Final Report

on

TOXICITY TESTING OF SEDIMENTS FROM CHESAPEAKE BAY AND SURROUNDING AREAS YEAR III

submitted to

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from

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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

INTRODUCTION

The National Status and Trends (NS&T) Program of NOAA has shown that some sampling sites in coastal Chesapeake Bay 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 multiyear multidisciplinary sediment quality survey conducted to determine the severity and spatial extent of the toxicity of surficial sediments of Chesapeake Bay and the adjoining tributaries and canals, toxicity of sediments collected from these sites was assessed using pore water in the sea urchin (*Arbacia punctulata*) fertilization 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; 1998; 1999a; 1999b; 2000a; 2000b; 2000c; 2001a; 2001b; 2001c; 2002). 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.

The specific objectives of this study were to:

- Extract sediment pore water from all 78 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) were run in conjunction with each test.
- Make statistical comparisons between test and reference stations/strata for the sea urchin assays.

MATERIALS AND METHODS

Sediment Sample Receipt and Tracking

Surficial sediment samples were collected from 78 stations in southern Chesapeake Bay and the surrounding tributaries and estuaries. Samples were collected by NOAA personnel during September 2001. Samples were placed in precleaned 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. Sample temperatures upon arrival were between 4 and 9°C with the exception of five samples that were delayed in shipment and were received at 19°C (Table 1). It is not known what effects elevated temperature may have had on the toxicity of these samples and this must be considered when interpreting the results. Shipments were accompanied by sample tracking sheets, and samples were logged into laboratory sample tracking systems. The samples were placed on blue ice and processed immediately upon receipt. All porewater samples were extracted within 12 days from the time of field collection of sediment, and within 24 hours of arrival at the MERS Corpus Christi laboratory (Table 1).

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, 1998; 1999a; 1999b; 2000a; 2000b; 2000c; 2001a; 2001b; 2001c; 2002). The apparatus and extraction procedures are detailed in SOP F10.9 (Attachment 1).

Sediment samples were held on blue ice (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 24 hours 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 completely 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® refractometer; and pH, sulfide (as S^{-2}), and total ammonia (TAN) were measured with Orion® meters and their respective probes. Unionized ammonia 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 78 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; 1998; 1999a; 1999b; 2000a; 2000b; 2000c; 2001a; 2001b; 2001c; 2002), 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 laboratory (Carr and Biedenbach, 1999). 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%.

Dissolved Organic Carbon Analysis

Dissolved organic carbon (DOC) was measured in the porewater samples using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer following the model 1010 operators manual (OI Analytical, 1998). Samples of freshly extracted pore water (20 ml approx.) were immediately filtered through a 0.45 µm Whatman® nylon syringe filter and preserved with approximately 0.5 ml of phosphoric acid. Samples were stored refrigerated before analysis.

Samples were analyzed in the TOC mode with 400 µl of acid (5% phosphoric acid) and 4000 µl of oxidant (200 g/L sodium persulfate). Total inorganic carbon react and detect times were 2:00 (min:sec) and 1:35 (min:sec), respectively. Total organic carbon react and detect times were 8:30 (min:sec) and 2:00 (min:sec), respectively. At least one blank was run with each batch of samples. In addition a laboratory control was run for every 10 to 15 samples. Sample analysis was repeated with dilution of the sample if concentrations were found to be in excess of the highest concentration used to calculate the calibration curve (50 mg/L). Analysis was also repeated if the percent recovery of the laboratory control failed to meet the 90-110% level.

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 station positions 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 133-172, and the second containing samples 173-211 To satisfy the test salinity requirement of 30 ± 1$^{o}/_{oo}$, all but five samples required salinity adjustments using a brine made from concentrating seawater. References porewater and three test samples required salinity adjustment with Milli-Q purified deionized water. Two test samples required no salinity adjustment.

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 but four samples (#140, #166, #209 and #211 sulfide measured 0.4, 0.01, 0.02 and 0.01, respectively). Porewater dissolved oxygen concentrations ranged from 6.22 to 7.57 mg/L (82.8 to 100.6%). Three samples (#138, #140 and #148) required aeration by stirring prior to testing to increase dissolved oxygen above 80% saturation. Values for pH ranged from 6.78 to 8.29. TAN concentrations ranged from 0.171 to 23.5 mg/L, and UAN ranged from 1.7 to 1068.3 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. Five 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, and the remaining two in the 50% dilution.

The EC_{50} value for the SDS positive control in the first fertilization assay was 6.88 mg/L, which is near the upper 95% confidence limit of our laboratories control chart. Despite this elevated level, a significant number of toxic responses were observed indicating little loss in sensitivity of the test. The EC_{50} for the second assay was 5.70 mg/L, well 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 133-172)

Thirty-nine samples representing 12 strata were tested in the first fertilization assay. Twelve stations (138-140, 142-145, 154, 166, 168, 169, and 171) were significantly different than the reference in the 100% water quality adjusted concentration and met the DSC. Four of the 12 samples were also statistically different and met the DSC at the 50% dilution (138, 140, 154, and 166) . Furthermore, two were toxic at the 25% dilution level (samples 138 and 140) (Figures 2 through 5). EC_{50} concentrations were calculated for seven of the 39 samples tested and are reported in Table 6. EC_{50} concentrations were >100% for 31 of the remaining samples and <25% for sample #140.

Analysis by strata revealed that strata 43 located in the center of southern Chesapeake Bay and 50 located in Broad Bay, were significantly different than the reference at an $\alpha \leq 0.05$ in the 100 and 50% concentration range (Table 7). Stratum 43 was also toxic at $\alpha \leq 0.05$ in the 25% concentration.

TEST #2, (Stations 173-211)

Thirty-nine samples located in 13 strata were tested in the second fertilization assay. Sixteen stations (174, 176, 177, 183, 185, 187, 188, 191, 194, 200, 201, 202, 206, 209, 210, 210 and 211) were significantly different than the reference in the 100% water quality adjusted concentrations and met the DSC at $\alpha \leq 0.01$. Seven stations (174, 176, 177, 200, 201, 209, and 211 were also statistically different than the reference at the 50% concentration and met the DSC. Finally, four stations (174, 200, 209, and 211) were considered toxic at the 25% concentration (Figures 2 through 5). EC_{50} concentrations were calculated for seven stations and are reported in Table 6. EC_{50} concentrations for the remaining stations were >100% with the exception of station #209 which was < 25%.

Statistical analysis at the strata level revealed four strata (53 and 54 located in the Rappanhannock river; 62 located in the Elizabeth River and 65 located in Mobjack Bay) were significantly different than the reference at the 100% adjusted porewater concentration (Table 7). Strata 62 and 65 were also found to be toxic at the 50% dilution level with stratum #65 still toxic at the 25% concentration.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) was measured in all porewater samples collected . Results of the (DOC) analysis can be found in Table 8 with associated quality control sample results in table 9. Samples were run in four batchs with associated quality control standards. All analyses were run in duplicate with 1 ml autosampled volumes. Blank values were acceptable and measured 0.049 to 011 mg/L. Percent recovery from laboratory controls and spiked duplicates run in conjuction with the batches were acceptable ranging from 90 to 102%. DOC values reflect the concentrations in the pore water before salinity adjustment at the time of extraction prior to freezing. Preliminary data from our laboratory indicates that DOC concentrations in freshly collected samples may range from 1 to 20% higher than samples that have been frozen. Sample

138 had to be reanalyzed in diluted form due to its concentration exceeding the highest standard tested. DOC concentrations ranged from 4.98 to 90 mg/L.

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TABLES 1-9

Table 1. Geographic locations, sample arrival temperatures and holding times of Chesapeake Bay sampling sites collected in 2001.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature (°C)	Elapse Time: Collection-Process (Days)
41	133	37° 44' 45.1"	-75° 56' 20.1"	7	5
41	134	37° 44' 30.8"	-75° 59' 16.7"	7	5
41	135	37° 41' 41.0"	-76° 01' 53.4"	5	7
42	136	37° 39' 54.1"	-76° 19' 36.5"	9	6
42	137	37° 36' 36.5"	-76° 12' 56.1"	7	7
42	138	37° 32' 34.8"	-76° 18' 21.1"	9	5
42	139	37° 19' 55.4"	-76° 13' 31.6"	4	3
43	140	37° 43' 26.9"	-75° 56' 25.3"	7	5
43	141	37° 36' 55.0"	-76° 06' 08.4"	9	6
43	142	37° 33' 59.6"	-76° 11' 39.5"	7	7
43	143	37° 27' 46.7"	-76° 06' 20.9"	19 [1]	10
43	144	37° 13' 30.2"	-76° 05' 08.0"	4	2
44	145	37° 43' 00.2"	-75° 48' 01.1"	9	5
44	146	37° 38' 10.1"	-75° 55' 30.7"	7	5
44	147	37° 24' 04.0"	-76° 02' 26.0"	7	8
44	148	37° 13' 27.7"	-76° 02' 07.1"	4	2
45	149	37° 10' 10.3"	-76° 00' 47.7"	4	2
45	150	37° 05' 01.8"	-76° 04' 49.7"	4	3
45	151	37° 02' 08.0"	-75° 58' 27.6"	4	2
46	152	37° 12' 55.9"	-76° 16' 14.6"	4	3
46	153	37° 04' 57.5"	-76° 09' 34.8"	4	3
46	154	36° 57' 33.4"	-76° 00' 29.8"	4	2
47	155A1	37° 08' 28.6"	-76° 14' 13.5"	19 [1]	10
47	156	36° 58' 15.4"	-76° 03' 29.2"	7	12

