Final Report

on

TOXICITY TESTING OF SEDIMENTS FROM DELAWARE BAY AND SURROUNDING AREAS

submitted to

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Table 4. Sea urchin fertilization test raw data and means for sediment porewater samples from Delaware Bay, and surrounding areas (stations 43-73, 84, 85, 87-92). Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test; + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

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Table 6. Summary of strata means and statistical significance for the sea urchin fertilization test from 22 strata in Delaware Bay and surrounding areas. Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, **$\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test; + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Table 7. Microtox® Basic Assay EC_{50} raw data and means for organic extracts of sediment samples from Delaware Bay and surrounding areas. Asterisks denote significant differences between test and reference stations (Dunnett's *t*-test; * $\alpha \leq 0.05$, **$\alpha \leq 0.01$).

Table 8. Strata means of Microtox® data (EC_{50}s; mg equivalent sediment wet weight) of sediment extracts from Delaware Bay and surrounding areas. Asterisks denote significant differences between test and reference stations (Dunnett's *t*-test; * $\alpha \leq 0.05$, **$\alpha \leq 0.01$).

FIGURES

Figure 1 Sample strata and stations in Delaware Bay and surrounding areas.

Figure 2. Sample stations in strata 1 through 4, 19 and 20. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata)* fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied), and Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

Figure 3. Sample stations in strata 5 through 12, and 21. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata)* fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied), and Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

Figure 4. Sample stations in strata 13, 14 and 22. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata)* fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied), and Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

Figure 5. Sample stations in strata 15 through 18. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata)* fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied), and Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

ATTACHMENTS

Attachment 1. (SOP F10.9) Extraction and Storage of Porewater Samples

Attachment 2. (SOP F10.12) Water Quality Adjustment of Samples

Attachment 3. (SOP F10.6) Sea Urchin Fertilization Toxicity Test

Attachment 4. (SOPB5.266) An *In Vitro* Acute Toxicity Test: Microtox® Basic Test

INTRODUCTION

The National Status and Trends (NS&T) Program of NOAA has shown that some sampling sites in coastal Delaware and New Jersey bays are relatively highly contaminated with a variety of chemicals, and the potential for adverse biological effects at these sites is among the highest of all of the sites in the USA. As part of a multi disciplinary sediment quality survey conducted to determine the severity and spatial extent of the toxicity of surficial sediments of Delaware Bay and the adjoining tributaries and canals, toxicity of sediments collected from these sites was assessed using porewater in the sea urchin (*Arbacia punctulata*) fertilization, and the Microtox® Basic Assay. Sediment samples were collected by NOAA and shipped to the U. S. Geological Survey (USGS) Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas where the tests were performed. Sediment pore water was extracted with a pneumatic apparatus similar to the one used in previous studies (Carr and Chapman, 1992; 1995; Carr et al., 1996a; 1996b; NBS, 1993; 1994; 1995a; 1995b, USGS 1997a; 1997b). Porewater samples were stored frozen until just prior to testing when water quality parameters were measured and adjusted, if necessary. A dilution series (100, 50 and 25%) test design was used to determine the toxicity of sediment porewater samples.

In addition to porewater toxicity testing, aliquots of each sediment were shipped separately to Columbia Analytical Systems Inc., in Kelso, Washington for organic solvent extraction. Extracts were transferred to the USGS Environmental and Contaminants Research Center, Columbia, Missouri and assayed for toxicity using the Microtox® assay (Microbics, 1992).

The specific objectives of this study were to:

- Extract sediment pore water from all 81 sediment samples as soon as possible after receipt of the samples using a pneumatic extraction device.

- Measure water quality parameters (salinity, dissolved oxygen, pH, sulfide, temperature, and ammonia) of thawed porewater samples prior to testing and adjust salinity, temperature, and dissolved oxygen, if necessary.

- Conduct the fertilization toxicity test with pore water using sea urchin (*Arbacia punctulata*) gametes and calculate EC_{50} values where possible. Quality control assays with reference pore water, dilution blanks and a positive control dilution series with sodium dodecyl sulfate (SDS) in conjunction with each test.

- Assay dichloromethane extracts of the 81 sediments with the Microtox® assay and determine EC_{50} values.

- Make statistical comparisons between test and reference stations/strata for the sea urchin and Microtox® assays.

MATERIALS AND METHODS

Sediment Sample Receipt and Tracking

Surficial sediment samples were collected from 81 stations in Delaware Bay and the surrounding areas. Samples were collected by NOAA personnel during September 1997. Samples were placed in presoaked one-gallon high density polyethylene containers, chilled, and shipped in insulated coolers with blue ice. Samples were received by the USGS in Corpus Christi, Texas, the day following shipment. Shipments were accompanied by sample tracking sheets, and samples were logged into laboratory sample tracking systems. The samples were either refrigerated (4 °C) or processed immediately upon receipt. All porewater samples were extracted within 8 days from the time of field collection of sediment, and within 24 hours of arrival at the Corpus Christi laboratory.

Porewater Toxicity Testing

Sediment Porewater Extraction Procedure

Pore water was extracted from the sediments using a pressurized pneumatic extraction device. This extractor is made of polyvinyl chloride (PVC) and uses a 5 μm polyester filter. It is the same device used in previous sediment quality assessment surveys (USFWS, 1992; Carr, 1993; NBS, 1993; 1994; 1995a, 1995b; USGS 1997a; 1997b). The apparatus and extraction procedures are detailed in SOP F10.9 (Attachment 1).

Sediment samples were held refrigerated (4 °C) until the pore water was extracted. Pore water was extracted as soon as possible after receipt of the samples but in no event were the sediments held longer than 2 days from the time of receipt before they were processed. After extraction, the porewater samples were centrifuged in polycarbonate bottles at 1200 x *g* for 20 min to remove any suspended particulate material; the supernatant was collected and frozen.

Two days before conducting a toxicity test, the samples were moved from the freezer to a refrigerator at 4 °C. One day prior to testing, samples were thawed in a tepid water bath. Temperature of the samples was maintained at 20 ± 1 °C. Sample salinity was measured and adjusted to 30 ± 1 $^{o}/_{oo}$, if necessary, using purified deionized water or concentrated brine (see SOP F10.12, Attachment 2). Other water quality measurements (dissolved oxygen, pH, sulfide and ammonia concentrations) were made. Temperature and dissolved oxygen (DO) were measured with YSI® meters; salinity was measured with a Reichert® or American Optical® refractometer; and pH, sulfide (as S^{-2}), and total ammonia (expressed as nitrogen; TAN) were measured with Orion® meters and their respective probes. Unionized ammonia (expressed as nitrogen) concentrations (UAN) were calculated for each sample using the respective salinity, temperature, pH, and TAN values. Any samples containing less than 80% DO saturation were gently aerated by stirring the sample on a magnetic stir plate. Following water quality measurements and adjustments, the samples were stored overnight at 4 °C but returned to 20 ± 1 °C before the start of the toxicity tests.

Porewater Toxicity Testing with Sea Urchins

Toxicity of the sediment pore water was determined using the fertilization test with the sea urchin *Arbacia punctulata* following the procedures outlined in SOP F10.6 (Attachment 3). The sea urchins used in this study were obtained from Gulf Specimen Company, Inc. (Panacea, Florida). Each of the 81 porewater samples was tested in a dilution series design at 100, 50, and 25% of the water quality adjusted sample with 5 replicates per treatment. Dilutions were made with 0.45 μm filtered seawater. A reference porewater sample collected from Redfish Bay, Texas, 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 previously as a reference site (Carr and Chapman, 1992; Carr, 1993a; 1993b; NBS, 1993; 1994; 1995a; 1995b; USGS, 1997a; 1997b), as noted previously. In addition, dilution blanks of filtered seawater and a reconstituted brine (brine with purified deionized water) were also included. A dilution series test with sodium dodecyl sulfate (SDS) was included as a positive control.

Fertilization test statistical comparisons among treatments were made using ANOVA and Dunnett's one-tailed *t*-test (which controls the experimentwise error rate) on the arcsine square root transformed data with the aid of SAS (SAS, 1989). The trimmed Spearman-Karber method (Hamilton et al., 1977) with Abbott's correction (Morgan, 1992) was used to calculate EC_{50} (50% effective concentration) values for dilution series tests when possible. Prior to 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: $cv = t(df_{Error}, .05/(2 \times n))$. After omitting outliers but prior to 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 to reference means. Detectable significance criteria (DSC) were developed to determine the 95% confidence value based on power analysis of all similar tests performed by our lab (Carr and Biedenbach, in press). This value is the percent minimum significant difference from the reference that is necessary to accurately detect a difference from the reference. The DSC value for the sea urchin fertilization assay at $\alpha = 0.05$ is 15.5%. At $\alpha = 0.01$, the DSC value is 19%.

Microtox® Testing

The Microtox® Basic toxicity test was performed with 81 sediment organic extracts from 22 regions in Delaware Bay and the surrounding tributaries, following similar procedures used in testing Puget Sound sediments (U.S. EPA, 1986, 1990, USGS 1997b), San Francisco Bay sediments (Long and Markel, 1992), and Pensacola Bay sediments (Johnson and Long, in press).

Organic Extraction of Sediments

The sediment samples were collected by the NOAA personnel or their contractors and were shipped to Columbia Analytical Services, Inc. of Kelso, Washington, where organic sediment extracts were prepared. The organic sediment extracts were shipped to the Environmental and Contaminants Research Center (ECRC) in Columbia, Missouri for testing. The extractions and transfers were conducted under a laminar flow hood to limit exposure of the samples to light. All sediment samples and extracts were stored in the dark at 4°C. Excess water was decanted and large debris (shells, pebbles, etc.) was discarded prior to initial homogenization of the sediment samples. Each sediment sample was centrifuged for five minutes at 1000 x *g*. Water was removed by decanting with a Pasteur pipette. The moisture content of each sample was determined and recorded. Ten g of sediment were weighed, recorded, and placed into a dichloromethane (DCM) rinsed 50 mL centrifuge tube. Sodium sulfate (approx. 15 g) was added to each centrifuge tube and mixed thoroughly. Spectral grade DCM (30 mL) was then added and mixed. The mixture was shaken for 10 seconds, vented, and tumbled overnight. Then each sample was centrifuged for 5 minutes at 1000 x *g*, and the extract poured into a Kuderna-Danish flask. A Snyder column was attached to the flask, and the DCM extract was concentrated with steam to a final volume of < 2 mL. Acetone (approx. 5 mL) was added to the flask and the volume was concentrated to approximately 2 mL. This acetone procedure was repeated. The extract was quantitatively transferred to a DCM-rinsed 10 mL volumetric flask using acetone to rinse the Kuderna-Danish flask. The extract was concentrated with a gentle stream of nitrogen gas to a volume of approximately 1 mL. Dimethylsulfoxide (DMSO) was added to make a final volume of 1 mL. The organic extracts were typically tested at concentrations from 50 mg equivalent wet wt/ mL to 1.5 mg equivalent wet wt/ mL. A negative control (extraction blank) was prepared using DMSO, the test carrier solvent.

Microtox® Assay

The Microtox® Basic assay (AZUR Environmental, Carlsbad, CA, USA formerly Microbics Corporation) was performed by ECRC in Columbia, Missouri, USA. The analyses of organic sediment extracts was conducted according to the Microtox® Basic protocol and QA/QC performance standards as described by Microbics Corporation (1992) and ECRC SOP B5.266 (Attachment 4) All essential test components (analyzer, liquid reagents, and freeze-dried bacteria) were obtained from AZUR Environmental.

Basic Test: A suspension of luminescent bacteria, *Vibrio fisheri*, formerly *Photobacterium phosphoreum*, (B-NRL 1117, Microbics Corp.) was thawed and hydrated. An aliquot of 10 µL of the bacterial suspension was transferred to a test vial containing the standard diluent (2% NaCl) and equilibrated to 15°C using a temperature-controlled photometer. The amount of light lost per sample was proportional to the toxicity of that test sample. Light loss was expressed as a gamma value and defined as the ratio of light lost to light remaining. The relative sensitivity of Microtox® has been reported by Kaiser and Palabrica (1991) and Johnson and Long (in press).

To determine sediment extract toxicity, each sample was diluted into four test concentrations. Because organic sediment extracts were obtained with DCM, a strong non-polar solvent, the final extract was evaporated and redissolved in DMSO. DMSO was compatible with the Microtox® system because of its low test toxicity and good solubility with a broad spectrum of apolar chemicals (Johnson and Long, in press). The log of gamma values from these four dilutions was plotted and compared with the log of the sample's concentrations. The concentration of the extract that inhibited luminescence by 50% after a 5 minute exposure period, the EC_{50} value was determined and expressed as mg equivalent sediment wet weight. Data were reduced using the Microtox® Data Reduction software package (Microtox® Manual, Microbics Corporation, 1992.). All EC_{50} reports were 5-minutes readings with 95% confidence intervals. All tests were performed in triplicate.

Microtox® Data Analysis

Summary EC_{50} values were reported as the mean of three replicates with variability expressed as standard deviations. Statistical comparisons among treatments were made using ANOVA and Dunnett's one-tailed *t*-test on the log transformed data with the aid of SAS (SAS, 1989).

Microtox® Data Interpretation

A sample was designated toxic using two criteria: the Sediment Reference Index and the Toxicity Reference Index. A whole sediment sample or a DCM sediment extract obtained from Redfish Bay in Texas, USA, was used as a reference standard designated Red Fish to develop a Sediment Reference Index. First, the Redfish Bay sample with an EC_{50} value of 102 mg eq/mL was given the Sediment Reference Index number of 1. Any sample with an EC_{50} value that was significantly *($P \leq 0.05$)* lower than that of the reference sample was designated toxic. For example, a sample with an EC_{50} value of 5.0 mg eq/mL would have a Sediment Reference Index (SRI) number of 20 (Redfish EC_{50} value/ test sample EC_{50} value = SRI number; 102/ 5 = 20). The SRI number is greater than 1, in this example 20-fold greater than the control sediment, and therefore this sample would be designated toxic if the means were also statistically significantly different. Second, to separate endogenous background toxicants in sediments and present a toxicological reference point to address the question "how toxic is toxic?" a common water-soluble chemical contaminant, phenol, was spiked into Redfish Bay sediment extract to develop a Toxicity Reference Index (TRI). The spiked sample with an EC_{50} value of 15 mg eq/mL for the Basic Test was given the Toxicity Reference Index number of 1. Samples with EC_{50} values less than the spiked control had a Toxicity Index number > 1 indicating the sample was more toxic than the model toxicant. For example, a sample with an EC_{50} value of 5.0 mg eq/mL would have a Toxicity Reference Index number of 3.0 (Spiked Reference Standard EC_{50} value/ test sample EC_{50} value = Toxicity Reference Index number; 15/5 = 3.0) suggesting that this sample was about three-fold more toxic.

RESULTS

Porewater Quality Measurements

The sea urchin fertilization test was performed with sediment pore water from all stations. Figure 1 illustrates the area of study, the stations sampled and the strata designations. Table 1 lists the geographic positions of each site in latitude and longitude. Two separate tests were performed, the first containing samples 1-42, and the second containing samples 43-73, 84,85, and 87-92. To satisfy the test salinity requirement of 30 ± 1‰, most samples required salinity adjustments using a 105‰ brine made from seawater or purified deionized water (Baker Chemical lot #K40318) A 20% addition of reference pore water was added to the brine prior to salinity adjustment to replace missing trace elements lost from brine storage or processing.

Table 2 reports the values obtained for the various water quality parameters measured. Sulfide concentrations were below the detection limit of 0.01 mg/L in all samples. Porewater dissolved oxygen concentrations ranged from 7.31 to 9.16 mg/L (96 to114%). Values for pH ranged from 6.76 to 8.23. TAN concentrations ranged from 0.06 to 32.1 mg/L, and UAN ranged from 0.8 to 465.3 μg/L.

Sea Urchin Toxicity Testing

Raw data and means from the fertilization tests are given in Tables 3 and 4. Data from both sea urchin tests is summarized by stations in Table 5 and by strata in Table 6. Seven data points were determined to be outliers (SAS, 1992) for both sea urchin fertilization tests with three of these occurring in the 100% water quality adjusted samples, three in the 50% dilutions, and one in the 2.5 mg/L concentration of the SDS positive control in the second test. No data points were excluded from the Microtox® data set.

EC_{50}s for the SDS positive controls in the fertilization assays were 4.88 and 4.51, and were within the acceptable range for these tests. Acceptable results were obtained from the dilution water blanks and the reconstituted brine blanks for both tests.

TEST #1, (Stations 1-42)

Forty-two samples located in 13 strata were tested in the first fertilization assay. Six stations (3, 21, 23, 25, and 29) were significantly different than the reference in the 100% water quality adjusted concentration of which two of those (21 and 23) were also statistically different at the 50% dilution. However, only stations 21, 23 and 25 met the significance criteria establish by this lab (Figure 3). Station 21 also met the significance criteria at the 50% dilution at $\alpha = 0.05$. EC_{50} concentrations for all treatments were greater than 100%.

TEST #2, (Stations 43-73, 84,85, 87-92)

Thirty-nine samples located in 9 strata were tested in the second fertilization assay. Five stations (56, 57, 60, 89, and 92) were significantly different than the reference in the 100% water quality adjusted concentrations. Two stations (56 and 89) were also statistically different than the reference at the 50% concentration. Of these, four stations (56, 57, 60, and 89) met the significance criteria at the 100% concentration. In addition, station #56 also met the significance criteria at the 50% dilution and was the only treatment for which an EC_{50} concentration (EC_{50} = 45.8 % (42.3-49.6)) could be calculated. EC_{50} concentrations for the remaining treatments were greater than 100% water quality adjusted sample.

Microtox® Toxicity Testing

Microtox® raw data, means and significant indices are presented in Table 7. Summarized Microtox® data by strata is presented in Table 8. Figures 2 through 5 illustrate the location and degree of toxicity observed in both tests. Three data points were determined to be outliers (samples 35, 65, and 69) and were not included in the statistical analysis. Statistical comparison to the non-spiked Redfish Bay reference indicated that 81.5% of the samples were found to be toxic. The most toxic stations included stations 26, 30, 56, and 92 with Sediment Reference Indices (SRI = number of times more toxic than the reference) in excess of 200. Toxic stations were scattered throughout the study area (Figures 2-5). Acute toxicity (stations significantly different than the spiked reference) as indicated by an elevated Toxicity Reference Index (TRI) value revealed that 59% of the samples were acutely toxic. Analysis by strata revealed that 12 of 22 strata (54.5%) were significantly different than the non-spiked reference in the basic test (Table 8). However, when comparing strata means against the spiked reference sediment extract, only 3 of 12 (13.6%) strata (strata #8, #21, and #22) were significantly different or considered acutely toxic.

LITERATURE CITED

Carr, R.S. 1993. Sediment quality assessment survey of the Galveston Bay System. Galveston Bay National Estuary Program report, GBNEP-30, 101 pp.

Carr, R.S. and J. M. Biedenbach. 1998. Use of power analysis to develop detectable significance criteria for sea urchin toxicity tests. In: Ecovision World Monograph Series: Development and Progress in Sediment Quality Assessment. SPB Academic Publishing, Amsterdam, The Netherlands. (in press)

Carr, R.S. and D.C. Chapman. 1992. Comparison of solid-phase and pore-water approaches for assessing the quality of marine and estuarine sediments. Chem. Ecol. 7:19-30.

