboratory experiments

toxicity at concentrations lower than the NOEC and must be covarying with other toxicants present in the porewater samples because there were numerous nontoxic samples considerably higher than the PERM.

The embryological development endpoint, however, is considerably more sensitive to ammonia with a NOEC in filtered seawater exposures of 30 μg/L unionized ammonia. The majority (approximately 75%) of field-collected porewater samples have unionized ammonia concentrations higher than this value. Other constituents of porewater may actually provide some additional protective effect in this test because porewater unionized ammonia concentrations as high as 200 μg/L have been measured in nontoxic samples (Fig. 2). A NOEC of 45 μg/L unionized ammonia has been reported for the embryological development endpoint of the sea urchin *Paracentrotus lividus* (Basuyaux *et al*. 1999). Other

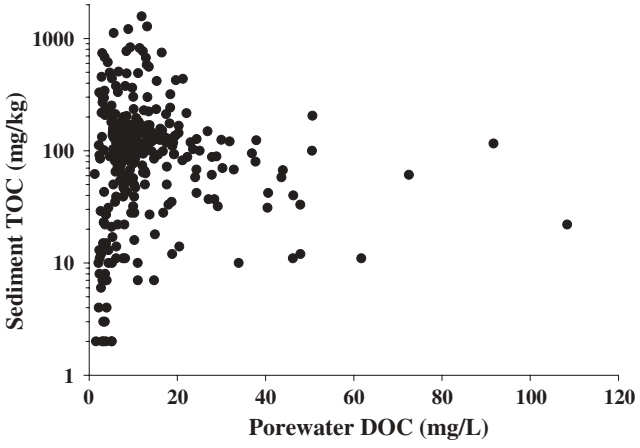

Fig. 9. Relationship between TOC and porewater DOC (n = 356)

researchers have shown that the NOEC for *P. lividus* embryotoxicity decreases from 11 to 4 μg/L unionized ammonia between pH 7.7 to 8.3, respectively (Novelli *et al*. 2003). Additional testing (*e.g.*, toxicity identification evaluation procedures) may be necessary to identify the contribution of ammonia to observed toxicity for the embryological development endpoint when concentrations are higher than the NOEC value for a particular species. By performing serial dilutions of the porewater sample, it is often possible to decrease the ammonia concentrations to an acceptable level while toxicity resulting from other toxicants is still evident. It is recommended that the fertilization test be performed in conjunction with the embryological development test to help interpret test results when ammonia is likely to be a confounding factor for the more sensitive endpoint.

Bay *et al*. (2003) reported pH tolerance limits for the fertilization and embryological development tests with the sea urchin *Strongylocentrotus purpuratus*. The acceptable pH range was 7.2 to 8.2 and 7.4 to 8.3 for the fertilization and embryological development tests, respectively. The acceptable pH range for the sea urchin *A. crassispina* was reported to be 6.6 to 9.4 and 7.4 to 9.0 for the fertilization and embryological development tests, respectively (Kobayashi 1973). Smith and Clowes (1924) reported that the acceptable tolerance range for fertilization with *A. punctulata* was 6.7 to >10.2. Data from the present study with water-only exposures indicated that an acceptable tolerance range for the fertilization and embryological development tests with *A. punctulata* were 6.9 to 8.8, which agrees well with the field data shown in Figures 4 and 6. These laboratory experiments were repeated using Texas reference pore water adjusted to different pHs (unpublished data, 2005), and the results were essentially identical to the filtered seawater pH experiments shown in Figures 3 and 5. Two populations of *A. punctulata* (from Woods Hole, MA, and Panacea, FL) were also compared for their pH tolerance, and it was observed that the Woods Hole population was slightly more sensitive (0.2 pH units) than the Panacea population on the acidic side for both the fertilization and embryological development endpoints. There is obviously a wide variation in pH tolerance ranges for different species of sea urchins for these endpoints. The acceptable tolerance ranges for any new test species and population needs to be determined during

methods development to aid in the interpretation of test results with porewater samples.