Table 1. Continued.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature (°C)	Elapse Time: Collection-Process (Days)
47	157	37° 01' 11.9"	-76° 15' 31.1"	9	5
48	158	36° 59' 41.0"	-76° 22' 24.3"	19 [1]	10
48	159	36° 58' 43.1"	-76° 23' 11.8"	19 [1]	10
48	160	36° 57' 41.2"	-76° 24' 10.2"	3	3
49	161	36° 59' 55.4"	-76° 15' 09.1"	4	2
49	162	36° 58' 53.4"	-76° 18' 46.8"	9	5
49	163	36° 57' 23.9"	-76° 05' 55.3"	9	5
50	164	36° 56' 01.5"	-76° 11' 30.3"	9	5
50	166	36° 51' 40.5"	-75° 59' 41.8"	9	5
51	167	36° 55' 56.9"	-76° 21' 45.5"	5	5
51	168	36° 55' 26.9"	-76° 26' 13.8"	9	4
51	169	36° 54' 17.6"	-76° 25' 10.7"	5	3
52	170	37° 44' 28.6"	-75° 31' 04.0"	4	7
52	171	37° 37' 48.6"	-76° 27' 19.0"	5	7
52	172	37° 36' 17.2"	-76° 22' 03.3"	5	7
53	173	37° 47' 31.1"	-76° 38' 46.8"	7	7
53	174	37° 42' 35.5"	-76° 33' 35.3"	7	7
53	175	37° 40' 03.3"	-76° 33' 17.0"	7	7
54	176	37° 53' 32.8"	-76° 46' 48.0"	5	6
54	177	37° 52' 24.0"	-76° 46' 12.0"	4	6
54	178	37° 50' 40.8"	-76° 45' 07.7"	4	6
55	179	37° 54' 58.6"	-76° 50' 04.2"	5	6
55	180	37° 50' 22.6"	-76° 45' 17.4"	4	6
55	181	37° 47' 58.8"	-76° 42' 47.8"	4	6

Table 1. Continued.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature (°C)	Elapse Time: Collection-Process (Days)
56	182	37° 24' 36.9"	-76° 40' 26.5"	7	11
56	183	37° 20' 13.3"	-76° 36' 21.1"	7	4
56	184	37° 18' 39.5"	-76° 33' 46.2"	7	11
57	185	37° 21' 29.1"	-76° 38' 01.5"	5	4
57	186	37° 18' 07.6"	-76° 34' 36.3"	7	4
57	187	37° 15' 43.0"	-76° 32' 04.7"	7	11
58	188	37° 20' 27.5"	-76° 38' 14.5"	5	4
58	189	37° 18' 23.6"	-76° 36' 39.9"	7	4
58	190	37° 17' 23.1"	-76° 35' 06.3"	7	4
59	191	37° 06' 23.1"	-76° 37' 52.8"	5	2
59	192A1	37° 05' 02.0"	-76° 35' 14.7"	4	4
59	193A1	37° 01' 18.1"	-76° 30' 21.3"	4-6	3
60	194	37° 05' 20.6"	-76° 38' 42.4"	4	4
60	195	37° 03' 50.6"	-76° 39' 33.9"	4	4
60	196	37° 02' 40.4"	-76° 38' 03.5"	4-6	4
61	197	37° 00' 27.5"	-76° 33' 36.1"	5	3
61	198	36° 59' 25.8"	-76° 31' 40.9"	3	3
61	199	36° 56' 20.0"	-76° 29' 37.9"	4	3
62	200	36° 54' 46.0"	-76° 20' 23.1"	5	5
62	201	36° 53' 50.7"	-76° 20' 17.0"	19 [1]	10
62	202	36° 51' 33.7"	-76° 19' 20.1"	5-9	4
63	203	36° 50' 17.7"	-76° 14' 17.1"	5	2
63	204	36° 50' 09.0"	-76° 15' 17.8"	6	2
63	205	36° 50' 03.0"	-76° 13' 06.7"	5	3

Table 1. Continued.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature (°C)	Elapse Time: Collection-Process (Days)
64	206	36° 49' 21.1"	-76° 17' 28.8"	5	3
64	207	36° 47' 25.7"	-76° 18' 19.6"	4	2
64	208	36° 44' 39.7"	-76° 17' 49.5"	5	3
65	209	37° 23' 06.1"	-76° 24' 01.3"	5	4
65	210	37° 19' 06.3"	-76° 21' 36.1"	7	4
65	211	37° 17' 33.1"	-76° 23' 31.5"	4	8

[1] Sample lost in transport; delivery delayed two days.

Table 2. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples from Chesapeake Bay and surrounding areas collected in 2001.

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[9]	34.5	6.71	91.6	8.23	1.4	72.9	<0.01	87
41-133	22	7.01	91.9	7.31	3.77	24.7	< 0.01	91
41-134	23	7.07	92.7	7.35	7.56	54.4	< 0.01	91
41-135	24	6.5	85.7	7.53	3.27	35.5	< 0.01	93
42-136	20	6.95	91.1	6.78	2.37	4.6	< 0.01	88
42-137	22	6.89	90.2	7.45	4.51	40.8	< 0.01	90
42-138	20	7.14	93.3	6.98	10.1	31.1	< 0.01	88
42-139	25	7.43	96.1	7.52	2.82	29.9	< 0.01	94
43-140	24	6.32	82.8	8.29	18.0	1068.3	0.40	93
43-141	26	6.77	88.6	7.53	2.49	27.0	< 0.01	95
43-142	24	6.91	90.6	7.46	3.59	33.2	< 0.01	93
43-143	26	6.87	90.8	7.63	7.16	97.5	< 0.01	95
43-144	28	6.86	91.2	7.70	3.18	50.8	< 0.01	98
44-145	21	6.73	89.5	7.93	7.5	201.0	< 0.01	73
44-146	24	6.56	87.0	8.07	4.34	159.0	< 0.01	93
44-147	26	6.34	84.2	7.83	3.87	82.9	< 0.01	95
44-148	26	6.46	84.6	7.67	1.51	22.5	< 0.01	95
45-149	28	6.6	87.7	7.69	2.05	32.0	< 0.01	98
45-150	30	6.69	88.7	7.40	2.14	17.3	< 0.01	100
45-151	32	6.55	87.3	7.45	0.658	5.9	< 0.01	94
46-152	24	6.69	88.9	7.73	1.72	29.4	< 0.01	93
46-153	28	6.74	88.9	7.51	2.96	30.7	< 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]
46-154	32	6.8	90.3	7.4	4.57	36.8	< 0.01	94
47-155	25	6.75	89.6	7.63	5.84	79.5	< 0.01	94
47-156	32	6.67	88.1	7.17	9.15	43.6	< 0.01	94
47-157	26	6.97	91.9	7.55	2.74	31.1	<0.01	95
48-158	26	6.91	90.5	7.37	4.96	37.3	< 0.01	95
48-159	26	6.5	86.7	7.59	4.75	59.1	< 0.01	95
48-160	26	6.22	82.9	7.57	1.81	21.5	< 0.01	95
49-161	26	6.66	88.6	7.83	0.171	3.7	< 0.01	95
49-162	26	6.78	89.2	7.6	2.49	31.7	< 0.01	95
49-163	30	6.55	86.7	7.37	3.32	25.0	<0.01	100
50-164	26	7.12	93.3	7.42	1.93	16.3	<0.01	93
50-166	24.5	6.7	88.2	7.31	4.98	32.7	0.01	93
51-167	25	7.04	92.9	7.64	4.27	59.5	<0.01	94
51-168	24	6.62	87.0	7.27	2.89	17.3	<0.01	93
51-169	24	6.87	90.3	7.52	2.64	28.0	<0.01	93
52-170	15	6.21	82.4	7.33	1.50	10.3	<0.01	85
52-171	20	7.25	95.0	7.89	5.89	144.3	<0.01	75
52-172	20	6.69	87.8	8.01	2.63	84.3	<0.01	89
53-173	16	6.86	90.2	7.60	1.90	24.2	<0.01	84
53-174	18	6.71	88.4	7.99	16.2	496.6	<0.01	87
53-175	20	7.01	92.4	8.04	2.22	76.1	<0.01	89