Carr, R.S. and D.C. Chapman. 1995. Comparison of methods for conducting marine and estuarine sediment porewater toxicity tests-extraction, storage, and handling techniques. Arch. Environ. Contam. Toxicol. 28:69-77.

Carr, R.S., E.R. Long, H.L. Windom, D.C. Chapman, G. Thursby, G.M. Slone, and D.A. Wolfe. 1996a. Sediment Quality Assessment Studies of Tampa Bay, Florida. Environ. Toxicol. and Chem. 15:1218-1231.

Carr, R.S., D.C. Chapman, C.L. Howard, and J.M. Biedenbach. 1996b. Sediment quality triad assessment survey of the Galveston Bay, Texas system. Ecotoxicology 5:341-364.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11:714-719; Correction 12:417 (1978).

Johnson, B.T. and E.R. Long. Rapid toxicity assessment of sediments from large estuarine ecosystems: a new tandem *In Vitro* testing approach. *Environ. Toxicol. Chem.* (In Press).

Kaiser, K.L.E., and V.S. Palabrica. 1991. *Photobacterium phosphoreum* toxicity data index. Water Poll. Res. J. Canada 26:361-431.

Long, E.R. and R. Markel. 1992. An evaluation of the extent and magnitude of biological effects associated with chemical contaminants in San Francisco Bay, California. NOAA Technical Memorandum, NOS ORCA 64, 86pp.

Microbics Corporation. 1992. Microtox® manual, Vol.III, condensed protocols. Microbics Corporation, Carlsbad, CA. 232p.

Morgan, B.J.T. 1992. *Analysis of Quantal Response Data,* London, England: Chapman and Hall, 511 pp.

National Biological Service. 1995a. Toxicity testing of sediments from Biscayne Bay and Surrounding Areas. Report submitted by the National Biological Service to the National Oceanic and Atmospheric Administration, Coastal Monitoring and Bioeffects Division, Seattle, WA, 11 pp. + 17 tables, 11 figures and 4 attachments.

National Biological Service. 1995b. Toxicity testing of sediments from western Florida and coastal South Carolina and Georgia. Report submitted by the National Biological Service to the National Oceanic and Atmospheric Administration, Coastal Monitoring and Bioeffects Division, Seattle, WA, 14 pp. + 35 tables, 10 figures and 4 attachments.

National Biological Survey. 1994. Survey of sediment toxicity in Pensacola Bay and St. Andrew Bay, Florida. Report submitted by the National Biological Survey to the National Oceanic and Atmospheric Administration, Ocean Assessment Division, Seattle, WA, 12 pp. + 24 tables and 5 attachments.

National Biological Survey. 1993. Toxicity testing of sediments from Charleston Harbor, South Carolina and vicinity. Report submitted by the National Biological Survey to the National Oceanic and Atmospheric Administration, Ocean Assessment Division, Seattle, WA, 7 pp. + 16 tables and 4 attachments.

SAS Institute Inc. 1992. *SAS/LAB® Software: User's Guide, Version 6, First Edition*, Cary, NC: SAS Institute Inc., 291 pp.

SAS Institute Inc. 1989. *SAS/STAT® User's Guide, Version 6, Fourth Edition, Version 6, Volume 2.* Cary, NC:SAS Institute Inc., 846 pp.

U.S. EPA. 1986. Recommended protocols for conducting laboratory bioassays on Puget Sound sediments. Final Report. 52pp. Prepared for U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

U.S. EPA. 1990. Recommended protocols for conducting laboratory bioassays on Puget Sound sediments. Final Report. 79 pp. Prepared for U.S. Environmental Protection Agency, Region 10, Puget Sound Estuary Program, Seattle, WA.

U.S. Fish and Wildlife Service. 1992. Amphipod solid-phase and sea urchin porewater toxicity tests of Tampa Bay, Florida sediments. Report submitted by the U.S. Fish and Wildlife Service to National Oceanic and Atmospheric Administration, Ocean Assessment Division, Seattle, WA, 9 pp. + 19 appendices.

U. S. Geological Survey. 1997a. Toxicity testing of sediments from Biscayne Bay, Florida and surrounding areas - Phase II. Report submitted by the USGS to National Oceanic and Atmospheric Administration, Coastal Monitoring and Biological Assessment Division, Seattle, WA, 10pp. + 8 tables, 10 figures and 4 attachments.

U. S. Geological Survey. 1997b. Toxicity testing of sediments from Puget Sound, Washington and surrounding areas. Report submitted by the USGS to National Oceanic and Atmospheric Administration, Coastal Monitoring and Biological Assessment Division, Seattle, WA, 15pp. + 13 tables, 9 figures and 7 attachments.

TABLES 1-8

Table 1. Geographic locations of sampling sites in Delaware Bay and the surrounding area.

Strata	Site Number	Longitude (°W)	Latitude (°N)	Strata	Site Number	Longitude (°W)	Latitude (°N)
19	1	74° 54.640	40° 04.106	8	28	75° 32.080	39° 34.309
19	2	74° 43.38	40° 08.82	8	29	75° 33.827	39° 32.575
19	3	74° 54.604	40° 04.120	8	30	75° 31.842	39° 32.178
20	4	74° 58.51	40° 03.207	9	31	75° 35.037	39° 28.851
20	5	75° 05.125	39° 58.481	9	32	75° 33.8481	39° 27.9860
20	6	75° 00.387	40° 01.645	9	33	75° 32.5342	39° 26.1213
1	7	75° 07.945	39° 56.737	9	34	75° 31.069	39° 22.864
1	8	75° 07.951	39° 54.431	10	35	75° 32.661	39° 28.460
1	9	75° 08.977	39° 52.700	10	36	75° 30.36	39° 26.82
2	10	75° 12.206	39° 51.970	10	37	75° 28.482	39° 25.639
2	11	75° 12.478	39° 51.950	10	38	75° 26.101	39° 22.823
2	12	75° 14.6417	39° 51.4021	11	39	75° 29.095	39° 20.627
3	13	75° 17.911	39° 51.644	11	40	75° 21.90	39° 16.69
3	14	75° 16.078	39° 51.131	11	41	75° 21.233	39° 10.783
3	15	75° 21.050	39° 50.227	11	42	75° 21.026	39° 09.756
4	16	75° 23.0548	39° 49.0789	12	43	75° 17.271	39° 14.993
4	17	75° 24.1906	39° 48.5311	12	44	75° 14.032	39° 14.381
4	18	75° 26.112	39° 47.059	12	45	75° 13.415	39° 12.309
5	19	75° 28.674	39° 46.320	12	46	75° 16.1058	39° 10.3868
5	20	75° 27.485	39° 46.245	13	47	75° 15.090	39° 07.707
5	21	75° 29.5081	39° 44.7136	13	48	75° 04.164	39° 03.127
6	22	75° 29.308	39° 43.234	13	49	75° 13.64	39° 02.51
6	23	75° 30.674	39° 41.566	13	50	75° 02.1773	38° 59.1423
6	24	75° 32.844	39° 40.070	13	51	75° 17.8790	38° 59.127
7	25	75° 32.9156	39° 39.4505	13	52	75° 07.4564	38° 55.3604
7	26	75° 35.329	39° 36.030	13	53	75° 12.597	38° 55.2
7	27	75° 35.367	39° 35.548	13	54	75° 01.8334	38° 52.8442

Table 1. Continued.

Strata	Site Number	Longitude (°W)	Latitude (°N)	Strata	Site Number	Longitude (°W)	Latitude (°N)
13	55	75° 08.2115	38° 52.6333	17	69	75° 01.7555	38° 42.0125
13	56	75° 07.02	38° 48.27	17	70	75° 03.5985	38° 40.1329
14	57	75° 06.457	39° 12.029	18	71	75° 02.5379	38° 33.7025
14	58	75° 01.00	39° 08.878	18	72	75° 00.24	38° 33.36
14	59	75° 02.291	39° 04.35	18	73	75° 01.9637	38° 31.0025
14	60	75° 59.484	39° 04.011	21	84	75° 34.26	39° 26.40
14	61	75° 00.855	39° 01.710	21	85	75° 36.4938	39° 23.6612
15	62	75° 48.8201	38° 55.5478	NA[1]	87	75° 25.44	39° 14.4
15	63	75° 53.9879	38° 53.2177	22	88	75° 24.2387	39° 04.0395
15	64	75° 56.386	38° 53.165	22	89	75° 30.1667	39° 07.757
16	65	75° 00.047	38° 50.718	22	90	75° 27.615	39° 05.1315
16	66	75° 04.6165	38° 49.4901	NA[1]	91	75° 34.2682	39° 32.5655
16	67	75° 02.7509	38° 46.3437	NA[1]	92	75° 35.8155	39° 32.1952
17	68	75° 04.055	38° 43.204				

[1] NA=none assigned

Table 2. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples from Delaware Bay and surrounding areas.

Designation[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
REF-1[9]	26	8.22	99.4	8.04	0.96	32.9	<0.01	95
19-1	0	8.40	101.2	7.90	13.7	343.3	<0.01	72
19-2	0	9.16	110.2	7.70	2.0	31.9	<0.01	71
19-3	1	8.27	103.8	6.90	16.2	41.5	<0.01	72
20-4	0	8.69	111.7	7.90	3.85	96.5	<0.01	71
20-5	0	8.55	109.8	8.23	0.77	40.1	<0.01	71
20-6	0	9.01	111.4	7.87	0.93	21.8	<0.01	71
1-7	0	7.88	99.8	7.07	9.44	35.8	<0.01	71
1-8	1	7.77	99.6	7.01	32.1	105.9	<0.01	72
1-9	0	8.12	103.1	8.16	0.81	36.2	<0.01	71
2-10	0	7.81	100.1	7.90	1.51	37.8	<0.01	71
2-11	0	7.70	98.9	8.16	0.70	31.3	<0.01	71
2-12	1	8.63	110.6	7.89	1.13	27.7	<0.01	72
3-13	1	7.96	102.7	8.21	9.33	465.2	<0.01	72
3-14	0	7.72	99.8	8.05	0.52	18.2	<0.01	71
3-15	0	7.67	99.9	7.85	0.73	16.3	<0.01	71
4-16	1	8.02	101.9	7.71	2.02	33.0	<0.01	72
4-17	1	7.95	101.2	7.96	4.08	117.0	<0.01	72
4-18	2	8.26	102.3	7.96	2.76	79.1	<0.01	73
5-19	2	8.06	102.5	7.25	4.05	23.2	<0.01	73
5-20	2	8.25	107.5	7.59	8.46	105.2	<0.01	73
5-21	2	8.01	104.6	7.60	12.7	161.5	<0.01	73
6-22	2	8.06	105.1	7.58	1.21	14.7	<0.01	73
6-23	4	7.76	100.9	7.28	7.74	47.4	<0.01	74

Table 2. Continued.

Designation[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
6-24	3	8.19	107.6	7.03	3.64	12.6	<0.01	74
7-25	4	8.17	107.4	7.4	6.97	56.2	<0.01	74
7-26	4	8.0	105.1	6.98	8.6	26.5	<0.01	74
7-27	6	8.15	106.9	7.64	2.02	28.1	<0.01	76
8-28	6	8.59	110.5	7.73	1.03	17.6	<0.01	76
8-29	8	7.94	101.8	6.76	4.21	7.8	<0.01	78
8-30	6	8.24	106.6	7.52	3.35	35.5	<0.01	76
9-31	8	7.46	97.3	7.82	0.85	17.8	<0.01	76
9-32	11	8.31	105.2	8.02	5.02	164.5	<0.01	80
9-33	12	7.99	104.9	7.59	8.43	104.8	<0.01	81
9-34	13	7.84	103.0	7.68	0.92	14.0	<0.01	82
10-35	9	7.61	100.7	7.81	0.21	4.3	<0.01	78
10-36	12	7.43	98.1	7.74	1.53	26.7	<0.01	81
10-37	12	7.95	103.6	7.71	1.36	22.2	<0.01	81
10-38	14	8.87	113.8	7.37	2.68	20.2	<0.01	83
11-39	14	8.01	104.7	7.72	0.8	13.4	<0.01	83
11-40	20	7.62	100.0	7.38	5.24	40.4	<0.01	89
11-41	22	7.53	99.4	7.7	2.74	43.7	<0.01	91
11-42	22	7.59	100.4	7.73	2.11	36.0	<0.01	91
12-43	22	7.35	96.3	7.62	3.56	47.4	<0.01	91
12-44	22	7.53	99.1	7.69	3.83	59.8	<0.01	91
12-45	23	7.75	102.1	7.67	2.53	37.7	<0.01	92
12-46	26	7.78	101.9	7.65	4.03	57.4	<0.01	95
13-47	26	7.31	96.3	7.76	3.55	64.9	<0.01	95
13-48	28	7.52	100.4	7.74	3.26	57.0	<0.01	98

Table 2. Continued.

Designation[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
13-49	29	8.01	102.3	7.62	2.51	33.4	<0.01	100
13-50	30	7.44	99.1	7.64	7.95	110.7	<0.01	100
13-51	26	7.33	97.4	7.63	2.0	27.2	<0.01	95
13-52	32	7.47	99.2	7.79	2.71	53.0	<0.01	94
13-53	30	7.46	98.6	6.98	7.48	23.0	<0.01	100
13-54	31	7.54	99.4	6.98	6.07	18.7	<0.01	100
13-55	31	7.58	100.1	7.70	8.86	141.4	<0.01	100
13-56	31	7.68	101.3	7.70	16.5	263.3	<0.01	100
14-57	21	8.22	105.7	7.14	10.7	47.6	<0.01	89
14-58	24	7.60	98.0	7.50	10.5	106.4	<0.01	93
14-59	26	7.90	102.3	7.89	2.72	66.6	<0.01	95
14-60	25	7.68	97.4	7.90	7.45	186.7	<0.01	94
14-61	27	7.68	99.5	7.56	3.45	40.1	<0.01	96
15-62	32	7.64	100.0	7.63	2.83	38.5	<0.01	94
15-63	32	7.46	98.1	7.60	9.85	125.3	<0.01	94
15-64	31	7.58	99.9	7.63	0.06	0.8	<0.01	100
16-65	32	7.39	97.3	7.64	2.87	40.0	<0.01	94
16-66	32	7.41	97.3	7.78	7.54	144.2	<0.01	94
16-67	32	7.48	98.2	7.56	8.28	96.2	<0.01	94
17-68	32	7.42	97.8	7.72	3.69	61.6	<0.01	94
17-69	32	7.37	97.8	7.58	2.77	33.7	<0.01	94
17-70	32	7.40	97.9	7.55	2.63	29.9	<0.01	94
18-71	32	7.87	101.2	7.62	2.55	33.9	<0.01	94
18-72	32	7.53	99.7	7.68	3.51	53.5	<0.01	94
18-73	32	7.39	97.8	7.66	4.4	64.6	<0.01	94

Table 2. Continued.

Designation[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
21-84	8	7.55	99.7	7.73	2.30	39.3	<0.01	78
21-85	6	7.73	101	7.02	12.4	41.9	<0.01	76
?-87	18	7.36	97.5	7.6	4.18	53.2	<0.01	86
22-88	22	7.46	99.2	7.11	8.96	37.2	<0.01	91
22-89	6	7.66	101.5	7.31	10.8	70.9	<0.01	76
22-90	18	7.84	103.2	7.04	3.49	12.3	<0.01	86
?-91	8	7.86	102.6	7.27	2.68	16.1	<0.01	78
?-92	4	7.99	104.6	7.25	24.8	141.9	<0.01	74

[1] Designation refers to strata and station, respectively.
[2] Salinity of sample prior to adjustment. Sample adjusted to 30±1‰.
[3] Dissolved oxygen
[4] Percent saturation of dissolved oxygen
[5] Total ammonia as nitrogen
[6] Un-ionized ammonia
[7] Measured as S^{-2}
[8] Percent of original sample after salinity adjustment
[9] Reference pore water extracted from sediment collected in Redfish Bay, Texas.

Table 3. Sea urchin fertilization test raw data and means for sediment porewater samples from Delaware Bay and surrounding areas (stations 1-42). Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	89	88	94	92	95	92.7 ± 3.0	100
		89	94	96	96	94		
REF [3]	50	93	89	97	97	95	94.3 ± 2.4	100
		95	94	96	93	94		
REF [3]	25	95	93	93	94	94	93.8 ± 2.2	100
		94	97	90	97	91		
19-1	100	94	94	90	86	90	90.8 ± 3.4	98
19-1	50	95	93	95	96	93	94.4 ± 1.3	100
19-1	25	91	93	96	95	98	94.6 ± 2.7	101
19-2	100	93	97	91	93	98	94.4 ± 3.0	102
19-2	50	97	94	96	93	95	95.0 ± 1.6	101
19-2	25	92	91	97	96	95	94.2 ± 2.6	100
19-3	100	89	86	81	88	84	85.6 ± 3.2 ++	92
19-3	50	89	88	98	91	92	91.6 ± 3.9	97
19-3	25	95	92	87	91	92	91.4 ± 2.9	97
20-4	100	91	98	94	94	95	94.4 ± 2.5	102
20-4	50	94	93	93	90	95	93.0 ± 1.9	99
20-4	25	97	98	97	97	95	96.8 ± 1.1	103
20-5	100	94	98	94	93	94	94.6 ± 2.0	102
20-5	50	94	96	97	95	90	94.4 ± 2.7	100
20-5	25	96	91	95	97	96	95.0 ± 2.4	101

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
20-6	100	93	94	97	92	95	94.2 ± 1.9	102
20-6	50	93	91	92	98	95	93.8 ± 2.8	99
20-6	25	96	93	95	89	93	93.2 ± 2.7	99
1-7	100	87	89	87	87	94	88.8 ± 3.0	96
1-7	50	96	97	91	95	97	95.2 ± 2.5	101
1-7	25	95	91	94	98	94	94.4 ± 2.5	101
1-8	100	89	92	87	91	51 [4]	89.8 ± 2.2	97
1-8	50	97	91	89	96	93	93.2 ± 3.4	99
1-8	25	91	94	93	94	92	92.8 ± 1.3	99
1-9	100	90	95	99	98	94	95.2 ± 3.6	103
1-9	50	88	94	94	95	98	93.8 ± 3.6	99
1-9	25	95	97	96	97	95	96.0 ± 1.0	102
2-10	100	90	92	91	94	94	92.2 ± 1.8	99
2-10	50	93	95	94	93	95	94.0 ± 1.0	100
2-10	25	89	95	98	97	95	94.8 ± 3.5	101
2-11	100	93	96	93	98	94	94.8 ± 2.2	102
2-11	50	93	93	97	96	96	95.0 ± 1.9	101
2-11	25	98	98	93	92	91	94.4 ± 3.4	101
2-12	100	91	94	93	91	94	92.6 ± 1.5	100
2-12	50	95	97	94	95	96	95.4 ± 1.1	101
2-12	25	92	92	91	95	97	93.4 ± 2.5	100
3-13	100	92	91	92	91	87	90.6 ± 2.1	98
3-13	50	92	92	93	92	94	92.6 ± 0.9	98
3-13	25	97	94	94	96	99	96.0 ± 2.1	102