Sulfide is rarely present at a concentration higher than the method detection limit of 10 μg/L in porewater samples collected by the methods described. When measurable concentrations of sulfide are present, the samples are usually toxic, particularly for the embryological development test. NOEC and LOEC values for *S. purpuratus* embryological development testing were reported as 0.10 and 0.13 mg/L total sulfides, respectively (Knezovitch *et al*. 1996). If the sulfide sensitivity of *A. punctulata* is similar to *S. purpuratus*, then sulfide concentrations lower than the detection limit are unlikely to be responsible for any observed toxicity. Because the toxic concentration is close to the method detection limit and we do not currently know the NOEC and LOEC values for *A. punctulata*, we cannot be absolutely sure that there was no influence of sulfide toxicity for our data set. This potentially confounding factor could easily be eliminated, however, by purging the H_2S from the samples by gentle stirring or aeration and verification of the sulfide concentration using a more sensitive method.

It was surprising to find that there was absolutely no correlation between the TOC concentrations of whole sediment and their respective porewater DOC concentrations. There is little information in the literature pertaining to the effects of DOC on sea urchin toxicity tests. Using humic acid as a representative for one of the most common types of organic carbon found in marine sediments, no toxicity was found at saturation concentrations with the fertilization test, but toxicity was observed for the embryological development endpoint at near-saturation concentrations. Although the DOC complex associated with specific porewater samples is likely to be composed of a complex variety of substances, it does not appear that DOC concentration is likely to be a major confounding factor in sea urchin porewater toxicity tests. However, its role in affecting the bioavailability of contaminants in pore waters should not be underestimated.

In 1899, Jacques Loeb published an article describing his experiments with *A. punctulata* in which he provided the first report of ''artificial parthenogenesis'' (Loeb 1899). Loeb found that he could initiate embryological development by treating sea urchin eggs with various inorganic salts. Further studies by Loeb and others found that this parthenogenesis could be induced by a wide variety of chemical and physical agents, which have been summarized by Harvey (1956). The list of agents that can initiate parthenogenesis in sea urchin eggs includes acids, alkali, amines, alcohols, solvents, alkaloids, surfactants, various proteins, ammonia, chlorine, sucrose, ultraviolet light, heat or cold, and many others (Harvey 1956). In 1913, Heilbrunn published the first of several papers in which he theorized that all of these different parthenogenetic or membrane-increasing agents act through a similar mechanism: They all decrease the surface tension of the vitelline membrane. When the surface tension decreases below a certain threshold, elevation occurs, which for many of these agents also initiates parthenogenesis (Heilbrunn 1913, 1915).

In light of the fact that this phenomenon appears to be initiated by a broad range of agents and stimuli, it would seem prudent to assess whether this artifactual response is occurring during the sea urchin fertilization test, which uses the elevation of the fertilization membrane as the endpoint. We recommend that a no-sperm control be included with every test sample to determine whether there are substances present in the sample, which may be producing this response in a sample that is spermatotoxic.

It is not uncommon to observe significant toxicity with sea urchin porewater tests from sediments which contain none, or very low, levels of the priority contaminants that are routinely measured in most coastal monitoring programs. Apart from the fact that only a small fraction of the anthropogenic contaminants and their degradation products are included in these routine analyses, another group of chemicals could be responsible for the observed toxicity that is never considered in sediment-quality assessment surveys. A number of different benthic organisms produce and exude a wide variety of haloaromatic compounds (*e.g.*, brominated phenols), presumably to inhibit recruitment by other species or for defensive purposes (Woodin *et al*. 1987, 1993; Woodin 1991). Common polychaete species (*e.g.*, capitellids, glycerids, cirratullids, pectinarids, spionids, terebellids, and nereids) have been shown to produce haloaromatics, which could be toxic in sea urchin porewater toxicity tests (Fielman *et al*. 1999). A number of other marine organisms (*e.g.*, sponges, corals, tunicates, gorgonians, fungi, algae, and bacteria) have also been shown to produce similar organobrominated compounds (Gribble 2000). Because benthic community information is often available for sediment-quality assessment studies, it would be wise to consider these naturally occurring compounds when interpreting sediment and porewater toxicity test results, if organisms known to produce them are present.

Based on the information presented in this article, it should be possible to use sea urchin porewater toxicity data with confidence when potentially confounding parameters fall within established acceptable ranges. When water-quality parameters reach or exceed levels known to produce a significant response, this must be taken into consideration when interpreting the data. Alternatively, additional experiments can be designed to avoid exceeding the acceptable ranges in water-quality parameters. Caveats regarding the occurrence of naturally produced haloaromatic compounds are not limited to porewater tests but could likely influence whole-sediment tests as well. With this knowledge, it is hoped that the reluctance of the regulatory community to include porewater toxicity tests as a useful tool for assessing the quality of sediments will be assuaged.

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