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]
54-176	12	6.94	91.7	7.81	5.18	106.0	<0.01	81
54-177	12	7	92.9	7.48	5.2	50.3	<0.01	81
54-178	13	7.46	98.6	7.08	2.82	10.9	<0.01	71
55-179	8	6.39	84.5	7.95	1.10	30.8	<0.01	78
55-180	13	6.8	89.7	7.70	1.52	24.3	<0.01	82
55-181	15	6.48	86.1	7.55	2.32	26.3	<0.01	84
56-182	20	6.6	87.1	7.56	2.34	27.2	<0.01	89
56-183	22	6.72	89.1	7.47	3.33	31.5	<0.01	91
56-184	23.5	6.6	88.0	7.43	2.15	18.6	<0.01	93
57-185	20	6.69	89.1	7.78	5.06	96.8	<0.01	89
57-186	24	6.68	88.7	7.40	1.64	13.2	<0.01	93
57-187	23	6.91	91.6	7.46	3.71	34.3	<0.01	92
58-188	21	7.21	95.4	7.48	2.64	25.5	<0.01	90
58-189	22	7.28	96.0	7.92	1.38	36.2	<0.01	91
58-190	22.5	7.46	97.5	7.74	1.27	22.2	<0.01	91
59-191	18	7.13	93.2	6.63	1.26	1.7	<0.01	87
59-192	21	7.07	92.1	7.25	2.21	12.6	<0.01	90
59-193	22	7.18	92.8	7.68	2.06	31.4	<0.01	91
60-194	15	6.89	90	7.59	3.64	45.3	<0.01	84
60-195	16	7.11	93.2	7.63	2.61	35.5	<0.01	86
60-196	16	7.13	93.5	7.48	3.8	36.8	<0.01	86
61-197	20	6.95	91.3	7.35	2.3	16.5	<0.01	89
61-198	20	6.92	91.9	7.67	1.08	16.1	<0.01	89
61-199	22	7.11	94.2	7.37	1.19	9.0	<0.01	91

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]
62-200	26	6.93	92.7	7.32	23.5	157.8	<0.01	95
62-201	25?	7.12	94.4	7.87	9.69	227.0	<0.01	94
62-202	25	7.57	100.6	7.4	1.73	13.9	<0.01	94
63-203	24	7.38	98.1	7.32	1.44	9.7	<0.01	93
63-204	24	7.04	93.7	7.39	1.29	10.2	<0.01	93
63-205	22.5	6.94	92.6	7.48	1.03	10.0	<0.01	91
64-206	24	6.8	91.5	7.44	3.58	31.6	<0.01	93
64-207	24	6.67	89.5	7.56	1.62	18.8	<0.01	93
64-208	23	6.94	92.5	7.76	1.18	21.6	<0.01	92
65-209	22	6.15	82.2	8.02	7.88	258.3	0.02	91
65-210	24	7.05	94.4	8.1	1.19	46.6	<0.01	93
65-211	24	7.05	94.2	7.04	2.44	8.6	0.01	93

[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 one raw data and means for sediment porewater samples in Chesapeake Bay and surrounding areas (stations 133-172). 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	85	69	81	89	66	83.7 ± 9.4	100
		91	84	93	93	86		
REF [3]	50	98	97	98	99	98	97.5 ± 1.4	100
		97	96	95	97	100		
REF [3]	25	100	99	99	99	97	98.5 ± 1.3	100
		98	100	96	98	99		
41-133	100	44 [4]	91	87	88	93	89.8 ± 2.8	107
	50	95	99	98	95	100	97.4 ± 2.3	100
	25	98	97	99	99	97	98.0 ± 1.0	99
41-134	100	99	100	94	95	92	96.0 ± 3.4	115
	50	100	97	98	97	96	97.6 ± 1.5	100
	25	99	98	100	95	99	98.2 ± 1.9	100
41-135	100	95	97	98	97	95	96.4 ± 1.3	115
	50	99	97	99	99	99	98.6 ± 0.9	101
	25	98	100	98	100	100	99.2 ± 1.1	101
42-136	100	100	99	100	99	99	99.4 ± 0.6	119
	50	97	100	100	99	99	99.0 ± 1.2	102
	25	98	99	98	99	98	98.4 ± 0.6	100
42-137	100	97	98	97	94	98	96.8 ± 1.6	116
	50	100	97	97	95	99	97.6 ± 2.0	100
	25	97	98	99	99	99	98.4 ± 0.9	100

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
42-138	100	0	0	0	0	0	0.0 ± 0.0 **	0
	50	3	4	4	7	0	3.6 ± 2.5 **	4
	25	39	45	61	48	35	45.6 ± 10.0 **	46
42-139	100	47	49	71	55	57	55.8 ± 9.4 **	67
	50	99	98	99	100	99	99.0 ± 0.7	102
	25	99	99	98	100	100	99.2 ± 0.8	101
43-140	100	0	0	1	0	0	0.2 ± 0.4 **	0
	50	0	0	3	3	0	1.2 ± 1.6 **	1
	25	9	11	13	7	10	10.0 ± 2.2 **	10
43-141	100	97	94	94	92	96	94.6 ± 2.0	113
	50	100	100	99	98	99	99.2 ± 0.8	102
	25	99	99	96	98	99	98.2 ± 1.3	100
43-142	100	50	35	38	45	41	41.8 ± 5.9 **	50
	50	89	95	95	94	93	93.2 ± 2.5	96
	25	96	100	97	98	98	97.8 ± 1.5	99
43-143	100	39	35	34	43	34	37.0 ± 3.9 **	44
	50	100	98	100	99	98	99.0 ± 1.0	102
	25	99	99	100	100	100	99.6 ± 0.6	101
43-144	100	18	19	17	30	32	23.2 ± 7.2 **	28
	50	87	89	81	90	93	88.0 ± 4.5 ++	90
	25	95	96	97	99	98	97.0 ± 1.6	98
44-145	100	45	55	67	78	60	61.0 ± 12.4 **	73
	50	98	99	93	94	81	93.0 ± 7.2	95
	25	99	97	96	98	96	97.2 ± 1.3	99

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
44-146	100	98	97	99	97	99	98.0 ± 1.0	117
	50	97	100	98	99	97	98.2 ± 1.3	101
	25	98	99	99	96	99	98.2 ± 1.3	100
44-147	100	99	100	99	96	96	98.0 ± 1.9	117
	50	100	98	100	98	100	99.2 ± 1.1	102
	25	99	98	99	97	96	97.8 ± 1.3	99
44-148	100	100	97	98	97	97	97.8 ± 1.3	117
	50	100	100	99	99	100	99.6 ± 0.6	102
	25	99	98	100	99	99	99.0 ± 0.7	101
45-149	100	95	97	96	95	97	96.0 ± 1.0	115
	50	100	97	98	98	96	97.8 ± 1.5	100
	25	97	98	100	98	100	98.6 ± 1.3	100
45-150	100	97	97	100	99	97	98.0 ± 1.4	117
	50	100	99	100	98	99	99.2 ± 0.8	102
	25	100	99	99	100	100	99.6 ± 0.6	101
45-151	100	98	97	99	100	100	98.8 ± 1.3	118
	50	100	100	98	99	100	99.4 ± 0.9	102
	25	100	98	97	100	98	98.6 ± 1.3	100
46-152	100	99	98	100	99	100	99.2 ± 0.8	119
	50	100	100	98	97	98	98.6 ± 1.3	101
	25	100	100	97	97	97	98.2 ± 1.6	100
46-153	100	95	95	98	97	98	96.6 ± 1.5	115
	50	96	96	99	97	98	97.2 ± 1.3	100
	25	99	97	99	100	100	99.0 ± 1.2	101

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
46-154	100	9	18	11	18	16	14.4 ± 4.2 **	17
	50	65	68	64	52	65	62.8 ± 6.2 **	64
	25	100	94	99	99	94	97.2 ± 3.0	99
47-155 A1	100	98	99	99	98	100	98.8 ± 0.8	118
	50	100	100	100	98	100	99.6 ± 0.9	102
	25	100	99	100	96	96	98.2 ± 2.0	100
47-156	100	94	97	100	100	99	98.0 ± 2.6	117
	50	100	99	100	100	99	99.6 ± 0.6	102
	25	99	98	99	100	100	99.2 ± 0.8	101
47-157	100	97	99	99	98	98	98.2 ± 0.8	117
	50	100	96	100	99	99	98.8 ± 1.6	101
	25	100	99	98	100	100	99.4 ± 0.9	101
48-158	100	99	100	99	100	100	99.6 ± 0.6	119
	50	100	98	100	99	100	99.4 ± 0.9	102
	25	99	100	100	99	99	99.4 ± 0.6	101
48-159	100	98	100	99	99	97	98.6 ± 1.1	118
	50	100	98	100	100	100	99.6 ± 0.9	102
	25	98	98	100	98	100	98.8 ± 1.1	100
48-160	100	99	100	99	99	99	99.2 ± 0.4	119
	50	98	100	98	100	100	99.2 ± 1.1	102
	25	98	98	98	98	100	98.4 ± 0.9	100
49-161	100	99	99	98	98	100	98.8 ± 0.8	118
	50	98	100	98	99	98	98.6 ± 0.9	101
	25	100	100	100	100	100	100.0 ± 0.0	102