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
3-14	100	95	94	88	91	93	92.2 ± 2.8	99
3-14	50	94	97	94	94	94	94.6 ± 1.3	100
3-14	25	96	93	96	91	95	94.2 ± 2.2	100
3-15	100	93	97	95	99	96	96.0 ± 2.2	104
3-15	50	96	90	93	94	96	93.8 ± 2.5	99
3-15	25	94	94	97	99	99	96.6 ± 2.5	103
4-16	100	95	90	91	97	91	92.8 ± 3.0	100
4-16	50	98	96	96	96	93	95.8 ± 1.8	102
4-16	25	97	93	96	91	96	94.6 ± 2.5	101
4-17	100	93	96	92	91	91	92.6 ± 2.1	100
4-17	50	96	94	97	98	90	95.0 ± 3.2	101
4-17	25	92	97	89	97	94	93.8 ± 3.4	100
4-18	100	92	95	88	94	89	91.6 ± 3.0	99
4-18	50	94	96	95	96	91	94.4 ± 2.1	100
4-18	25	97	97	91	95	93	94.6 ± 2.6	101
5-19	100	93	94	93	91	96	93.4 ± 1.8	101
5-19	50	91	95	95	91	87	91.8 ± 3.4	97
5-19	25	93	99	96	93	96	95.4 ± 2.5	102
5-20	100	97	92	90	97	96	94.4 ± 3.2	102
5-20	50	90	93	95	97	94	93.8 ± 2.6	99
5-20	25	95	95	89	95	90	92.8 ± 3.0	99
5-21	100	65	60	42	54	44	53.0 ± 10.0 **	57
5-21	50	30 [4]	80	75	74	78	78.6 ± 2.8 *	83
5-21	25	89	92	96	94	95	93.2 ± 2.8	99

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
6-22	100	98	92	94	96	92	94.4 ± 2.6	102
6-22	50	89	92	94	99	96	94.0 ± 3.8	100
6-22	25	95	97	90	91	88	92.2 ± 3.7	98
6-23	100	66	70	63	59	65	64.6 ± 4.0 **	70
6-23	50	87	80	82	76	88	82.6 ± 5.0 ++	88
6-23	25	93	91	93	95	93	93.0 ± 1.4	99
6-24	100	86	87	87	89	84	86.6 ± 1.8 +	93
6-24	50	91	93	94	89	91	91.6 ± 2.0	97
6-24	25	92	97	93	93	85	92.0 ± 4.4	98
7-25	100	66	72	61	68	57	64.8 ± 5.9 **	70
7-25	50	89	87	91	91	91	89.8 ± 1.8	95
7-25	25	97	88	90	92	94	92.2 ± 3.5	98
7-26	100	91	89	92	94	91	91.4 ± 1.8	99
7-26	50	87	91	90	91	93	90.4 ± 2.2	96
7-26	25	97	97	96	92	96	95.6 ± 2.1	102
7-27	100	94	66 [4]	89	96	92	92.8 ± 3.0	100
7-27	50	96	94	95	91	93	93.8 ± 1.9	99
7-27	25	98	97	94	92	93	94.8 ± 2.6	101
8-28	100	89	92	89	94	98	92.4 ± 3.8	100
8-28	50	93	95	92	92	96	93.6 ± 1.8	99
8-28	25	93	94	98	94	95	94.8 ± 1.9	101
8-29	100	79	82	78	79	81	79.8 ± 1.6 ++	86
8-29	50	92	93	93	94	95	93.4 ± 1.1	99
8-29	25	91	94	95	94	90	92.8 ± 2.2	99

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
8-30	100	88	95	93	88	87	90.2 ± 3.6	97
8-30	50	92	96	96	95	95	94.8 ± 1.6	101
8-30	25	99	94	95	94	93	95.0 ± 2.4	101
9-31	100	96	97	94	98	100	97.0 ± 2.2	105
9-31	50	96	97	96	95	96	96.0 ± 0.7	102
9-31	25	89	95	98	98	95	95.0 ± 3.7	101
9-32	100	93	89	95	96	100	94.6 ± 4.0	102
9-32	50	91	94	96	95	99	95.0 ± 2.9	101
9-32	25	98	93	97	95	98	96.2 ± 2.2	103
9-33	100	95	99	96	93	95	95.6 ± 2.2	103
9-33	50	92	100	93	95	92	94.4 ± 3.4	100
9-33	25	94	96	98	94	95	95.4 ± 1.7	102
9-34	100	93	92	92	95	94	93.2 ± 1.3	101
9-34	50	92	94	99	92	95	94.4 ± 2.9	100
9-34	25	97	92	93	96	96	94.8 ± 2.2	101
10-35	100	97	94	96	94	95	95.2 ± 1.3	103
10-35	50	91	98	96	96	94	95.0 ± 2.6	101
10-35	25	97	94	96	97	93	95.4 ± 1.8	102
10-36	100	93	95	89	91	89	91.4 ± 2.6	99
10-36	50	94	93	97	92	93	93.8 ± 1.9	99
10-36	25	94	97	94	89	91	93.0 ± 3.1	99
10-37	100	93	92	95	92	89	92.2 ± 2.2	99
10-37	50	97	90	97	96	94	94.8 ± 3.0	101
10-37	25	94	96	91	93	95	93.8 ± 1.9	100

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
10-38	100	88	96	89	93	91	91.4 ± 3.2	99
10-38	50	97	98	92	93	94	94.8 ± 2.6	101
10-38	25	94	92	94	90	96	93.2 ± 2.3	99
11-39	100	92	95	93	96	95	94.2 ± 1.6	102
11-39	50	92	93	93	96	94	93.6 ± 1.5	99
11-39	25	96	99	98	97	96	97.2 ± 1.3	104
11-40	100	90	95	94	95	91	93.0 ± 2.4	100
11-40	50	90	96	97	99	96	95.6 ± 3.4	101
11-40	25	96	94	95	95	94	94.8 ± 0.8	101
11-41	100	91	97	94	97	94	94.6 ± 2.5	102
11-41	50	98	97	96	96	96	96.6 ± 0.9	102
11-41	25	92	95	94	95	100	95.2 ± 3.0	101
11-42	100	96	92	85	92	90	91.0 ± 4.0	98
11-42	50	88	94	94	96	91	92.6 ± 3.1	98
11-42	25	92	91	93	94	93	92.6 ± 1.1	99

[1] Designation refers to strata and sample ID, respectively.

[2] Percent of water quality adjusted porewater sample.

[3] Reference pore water extracted from sediment collected in Redfish Bay, Texas.

[4] Value is an outlier and was omitted from statistical analysis.

Table 4. Sea urchin fertilization test raw data and means for sediment porewater samples from Delaware Bay and surrounding areas (stations 43-73, 84, 85, and 87-92). Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	95	98	95	93	94	94.6 ± 2.5	100
		99	91	94	92	95		
REF [3]	50	94	96	93	97	96	95.4 ± 1.4	100
		96	95	96	97	94		
REF [3]	25	96	97	96	99	97	96.3 ± 1.6	100
		96	97	93	95	97		
12-43	100	97	96	97	98	94	96.4 ± 1.5	102
12-43	50	94	96	96	96	96	95.6 ± 0.9	100
12-43	25	97	96	97	94	96	96.0 ± 1.2	100
12-44	100	96	99	98	100	96	97.8 ± 1.8	103
12-44	50	96	96	98	95	99	96.8 ± 1.6	101
12-44	25	97	96	96	100	99	97.6 ± 1.8	101
12-45	100	98	96	98	97	97	97.2 ± 0.8	103
12-45	50	97	94	99	99	96	97.0 ± 2.1	102
12-45	25	97	97	98	97	96	97.0 ± 0.7	101
12-46	100	95	95	97	97	98	96.4 ± 1.3	102
12-46	50	97	97	97	99	97	97.4 ± 0.9	102
12-46	25	92	93	99	99	97	96.0 ± 3.3	100
13-47	100	97	97	99	99	97	97.8 ± 1.1	103
13-47	50	98	97	98	96	97	97.2 ± 0.8	102
13-47	25	96	98	97	98	100	97.8 ± 1.5	102

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
13-48	100	98	98	95	99	98	97.6 ± 1.5	103
13-48	50	98	98	94	98	97	97.0 ± 1.7	102
13-48	25	97	93	98	95	95	95.6 ± 2.0	99
13-49	100	94	97	97	95	95	95.6 ± 1.3	101
13-49	50	97	97	94	95	97	96.0 ± 1.4	101
13-49	25	99	98	98	97	97	97.8 ± 0.8	102
13-50	100	96	91	90	98	96	94.2 ± 3.5	100
13-50	50	99	99	95	96	96	97.0 ± 1.9	102
13-50	25	97	97	98	98	97	97.4 ± 0.6	101
13-51	100	96	93	95	96	100	96.0 ± 2.6	101
13-51	50	99	99	97	96	99	98.0 ± 1.4	103
13-51	25	100	97	98	96	98	97.8 ± 1.5	102
13-52	100	96	97	98	98	96	97.0 ± 1.0	103
13-52	50	96	97	100	96	100	97.8 ± 2.0	103
13-52	25	95	96	96	97	94	95.6 ± 1.1	99
13-53	100	95	95	96	95	91	94.4 ± 2.0	100
13-53	50	95	95	97	92	96	95.0 ± 1.9	100
13-53	25	95	97	96	95	94	95.4 ± 1.1	99
13-54	100	94	94	92	98	97	95.0 ± 2.4	100
13-54	50	98	94	97	99	98	97.2 ± 1.9	102
13-54	25	95	98	97	93	96	95.8 ± 1.9	99
13-55	100	93	88	95	97	96	93.8 ± 3.6	99
13-55	50	97	97	93	99	92	95.6 ± 3.0	100
13-55	25	97	97	100	92	96	96.4 ± 2.9	100

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
13-56	100	5	4	6	52 [4]	9	6.0 ± 2.2 **	6
13-56	50	33	50 [4]	32	20 [4]	40	35.0 ± 4.4**	37
13-56	25	98	89	91	96	92	93.2 ± 3.7	97
14-57	100	72	49	58	74	58	62.2 ± 10.6 **	66
14-57	50	91	90	95	88	91	91.0 ± 2.6 +	95
14-57	25	97	96	98	96	98	97.0 ± 1.0	101
14-58	100	98	96	88	95	94	94.2 ± 3.8	100
14-58	50	97	94	95	97	95	95.6 ± 1.3	100
14-58	25	95	98	98	96	98	97.0 ± 1.4	101
14-59	100	96	96	98	97	95	96.4 ± 1.1	102
14-59	50	97	97	97	95	98	96.8 ± 1.1	101
14-59	25	97	96	98	96	99	97.2 ± 1.3	101
14-60	100	57	56	55	51	54	54.6 ± 2.3 **	58
14-60	50	96	94	93	94	97	94.8 ± 1.6	99
14-60	25	97	96	93	96	98	96.0 ± 1.9	100
14-61	100	99	90	97	97	98	96.2 ± 3.6	102
14-61	50	99	96	95	98	95	96.6 ± 1.8	101
14-61	25	95	97	97	97	95	96.2 ± 1.1	100
15-62	100	97	98	89	96	96	95.2 ± 3.6	101
15-62	50	99	97	98	95	100	97.8 ± 1.9	103
15-62	25	96	94	97	95	97	95.8 ± 1.3	99
15-63	100	96	94	97	94	95	95.2 ± 1.3	101
15-63	50	98	96	95	96	96	96.2 ± 1.1	101
15-63	25	96	98	99	99	98	98.0 ± 1.2	102

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
15-64	100	97	94	97	98	94	96.0 ± 1.9	101
15-64	50	99	96	97	99	93	96.8 ± 2.5	101
15-64	25	97	99	99	99	95	97.8 ± 1.8	102
16-65	100	94	99	100	93	97	96.6 ± 3.0	102
16-65	50	96	97	97	94	96	96.0 ± 1.2	101
16-65	25	93	95	98	98	99	96.6 ± 2.5	100
16-66	100	97	99	97	96	98	97.4 ± 1.1	103
16-66	50	99	95	97	98	97	97.2 ± 1.5	102
16-66	25	98	98	97	95	98	97.2 ± 1.3	101
16-67	100	89	97	95	97	97	95.0 ± 3.5	100
16-67	50	97	97	97	96	98	97.0 ± 0.7	102
16-67	25	98	97	99	95	98	97.4 ± 1.5	101
17-68	100	97	95	97	96	96	96.2 ± 0.8	102
17-68	50	96	96	96	98	97	96.6 ± 0.9	101
17-68	25	94	98	97	99	98	97.2 ± 1.9	101
17-69	100	99	96	97	97	97	97.2 ± 1.1	103
17-69	50	96	99	94	98	99	97.2 ± 2.2	102
17-69	25	99	99	96	97	97	97.6 ± 1.3	101
17-70	100	96	97	98	94	98	96.6 ± 1.7	102
17-70	50	96	98	97	98	99	97.6 ± 1.1	102
17-70	25	96	99	98	95	99	97.4 ± 1.8	101
18-71	100	97	96	96	97	99	97.0 ± 1.2	103
18-71	50	99	98	97	99	96	97.8 ± 1.3	103
18-71	25	96	99	98	96	100	97.8 ± 1.8	102

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
18-72	100	94	97	98	95	100	96.8 ± 2.4	102
18-72	50	100	97	99	98	99	98.6 ± 1.1	103
18-72	25	98	98	98	95	96	97.0 ± 1.4	101
18-73	100	98	95	99	97	99	97.6 ± 1.7	103
18-73	50	99	99	97	100	100	99.0 ± 1.2	104
18-73	25	99	96	97	95	98	97.0 ± 1.6	101
21-84	100	97	98	97	99	97	97.6 ± 0.9	103
21-84	50	96	95	95	94	99	95.8 ± 1.9	100
21-84	25	96	93	95	98	97	95.8 ± 1.9	99
21-85	100	92	93	91	95	91	92.4 ± 1.7	98
21-85	50	97	96	96	96	94	95.8 ± 1.1	100
21-85	25	98	98	97	97	98	97.6 ± 0.6	101
87	100	97	95	98	96	97	96.6 ± 1.1	102
87	50	94	97	98	97	98	96.8 ± 1.6	101
87	25	97	95	96	96	99	96.6 ± 1.5	100
22-88	100	97	97	92	94	98	95.6 ± 2.5	101
22-88	50	96	95	97	93	95	95.2 ± 1.5	100
22-88	25	98	98	99	97	98	98.0 ± 0.7	102
22-89	100	81	64	60	82	80	73.4 ± 10.5 **	78
22-89	50	80	90	88	84	94	87.2 ± 5.4 ++	91
22-89	25	97	95	97	98	95	96.4 ± 1.3	100
22-90	100	100	94	97	98	98	97.4 ± 2.2	103
22-90	50	97	98	94	96	97	96.4 ± 1.5	101
22-90	25	98	98	93	95	95	95.8 ± 2.2	99

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
91	100	97	96	99	96	98	97.2 ± 1.3	103
91	50	94	95	95	96	98	95.6 ± 1.5	100
91	25	97	98	97	98	96	97.2 ± 0.8	101
92	100	85	82	80	90	88	85.0 ± 4.1 ++	90
92	50	94	89	90	93	93	91.8 ± 2.2	96
92	25	96	97	93	98	96	96.0 ± 1.9	100

[1] Designation refers to strata and sample ID, respectively.

[2] Percent of water quality adjusted porewater sample.

[3] Reference pore water extracted from sediment collected in Redfish Bay, Texas.

[4] Value is an outlier and was omitted from statistical analysis.

Table 5. Summary of station means and statistical significance for the sea urchin fertilization test from 81 stations in Delaware Bay and surrounding areas. Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, $\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).**