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
49-162	100	99	99	100	99	100	99.4 ± 0.6	119
	50	97	100	97	100	100	98.8 ± 1.6	101
	25	99	100	99	100	100	99.6 ± 0.6	101
49-163	100	99	100	100	99	100	99.6 ± 0.6	119
	50	97	98	99	98	98	98.0 ± 0.7	101
	25	97	97	100	98	100	98.4 ± 1.5	100
50-164	100	97	100	96	100	99	98.4 ± 1.8	118
	50	99	100	99	100	97	99.0 ± 1.2	102
	25	99	94	100	98	100	98.2 ± 2.5	100
50-166	100	0	0	1	0	0	0.2 ± 0.4 **	0
	50	19	8	34	28	21	22.0 ± 9.8 **	23
	25	83	77	90	84	86	84.0 ± 4.7 ++	85
51-167	100	93	80	71	81	87	82.4 ± 8.2	98
	50	97	99	100	98	100	98.8 ± 1.3	101
	25	98	99	99	99	99	98.8 ± 0.4	100
51-168	100	68	77	65	60	82	70.4 ± 9.0 *	84
	50	98	100	98	98	99	98.6 ± 0.9	101
	25	100	98	95	97	98	97.6 ± 1.8	99
51-169	100	74	67	78	49	65	66.6 ± 11.2 **	80
	50	99	98	98	97	98	98.0 ± 0.7	101
	25	99	99	97	99	99	98.6 ± 0.9	100
52-170	100	99	97	98	100	97	98.2 ± 1.3	117
	50	97	100	100	99	98	98.8 ± 1.3	101
	25	97	100	99	100	98	98.8 ± 1.3	100

Table 3. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
52-171	100	31	34	20	31	23	27.8 ± 6.0 **	33
	50	97	94	90	92	92	93.0 ± 2.7	95
	25	97	98	99	98	100	98.4 ± 1.1	100
52-172	100	98	100	100	99	99	99.2 ± 0.8	119
	50	100	99	97	99	100	99.0 ± 1.2	102
	25	97	100	97	98	99	98.2 ± 1.3	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 4. Sea urchin fertilization test two raw data and means for sediment porewater samples in Chesapeake Bay and surrounding areas (stations 173-211). 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	98	87	96	99	99	95.1 ± 3.6	100
		96	95	95	92	94		
REF [3]	50	95	98	93	98	96	96.4 ± 1.8	100
		97	98	94	98	97		
REF [3]	25	94	99	98	100	100	97.7 ± 2.3	100
		94	97	100	97	98		
53-173	100	95	100	100	99	99	98.6 ± 2.1	104
	50	99	100	97	100	100	99.2 ± 1.3	103
	25	97	98	98	99	99	98.2 ± 0.8	101
53-174	100	0	0	0	0	0	0.0 ± 0.0 **	0
	50	13	18	16	36	19	20.4 ± 9.0 **	21
	25	88	72	31	61	41	58.6 ± 23.0 **	60
53-175	100	81	89	92	97	95	90.8 ± 6.3	95
	50	97	97	96	96	95	96.2 ± 0.8	100
	25	99	99	98	100	97	98.6 ± 1.1	101
54-176	100	69	40	36	37	92 [4]	45.5 ± 15.8 **	48
	50	53	65	69	95	94	75.2 ± 18.6 **	78
	25	92	100	100	96	97	97.0 ± 3.3	99
54-177	100	44	31	47	52	26	40.0 ± 11.0 **	42
	50	43	71	44	61	65	56.8 ± 12.7 **	59
	25	99	79	99	71	98	89.2 ± 13.3	91

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
54-178	100	69	98	93	65	90	83.0 ± 15.0 +	87
	50	99	100	95	96	97	97.4 ± 2.1	101
	25	99	99	97	98	97	98.0 ± 1.0	100
55-179	100	98	99	99	98	96	98.0 ± 1.2	103
	50	100	99	100	98	97	98.8 ± 1.3	102
	25	99	100	100	100	100	99.8 ± 0.4	102
55-180	100	99	98	99	98	99	98.6 ± 0.6	104
	50	98	99	99	99	99	98.8 ± 0.4	102
	25	99	100	100	99	99	99.4 ± 0.6	102
55-181	100	99	95	100	99	98	98.2 ± 1.9	103
	50	100	99	100	99	98	99.2 ± 0.8	103
	25	99	100	100	98	100	99.4 ± 0.9	102
56-182	100	99	98	98	99	98	98.4 ± 0.6	103
	50	100	98	100	100	100	99.6 ± 0.9	103
	25	99	99	99	100	99	99.2 ± 0.4	102
56-183	100	36	48	68	59	59	54.0 ± 12.3 **	57
	50	93	90	92	97	97	93.8 ± 3.1	97
	25	98	99	100	100	100	99.4 ± 0.9	102
56-184	100	97	96	100	97	95	97.0 ± 1.9	102
	50	99	96	98	96	99	97.6 ± 1.5	101
	25	100	99	97	98	98	98.4 ± 1.1	101
57-185	100	82	64	64	56	41	61.4 ± 14.9 **	65
	50	94	78	97	94	95	91.6 ± 7.7	95
	25	98	97	96	100	95	97.2 ± 1.9	99

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
57-186	100	85	92	97	96	91	92.2 ± 4.8	97
	50	97	99	100	96	98	98.0 ± 1.6	102
	25	98	97	99	91	92	95.4 ± 3.6	98
57-187	100	74	97	65	50	94	76.0 ± 19.8 **	80
	50	100	95	94	99	96	96.8 ± 2.6	100
	25	98	97	100	99	96	98.0 ± 1.6	100
58-188	100	47	77	57	45	49	55.0 ± 13.1 **	58
	50	97	97	95	99	95	96.6 ± 1.7	100
	25	99	96	100	98	98	98.2 ± 1.5	101
58-189	100	100	98	99	100	99	99.2 ± 0.8	104
	50	99	99	98	93	98	97.4 ± 2.5	101
	25	99	99	100	99	99	99.2 ± 0.4	102
58-190	100	9 [4]	75	90	85	94	86.0 ± 8.2	90
	50	98	100	98	98	99	98.6 ± 0.9	102
	25	99	98	98	99	95	97.8 ± 1.6	100
59-191	100	57	64	48	57	41	53.4 ± 9.0 **	56
	50	98	97	96	93	100	96.8 ± 2.6	100
	25	98	99	99	99	97	98.4 ± 0.9	101
59-192 A1	100	85	91	79	86	79	84.0 ± 5.1 ++	88
	50	99	100	97	100	98	98.8 ± 1.3	102
	25	98	98	98	99	94	97.4 ± 2.0	100
59-193 A1	100	100	98	96	99	100	98.6 ± 1.7	104
	50	100	98	100	98	100	99.2 ± 1.1	103
	25	100	98	100	98	100	99.2 ± 1.1	102

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
60-194	100	54	53	47	51	53	51.6 ± 2.8 **	54
	50	97	95	98	98	97	97.0 ± 1.2	101
	25	99	100	100	98	98	99.0 ± 1.0	101
60-195	100	89	97	96	86	96	92.8 ± 5.0	98
	50	98	98	99	99	97	98.2 ± 0.8	102
	25	97	97	100	99	98	98.2 ± 1.3	101
60-196	100	74	84	98	99	94	89.8 ± 10.6	94
	50	98	98	95	97	96	96.8 ± 1.3	100
	25	99	100	100	100	97	99.2 ± 1.3	102
61-197	100	79	77	86	78	84	80.8 ± 4.0 ++	85
	50	98	99	100	98	100	99.0 ± 1.0	103
	25	100	100	99	99	100	99.6 ± 0.6	102
61-198	100	97	98	97	94	98	96.8 ± 1.6	102
	50	99	100	99	100	99	99.4 ± 0.6	103
	25	97	100	100	99	98	98.8 ± 1.3	101
61-199	100	99	100	100	100	100	99.8 ± 0.4	105
	50	99	100	99	97	100	99.0 ± 1.2	103
	25	100	99	99	99	99	99.2 ± 0.4	102
62-200	100	0	0	0	1	0	0.2 ± 0.4 **	0
	50	36	10	11	18	15	18.0 ± 10.6 **	19
	25	85	39	78	64	42	61.6 ± 20.7 **	63
62-201	100	4	6	5	2	7	4.8 ± 1.9 **	5
	50	38 [4]	87	52	60	93 [4]	66.3 ± 18.3 **	69
	25	100	98	83	98	100	95.8 ± 7.2	98