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
19	1	100	90.8	3.4	98	92.7	3.0	
		50	94.4	1.3	100	94.3	2.4	
		25	94.6	2.7	101	93.8	2.2	
	2	100	94.4	3.0	102	92.7	3.0	
		50	95.0	1.6	101	94.3	2.4	
		25	94.2	2.6	100	93.8	2.2	
	3	100	85.6	3.2	92	92.7	3.0	
		50	91.6	3.9	97	94.3	2.4	
		25	91.4	2.9	97	93.8	2.2	
20	4	100	94.4	2.5	102	92.7	3.0	
		50	93.0	1.9	99	94.3	2.4	
		25	96.8	1.1	103	93.8	2.2	
	5	100	94.6	2.0	102	92.7	3.0	
		50	94.4	2.7	100	94.3	2.4	
		25	95.0	2.4	101	93.8	2.2	
	6	100	94.2	1.9	102	92.7	3.0	
		50	93.8	2.8	99	94.3	2.4	
		25	93.2	2.7	99	93.8	2.2	
1	7	100	88.8	3.0	96	92.7	3.0	
		50	95.2	2.5	101	94.3	2.4	
		25	94.4	2.5	101	93.8	2.2	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
1	8	100	89.8	2.2	97	92.7	3.0	
		50	93.2	3.4	99	94.3	2.4	
		25	92.8	1.3	99	93.8	2.2	
	9	100	95.2	3.6	103	92.7	3.0	
		50	93.8	3.6	99	94.3	2.4	
		25	96.0	1.0	102	93.8	2.2	
2	10	100	92.2	1.8	99	92.7	3.0	
		50	94.0	1.0	100	94.3	2.4	
		25	94.8	3.5	101	93.8	2.2	
	11	100	94.8	2.2	102	92.7	3.0	
		50	95.0	1.9	101	94.3	2.4	
		25	94.4	3.4	101	93.8	2.2	
	12	100	92.6	1.5	100	92.7	3.0	
		50	95.4	1.1	101	94.3	2.4	
		25	93.4	2.5	100	93.8	2.2	
3	13	100	90.6	2.1	98	92.7	3.0	
		50	92.6	0.9	98	94.3	2.4	
		25	96.0	2.1	102	93.8	2.2	
	14	100	92.2	2.8	99	92.7	3.0	
		50	94.6	1.3	100	94.3	2.4	
		25	94.2	2.2	100	93.8	2.2	
	15	100	96.0	2.2	104	92.7	3.0	
		50	93.8	2.5	99	94.3	2.4	
		25	96.6	2.5	103	93.8	2.2	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
4	16	100	92.8	3.0	100	92.7	3.0	
		50	95.8	1.8	102	94.3	2.4	
		25	94.6	2.5	101	93.8	2.2	
	17	100	92.6	2.1	100	92.7	3.0	
		50	95.0	3.2	101	94.3	2.4	
		25	93.8	3.4	100	93.8	2.2	
	18	100	91.6	3.0	99	92.7	3.0	
		50	94.4	2.1	100	94.3	2.4	
		25	94.6	2.6	101	93.8	2.2	
5	19	100	93.4	1.8	101	92.7	3.0	
		50	91.8	3.4	97	94.3	2.4	
		25	95.4	2.5	102	93.8	2.2	
	20	100	94.4	3.2	102	92.7	3.0	
		50	93.8	2.6	99	94.3	2.4	
		25	92.8	3.0	99	93.8	2.2	
	21	100	53.0	10.0	57	92.7	3.0	**
		50	78.6	2.8	83	94.3	2.4	*
		25	93.2	2.8	99	93.8	2.2	
6	22	100	94.4	2.6	102	92.7	3.0	
		50	94.0	3.8	100	94.3	2.4	
		25	92.2	3.7	98	93.8	2.2	
	23	100	64.6	4.0	70	92.7	3.0	**
		50	82.6	5.0	88	94.3	2.4	++
		25	93.0	1.4	99	93.8	2.2	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
6	24	100	86.6	1.8	93	92.7	3.0	+
		50	91.6	2.0	97	94.3	2.4	
		25	92.0	4.4	98	93.8	2.2	
7	25	100	64.8	5.9	70	92.7	3.0	**
		50	89.8	1.8	95	94.3	2.4	
		25	92.2	3.5	98	93.8	2.2	
	26	100	91.4	1.8	99	92.7	3.0	
		50	90.4	2.2	96	94.3	2.4	
		25	95.6	2.1	102	93.8	2.2	
	27	100	92.8	3.0	100	92.7	3.0	
		50	93.8	1.9	99	94.3	2.4	
		25	94.8	2.6	101	93.8	2.2	
8	28	100	92.4	3.8	100	92.7	3.0	
		50	93.6	1.8	99	94.3	2.4	
		25	94.8	1.9	101	93.8	2.2	
	29	100	79.8	1.6	86	92.7	3.0	++
		50	93.4	1.1	99	94.3	2.4	
		25	92.8	2.2	99	93.8	2.2	
	30	100	90.2	3.6	97	92.7	3.0	
		50	94.8	1.6	101	94.3	2.4	
		25	95.0	2.4	101	93.8	2.2	
9	31	100	97.0	2.2	105	92.7	3.0	
		50	96.0	0.7	102	94.3	2.4	
		25	95.0	3.7	101	93.8	2.2	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
9	32	100	94.6	4.0	102	92.7	3.0	
		50	95.0	2.9	101	94.3	2.4	
		25	96.2	2.2	103	93.8	2.2	
	33	100	95.6	2.2	103	92.7	3.0	
		50	94.4	3.4	100	94.3	2.4	
		25	95.4	1.7	102	93.8	2.2	
	34	100	93.2	1.3	101	92.7	3.0	
		50	94.4	2.9	100	94.3	2.4	
		25	94.8	2.2	101	93.8	2.2	
10	35	100	95.2	1.3	103	92.7	3.0	
		50	95.0	2.6	101	94.3	2.4	
		25	95.4	1.8	102	93.8	2.2	
	36	100	91.4	2.6	99	92.7	3.0	
		50	93.8	1.9	99	94.3	2.4	
		25	93.0	3.1	99	93.8	2.2	
	37	100	92.2	2.2	99	92.7	3.0	
		50	94.8	3.0	101	94.3	2.4	
		25	93.8	1.9	100	93.8	2.2	
	38	100	91.4	3.2	99	92.7	3.0	
		50	94.8	2.6	101	94.3	2.4	
		25	93.2	2.3	99	93.8	2.2	
11	39	100	94.2	1.6	102	92.7	3.0	
		50	93.6	1.5	99	94.3	2.4	
		25	97.2	1.3	104	93.8	2.2	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
11	40	100	93.0	2.4	100	92.7	3.0	
		50	95.6	3.4	101	94.3	2.4	
		25	94.8	0.8	101	93.8	2.2	
	41	100	94.6	2.5	102	92.7	3.0	
		50	96.6	0.9	102	94.3	2.4	
		25	95.2	3.0	101	93.8	2.2	
	42	100	91.0	4.0	98	92.7	3.0	
		50	92.6	3.1	98	94.3	2.4	
		25	92.6	1.1	99	93.8	2.2	
12	43	100	96.4	1.5	102	94.6	2.5	
		50	95.6	0.9	100	95.4	1.4	
		25	96.0	1.2	100	96.3	1.6	
	44	100	97.8	1.8	103	94.6	2.5	
		50	96.8	1.6	101	95.4	1.4	
		25	97.6	1.8	101	96.3	1.6	
	45	100	97.2	0.8	103	94.6	2.5	
		50	97.0	2.1	102	95.4	1.4	
		25	97.0	0.7	101	96.3	1.6	
	46	100	96.4	1.3	102	94.6	2.5	
		50	97.4	0.9	102	95.4	1.4	
		25	96.0	3.3	100	96.3	1.6	
13	47	100	97.8	1.1	103	94.6	2.5	
		50	97.2	0.8	102	95.4	1.4	
		25	97.8	1.5	102	96.3	1.6	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
13	48	100	97.6	1.5	103	94.6	2.5	
		50	97.0	1.7	102	95.4	1.4	
		25	95.6	2.0	99	96.3	1.6	
	49	100	95.6	1.3	101	94.6	2.5	
		50	96.0	1.4	101	95.4	1.4	
		25	97.8	0.8	102	96.3	1.6	
	50	100	94.2	3.5	100	94.6	2.5	
		50	97.0	1.9	102	95.4	1.4	
		25	97.4	0.6	101	96.3	1.6	
	51	100	96.0	2.6	101	94.6	2.5	
		50	98.0	1.4	103	95.4	1.4	
		25	97.8	1.5	102	96.3	1.6	
	52	100	97.0	1.0	103	94.6	2.5	
		50	97.8	2.0	103	95.4	1.4	
		25	95.6	1.1	99	96.3	1.6	
	53	100	94.4	2.0	100	94.6	2.5	
		50	95.0	1.9	100	95.4	1.4	
		25	95.4	1.1	99	96.3	1.6	
	54	100	95.0	2.4	100	94.6	2.5	
		50	97.2	1.9	102	95.4	1.4	
		25	95.8	1.9	99	96.3	1.6	
	55	100	93.8	3.6	99	94.6	2.5	
		50	95.6	3.0	100	95.4	1.4	
		25	96.4	2.9	100	96.3	1.6	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
13	56	100	6.0	2.2	6	94.6	2.5	**
		50	35.0	4.4	37	95.4	1.4	**
		25	93.2	3.7	97	96.3	1.6	
14	57	100	62.2	10.6	66	94.6	2.5	**
		50	91.0	2.6	95	95.4	1.4	+
		25	97.0	1.0	101	96.3	1.6	
	58	100	94.2	3.8	100	94.6	2.5	
		50	95.6	1.3	100	95.4	1.4	
		25	97.0	1.4	101	96.3	1.6	
	59	100	96.4	1.1	102	94.6	2.5	
		50	96.8	1.1	101	95.4	1.4	
		25	97.2	1.3	101	96.3	1.6	
	60	100	54.6	2.3	58	94.6	2.5	**
		50	94.8	1.6	99	95.4	1.4	
		25	96.0	1.9	100	96.3	1.6	
	61	100	96.2	3.6	102	94.6	2.5	
		50	96.6	1.8	101	95.4	1.4	
		25	96.2	1.1	100	96.3	1.6	
15	62	100	95.2	3.6	101	94.6	2.5	
		50	97.8	1.9	103	95.4	1.4	
		25	95.8	1.3	99	96.3	1.6	
	63	100	95.2	1.3	101	94.6	2.5	
		50	96.2	1.1	101	95.4	1.4	
		25	98.0	1.2	102	96.3	1.6	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
15	64	100	96.0	1.9	101	94.6	2.5	
		50	96.8	2.5	101	95.4	1.4	
		25	97.8	1.8	102	96.3	1.6	
16	65	100	96.6	3.0	102	94.6	2.5	
		50	96.0	1.2	101	95.4	1.4	
		25	96.6	2.5	100	96.3	1.6	
	66	100	97.4	1.1	103	94.6	2.5	
		50	97.2	1.5	102	95.4	1.4	
		25	97.2	1.3	101	96.3	1.6	
	67	100	95.0	3.5	100	94.6	2.5	
		50	97.0	0.7	102	95.4	1.4	
		25	97.4	1.5	101	96.3	1.6	
17	68	100	96.2	0.8	102	94.6	2.5	
		50	96.6	0.9	101	95.4	1.4	
		25	97.2	1.9	101	96.3	1.6	
	69	100	97.2	1.1	103	94.6	2.5	
		50	97.2	2.2	102	95.4	1.4	
		25	97.6	1.3	101	96.3	1.6	
	70	100	96.6	1.7	102	94.6	2.5	
		50	97.6	1.1	102	95.4	1.4	
		25	97.4	1.8	101	96.3	1.6	
18	71	100	97.0	1.2	103	94.6	2.5	
		50	97.8	1.3	103	95.4	1.4	
		25	97.8	1.8	102	96.3	1.6	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
18	72	100	96.8	2.4	102	94.6	2.5	
		50	98.6	1.1	103	95.4	1.4	
		25	97.0	1.4	101	96.3	1.6	
	73	100	97.6	1.7	103	94.6	2.5	
		50	99.0	1.2	104	95.4	1.4	
		25	97.0	1.6	101	96.3	1.6	
21	84	100	97.6	0.9	103	94.6	2.5	
		50	95.8	1.9	100	95.4	1.4	
		25	95.8	1.9	99	96.3	1.6	
	85	100	92.4	1.7	98	94.6	2.5	
		50	95.8	1.1	100	95.4	1.4	
		25	97.6	0.6	101	96.3	1.6	
	87	100	96.6	1.1	102	94.6	2.5	
		50	96.8	1.6	101	95.4	1.4	
		25	96.6	1.5	100	96.3	1.6	
22	88	100	95.6	2.5	101	94.6	2.5	
		50	95.2	1.5	100	95.4	1.4	
		25	98.0	0.7	102	96.3	1.6	
	89	100	73.4	10.5	78	94.6	2.5	**
		50	87.2	5.4	91	95.4	1.4	++
		25	96.4	1.3	100	96.3	1.6	
	90	100	97.4	2.2	103	94.6	2.5	
		50	96.4	1.5	101	95.4	1.4	
		25	95.8	2.2	99	96.3	1.6	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
	91	100	97.2	1.3	103	94.6	2.5	
		50	95.6	1.5	100	95.4	1.4	
		25	97.2	0.8	101	96.3	1.6	
	92	100	85.0	4.1	90	94.6	2.5	++
		50	91.8	2.2	96	95.4	1.4	
		25	96.0	1.9	100	96.3	1.6	

[1] Percent of water quality adjusted sample.

[2] Test mean as a percentage of reference (control). Reference sediment collected from Redfish Bay, Texas.

[3] Mean of reference to which statistical comparison of test mean was made.

[4] Significant difference from reference denoted as asterisk or plus sign.

Table 6. Summary of strata means and statistical significance for the sea urchin fertilization test from 22 strata in Delaware Bay and surrounding areas. Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
19	1, 2, 3	100	90.3	4.8	97	92.7	3.0	
		50	93.7	2.8	99	94.3	2.4	
		25	93.4	2.9	100	93.8	2.2	
20	4, 5, 6	100	94.4	2.0	102	92.7	3.0	
		50	93.7	2.4	99	94.3	2.4	
		25	95.0	2.5	101	93.8	2.2	
1	7, 8, 9	100	91.4	4.1	98	92.7	3.0	
		50	94.1	3.1	100	94.3	2.4	
		25	94.4	2.1	101	93.8	2.2	
2	10, 11, 12	100	93.2	2.1	100	92.7	3.0	
		50	94.8	1.4	100	94.3	2.4	
		25	94.2	3.0	100	93.8	2.2	
3	13, 14, 15	100	92.9	3.2	100	92.7	3.0	
		50	93.7	1.8	99	94.3	2.4	
		25	95.6	2.4	102	93.8	2.2	
4	16, 17, 18	100	92.3	2.6	100	92.7	3.0	
		50	95.1	2.3	101	94.3	2.4	
		25	94.3	2.7	100	93.8	2.2	
5	19, 20, 21	100	80.3	20.8	87	92.7	3.0	++
		50	88.2	8.0	94	94.3	2.4	++
		25	93.8	2.8	100	93.8	2.2	

Table 6. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
6	22, 23, 24	100	81.9	13.3	88	92.7	3.0	++
		50	89.4	6.2	95	94.3	2.4	+
		25	92.4	3.2	98	93.8	2.2	
7	25, 26, 27	100	82.3	14.0	89	92.7	3.0	+
		50	91.3	2.6	97	94.3	2.4	
		25	94.2	3.0	100	93.8	2.2	
8	28, 29, 30	100	87.5	6.4	94	92.7	3.0	
		50	93.9	1.6	100	94.3	2.4	
		25	94.2	2.2	100	93.8	2.2	
9	31, 32, 33, 34	100	95.1	2.8	99	92.7	3.0	
		50	95.0	2.5	101	94.3	2.4	
		25	95.4	2.4	102	93.8	2.2	
10	35, 36, 37, 38	100	92.6	2.7	100	92.7	3.0	
		50	94.6	2.4	100	94.3	2.4	
		25	93.8	2.3	100	93.8	2.2	
11	39, 40, 41, 42	100	93.2	2.9	100	92.7	3.0	
		50	94.6	2.8	100	94.3	2.4	
		25	95.0	2.3	101	93.8	2.2	
12	43, 44, 45, 46	100	97.0	1.4	102	94.6	2.5	
		50	96.7	1.5	101	95.4	1.4	
		25	96.6	2.0	100	96.3	1.6	
13	47, 48, 49, 50, 51, 52, 53, 54, 55, 56	100	88.4	24.9	93	94.6	2.5	
		50	92.9	15.2	97	95.4	1.4	
		25	96.3	2.2	100	96.3	1.6	

Table 6. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
14	57, 58, 59, 60, 61	100	80.7	19.4	85	94.6	2.5	
		50	95.0	2.7	100	95.4	1.4	
		25	96.7	1.3	100	96.3	1.6	
15	62, 63, 64	100	95.5	2.3	101	94.6	2.5	
		50	96.9	1.9	102	95.4	1.4	
		25	97.2	1.7	101	96.3	1.6	
16	65, 66, 67	100	96.3	2.7	102	94.6	2.5	
		50	96.7	1.2	101	95.4	1.4	
		25	97.1	1.8	101	96.3	1.6	
17	68, 69, 70	100	96.7	1.2	102	94.6	2.5	
		50	97.1	1.4	102	95.4	1.4	
		25	97.4	1.6	101	96.3	1.6	
18	71, 72, 73	100	97.1	1.7	103	94.6	2.5	
		50	98.5	1.2	103	95.4	1.4	
		25	97.3	1.5	101	96.3	1.6	
21	84, 85	100	95.0	3.0	100	94.6	2.5	
		50	95.8	1.5	100	95.4	1.4	
		25	96.7	1.6	100	96.3	1.6	
	87	100	96.6	1.1	102	94.6	2.5	
		50	96.8	1.6	101	95.4	1.4	
		25	96.6	1.5	100	96.3	1.6	
22	88, 89, 90	100	88.8	12.7	94	94.6	2.5	
		50	92.9	5.2	97	95.4	1.4	
		25	96.7	1.7	100	96.3	1.6	

Table 6. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
	91,92	100	91.1	7.0	96	94.6	2.5	
		50	93.7	2.7	98	95.4	1.4	
		25	96.6	1.5	100	96.3	1.6	

[1] Percent of water quality adjusted sample.

[2] Test mean as a percentage of reference (control). Reference sediment collected from Redfish Bay, Texas.

[3] Mean of reference to which statistical comparison of test mean was made.

[4] Significant difference from reference denoted as asterisks or plus signs.

Table 7. Microtox® Basic Assay EC_{50} raw data and means for organic extracts of sediment samples taken from Delaware Bay and surrounding areas. Asterisks denote significant differences between test and reference stations (Dunnett's *t*- test, * $\alpha \leq 0.05$, ** $\alpha \leq 0.01$).

Designation[1]	EC_{50} (mg equivalent sediment weight)			Mean ± SD	SR Index[2]	Phenol Index[3]
	Rep 1	Rep 2	Rep 3			
REF[4]	102.7	100.0	106.0	102.9 ± 3.0	1	0.2
REF + Phenol	15.3	14.5	15.9	15.2 ± 0.7	7**	1
19-1	16.4	16.0	9.9	14.1 ± 3.6	7**	1
19-2	13.1	12.8	7.5	11.1 ± 3.2	9**	1
19-3	32	29.3	24.4	28.6 ± 3.8	4**	0.5
20-4	2.8	2.7	1.8	2.4 ± 0.6	43**	6**
20-5	77.7	104.9	91.9	91.5 ± 13.6	1	0.2
20-6	101.3	108.9	82.8	97.7 ± 13.4	1	0.2
1-7	3.9	5.0	4.4	4.4 ± 0.6	23**	4**
1-8	8.3	8.8	7.1	8.1 ± 0.9	13**	2**
1-9	5.3	4.5	5.2	5.0 ± 0.4	21**	3**
2-10	2.7	2.2	1.5	2.1 ± 0.6	49**	7**
2-11	2.8	2.5	1.7	2.3 ± 0.6	45**	7**
2-12	39.1	34.6	34.4	36.0 ± 2.7	3**	0.4
3-13	2.3	2.7	1.4	2.1 ± 0.7	49**	7**
3-14	33	30.3	29.6	31.0 ± 1.8	3**	0.5
3-15	34.6	33.6	32.5	33.6 ± 1.0	3**	0.4
4-16	7.3	6.6	4.4	6.1 ± 1.5	17**	3**
4-17	7.9	10.5	11.6	10.0 ± 1.9	10**	2
4-18	15.2	14.5	14.8	14.8 ± 0.4	7**	1
5-19	4.3	5.4	3.8	4.5 ± 0.8	23**	3**

Table 7. Continued.

Designation[1]	EC_{50} (mg equivalent sediment weight)			Mean ± SD	SR Index[2]	Phenol Index[3]
	Rep 1	Rep 2	Rep 3			
5-20	1.0	1.3	1.2	1.2 ± 0.2	86**	13**
5-21	177.8	115.7	268	187.2 ± 76.6	0.5	0.1
6-22	9.8	8.8	10.7	9.8 ± 1.0	10**	2*
6-23	10.0	12.0	13.9	12.0 ± 2.0	9**	1
6-24	5.6	6.5	6.8	6.3 ± 0.6	16**	2**
7-25	9.0	10.3	9.7	9.7 ± 0.6	11**	2*
7-26	0.42	0.39	0.32	0.4 ± 0.7	257**	38**
7-27	1.3	1.2	1.3	1.3 ± 0.06	79**	12**
8-28	1.3	0.9	0.9	1.0 ± 0.2	103**	15**
8-29	4.5	4.6	4.2	4.4 ± 0.2	23**	3**
8-30	0.32	0.28	0.27	0.3 ± 0.03	343**	51**
9-31	62.1	60.0	72.0	64.7 ± 6.4	2*	0.2
9-32	16.9	23.3	16.8	19.0 ± 3.7	5**	0.8
9-33	6.1	7.0	5.8	6.3 ± 0.6	16**	2**
9-34	2.5	1.9	1.1	1.8 ± 0.7	57**	8**
10-35	97.4	95.6	123.2[5]	96.5 ± 1.3	1	0.2
10-36	1.7	1.9	0.9	1.5 ± 0.5	69**	10**
10-37	1.6	1.6	1.7	1.6 ± 0.06	64**	9**
10-38	2.5	2.6	2.3	2.5 ± 0.2	41**	6**
11-39	24.7	22.6	25.3	24.2 ± 1.4	4**	0.6
11-40	2.6	2.2	2.8	2.5 ± 0.3	41**	6**
11-41	7.7	6.5	6.6	6.9 ± 0.7	15**	2**
11-42	1.2	1.8	2.2	1.7 ± 0.5	60**	9**

Table 7. Continued.

Designation[1]	EC_{50} (mg equivalent sediment weight)			Mean ± SD	SR Index [2]	Phenol Index [3]
	Rep 1	Rep 2	Rep 3			
12-43	2.9	2.1	1.8	2.3 ± 0.6	45**	6**
12-44	3.2	3.2	2.4	2.9 ± 0.5	36**	5**
12-45	36.6	36.7	29.2	34.2 ± 4.3	3**	0.4
12-46	75.4	61.5	81.7	72.9 ± 10.3	1	0.2
13-47	52.4	45.7	48.7	48.9 ± 3.4	2**	0.3
13-48	11.5	14.0	8.1	11.2 ± 3.0	9**	1
13-49	161.4	153.6	168.0	161.0 ± 7.2	0.6	0.1
13-50	1.3	1.3	1.5	1.4 ± 0.1	74**	11**
13-51	195.9	196.6	176.1	189.5 ± 11.6	0.5	0.08
13-52	32.8	32.0	30.4	31.7 ± 1.2	3**	0.5
13-53	4.7	5.0	4.7	4.8 ± 0.2	21**	3**
13-54	4.5	4.6	4.4	4.5 ± 0.1	23**	3**
13-55	1.6	2.0	1.9	1.8 ± 0.2	57**	8**
13-56	0.23	0.33	0.27	0.28 ± 0.05	368**	66**
14-57	3.1	3.0	3.1	3.1 ± 0.06	33**	5**
14-58	2.3	2.4	1.9	2.2 ± 0.3	47**	7**
14-59	4.5	3.8	4.2	4.2 ± 0.4	24**	4**
14-60	4.0	3.4	2.4	3.3 ± 0.8	21**	5**
14-61	78.1	62.1	74.0	71.4 ± 8.3	1	0.2
15-62	288.4	184.6	212	228.3 ± 53.8	0.4	0.07
15-63	4.2	5.4	4.7	4.8 ± 0.6	21**	3**
15-64	300.8	257.9	258.8	272.5 ± 24.5	0.4	0.06
16-65	180.5	175.8	242.7 [5]	178.2 ± 3.3	0.6	0.08
16-66	1.4	1.6	1.5	1.5 ± 0.1	69**	10**
16-67	1.4	1.6	1.3	1.4 ± 0.2	74**	11**

Table 7. Continued.

Designation[1]	EC_{50} (mg equivalent sediment weight)			Mean ± SD	SR Index[2]	Phenol Index[3]
	Rep 1	Rep 2	Rep 3			
17-68	110.7	93.9	101.7	102.1 ± 8.4	1	0.2
17-69	137.9	95.5[5]	135.8	136.8 ± 1.5	0.8	0.1
17-70	32.9	22.9	23.5	26.4 ± 5.6	4**	0.6
18-71	100	100	97.5	99.2 ± 1.4	1	0.2
18-72	120	128.6	134.5	127.7 ± 7.3	0.8	0.1
18-73	11.0	13.5	15.0	13.2 ± 2.0	8**	1
21-84	0.69	0.45	0.50	0.55 ± 0.1	187**	28**
21-85	0.75	0.74	0.73	0.74 ± 0.01	139**	21**
87	3.7	3.6	3.7	3.7 ± 0.06	28**	4**
22-88	0.94	0.68	0.89	0.84 ± 0.1	122**	18**
22-89	1.2	0.7	1.7	1.2 ± 0.5	85**	13**
22-90	1.2	1.1	0.6	1.0 ± 0.3	107**	16**
91	1.5	1.4	1.0	1.3 ± 0.3	79**	12**
92	0.45	0.38	0.36	0.4 ± 0.04	257**	38**

[1] Designation refers to strata, station and sample ID, respectively.

[2] Sediment Reference Index (i.e., Reference EC_{50} mean/sample EC_{50} mean).

[3] Phenol Spiked Sediment Control Index (Phenol Spiked control EC_{50} value (15.23)/sample EC_{50} value).

[4] Reference sediment collected from Redfish Bay, Texas.

[5] Value is an outlier and was omitted from statistical analysis.

Table 8. Strata means of Microtox® data (EC50s; mg equivalent sediment wet weight) of sediment extracts from Delaware Bay and surrounding areas. Asterisks denote significant differences between test and reference stations (Dunnett's *t*-test, * $\alpha \leq 0.05$, ** $\alpha \leq 0.01$).

Strata	Sample ID	Mean	SD	% REF [1]	Sig. [2]	% Spiked REF[3]	Sig. [2]
19	1, 2, 3	17.9	8.6	17.40		117.53	
20	4, 5, 6	63.9	47.1	62.10		419.57	
1	7, 8, 9	5.8	1.8	5.64	*	38.08	
2	10, 11, 12	13.5	17.0	13.12	*	88.64	
3	13, 14, 15	22.2	15.1	21.57		145.76	
4	16, 17, 18	10.3	4.0	10.00		67.63	
5	19, 20, 21	64.3	99.8	62.49		422.19	
6	22, 23, 24	9.3	2.7	9.04	*	61.06	
7	25, 26, 27	3.8	4.4	3.69	**	24.95	
8	28, 29, 30	1.9	1.9	1.85	**	12.48	*
9	31, 32, 33, 34	23.0	26.2	22.35		151.02	
10	35, 36, 37,38	19.1	38.3	18.56	**	125.41	
11	39, 40, 41,42	8.8	9.5	8.55	**	57.78	
12	43, 44, 45, 46	28.1	30.6	27.31		184.50	
13	47, 48, 49,50, 51, 52, 53, 54, 55, 56	45.5	68.1	44.22	*	298.75	
14	57, 58, 59, 60,61	16.8	28.4	16.33	**	110.31	
15	62, 63, 64	168.5	127.8	163.75		1106.37	
16	65, 66, 67	45.6	81.8	44.31	**	299.41	
17	68, 69, 70	82.4	48.8	80.08		541.04	
18	71, 72, 73	80.0	51.8	77.45		525.28	

Table 8. Continued.

Strata	Sample ID	Mean	SD	% REF [1]	Sig. [2]	% Spiked REF[3]	Sig. [2]
21	84, 85	0.6	0.1	0.58	**	3.94	**
	87	3.7	0.06	3.60	*	24.29	
22	88, 89, 90	1.0	0.3	0.97	**	6.56	*
	91,92	0.8	0.5	0.78	**	5.25	*

[1] Test means as a percentage of reference value (102.9 ± 3.0). Reference sediment collected from Redfish Bay, Texas.

[2] Significant difference from reference denoted as asterisks.

[3] Test mean as a percentage of phenol spiked sediment reference value (15.2 ± 8.0). Spiked sediment prepared with Redfish Bay reference sediment and phenol.

FIGURES 1-5

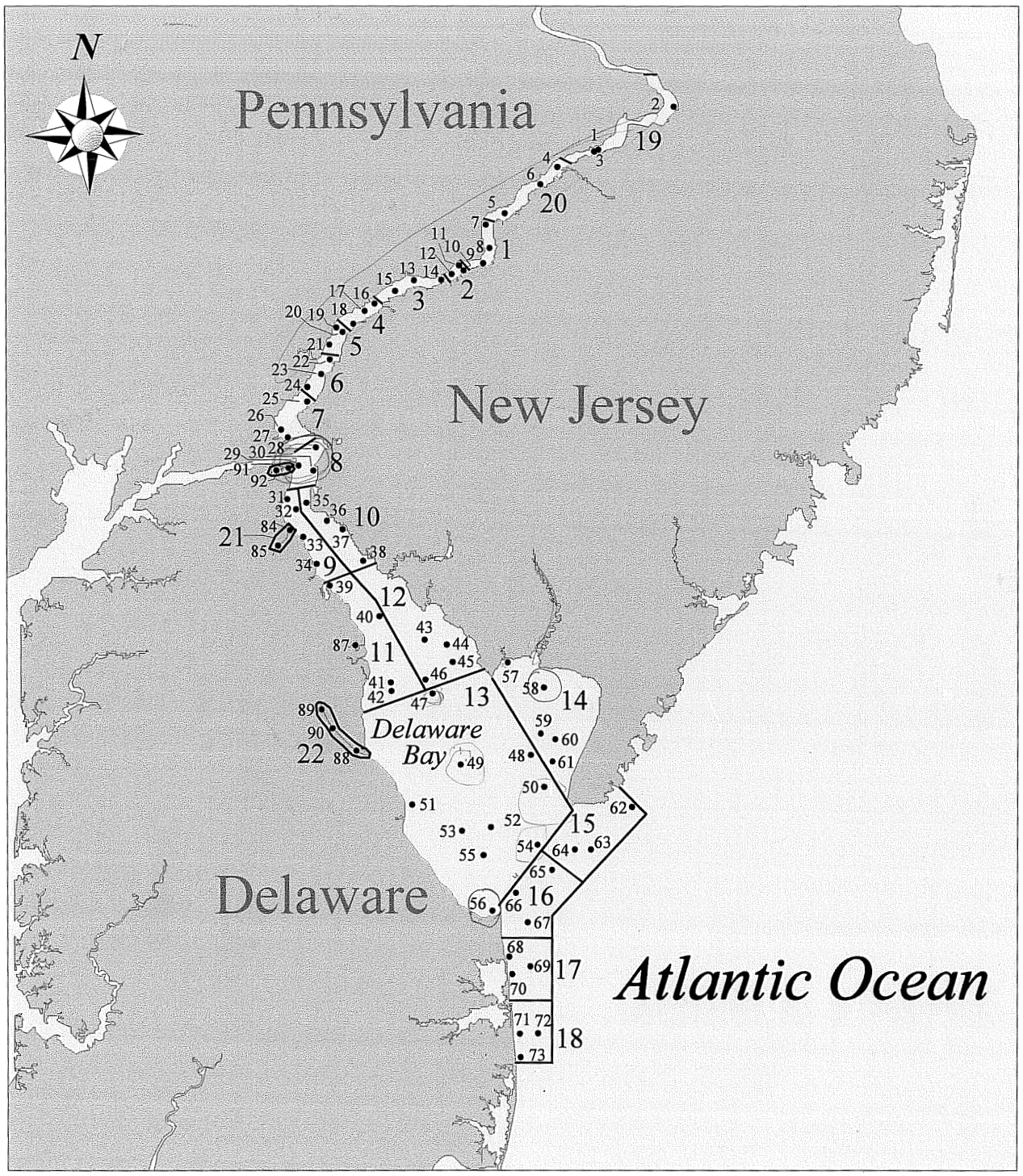

Figure 1. Sample strata and stations in Delaware Bay and surrounding areas.

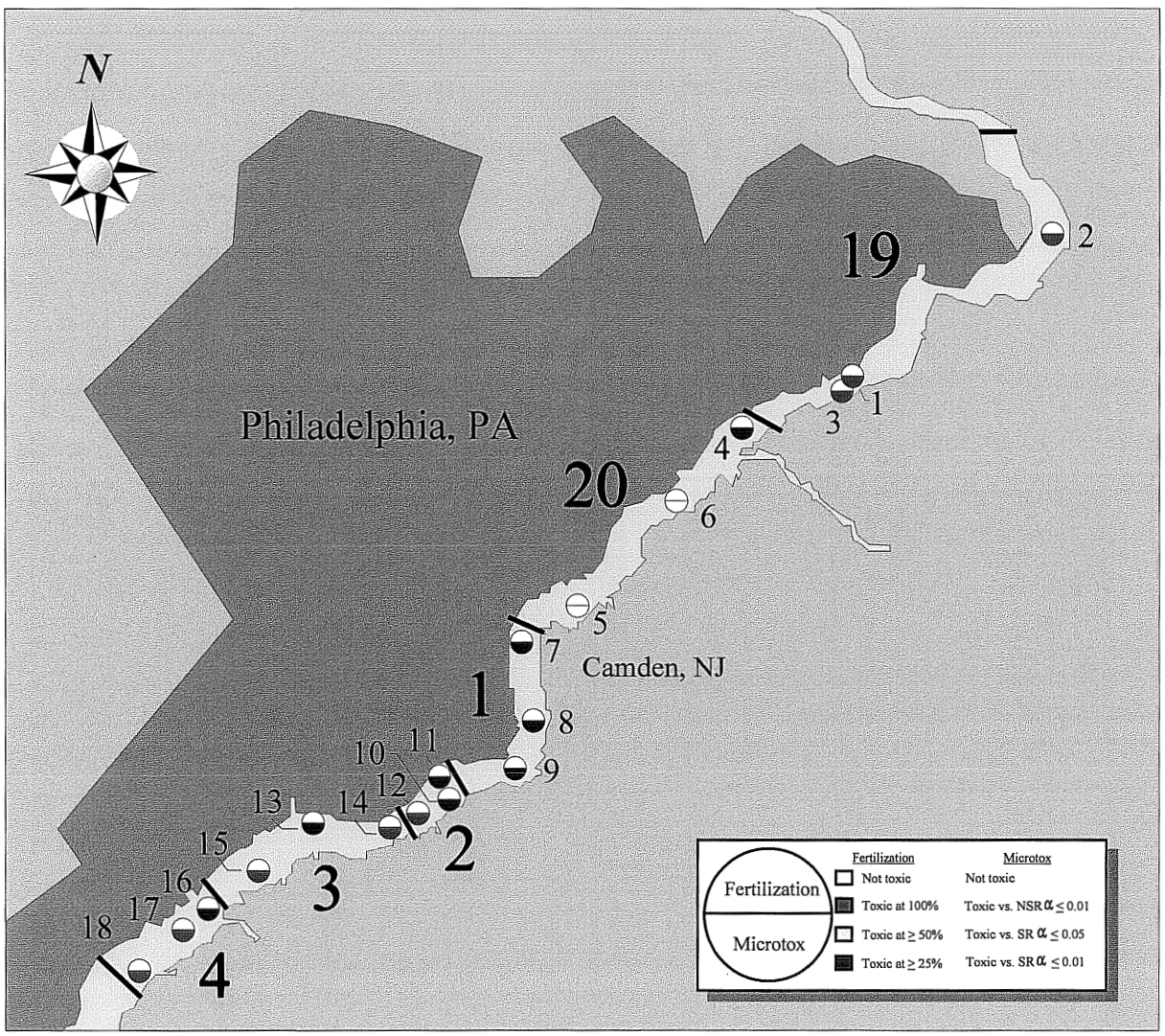

Figure 2. Sample stations in strata 1 through 4, 19 and 20. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied) and the Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

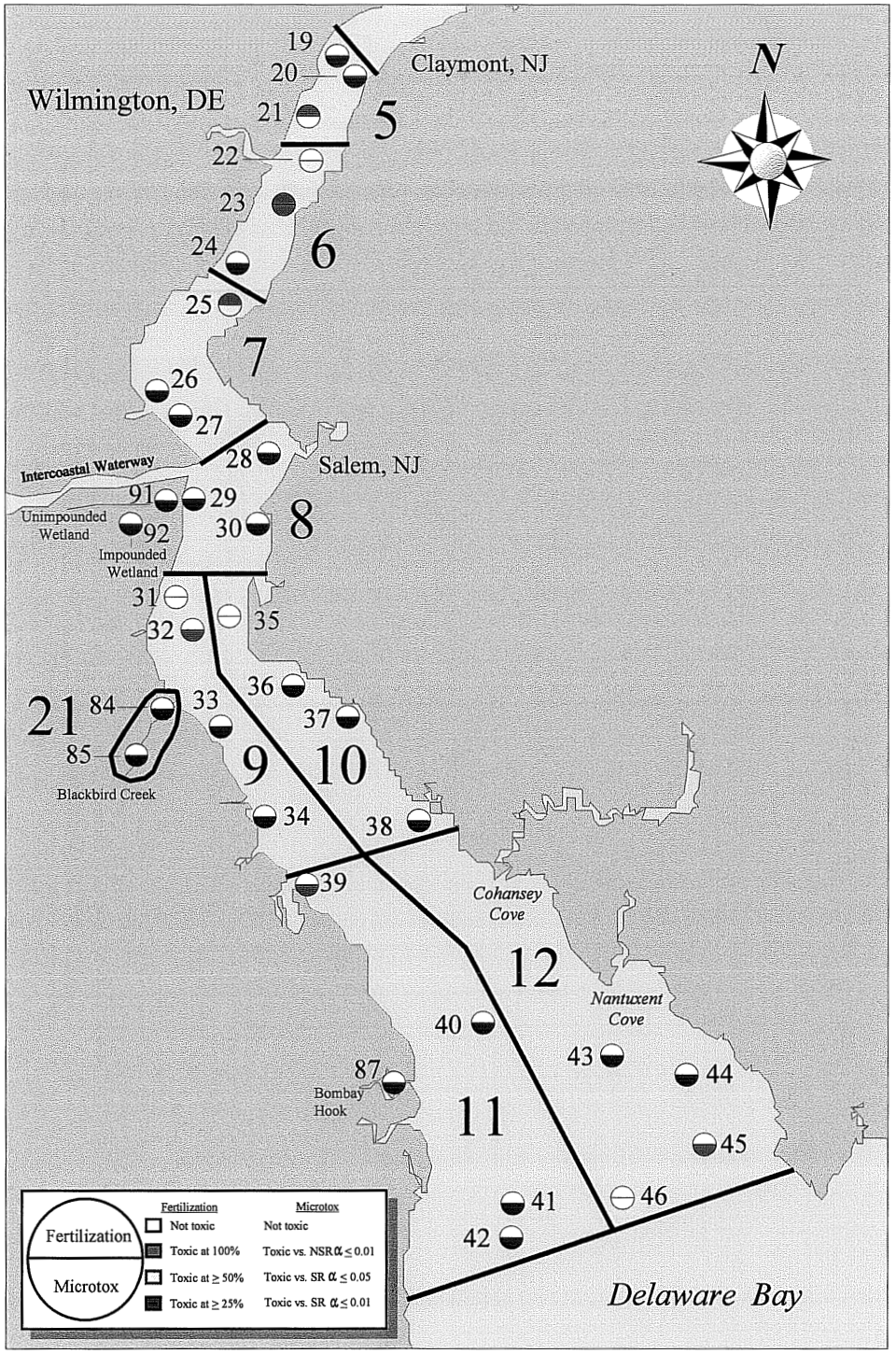

Figure 3. Sample stations in strata 5 through 12 and 21. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied) and the Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

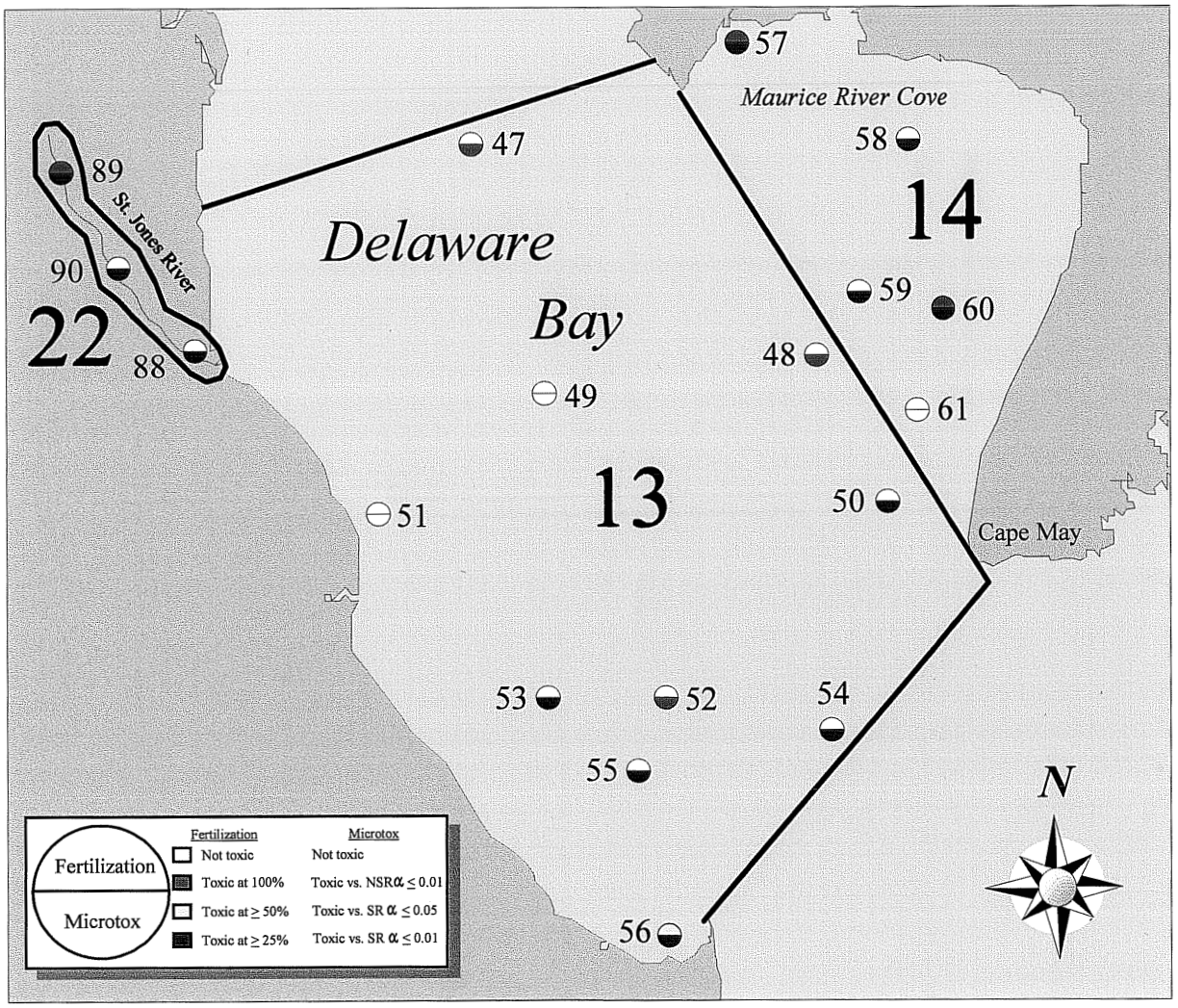

Figure 4. Sample stations in strata 13, 14 and 22. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied) and the Microtox® Basic assay (Dunnett's *t*-test comparison with nonspiked reference (NSR) and spiked reference SR)).