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
62-202	100	66	78	85	88	67	76.8 ± 10.1 **	81
	50	100	100	99	99	97	99.0 ± 1.2	103
	25	98	100	100	99	99	99.2 ± 0.8	102
63-203	100	99	99	99	96	97	98.0 ± 1.4	103
	50	100	98	100	100	99	99.4 ± 0.9	103
	25	100	96	99	99	100	98.8 ± 1.6	101
63-204	100	100	99	100	98	99	99.2 ± 0.8	104
	50	99	100	99	99	99	99.2 ± 0.4	103
	25	99	100	98	97	96	98.0 ± 1.6	100
63-205	100	98	100	99	100	99	99.2 ± 0.8	104
	50	99	99	100	99	99	99.2 ± 0.4	103
	25	98	99	100	99	99	99.0 ± 0.7	101
64-206	100	37	34	50	23	38	36.4 ± 9.7 **	38
	50	89	91	97	95	96	93.6 ± 3.4	97
	25	99	98	96	99	100	98.4 ± 1.5	101
64-207	100	99	98	98	100	100	99.0 ± 1.0	104
	50	99	98	100	100	99	99.2 ± 0.8	103
	25	100	99	98	98	99	98.8 ± 0.8	101
64-208	100	99	100	97	99	99	98.8 ± 1.1	104
	50	100	99	97	100	98	98.8 ± 1.3	102
	25	100	100	97	96	100	98.6 ± 2.0	101
65-209	100	0	1	0	1	0	0.4 ± 0.6 **	0
	50	0	1	0	0	0	0.2 ± 0.4 **	0
	25	11	19	14	8	9	12.2 ± 4.4 **	12

Table 4. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
65-210	100	82	79	60	71	67	71.8 ± 8.9 **	75
	50	98	99	97	99	97	98.0 ± 1.0	102
	25	100	99	99	99	99	99.2 ± 0.4	102
65-211	100	0	1	3	2	2	1.6 ± 1.1 **	2
	50	13	7	9	10	10	9.8 ± 2.2 **	10
	25	41	75	55	74	61	61.2 ± 14.2 **	63

[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 78 stations sampled in Chesapeake Bay and surrounding areas collected in 2001. 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]
41	133	100	89.8	2.8	107	83.7	9.4	
		50	97.4	2.3	100	97.5	1.4	
		25	98.0	1.0	99	98.5	1.3	
	134	100	96.0	3.4	115	83.7	9.4	
		50	97.6	1.5	100	97.5	1.4	
		25	98.2	1.9	100	98.5	1.3	
	135	100	96.4	1.3	115	83.7	9.4	
		50	98.6	0.9	101	97.5	1.4	
		25	99.2	1.1	101	98.5	1.3	
42	136	100	99.4	0.6	119	83.7	9.4	
		50	99.0	1.2	102	97.5	1.4	
		25	98.4	0.6	100	98.5	1.3	
	137	100	96.8	1.6	116	83.7	9.4	
		50	97.6	2.0	100	97.5	1.4	
		25	98.4	0.9	100	98.5	1.3	
	138	100	0.0	0.0	0	83.7	9.4	**
		50	3.6	2.5	4	97.5	1.4	**
		25	45.6	10.0	46	98.5	1.3	**
	139	100	55.8	9.4	67	83.7	9.4	**
		50	99.0	0.7	102	97.5	1.4	
		25	99.2	0.8	101	98.5	1.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
43	140	100	0.2	0.4	0	83.7	9.4	**
		50	1.2	1.6	1	97.5	1.4	**
		25	10.0	2.2	10	98.5	1.3	**
	141	100	94.6	2.0	113	83.7	9.4	
		50	99.2	0.8	102	97.5	1.4	
		25	98.2	1.3	100	98.5	1.3	
	142	100	41.8	5.9	50	83.7	9.4	**
		50	93.2	2.5	96	97.5	1.4	
		25	97.8	1.5	99	98.5	1.3	
	143	100	37.0	3.9	44	83.7	9.4	**
		50	99.0	1.0	102	97.5	1.4	
		25	99.6	0.6	101	98.5	1.3	
	144	100	23.2	7.2	28	83.7	9.4	**
		50	88.0	4.5	90	97.5	1.4	++
		25	97.0	1.6	98	98.5	1.3	
44	145	100	61.0	12.4	73	83.7	9.4	**
		50	93.0	7.2	95	97.5	1.4	
		25	97.2	1.3	99	98.5	1.3	
	146	100	98.0	1.0	117	83.7	9.4	
		50	98.2	1.3	101	97.5	1.4	
		25	98.2	1.3	100	98.5	1.3	
	147	100	98.0	1.9	117	83.7	9.4	
		50	99.2	1.1	102	97.5	1.4	
		25	97.8	1.3	99	98.5	1.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
44	148	100	97.8	1.3	117	83.7	9.4	
		50	99.6	0.6	102	97.5	1.4	
		25	99.0	0.7	101	98.5	1.3	
45	149	100	96.0	1.0	115	83.7	9.4	
		50	97.8	1.5	100	97.5	1.4	
		25	98.6	1.3	100	98.5	1.3	
	150	100	98.0	1.4	117	83.7	9.4	
		50	99.2	0.8	102	97.5	1.4	
		25	99.6	0.6	101	98.5	1.3	
	151	100	98.8	1.3	118	83.7	9.4	
		50	99.4	0.9	102	97.5	1.4	
		25	98.6	1.3	100	98.5	1.3	
46	152	100	99.2	0.8	119	83.7	9.4	
		50	98.6	1.3	101	97.5	1.4	
		25	98.2	1.6	100	98.5	1.3	
	153	100	96.6	1.5	115	83.7	9.4	
		50	97.2	1.3	100	97.5	1.4	
		25	99.0	1.2	101	98.5	1.3	
	154	100	14.4	4.2	17	83.7	9.4	**
		50	62.8	6.2	64	97.5	1.4	**
		25	97.2	3.0	99	98.5	1.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
47	155A1	100	98.8	0.8	118	83.7	9.4	
		50	99.6	0.9	102	97.5	1.4	
		25	98.2	2.0	100	98.5	1.3	
	156	100	98.0	2.6	117	83.7	9.4	
		50	99.6	0.6	102	97.5	1.4	
		25	99.2	0.8	101	98.5	1.3	
	157	100	98.2	0.8	117	83.7	9.4	
		50	98.8	1.6	101	97.5	1.4	
		25	99.4	0.9	101	98.5	1.3	
48	158	100	99.6	0.6	119	83.7	9.4	
		50	99.4	0.9	102	97.5	1.4	
		25	99.4	0.6	101	98.5	1.3	
	159	100	98.6	1.1	118	83.7	9.4	
		50	99.6	0.9	102	97.5	1.4	
		25	98.8	1.1	100	98.5	1.3	
	160	100	99.2	0.4	119	83.7	9.4	
		50	99.2	1.1	102	97.5	1.4	
		25	98.4	0.9	100	98.5	1.3	
49	161	100	98.8	0.8	118	83.7	9.4	
		50	98.6	0.9	101	97.5	1.4	
		25	100	0.0	102	98.5	1.3	
	162	100	99.4	0.6	119	83.7	9.4	
		50	98.8	1.6	101	97.5	1.4	
		25	99.6	0.6	101	98.5	1.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
49	163	100	99.6	0.6	119	83.7	9.4	
		50	98.0	0.7	101	97.5	1.4	
		25	98.4	1.5	100	98.5	1.3	
50	164	100	98.4	1.8	118	83.7	9.4	
		50	99.0	1.2	102	97.5	1.4	
		25	98.2	2.5	100	98.5	1.3	
	166	100	0.2	0.4	0	83.7	9.4	**
		50	22.0	9.8	23	97.5	1.4	**
		25	84.0	4.7	85	98.5	1.3	++
51	167	100	82.4	8.2	98	83.7	9.4	
		50	98.8	1.3	101	97.5	1.4	
		25	98.8	0.4	100	98.5	1.3	
	168	100	70.4	9.0	84	83.7	9.4	**
		50	98.6	0.9	101	97.5	1.4	
		25	97.6	1.8	99	98.5	1.3	
	169	100	66.6	11.2	80	83.7	9.4	**
		50	98.0	0.7	101	97.5	1.4	
		25	98.6	0.9	100	98.5	1.3	
52	170	100	98.2	1.3	117	83.7	9.4	
		50	98.8	1.3	101	97.5	1.4	
		25	98.8	1.3	100	98.5	1.3	
	171	100	27.8	6.0	33	83.7	9.4	**
		50	93.0	2.7	95	97.5	1.4	
		25	98.4	1.1	100	98.5	1.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
52	172	100	99.2	0.8	119	83.7	9.4	
		50	99.0	1.2	102	97.5	1.4	
		25	98.2	1.3	100	98.5	1.3	
53	173	100	98.6	2.1	104	95.1	3.6	
		50	99.2	1.3	103	96.4	1.8	
		25	98.2	0.8	101	97.7	2.3	
	174	100	0.0	0.0	0	95.1	3.6	**
		50	20.4	9.0	21	96.4	1.8	**
		25	58.6	23.0	60	97.7	2.3	**
	175	100	90.8	6.3	95	95.1	3.6	
		50	96.2	0.8	100	96.4	1.8	
		25	98.6	1.1	101	97.7	2.3	
54	176	100	45.5	15.8	48	95.1	3.6	**
		50	75.2	18.6	78	96.4	1.8	**
		25	97.0	3.3	99	97.7	2.3	
	177	100	40.0	11.0	42	95.1	3.6	**
		50	56.8	12.7	59	96.4	1.8	**
		25	89.2	13.3	91	97.7	2.3	
	178	100	83.0	15.0	87	95.1	3.6	+
		50	97.4	2.1	101	96.4	1.8	
		25	98.0	1.0	100	97.7	2.3	
55	179	100	98.0	1.2	103	95.1	3.6	
		50	98.8	1.3	102	96.4	1.8	
		25	99.8	0.4	102	97.7	2.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
55	180	100	98.6	0.6	104	95.1	3.6	
		50	98.8	0.4	102	96.4	1.8	
		25	99.4	0.6	102	97.7	2.3	
	181	100	98.2	1.9	103	95.1	3.6	
		50	99.2	0.8	103	96.4	1.8	
		25	99.4	0.9	102	97.7	2.3	
56	182	100	98.4	0.6	103	95.1	3.6	
		50	99.6	0.9	103	96.4	1.8	
		25	99.2	0.4	102	97.7	2.3	
	183	100	54.0	12.3	57	95.1	3.6	**
		50	93.8	3.1	97	96.4	1.8	
		25	99.4	0.9	102	97.7	2.3	
	184	100	97.0	1.9	102	95.1	3.6	
		50	94.6	1.5	101	96.4	1.8	
		25	98.4	1.1	101	97.7	2.3	
57	185	100	61.4	14.9	65	95.1	3.6	**
		50	91.6	7.7	95	96.4	1.8	
		25	97.2	1.9	99	97.7	2.3	
	186	100	92.2	4.8	97	95.1	3.6	
		50	98.0	1.6	102	96.4	1.8	
		25	95.4	3.6	98	97.7	2.3	
	187	100	76.0	19.8	80	95.1	3.6	**
		50	96.8	2.6	100	96.4	1.8	
		25	98.0	1.6	100	97.7	2.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
58	188	100	55.0	13.1	58	95.1	3.6	**
		50	96.6	1.7	100	96.4	1.8	
		25	98.2	1.5	101	97.7	2.3	
	189	100	99.2	0.8	104	95.1	3.6	
		50	97.4	2.5	101	96.4	1.8	
		25	99.2	0.4	102	97.7	2.3	
	190	100	86.0	8.2	90	95.1	3.6	
		50	98.6	0.9	102	96.4	1.8	
		25	97.8	1.6	100	97.7	2.3	
59	191	100	53.4	9.0	56	95.1	3.6	**
		50	96.8	2.6	100	96.4	1.8	
		25	98.4	0.9	101	97.7	2.3	
	192	100	84.0	5.1	88	95.1	3.6	++
		50	98.8	1.3	102	96.4	1.8	
		25	97.4	2.0	100	97.7	2.3	
	193	100	98.6	1.7	104	95.1	3.6	
		50	99.2	1.1	103	96.4	1.8	
		25	99.2	1.1	102	97.7	2.3	
60	194	100	51.6	2.8	54	95.1	3.6	**
		50	97.0	1.2	101	96.4	1.8	
		25	99.0	1.0	101	97.7	2.3	
	195	100	92.8	5.0	98	95.1	3.6	
		50	98.2	0.8	102	96.4	1.8	
		25	98.2	1.3	101	97.7	2.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
60	196	100	89.8	10.6	94	95.1	3.6	
		50	96.8	1.3	100	96.4	1.8	
		25	99.2	1.3	102	97.7	2.3	
61	197	100	80.8	4.0	85	95.1	3.6	++
		50	99.0	1.0	103	96.4	1.8	
		25	99.6	0.6	102	97.7	2.3	
	198	100	96.8	1.6	102	95.1	3.6	
		50	99.4	0.6	103	96.4	1.8	
		25	98.8	1.3	101	97.7	2.3	
	199	100	99.8	0.4	105	95.1	3.6	
		50	99.0	1.2	103	96.4	1.8	
		25	99.2	0.4	102	97.7	2.3	
62	200	100	0.2	0.4	0	95.1	3.6	**
		50	18.0	10.6	19	96.4	1.8	**
		25	61.6	20.7	63	97.7	2.3	**
	201	100	4.8	1.9	5	95.1	3.6	**
		50	66.3	18.3	69	96.4	1.8	**
		25	95.8	7.2	98	97.7	2.3	
	202	100	76.8	10.1	81	95.1	3.6	**
		50	99.0	1.2	103	96.4	1.8	
		25	99.2	0.8	102	97.7	2.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
63	203	100	98.0	1.4	103	95.1	3.6	
		50	99.4	0.9	103	96.4	1.8	
		25	98.8	1.6	101	97.7	2.3	
	204	100	99.2	0.8	104	95.1	3.6	
		50	99.2	0.4	103	96.4	1.8	
		25	98.0	1.6	100	97.7	2.3	
	205	100	99.2	0.8	104	95.1	3.6	
		50	99.2	0.4	103	96.4	1.8	
		25	99.0	0.7	101	97.7	2.3	
64	206	100	36.4	9.7	38	95.1	3.6	**
		50	93.6	3.4	97	96.4	1.8	
		25	98.4	1.5	101	97.7	2.3	
	207	100	99.0	1.0	104	95.1	3.6	
		50	99.2	0.8	103	96.4	1.8	
		25	98.8	0.8	101	97.7	2.3	
	208	100	98.8	1.1	104	95.1	3.6	
		50	98.8	1.3	102	96.4	1.8	
		25	98.6	2.0	101	97.7	2.3	
65	209	100	0.4	0.6	0	95.1	3.6	**
		50	0.2	0.4	0	96.4	1.8	**
		25	12.2	4.4	12	97.7	2.3	**
	210	100	71.8	8.9	75	95.1	3.6	**
		50	98.0	1.0	102	96.4	1.8	
		25	99.2	0.4	102	97.7	2.3	