14

13

12

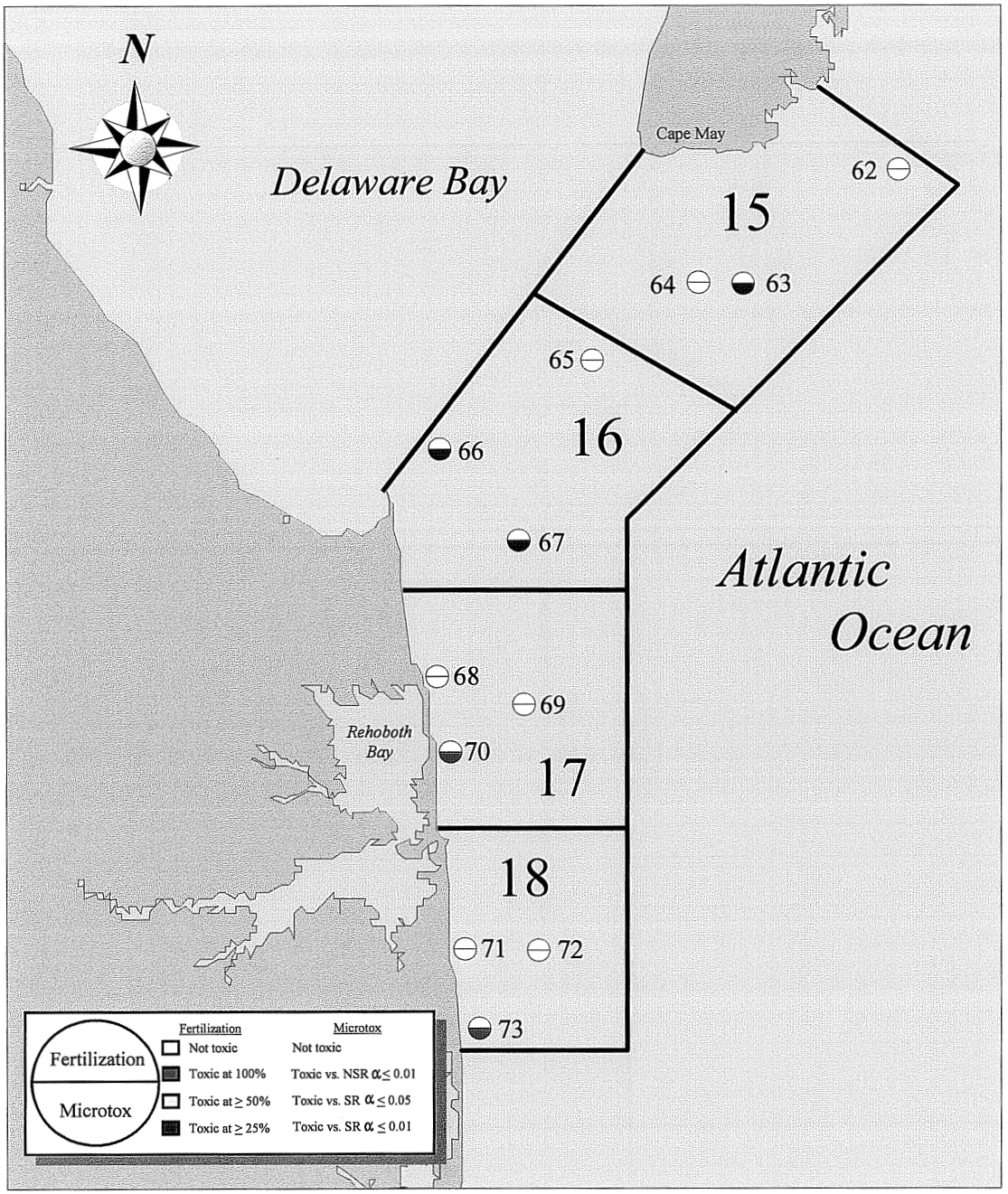

Figure 5. Sample stations in strata 15 through 18. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's *t*-test, $\alpha \leq 0.05$ and detectable significance criteria applied) and the Microtox® Basic assay (Dunnett's*t*-test comparison with nonspiked reference (NSR) and spiked reference (SR)).

ATTACHMENTS 1-4

Date Prepared: May 5, 1990

Date Revised: June 10, 1994

EXTRACTION AND STORAGE OF POREWATER SAMPLES

1.0 OBJECTIVE

This protocol describes a procedure for extracting and storing porewater samples from marine, estuarine, or freshwater sediments for use in toxicity testing. A pressurized extraction device is used to force the pore water from sediment samples. This procedure may be performed in the laboratory or it may be performed at or near the site of sample collection since the sampling apparatus is portable.

2.0 PREPARATION

2.1 Description of the Porewater Extraction System

In earlier studies (Carr et al., 1989; Carr and Chapman, 1992) pore water was extracted from sediments using a device constructed of Teflon®. Since then, the design has been improved (Carr and Chapman, 1994) The polyvinyl chloride (PVC) extractors in current use are less costly to construct and easier to operate. This device has been used in numerous sediment quality assessment surveys (Carr, 1993; NBS, 1993; NBS, 1994a; NBS, 1994b; USFWS, 1992).

The extractor is constructed from a PVC compression coupling for 4" I.D. schedule 40 PVC pipe. These commercially-available couplings (Lascotite®) consist of a cylinder (25 cm height and 13 cm diameter) with threaded ends and threaded open compression nuts (Figure 1). The coupling is fitted with end plates cut from 7/16" thick PVC sheeting that are held in place by the threaded end nuts. The gaskets provided with the coupling are discarded and silicon O-rings are used to seal the top and bottom connections. The top end plate is fitted with a quick-release fitting where the pressurized air is supplied, and a safety pressure relief valve. Like the original Teflon® extractor, the bottom end plate (Figure 1) has several interconnected concentric grooves to facilitate flow of the pore water to the central exit port. A 5 μm polyester filter is situated between the bottom end plate and the silicon O-ring. Before a sediment sample is loaded, the bottom end nut is tightened in place by using the stationary bottom wrench (Figure 1) and a standard strap wrench.

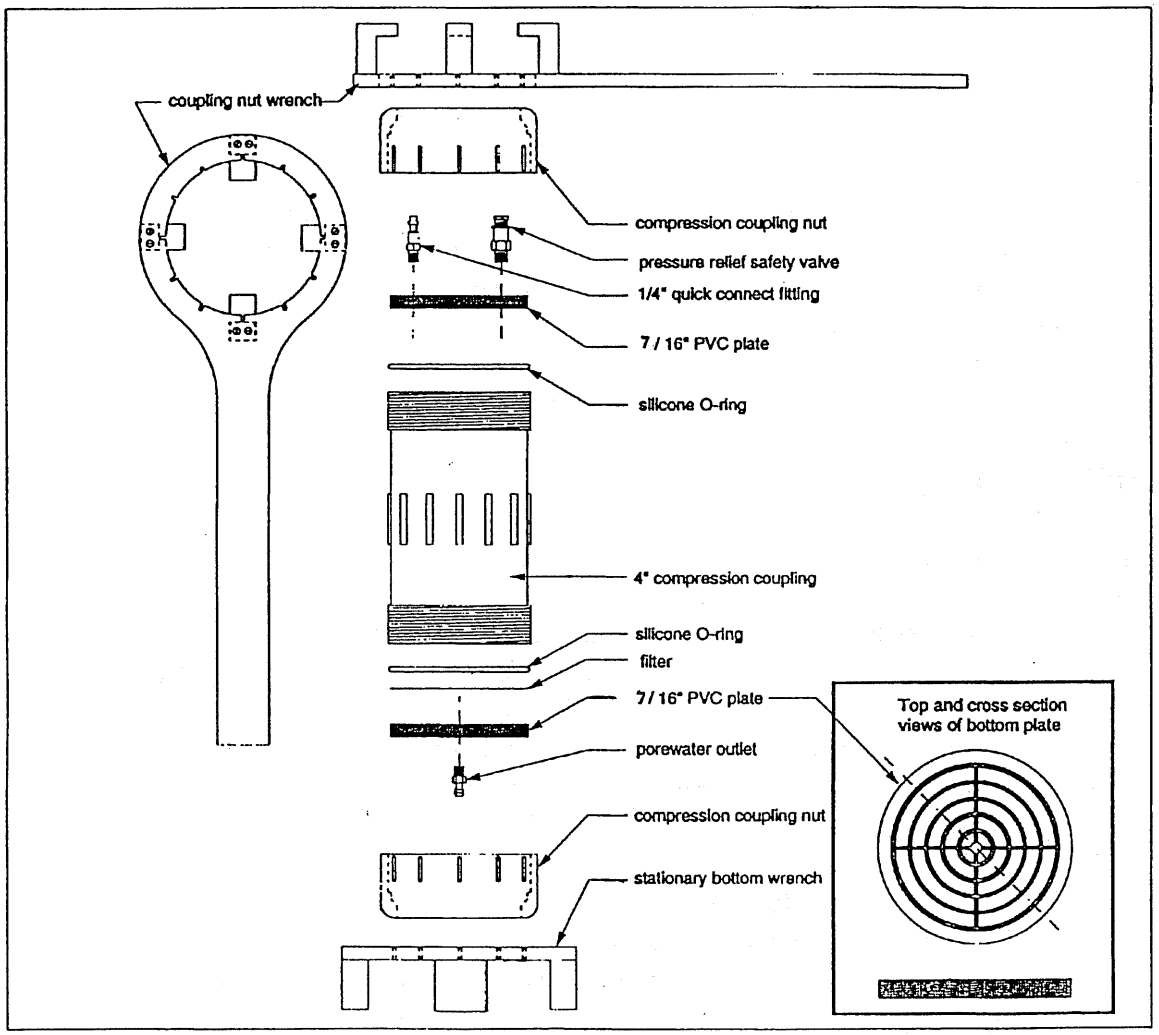

Figure 1. Sediment pore water squeeze extraction device.

The extractors are pressurized with air supplied from a standard SCUBA cylinder via a SCUBA first stage regulator which delivers air to a manifold with a valving system (Figure 2). With this system, multiple cylinders can be pressurized simultaneously, using the same SCUBA cylinder.

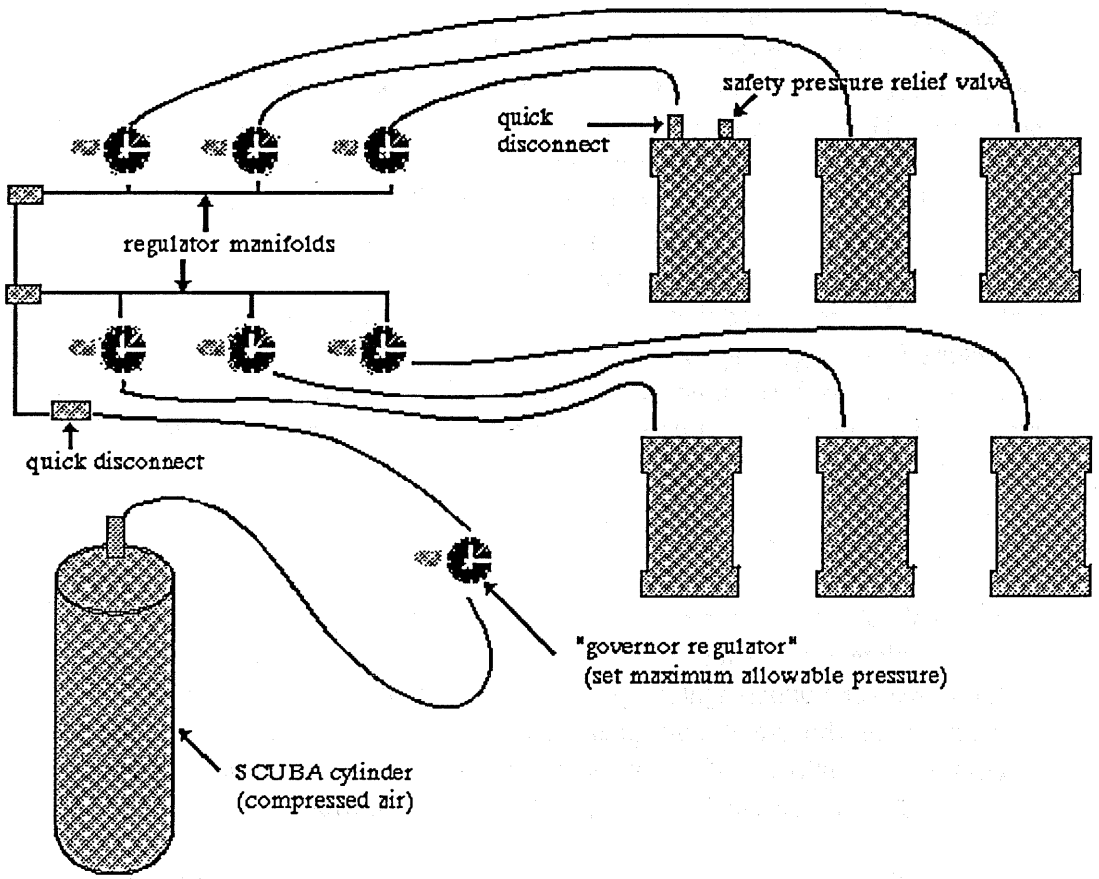

Figure 2. Schematic of sediment porewater pressure extraction system.

2.2 Equipment List

Supplies and equipment needed are listed in Attachment 1.

3.0 PROCEDURE

3.1 Sediment Collection and Storage Considerations

Generally, surficial sediment samples are collected for porewater extraction. A homogenate of the upper ~2-10 cm sediment may be collected by multiple cores or grabs at a particular sampling station. (Further details of sediment sampling procedures are not within the scope of this SOP.) One liter of sediment will typically provide 100-200 mL pore water. However, a larger volume of course sand sediments may be required since they contain less water, and a larger volume of fine clay sediments may be required since they are difficult to extract. The sample composites are kept in suitable containers (e.g., clean high density polyethelene containers or Zip-Lock® bags), labelled, and stored on ice, in a cooler, or in a refrigerator until the samples are delivered and processed. Pore water should be extracted from the samples as soon as possible because the toxicity of sediments in storage may change over time. A sample tracking system should be maintained for each sediment sample collected and porewater sample extracted. All manipulations made on samples are recorded on the Sample History Data Form (Attachment 2).

3.2 Load Extraction Cylinder

1. Assemble all parts of extraction cylinder except the top end compression coupling nut, top end plate and O-ring. Make sure filter is snugly in place beneath bottom O-ring (both over- and under-tightening will result in an improper seal). Place the extractor cylinder on the stand and positon an appropriately labelled porewater sample container (usually an I-Chem® amber 250 mL or 125 mL glass jar cleaned to EPA standards, with Teflon® lid liner) underneath the outlet.

2. Ensure that the sediment sample is homogenized, by shaking, stirring with a clean Teflon® or plastic spatula or spoon, or by both.

3. Transfer sediment from the sample container/bag to the extractor by pouring and/or using a clean Teflon® or plastic spatula or spoon. If necessary, particularly when extracting pore water from sandy or shelly sediments, the spatula may be used to compress the sample in the cylinder to eliminate channelization. The amount of sediment to be transferred will depend on the texture of the sample. The cylinder may be filled nearly full with a sandy sediment. However, when extracting pore water from a clay sediment, a relatively impermeable layer of compressed clay will eventually form on the filter, so that extraction of a large volume of clay sediment at once would take an extremely long time. When extracting pore water from extremely fine grained sediments, the cylinder should be less than one-third filled. If additional pore water is needed, this process can be repeated by removing the sediment including

sediment including removing or "peeling" the impermeable layer, and reintroducing more of the original sediment sample.

4. After sediment is loaded, the top end plate within the top compression coupling nut is installed . To tighten the top nut, the strap wrench and the coupling nut wrench (Figure 1) are used.

3.3 Porewater Extraction

After the extractor is sealed, a high-pressure hose is attached to the quick disconnect fitting on the top end plate, and the extractor is pressurized with air from a SCUBA tank. Pressure is controlled with a first-stage regulator on the SCUBA tank, an intermediate "governor" regulator, and final second stage regulators attached to a manifold that services multiple extractors (Figure 2).

1. Turn the SCUBA valve counter clockwise, pressurizing the first stage regulator and the intermediate-pressure hose (approximately 150 psi). An additional "governor" pressure regulator between the SCUBA tanks and the final second stage regulators which control pressure to the individual extractors should be set at maximum extractor pressure (~40 psi).

2. Ensure that all final pressure regulators are set to zero. Attach the hose from one of the pressure regulators on the pressure regulator manifold to the air inlet, using the quick disconnect fitting.