Table 5. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
65	211	100	1.6	1.1	2	95.1	3.6	**
		50	9.8	2.2	10	96.4	1.8	**
		25	61.2	14.2	63	97.7	2.3	**

[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. EC_{50} values of sediment porewater samples from Chesapeake Bay and surrounding areas assayed in the sea urchin (*Arbacia punctulata*) fertilization test in 2001.

Designation [1]	Fertilization Test	
	EC_{50} [2]	95% Confidence Limits
41-133	>100	-
41-134	>100	-
41-135	>100	-
42-136	>100	-
42-137	>100	-
42-138	25.37	21.96 - 29.31
42-139	>100	-
43-140	<25	-
43-141	>100	-
43-142	95.00	84.38 - 106.94
43-143	89.97	81.57 - 99.22
43-144	78.61	74.36 - 83.10
44-145	>100	-
44-146	>100	-
44-147	>100	-
44-148	>100	-
45-149	>100	-
45-150	>100	-
45-151	>100	-
46-152	>100	-
46-153	>100	-

Table 6. Continued.

Designation [1]	Fertilization Test	
	EC_{50} [2]	95% Confidence Limits
46-154	62.71	56.27 - 69.88
47-155	>100	-
47-156	>100	-
47-157	>100	-
48-158	>100	-
48-159	>100	-
48-160	>100	-
49-161	>100	-
49-162	>100	-
49-163	>100	-
50-164	>100	-
50-166	39.47	36.79 - 42.34
51-167	>100	-
51-168	>100	-
51-169	>100	-
52-170	>100	-
52-171	82.62	77.24 - 88.38
52-172	>100	-
53-173	>100	-
53-174	30.25	26.60 - 34.40
53-175	>100	-

Table 6. Continued.

Designation [1]	Fertilization Test	
	EC_{50} [2]	95% Confidence Limits
54-176	91.44	75.20 - 111.20
54-177	70.71	54.10 - 92.43
54-178	>100	-
55-179	>100	-
55-180	>100	-
55-181	>100	-
56-182	>100	-
56-183	>100	-
56-184	>100	-
57-185	>100	-
57-186	>100	-
57-187	>100	-
58-188	>100	-
58-189	>100	-
58-190	>100	-
59-191	>100	-
59-192	>100	-
59-193	>100	-
60-194	>100	-
60-195	>100	-
60-196	>100	-
61-197	>100	-
61-198	>100	-
61-199	>100	-

Table 6. Continued.

Designation [1]	Fertilization Test	
	EC_{50} [2]	95% Confidence Limits
62-200	31.02	27.83 - 34.57
62-201	58.21	54.03 - 62.72
62-202	>100	-
63-203	>100	-
63-204	>100	-
63-205	>100	-
64-206	86.06	78.82 - 93.95
64-207	>100	-
64-208	>100	-
65-209	>100	-
65-210	>100	-
65-211	29.63	26.89 - 32.66

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

[2] EC_{50} value calculated from percent of original sample.

Table 7. Summary of strata means and statistical significance for the sea urchin fertilization test from 25 strata sampled in Chesapeake Bay and surrounding areas in 2001. Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$).

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
41	133, 134, 135	100	94.4	3.9	113	83.7	9.4	
		50	97.9	1.6	100	97.5	1.4	
		25	98.5	1.4	100	98.5	1.3	
42	136, 137, 138, 139	100	63.0	41.6	75	83.7	9.4	
		50	74.8	42.2	77	97.5	1.4	
		25	85.4	24.0	87	98.5	1.3	
43	140, 141, 142, 143, 144	100	39.4	32.1	47	83.7	9.4	**
		50	76.1	38.5	78	97.5	1.4	*
		25	80.5	36.0	82	98.5	1.3	*
44	145, 146, 147, 148	100	88.7	17.4	106	83.7	9.4	
		50	97.5	4.3	100	97.5	1.4	
		25	98.0	1.3	99	98.5	1.3	
45	149, 150, 151	100	97.6	1.7	117	83.7	9.4	
		50	98.8	1.3	101	97.5	1.4	
		25	98.9	1.2	100	98.5	1.3	
46	152, 153, 154	100	70.1	40.8	84	83.7	9.4	
		50	86.2	17.5	88	97.5	1.4	
		25	98.1	2.1	100	98.5	1.3	
47	155, 156, 157	100	98.3	1.5	117	83.7	9.4	
		50	99.3	1.1	102	97.5	1.4	
		25	98.9	1.4	100	98.5	1.3	

Table 7. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
48	158, 159, 160	100	99.1	0.8	118	83.7	9.4	
		50	99.4	0.9	102	97.5	1.4	
		25	98.9	0.9	100	98.5	1.3	
49	161, 162, 163	100	99.3	0.7	119	83.7	9.4	
		50	98.5	1.1	101	97.5	1.4	
		25	99.3	1.1	101	98.5	1.3	
50	164, 166	100	49.3	51.8	59	83.7	9.4	*
		50	60.5	41.1	62	97.5	1.4	**
		25	91.1	8.3	92	98.5	1.3	
51	167, 168, 169	100	73.1	11.2	87	83.7	9.4	
		50	98.4	1.0	101	97.5	1.4	
		25	98.3	1.2	100	98.5	1.3	
52	170, 171, 172	100	75.1	34.8	90	83.7	9.4	
		50	96.9	3.3	99	97.5	1.4	
		25	98.5	1.2	100	98.5	1.3	
53	173, 174, 175	100	63.1	46.5	66	95.1	3.6	**
		50	71.9	38.1	75	96.4	1.8	
		25	85.1	23.0	87	97.7	2.3	
54	176, 177, 178	100	56.9	24.0	60	95.1	3.6	**
		50	76.5	30.0	79	96.4	1.8	
		25	94.7	8.4	97	97.7	2.3	
55	179, 180, 181	100	98.3	1.3	103	95.1	3.6	
		50	98.9	0.9	103	96.4	1.8	
		25	99.5	0.6	102	97.7	2.3	

Table 7. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
56	182, 183, 184	100	83.1	22.4	87	95.1	3.6	
		50	97.0	3.1	101	96.4	1.8	
		25	99.0	0.9	101	97.7	2.3	
57	185, 186, 187	100	76.5	18.7	80	95.1	3.6	
		50	95.5	5.3	99	96.4	1.8	
		25	96.9	2.6	99	97.7	2.3	
58	188, 189, 190	100	79.6	21.5	84	95.1	3.6	
		50	97.5	1.9	101	96.4	1.8	
		25	98.4	1.4	101	97.7	2.3	
59	191, 192, 193	100	78.7	20.3	83	95.1	3.6	
		50	98.3	2.0	102	96.4	1.8	
		25	98.3	1.5	101	97.7	2.3	
60	194, 195, 196	100	78.1	20.5	82	95.1	3.6	
		50	97.3	1.2	101	96.4	1.8	
		25	98.8	1.2	101	97.7	2.3	
61	197, 198, 199	100	92.5	8.9	97	95.1	3.6	
		50	99.1	0.9	103	96.4	1.8	
		25	99.2	0.9	102	97.7	2.3	
62	200, 201, 202	100	27.3	36.7	29	95.1	3.6	**
		50	60.3	38.4	63	96.4	1.8	**
		25	85.5	21.1	88	97.7	2.3	
63	203, 204, 205	100	98.8	1.2	104	95.1	3.6	
		50	99.3	0.6	103	96.4	1.8	
		25	98.6	1.3	101	97.7	2.3	

Table 7. Continued.

Strata	Sample ID	WQAS [1]	% Fertilized					
			Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
64	206, 207, 208	100	78.1	30.9	82	95.1	3.6	
		50	97.2	3.3	101	96.4	1.8	
		25	98.6	1.4	101	97.7	2.3	
65	209, 210, 211	100	24.6	34.9	26	95.1	3.6	**
		50	36.0	45.6	37	96.4	1.8	**
		25	57.5	37.7	59	97.7	2.3	**

[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 8. Dissolved organic carbon (DOC) measurements (mg/L) of porewater samples collected from sediments from Chesapeake Bay and surrounding areas in 2001.

Sample Id	Sample Date	Batch #	Run #	Rep 1	Rep 2	Mean	Relative SD
133	20-Sep-01	2	3	7.9288	8.6164	8.2726	5.54
134	20-Sep-01	2	4	14.108	13.8596	13.9838	1.21
135	20-Sep-01	2	6	11.9065	11.9726	11.9395	0.38
136	20-Sep-01	2	7	14.1496	14.4811	14.3154	1.58
137	20-Sep-01	2	8	13.8201	14.4556	14.1379	3.07
138	20-Sep-01	2	10	92.2868	91.9275	92.1071	0.27
138 2X dilution	24-Sep-01	3	3	44.3294	45.6077	44.9685	1.99
139	14-Sep-01	1	3	7.2134	8.1324	7.6729	7.98
140	20-Sep-01	2	11	15.9833	17.2499	16.6166	5.23
141	20-Sep-01	2	12	5.6696	6.095	5.8823	4.71
142	20-Sep-01	2	13	5.4863	5.7869	5.6366	3.47
143	20-Sep-01	2	39	13.7093	14.2519	13.9806	2.65
144	14-Sep-01	1	4	23.6828	25.8023	24.7426	5.94
145	20-Sep-01	2	14	9.1431	9.593	9.368	3.22
146	20-Sep-01	2	16	7.6388	8.1569	7.8978	4.36
147	20-Sep-01	2	24	10.3307	10.4544	10.3926	0.8

Table 8. Continued.

Sample Id	Sample Date	Batch #	Run #	Rep 1	Rep 2	Mean	Relative SD
148	14-Sep-01	1	5	15.623	15.9097	14.7664	1.25
149	14-Sep-01	1	6	16.8799	16.6304	16.7552	1.02
150	14-Sep-01	1	7	17.1496	17.0164	17.083	0.54
151	14-Sep-01	1	9	8.7923	9.1409	8.9666	2.61
152	14-Sep-01	1	10	21.105	20.7617	20.9333	1.13
153	14-Sep-01	1	11	22.0101	20.6028	21.3065	4.57
154	14-Sep-01	1	13	30.0487	29.4516	29.7501	1.40
155	20-Sep-01	2	17	11.142	11.8766	11.5093	4.33
156	20-Sep-01	2	18	21.2532	22.2255	21.7393	3.09
157	14-Sep-01	1	14	9.5311	9.5759	9.5535	0.32
158	20-Sep-01	2	19	8.4384	8.6089	8.5236	1.34
159	20-Sep-01	2	20	8.1878	8.4298	8.3088	1.94
160	24-Sep-01	3	4	10.8297	10.8147	10.8222	0.09
161	14-Sep-01	1	15	7.1249	7.1878	7.1563	0.58
162	14-Sep-01	1	16	9.9714	10.1516	10.0615	1.21
163	14-Sep-01	1	18	9.5535	9.9267	9.7401	2.58
164	14-Sep-01	1	19	8.4618	8.5236	8.4927	0.49

Table 8. Continued.

Sample Id	Sample Date	Batch #	Run #	Rep 1	Rep 2	Mean	Relative SD
166	14-Sep-01	1	21	11.3254	11.7668	11.5461	2.6
167	14-Sep-01	1	22	11.2657	12.1836	11.7247	5.32
168	24-Sep-01	3	6	4.9745	5.2933	5.1339	3.99
169	24-Sep-01	3	7	15.1848	15.6549	15.4199	2.09
170	20-Sep-01	2	21	6.5321	6.708	6.6201	1.75
171	20-Sep-01	2	22	7.062	7.1196	7.0908	0.54
172	20-Sep-01	2	23	8.5567	8.836	8.6964	2.15
173	20-Sep-01	2	26	6.6078	6.8402	6.724	2.28
174	20-Sep-01	2	27	17.0686	18.0228	17.5457	3.74
175	20-Sep-01	2	28	9.8989	10.0663	9.9826	1.13
176	20-Sep-01	2	29	15.7978	15.5942	15.696	0.89
177	20-Sep-01	2	30	21.5389	22.7159	22.1274	3.68
178	20-Sep-01	2	31	8.9416	9.0205	8.981	0.59
179	20-Sep-01	2	32	7.4735	7.9373	7.7054	4.00
180	20-Sep-01	2	33	6.5503	7.385	6.9676	7.91
181	20-Sep-01	2	34	7.369	7.482	7.4255	1.01
182	20-Sep-01	2	35	24.4046	23.0986	23.7516	3.81

Table 8. Continued.