3. Slowly open the corresponding pressure regulator to a pressure of 5-10 psi. Check the first drops of porewater passing from the outlet for cloudiness. Occasionally, a small amount of sediment will pass through the porewater outlet, presumably around the filter. If this happens, wait until the pore water clears, discard the initial pore water collected, and continue.

4. Check the cylinder for leaks and if necessary tighten clamping nuts slightly.

5. As the flow of pore water decreases, pressure may be increased gradually to a maximum of 35-40 psi. When flow is less than or slows to less than 1-3 drops per minute, increase the pressure in 5-10 psi increments to maintain the flow. Allow the extraction to continue until sufficient pore water has been collected.

6. Disassemble the extractor, discard sediment, and rinse and wash appropriately all parts contacting sediment before placing a different sediment sample into the extractor.

7. Repeat these procedures until all available extractors are in use or until all sediment samples have been processed.

3.4 Centrifugation of Porewater Samples

Porewater samples extracted at this field station are usually stored frozen until tested. Under most circumstances, the porewater samples are centrifuged after they are collected and before they are frozen.

1. After collection, keep the porewater samples refrigerated or chilled on ice until they are centrifuged.

2. Transfer the pore water from the glass sample jar to an appropriate centrifuge bottle (e.g., polycarbonate). Centrifuge at ≥1200 *g* for 20 minutes. Return the centrifuged sample to a rinsed and labelled glass jar, taking care not to disturb any material that may have settled on the bottom/sides of the centrifuge bottle.

3. If multiple jars of pore water were collected from a single sediment sample, they should be composited after centrifugation and redistributed to the glass jars before testing or storage.

3.5 Storage of Porewater Samples

If the porewater samples are not to be used on the day of collection, they should be frozen for storage. Sufficient room for freeze expansion should be left in the jars (for example, 200 mL maximum sample in a 250 mL jar). If the volume needed for testing is known in advance, it is prudent to allocate only that specific volume plus a little excess (~10 mL) to each jar in order to conserve pore water (once thawed, the pore water cannot be refrozen and reused), and to simplify the volume measurements required for Water Quality Adjustment of Samples (SOP F10.12) performed the day prior to testing. Frozen porewater samples may be shipped with dry ice.

4.0 QUALITY CONTROL

A sample tracking system is maintained for each sediment sample collected and porewater sample extracted. All actions taken with that respective sample are recorded on the Sample History Data Form (Attachment 2). This information includes, but not exclusively, : a) the date of collection or receipt, b) the date of porewater extraction, c) the volume or number of jars (I-Chem® amber glass jars) of pore water collected, d) centrifugation information, if performed, e) date frozen and location (freezer no.), and e) date and jar no. thawed and used in which test. The Sample History Forms are kept in a three-ring binder at the same location where the samples are stored.

5.0 TRAINING

Persons who will perform this procedure should first read this SOP and then operate under the supervision of an experienced individual for at least one series of extractions.

6.0 SAFETY

The sediment and porewater samples handled may contain contaminants. Care should be taken to avoid contact with the samples. Protective gloves, glasses and clothing may be worn. Waste sediment should be properly disposed. SCUBA cylinders should be securely mounted before, during, and after use. The pressure limit (40 psi) of the extraction cylinders should not be exceeded. Before disconnecting any pressure hoses, ensure that the pressure has been released or that the controlling regulator has been closed. The pressure relief valves should be set to leak at just above maximum operating pressure, and they should be checked regularly to ensure that they are performing. Pressure relief valves should be disassembled and cleaned yearly.

7.0 ATTACHMENTS

Attachment 1. Required Equipment and Materials
Attachment 2. Sample History Form

8.0 REFERENCES

Carr, R.S. 1993. Sediment quality assessment survey of the Galveston Bay System. Galveston Bay National Estuary Program report, GBNEP-30, 101 pp.

Carr, R.S., J.W. Williams, and C.T.B. Fragata. 1989. Development and evaluation of a novel marine sediment pore water toxicity test with the polychaete *Dinophilus gyrociliatus*. Environ. Toxicol. Chem. 8:533-543.

Carr, R.S. and D.C. Chapman. 1992. Comparison of solid-phase and pore-water approaches for assessing the quality of marine and estuarine sediments. Chem. Ecol. 7:19-30.

Carr, R.S. and Chapman. 1994. Improved device for extracting sediment pore water. National Biological Survey, Research Information Bulletin No. 38.

National Biological Survey (NBS). 1993. Toxicity testing of sediments from Charleston Harbor, South Carolina and vicinity. Report submitted by the National Biological Survey to the National Oceanic and Atmospheric Administration, Ocean Assessment Division, Seattle, WA, 7 pp. + 16 tables and 4 attachments.

National Biological Survey (NBS). 1994a. Survey of sediment toxicity in Pensacola Bay and St. Andrew Bay, Florida. Report submitted by the National Biological Survey to the National Oceanic and Atmospheric Administration, Ocean Assessment Division, Seattle, WA, 12 pp. + 24 tables and 5 attachments.

National Biological Survey (NBS). 1994b. Toxicity testing of sediments from Boston Harbor, Massachusetts. Final report submitted to National Oceanic and Atmospheric Administration, 6 pp. + 10 tables and 4 attachments.

US Fish and Wildlife Service (USFWS) 1992. Amphipod solid-phase and sea urchin porewater toxicity tests with Tampa Bay, Florida sediments. Final report submitted to National Oceanic and Atmospheric Administration, 9 pp. + 16 tables and 3 attachments.

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Joseph B. Hunn 6-28-94
Quality Assurance Officer

Attachment 1

REQUIRED EQUIPMENT AND MATERIALS

To construct a sediment pore water extraction device:

1-PVC cylinder (center portion of 4" compression coupling)
2-PVC end nuts (ends of 4" compression fitting)
1-PVC top end plate (7/16" width)
1-PVC bottom end plate (7/16" width)
1-Quick disconnect brass air fitting
1-Pressure relief valve
1-Teflon® 1/8" npt male connector for exit port

To use a pore water extraction device:

1-Filter, polyester material, 5 µm pore size
1-Wooden stand (1 stand per 3 cylinders)
1-Custom wrench for 4" compression coupling end nuts
1-Custom wrench head attached to table
1-Plastic or Teflon® spatula or spoon
1-SCUBA cylinder
1-SCUBA regulator with high pressure gauge
1-SCUBA intermediate pressure hose (~10 ft length)
with governor pressure gauge set to ~40 psi
1-Air pressure control manifold that includes:
Final pressure regulator valves (several per manifold)
Pressure gauges (1 per valve)
Low pressure hose, 6' length (1 per manifold)

Other required supplies/equipment:

Sediment sample containers or bags
Pore water sample jars
Sample labels or labeling tape
Beakers
Deionized water (DI)
Wash bottles, 500 ml
Protective gloves, glasses, clothing
Pens, pencils, markers
Centrifuge and centrifugation materials
Refrigerator
Freezer

Attachment 2

SAMPLE HISTORY DATA FORM

Sample Designation: _____ Study Protocol: _____ Initials: _____

Date of acquisition: _____ Sample type: _____

How acquired (refer to sample site data sheet number, if appropriate): _____

_____

_____

_____

Initials	Date	Action Taken

Date Prepared: March 14, 1991

Date Revised: May 17, 1994

WATER QUALITY ADJUSTMENT OF SAMPLES

1.0 OBJECTIVE

In order to perform toxicity tests with saline samples, all test and reference samples should be similar in salinity so that salinity is not a factor in survival of test organisms. Additionally, dissolved oxygen (DO) concentrations should be sufficiently high to ensure that low DO is not a source of stress to the test organisms. At the Corpus Christi field station, toxicity tests are performed using a variety of marine and estuarine organisms, including the sea urchin *Arbacia punctulata*, the polychaete *Dinophilus gyrociliatus*, the harpacticoid copepod *Longipedia* sp., and the red drum *Sciaenops ocellatus*. The aqueous samples tested may be pore water, different kinds of discharges and effluents, surface microlayer, or subsurface water samples that may range in salinity from 0-36‰. Although from test to test salinities used in the different toxicity tests may vary, the individual toxicity tests performed on a particular day are run at a single target salinity. Since initial salinities of the porewater or water samples to be tested commonly vary, they will require salinity adjustment to within 1‰ of the target salinity. Additionally, DO should normally be ≥80% saturation in all samples tested.

2.0 PREPARATION

2.1 Equipment and Labware

The supplies and equipment needed are listed in Attachment 1.

2.2 Source of Dilution Water

For samples lower in salinity than target salinity, concentrated brine (~100‰) is added to increase salinity. Concentrated brine is prepared by heating (to 35-40°C) and gently aerating filtered natural seawater (1 μm) to concentrate the salts by evaporation. For samples higher in salinity than target salinity, HPLC ultrapure sterile water (J.T. Baker® Cat. #JT4218-2) is added to decrease salinity.

3.0 PROCEDURES

The following describes the procedures required for the adjustment and determination of specific water quality parameters of a sample.

3.1 Preparation for Salinity Adjustment

1. Although fresh samples are routinely tested at the Corpus Christi field station, most of the samples tested are stored frozen in amber I-Chem® jars. If frozen, remove samples from freezer and allow them to thaw at room temperature or immerse them in a tepid water bath to thaw, ensuring that sample temperature does not exceed 25°C. The samples may be thawed the day of water quality adjustment (WQA) or may be transferred from the freezer to a refrigerator (4°C) the day before WQA and then completely thawed the following day. After thawing, allow the samples to come to room temperature. Generally, the samples should be maintained at the same temperature required for the toxicity test that will be conducted. The temperature requirement for most toxicity tests performed at this field station is 20±1°C, and room temperature should be maintained accordingly.

2. Turn bottled sample end over end a few times to mix thoroughly before measuring salinity. Using a salinity refractometer, measure salinity and record on Water Quality Adjustment Data Form (Attachment 2).

3. In order to make calculations for the salinity adjustment, the volume of the sample must be known. When porewater or other water samples are collected and transferred to amber jars for storage, they are commonly measured to an approximate volume (~110 mL, for example) prior to freezing. On the day of WQA, this volume should be recorded on the WQA data form for the respective samples. If the volume is unknown at this point, it should be measured using a graduated cylinder of appropriate size, and recorded on the data sheet.

3.2 Salinity Adjustment

3.21 Reducing the salinity of aqueous samples

Refer to the formulas below to calculate the volume of HPLC water needed to reduce the initial sample salinity to the target salinity. Add the volume calculated, mix the bottle thoroughly, check the salinity with a refractometer, and record the volume of HPLC water added as well as the final salinity.

(i) (target $^{o}/_{oo}$ ÷ sample $^{o}/_{oo}$) × sample vol. in mL = A
(ii) sample vol. - A = B
(iii) sample vol. ÷ A = C
(iv) B × C = volume of HPLC water to add

3.22 Increasing the salinity of aqueous samples

Refer to the formula below to calculate the volume of concentrated brine needed to increase the initial sample salinity to the target salinity. Add the volume calculated, mix the bottle thoroughly, check the salinity with a refractometer, and record the volume of brine added as well as the final salinity.

(i) ((target $^{o}/_{oo}$ - sample $^{o}/_{oo}$) × sample vol. in mL) ÷ (brine $^{o}/_{oo}$ - target $^{o}/_{oo}$) = vol. of brine to add

3.3 Dissolved Oxygen Adjustment

Measure and record DO and percent DO saturation of sample (SOP F10.13). Occasionally, a sample will have DO of less than 80% saturation. Any such samples should be gently stirred on a magnetic stirrer to increase the DO level above 80%. Record initial DO, the elapsed mixing time, and final DO in the comments section of the Water Quality Adjustment Data Form. (On the following day, DO should be rechecked and brought to >80% by stirring again if necessary before the toxicity test is performed.)

3.4 Other Water Quality Determinations

1. Measure pH (SOP F10.21) and record on the Water Quality Adjustment Data Form.

2. Measure and record ammonia concentration (SOP F10.4).

3. Measure and record sulfide concentration if required.

4.0 DATA COLLECTION

All raw data are entered on one standardized form, the Water Quality Adjustment Data Form (see Attachment 2) at the time the determinations or adjustments are made.

5.0 QUALITY CONTROL

A data form (Attachment 2) will be used to document all sample handling procedures for each sample. The person(s) recording data on the sheet will initial each sheet. Original data forms after completion will be stored in a three-ring file in the possession of the field station leader. Copies will be kept in the lab.

6.0 TRAINING

Personnel who will perform this task should first read this protocol and then operate under supervision during the preparation of at least two samples.

7.0 SAFETY

The NaOH solution used in the ammonia determination procedure is a highly caustic liquid. Care should be taken to avoid its contact with skin or clothing. Should such contact occur, quickly flush affected with water. A sink is present along the west wall of the dry lab, another is present along the east wall of the wet lab, and an eye flushing station is present in the northwest corner of the wet lab near the entrance door. The samples handled may be pore water, effluent, discharges, or other water samples that may contain contaminants. Care should be taken to avoid contact with the samples.

8.0 ATTACHMENTS

Attachment 1. Equipment List for Water Quality Adjustment
Attachment 2. Water Quality Adjustment Data Form

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ATTACHMENT 1

EQUIPMENT LIST FOR WATER QUALITY ADJUSTMENT

Graduated cylinders
Pipetters
Latex gloves
Magnetic stirrer and stir bars
10 M NaOH
Concentrated brine (See section 2.2 for preparation)
HPLC ultrapure sterile water (J.T. Baker® #JT4218-2)
Salinity refractometer
Dissolved oxygen meter
pH electrode, buffer solutions, and meter
Ammonia electrode, standard solutions, and meter
Sulfide electrode, standard solutions, and meter
Data sheets
Hand calculator

ATTACHMENT 2

WATER QUALITY ADJUSTMENT DATA FORM

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ DATE_____

A. Salinity Adjustment:

Initial volume (mL)	_____
Initial salinity (°/$_{oo}$)	_____
Vol. Baker® HPLC water added (mL)	_____
Vol. ____°/$_{oo}$ brine added (mL)	_____
% of original sample (initial vol./final vol. x 100)	_____

B. Character of Sample (after salinity adjustment):

Final Volume (mL)	_____
Final Salinity (°/$_{oo}$)	_____
pH	_____
Dissolved oxygen (mg/L)	_____
DO saturation (%)	_____
Total ammonia (mg/L)	_____
Sulfide (mg/L)	_____

COMMENTS_____

Date Prepared : April 10, 1990

Date Revised: March 10, 1995

SEA URCHIN FERTILIZATION TOXICITY TEST

1.0 OBJECTIVE

The purpose of the fertilization toxicity test with the sea urchin, *Arbacia punctulata*, is to determine if a sea water, pore water, sea surface microlayer, or other sample reduces fertilization of exposed gametes relative to that of gametes exposed to a reference sample. The test may also be used to determine the concentration of a test substance which reduces fertilization. Test results are reported as treatment (or concentration) which produces statistically significant reduced fertilization or as concentration of test substance which reduces fertilization by 50 percent (EC_{50}). This test can be performed concurrently with Sea Urchin Embryological Development Toxicity Test (SOP 10.7) and/or Sea Urchin Genotoxicity/Teratogenicity Test (SOP 10.8), using the same pretest and sperm and egg collection.

2.0 TEST PREPARATION

2.1 Test Animals

Gametes from the sea urchin, *Arbacia punctulata* are used in the sea urchin fertilization toxicity test. Animals can be collected in the field or obtained from a commercial supplier. *A. punctulata* can be differentiated from other species of urchins which are found in Texas by the five plates surrounding the anal opening, and by round sharp spines on the dorsal surface of the test and flattened spines surrounding the Aristotle's lantern. Urchins can be maintained easily in aquaria or other tanks with running seawater or an aquarium filter. Urchins will eat a wide variety of marine vegetation. A good diet may be provided by placing rocks from jetties (which have been colonized by diatoms and macroalgae) into the tank with the urchins or romaine lettuce may be provided as a substitute. Temperature manipulations of the cultures will prolong the useful life of the urchins. Cultures are maintained at 16 ± 1°C when gametes are not required. Temperature is gradually increased to 19 ± 1°C at least one week prior to gamete collection and subsequently decreased if no further tests are planned. Photoperiod is maintained at 16 hours of light per day. Water quality parameters should be monitored weekly and salinity maintained at 30 ± 3 $^{o}/_{oo}$. Males and females should be kept in separate tanks.

2.2 Dilution Water

HPLC reagent grade purified water or concentrated seawater brine is used to adjust samples to 30 $^{o}/_{oo}$ as described in Water Quality Adjustment of Samples (SOP 10.12). Concentrated seawater brine (90-110 $^{o}/_{oo}$) is made in large batches by heating seawater to 40°C or less in large tanks with aeration for 3-4 weeks. Brine quality will remain constant over long periods with no refrigeration. At the time of salinity adjustment, pH, ammonia, and dissolved oxygen are also measured. Salinity adjustment and water quality data are recorded on prepared data forms.

Filtered (0.45 µm) seawater adjusted to 30 $^{o}/_{oo}$ is used to wash eggs and is also used for sperm and egg dilutions. The acronym MFS (for Millipore® filtered seawater) is used for this filtered and salinity adjusted seawater.

2.3 Test System: Equipment

When testing samples for potential toxicity, five replicates per treatment are recommended. One replicate is a 5 mL volume of sample in a disposable glass scintillation vial. When conducting a dilution series test, fifty percent serial dilutions may be made in the test vials, using MFS as the diluent.

2.3.1 Equipment

A list of equipment necessary for conducting this test is given in Attachment 1 (Equipment List for Fertilization Toxicity Test).

2.3.2 Solutions

<u>10% Buffered Formalin</u>:

1,620 mL sea water
620 mL formaldehyde
6.48 g NaH_2P0_4 or KH_2PO_4 (mono)
10.5 g Na_2HPO_4 or K_2HPO_4 (dibasic)

1 mL needed for each replicate. Fill the dispenser.

2.4 Collection and Preparation of Gametes

Quality gametes must first be collected, and then diluted to the appropriate concentration for addition to the test vials.

2.4.1 Selection of Urchins to be Used in Toxicity Test.

1. Take two or three females and place in shallow bowl, barely covering tests with seawater.

2. Stimulate release of eggs from gonopores of a female by touching test with electrodes from a 12V transformer.

3. Collect a few eggs from between spines using a 10 mL disposable syringe with a large gauge blunt-tipped needle attached. Discard the first small quantity of eggs expelled from each gonopore and continue collecting. Place a 2 to 5 drops of eggs onto a scintillation vial containing 10ml of filtered seawater. Rinse syringe and repeat for each female.

4. Select females which have round, well developed eggs, and which do not release clumps of eggs or undeveloped ovarian tissue.

5. Place 2-4 males in shallow bowl(s) with a small amount of seawater, leaving the upper $^1/_2$ to $^1/_3$ of the animals uncovered.

6. Stimulate release of sperm from gonopores by touching test with electrodes from 12V transformer (about 30 seconds each time). If sperm is watery, reject the animal and choose another. Sperm should be the consistency of condensed milk. Collect sperm using a pastuere pipette with a rubber bulb attached.

Generally, a gamete check is performed in order to ensure that both the male and the female urchins used in the test have gametes with a high degree of viability. If the gamete check is performed, two to five females (depending on confidence in the proportion of urchins in the holding facility in good reproductive status) and at least two males should be selected using the above procedures. The check is performed by adding 5 to 7 drops of a concentrated dilution of sperm to the eggs in the scintillation vials (collected as described above) and observing the eggs under the microscope after 10 minutes. The concentrated dilution of sperm is usually made by diluting 20-50µl of sperm in 10 ml of filtered seawater. If the proportion of eggs fertilized is high (95-100%), that female and male may be used in the pretest and test. Sperm from a number of males or females may be combined in the beginning if the gamete check reveals a number of high quality animals or the confidence is high in the quality of the gametes Once a good male and female are selected a pretest can be conducted to determine the correct dilution of sperm to use in the test (Attachment 2).

2.4.2 Obtain Eggs

1. Place selected female in large Carolina dish and add enough water to cover the urchin's test with approximately 1 cm of seawater. Stimulate release of eggs from female with 12V transformer.

2. Collect eggs as above using the 10 mL syringe. Remove needle before dispensing eggs into a disposable shell vial or other clean container capable of holding 25-50 mL. Collect enough eggs for pretest and test. If female stops giving eggs readily or starts giving chunky material, cease stimulation and collection of eggs from that female.

3. Add MFS to fill shell vials, gently mixing eggs. Allow eggs to settle to bottom of vial. Remove water with a pipette. Replace water, again gently mixing the eggs.

4. Repeat washing procedure.

2.4.3 Prepare Appropriate Egg Concentration

1. Put approximately 100 mL of 30 $^{o}/_{oo}$ MFS in a 250 mL beaker, and add enough washed eggs to bring the egg density to approximately 10,000 per mL . If more than 400 total replicates (27 treatments) are to be tested, a larger amount of water and a correspondingly larger amount of eggs should be used. Two hundred µL of this egg solution will be used per replicate, and it is easier to maintain proper mixing and uniform egg density if there is an excess of at least 50%.

2. Check egg density and adjust to within approximately 9000 to 11,000 eggs per mL, as follows. Gently swirl egg solution until evenly mixed. Using a pipette, add 1 mL of the solution to a vial containing nine mL seawater. Mix and transfer 1 mL of this diluted solution to a second vial containing 4 mL of seawater. Again, mix and transfer 1 mL of this diluted solution to a counting slide such as a Sedgewick-Rafter slide.

3. Using a microscope (either a compound microscope with a 10x objective or a dissecting scope may be used here), count the number of eggs on the slide. If the number is not between 180 and 220, then adjust by adding eggs or water. If egg count is > 220 use the following formula to calculate the amount of water to add:

   ("egg count" - 200/200) x Current Volume of Eggs = Volume seawater to add to stock (mLs)

   If egg count < 200 add a small amount of eggs. Since it is less arbitrary and more likely to arrive at an acceptable count when using the water addition formula, it is better to originally overestimate the amount of eggs to add to the 100 mL of water.

4. Repeat steps 2 and 3 until an acceptable egg count (between 180 and 220) is obtained.

2.4.4 Obtain Sperm

Place selected male urchin in a large Carolina dish containing 1-2 cm of water. About half of test should be above water level. Stimulate male with 12V transformer, and collect about 0.5 mL of unwetted sperm from between spines using a pasteur pipette. Place sperm into a plastic microcentrifuge tube. Keep on ice until used. Be careful not to add any water or sperm which has contacted water to the vials. High quality sperm collected dry and kept on ice will last at least eight hours without measurable decline in viability.

2.4.5 Prepare Appropriate Sperm Dilution

It is desirable for control fertilization to be within 60-90%. Although controls outside these bounds do not automatically disqualify a test, particularly if a valuable dose response is generated, the sensitivity of the test is reduced by fertilization rates greater than 90% and good dose responses may be difficult to obtain with less than 60% fertilization in controls. Density of sperm in the sperm solution should be determined with this goal in mind. Condition of the animals and length of acclimation to the aquarium may effect the chosen sperm density. The pretest (Attachment 2) may be used to calculate an appropriate sperm dilution. Generally, a dilution of between 1:10,000 and 1:2500 will result in desirable fertilization rates, if the animals are in good condition.

For example, if a sperm dilution of 1:5000 is required (as determined from the pretest), add 20 µL sperm to 10 mL MFS. Mix thoroughly, then add 1 mL of this solution to 9 mL MFS. Sperm should not be wetted until just before starting the test. Sperm wetted more than 30 minutes before the test has begun, including sperm dilutions used in any pretest, should be discarded and a new dilution made from sperm kept on ice.

3.0 TEST PROCEDURES

1. Add 50 µL appropriately diluted sperm to each vial. Record time of sperm addition. Sperm should be used within 30 minutes of wetting.

2. Incubate all test vials at 20 ± 2°C for 30 minutes. At this point it is useful to set a timer for five to ten minutes prior to the end of the incubation period. This will notify the worker early enough to be ready to start the next step exactly on time.

3. While gently swirling the egg solution to maintain even mixing of eggs, use a 200 µL pipetter to add 200 µL diluted egg suspension to each vial. Pipette tips are cut back using

a clean razor blade to prevent crushing the eggs during pipetting. Record time of egg addition.

4. Incubate for 30 minutes at 20 ± 2°C. The timer may be used again at this point.

5. Using the dispenser, add 1 mL of 10% buffered formalin to each sample.

6. Vials may now be capped and stored overnight or for several days until evaluated. Fertilization membranes are easiest to see while eggs are fairly fresh, so evaluation within two to three days may decrease the time required for evaluation.

7. If it is not possible to make the evaluations within several days or the membranes are difficult to discern, an optional technique may be employed. Make up a 200 $^{o}/_{oo}$ NaCl solution (pickling salt) and add 2 to 4 drops of the solution to a 1 mL egg sample on a microscope slide. This solution causes the egg, but not the membrane, to shrink briefly thereby making the membrane easier to see. The effect only lasts for a short time (~5 min.) so the observations must be made immediately after the NaCl solution is added. If this optional technique is employed, it must be used on all samples in that test series.

4.0 DATA COLLECTION AND TABULATION

1. Transfer approximately 1 mL eggs and water from bottom of test vials to counting slide. Observe eggs using compound microscope under 100X magnification. Dark field viewing is useful here in identifying fertilization membranes.

2. Count 100 eggs/sample using hand counter with multiple keys (such as a blood cell counter), using one key to indicate fertilized eggs and another to indicate unfertilized eggs. Fertilization is defined by the presence of fertilization membrane surrounding egg.

3. Calculate fertilization percentage for each replicate test:

$$\frac{\text{Total No. Eggs - No. Eggs Unfertilized}}{\text{Total No. Eggs}} \times 100 = \text{Percent Eggs Fertilized}$$

5.0 DATA ANALYSIS

Data are recorded on standardized data sheets (See Attachments 3-7). Normally, percent fertilization in each treatment is compared to an appropriate reference treatment (seawater, pore water or sea surface microlayer from an uncontaminated environment). Statistical comparisons are made using analysis of variance (ANOVA) and Dunnett's *t*-test (Sokal and Rohlf 1981) on the arc sine square root transformed data. For multiple comparisons among treatments, Ryan's Q test (Day and Quinn 1989) with the arc sine square root transformed data is recommended. The trimmed Spearman-Karber method with Abbott's correction is recommended to calculate EC_{50} values for dilution series tests (Hamilton et al. 1977)

6.0 QUALITY CONTROL

Quality control tests may be run using both positive and negative controls with multiple replicates (as many as desired). Typically, a reference toxicant dilution series (sodium dodecyl sulfate) is tested with each test to evaluate the effectiveness of the sperm dilution chosen. Negative controls may include a reference porewater, filtered seawater, and/or a reconstituted brine.

7.0 TRAINING

A trainee will conduct the test with supervision initially. Determining egg concentrations and fertilization counts are test specific activities. These functions can be performed independently after a trainee has demonstrated he or she can accurately reproduce the test.

8.0 SAFETY

The sea urchin fertilization toxicity test poses little risk to those performing it. Care should be taken when making and dispensing the 10% buffered formalin solution; use a hood if available, but make sure the test area is well ventilated. Protective gloves can be worn when pipetting or dispensing formalin or potentially toxic samples.

Care should be taken when collecting or otherwise handling sea urchins. Urchin spines are sharp and fragile and may puncture the skin and break off if handled roughly. First aid similar to treatment of wood splinters is effective in this case (removal of spine and treatment with antiseptic). Collection of sea urchins by snorkeling should not be done alone.

9.0 ATTACHMENTS

Attachment l. Equipment List for Fertilization Toxicity Test
Attachment 2. Pretest to Insure Selection of Quality Gametes
Attachment 3. Water Quality Adjustment Data Form
Attachment 4. Sea Urchin Pretest Data Sheet
Attachment 5. Sea Urchin Pretest Continuation Data Sheet
Attachment 6. Sea Urchin Fertilization/Embryological Development Toxicity Test Gamete Data Sheet
Attachment 7. Sea Urchin Fertilization Toxicity Test Fertilization Data Sheet

10.0 REFERENCES

Day, R.W. and G.P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59:433-463.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11(7):714-719; Correction 12(4):417 (1978)

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. 2nd edition. W.H. Freeman and Company, San Francisco, CA 859 pp.

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Attachment 1

EQUIPMENT LIST FOR FERTILIZATION TOXICITY TEST

Large Carolina dishes (at least 2)
20 mL KIMBLE scintillation vials (These should be type shipped with caps off, and without cap liners. If other brand or type is used, the vials should be tested for toxicity prior to use.)
400 mL beaker or wide-mouthed thermos for holding vials of sperm
250 mL beakers (4)
Pasteur pipettes and latex bulbs
plastic microcentrifuge tubes
25 mL shell vials or equivalent
Test tube rack (to hold shell vials)
12V transformer with pencil type electrodes
Styrofoam (or something to hold electrode tips)
10 cc syringe with large diameter blunt ended needle (make by grinding sharp point off the needle with a grinding stone)
Marking pens
Ice
10-100 µL pipetter
50-200 µL pipetter
5 mL pipetters (2)
Counting slide such as Sedgewick-Rafter chamber
Compound microscope with 10x objective and dark field capability
Hand tally counter
Calculator
Timer for exposure / incubation periods
Buffered formalin and dispenser
Filtered (0.45 µm) seawater, adjusted to 30 $^{o}/_{oo}$
Data sheets
Baker reagent grade water
Approximately 100 $^{o}/_{oo}$ concentrated brine

Attachment 2
PRETEST TO INSURE SELECTION OF QUALITY GAMETES

1. Using the procedure in section 2.4.1, select 2 to 5 females and at least 2 male urchins to be used in the pretest.

2. Fill pretest vials with five mL of **reference** water. There should be at least two vials for each combination of male, female, and pretest sperm concentration (step 4 below). For example, in a pretest with two females, one male, and six pretest sperm concentrations, 24 vials (2 X 2 X 6) would be needed. Arrange and mark vials accordingly in a rack.

3. Perform steps 2.4.2 (egg collection) and 2.4.3 (egg dilution) for each female urchin. Make enough volume of the egg suspension to perform the pretest and the test.

4. Perform step 2.4.4 (sperm collection) for each male urchin or male combination. Prepare a dilution series of sperm concentrations which will bracket the 60-90% fertilization rate in the test. Sperm dilution will depend on the health and reproductive status of the male urchin, but in most cases the following "standard dilution" should be used:

1: 250 (20 µL dry sperm added to 5 mL MFS. This concentration is used only as stock solution to make up the rest of the dilution series and is not used full strength in the pretest.)
1: 1250 (1 mL of 1:250 and 4 mL MFS)
1: 2500 (1 mL of 1:250 and 9 mL MFS)
1: 5000 (2 mL of 1:2500 and 2 mL MFS)
1: 7500 (2 mL of 1:2500 and 4 mL MFS)
1:10000 (3 mL of 1:7500 and 1 mL MFS)
1:12500 (1 mL of 1:2500 and 4 mL MFS)

Sperm must be used within 30 minutes of dilution. Leave undiluted sperm on ice and retain, because a new sperm dilution of the concentration determined in this pretest will be needed for the toxicity test. **Sperm diluted for use in the pretest may not be used in the toxicity test, because the time elapsed since the addition of water is too great.**

5. As in section 3.0 add 50 µL of the diluted sperm to each pretest vial. Incubate for 30 minutes at approximately 20°C, and add 200 µL of the egg suspension. Incubate for another 30 minutes, then fix with 1 mL of the buffered formalin solution.

6. As in section 4.0, obtain a fertilization rate for the vials. There is no need to count all vials, enough vials should be counted to determine a good male/female combination, and an appropriate sperm dilution factor. If more than one male/female combination is acceptable, this is a good opportunity to choose a female which exhibits easily visible fertilization membranes or in cases where there are many samples, to combine eggs from different females . The appearance of the fertilization membranes may vary among female urchins, and presence of easily visible membranes facilitates counting.

Attachment 3

WATER QUALITY ADJUSTMENT DATA FORM

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ DATE_____

A. Salinity Adjustment:

Initial volume (mL)	_____
Initial salinity (‰)	_____
Vol. Milli-Q water added (mL)	_____
Vol. ___‰ brine added (mL)	_____
% of original sample (initial vol./final vol. x 100)	_____

B. Character of Sample (after salinity adjustment):

Volume (mL)	_____
Salinity (‰)	_____
pH	_____
Dissolved oxygen (mg/L)	_____
DO saturation (%)	_____
Total ammonia (mg/L)	_____
Sulfide (mg/L)	_____

COMMENTS_____

_____

_____

_____

_____

Attachment 4

SEA URCHIN PRETEST DATA SHEET

TEST ID_____ INITIALS_____

STUDY PROTOCOL_____ DATE_____

EGGS

Female number:	____	____	____	____
Collection time:	____	____	____	____
Count:	____	____	____	____

SPERM

Male number:	____	____	____	____
Collection time:	____	____	____	____
Dilution start time:	____	____	____	____

TEST TIMES

Sperm in:_____ Eggs in:_____ Formalin in:_____

SPERM DILUTION _____

COMMENTS _____

_____

% FERTILIZATION Reference sample designation:_____

Female #_____ Male #_____

Sperm Dilution	REP 1	REP 2	REP 3	REP 4
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

% FERTILIZATION Reference sample designation:_____

Female #_____ Male #_____

Sperm dilution	REP 1	REP 2	REP 3	REP 4
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

Attachment 5

SEA URCHIN PRETEST CONTINUATION DATA SHEET

TEST ID________________________________ INITIALS________________

STUDY PROTOCOL__________________________ DATE________________

% FERTILIZATION Reference sample designation:________________

Female #________________ Male #________________

Sperm dilution	REP 1	REP 2	REP 3	REP 4
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

% FERTILIZATION Reference sample designation:________________

Female #________________ Male #________________

Sperm dilution	REP 1	REP 2	REP 3	REP 4
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

% FERTILIZATION Reference sample designation:________________

Female #________________ Male #________________

Sperm dilution	REP 1	REP 2	REP 3	REP 4
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

% FERTILIZATION Reference sample designation:________________

Female #________________ Male #________________

Sperm dilution	REP 1	REP 2	REP 3	REP 4
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____
____	____	____	____	____

Attachment 6

SEA URCHIN FERTILIZATION/EMBRYOLOGICAL DEVELOPMENT

TOXICITY TEST GAMETE DATA SHEET

TEST ID_____ INITIALS_____

STUDY PROTOCOL_____ DATE_____

EGGS

Collection time:_____

Initial count/volume:_____

Final count:_____

SPERM

Collection time:_____ Dilution start time:_____

Sperm dilution:_____

_____

Test start temperature:_____

TEST TIMES

Box #	Sperm in:	Eggs in:	Formalin in:
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

COMMENTS_____

_____

_____

Attachment 7

SEA URCHIN FERTILIZATION TOXICITY TEST

FERTILIZATION DATA SHEET

TEST ID_____ INITIALS_____

STUDY PROTOCOL_____ DATE_____

PERCENT FERTILIZED

	Replicate						
Treatment	1	2	3	4	5	Mean±SD	Unfert.

COMMENTS _____

Date Prepared: 2-28-97

Date Revised:

AN IN VITRO ACUTE TOXICITY TEST: MICROTOX® BASIC TEST

I. GENERAL

This document describes the procedure for performing acute toxicity tests with the Microtox® Acute Toxicity System (commonly referred to as the Basic Test). A temperature-controlled luminometer provided by the manufacturer measures the light output of luminescent bacteria (supplied by the manufacturer) before and after they are challenged by serial dilutions of a sample of unknown toxicity. A Reagent Blank containing no toxicant is used to normalize the responses of the sample test concentrations during data reduction. The degree of light loss resulting from metabolic inhibition in the test organisms indicates the toxicity of the sample and is used to determine a dose-response curve and the sample's effective concentration (EC50).

II. REQUIRED MATERIALS

1. Microtox® Reagent (selected bacterial strains).
   The reagent is a freeze-dried culture of a specially developed strain of the marine bacterium *Photobacterium phosphoreum*. Self-defrosting freezers should not be used for long term storage of the reagent because periodical warming to prevent frost accumulation may decrease storage time and viability of the cultures.
2. Microtox® Reconstitution Solution (distilled water).
   Distilled water is used to reconstitute frozen reagent. Reconstitution Solutions are stored indefinitely in a tightly stoppered glass-container at refrigerator temperatures.
3. Microtox® Diluent (2% NaCL solution).
   Diluent is used for diluting the test sample. Diluent stock is stored indefinitely at refrigerator temperatures.

III. REQUIRED EQUIPMENT

1. Microtox® disposable cuvettes
2. Adjustable pipettes: 10 µL, 500 µL, 1000 µL, 10000 µL
3. Disposable pipette tips: 10 µL, 500 µL, 1000 µL, 10000 µL
4. 10 mL electronic adjustable pipette
5. Vortex mixer

6. Microbics Microtox® Model 500 Analyzer (luminometer)
7. Microbics data capture and reduction program (PC software)
8. Micro-computer with one serial port capable of running Micro Soft Basic or Basic A software.

IV. ANALYZER PREPARATION

The Model 500 Analyzer layout consists of a block of 30 Wells with Columns designated 1 through 5 and Rows A through F, and a single isolated Reagent Well, and a Reading Well. All wells are temperature controlled for 15°C.

1. Place clean, new cuvettes in the Reagent Well and in all 30 block wells.

2. Pipette 1.0 mL Reconstitution Solution into the cuvette in the REAGENT Well.

3. Pipette 0.5 mL Diluent into each cuvette in block wells:

   B1 through B5
   D1 through D5
   F1 through F5

4. Pipette 1.0 mL Diluent into each cuvette in block wells:

   A1 through A4
   C1 through C4
   E1 through E4

5. Pipette 1.9 mL Diluent into each cuvette in block wells:

   A5, C5, and E5

V. SAMPLE PREPARATION

Protocol for three simultaneous test analyses of organic extracts with dimethylsulfoxide (DMSO) as carrier solvent.

1. Pipette 100 µL of the prepared organic extract of a test sample into the cuvette containing the Diluent in Well A5.
2. Place cuvette containing sample extract and Diluent on a vortex mixer for 5 sec.
3. Repeat the same procedure placing test sample 2 in Well C5 and sample 3 in Well E5.

VI. SAMPLE DILUTION (Dose-Response Series)

Protocol for three test samples using one control and four tube dilution series.