Sample Id	Sample Date	Batch #	Run #	Rep 1	Rep 2	Mean	Relative SD
183	14-Sep-01	1	23	7.3914	7.2059	7.2987	1.69
184	20-Sep-01	2	36	6.6803	6.3392	6.5097	3.44
185	14-Sep-01	1	24	9.0279	9.0492	9.0386	0.16
186	14-Sep-01	1	25	5.3946	5.6419	5.5182	2.92
187	20-Sep-01	2	37	12.3958	12.4672	12.4315	0.39
188	14-Sep-01	1	26	13.1464	13.9566	13.5515	4.09
189	14-Sep-01	1	27	7.723	7.5279	7.6254	1.7
190	14-Sep-01	1	28	5.2421	5.2698	5.256	0.34
191	24-Sep-01	3	8	9.8041	10.0407	9.9224	1.6
192	24-Sep-01	3	9	4.9713	5.7539	5.3626	9.41
193	24-Sep-01	3	11	16.7861	17.349	17.0676	2.26
194	24-Sep-01	3	12	9.5397	9.6985	9.6191	1.11
195	24-Sep-01	3	13	8.577	8.7614	8.6692	1.42
196	24-Sep-01	3	14	10.6399	10.7582	10.6991	0.75
197	24-Sep-01	3	15	5.6195	5.8466	5.7331	2.57
198	24-Sep-01	3	16	7.5236	7.8115	7.6676	2.49
199	24-Sep-01	3	17	6.6142	6.788	6.7011	1.7

Table 8. Continued.

Sample Id	Sample Date	Batch #	Run #	Rep 1	Rep 2	Mean	Relative SD
200	14-Sep-01	1	30	27.3013	27.5891	27.4452	0.73
201	20-Sep-01	2	38	15.4375	17.9567	16.6971	10.36
202	24-Sep-01	3	18	8.1761	8.3062	8.2411	1.05
203	24-Sep-01	3	19	6.0492	6.0876	6.0684	0.41
204	24-Sep-01	3	20	6.7731	6.9980	6.8856	2.15
205	24-Sep-01	3	22	6.6995	6.8850	6.7923	1.79
206	24-Sep-01	3	23	8.8275	9.0130	8.9202	1.39
207	24-Sep-01	3	24	12.3382	12.2956	12.3169	0.23
208	24-Sep-01	3	25	9.6441	9.7657	9.7049	0.84
209	14-Sep-01	1	31	11.2092	11.6634	11.4363	2.70
210	14-Sep-01	1	32	5.0172	4.9596	4.9884	0.75
211	24-Sep-01	3	26	5.724	5.9212	5.8226	2.20

Table 9. Dissolved organic carbon (DOC) concentrations of quality assurance samples run in conjunction with porewater samples from Chesapeake Bay and surrounding areas.

Sample Id	Date of Analysis	Batch #	Rep 1	Rep 2	Mean	Relative SD	Evaluation Criteria
Procedure Blanks							
Blank	14-Sep-01	1	0.0198	0.0785	0.0491	43.16	-
Blank	14-Sep-01	1	0.0957	-	0.0957	-	-
Blank	20-Sep-01	2	0.0369	0.0802	0.0585	28.26	-
Blank	20-Sep-01	2	0.1096	-	0.1096	-	-
Blank	24-Sep-01	3	0.0324	0.0858	0.0591	33.98	-
Blank	24-Sep-01	3	0.0831	-	0.0831	-	-
Duplicates							**% Difference [1]**
150	14-Sep-01	1	16.9716	16.9684	16.97	0.01	0.7
162	14-Sep-01	1	10.1015	10.3211	10.2113	1.45	1.5
190	14-Sep-01	1	5.0588	5.1611	5.1099	1.3	2.8
134	20-Sep-01	2	13.141	13.4566	13.2988	1.62	4.9
145	20-Sep-01	2	9.2742	9.6132	9.4437	2.41	0.8
160	24-Sep-01	3	9.6537	10.4373	10.0455	5.24	7.2
204	24-Sep-01	3	6.5758	6.8509	6.7134	2.69	2.5

Table 9 Continued.

Sample Id	Date of Analysis	Batch #	Rep 1	Rep 2	Mean	Relative SD	Evaluation Criteria
Spiked Duplicates							**% Recovery**
153	14-Sep-01	1	29.4431	30.1318	29.7875	1.61	95
164	14-Sep-01	1	17.6411	18.1689	17.905	2.03	97
137	20-Sep-01	2	22.3811	23.1679	22.7745	2.39	94
147	20-Sep-01	2	19.3512	18.9876	19.1694	1.31	94
143	20-Sep-01	2	22.331	23.0293	22.6803	2.13	95
192	24-Sep-01	3	13.4992	14.1794	13.8393	3.35	90
Laboratory Control Samples							**% Recovery**
50 ppm check	14-Sep-01	1	49.2569	51.3433	50.3001	2.9	101
25 ppm check	24-Sep-01	3	24.9451	26.2724	25.6087	3.58	102

[1] Percent relative difference between duplicate and original sample.

FIGURES 1-5

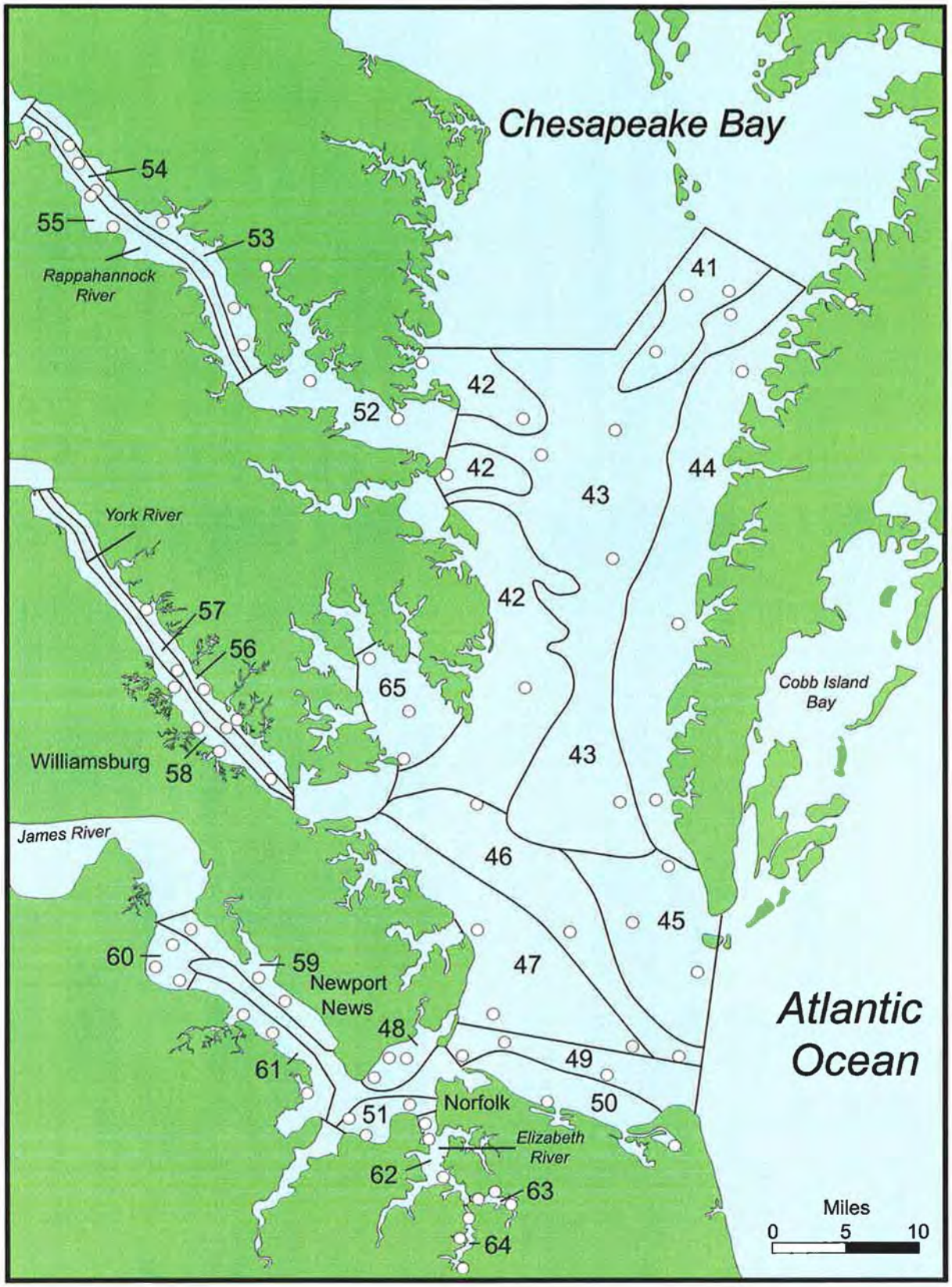

Figure 1. Sampling strata and station locations in Chesapeake Bay and surrounding areas collected in 2001.

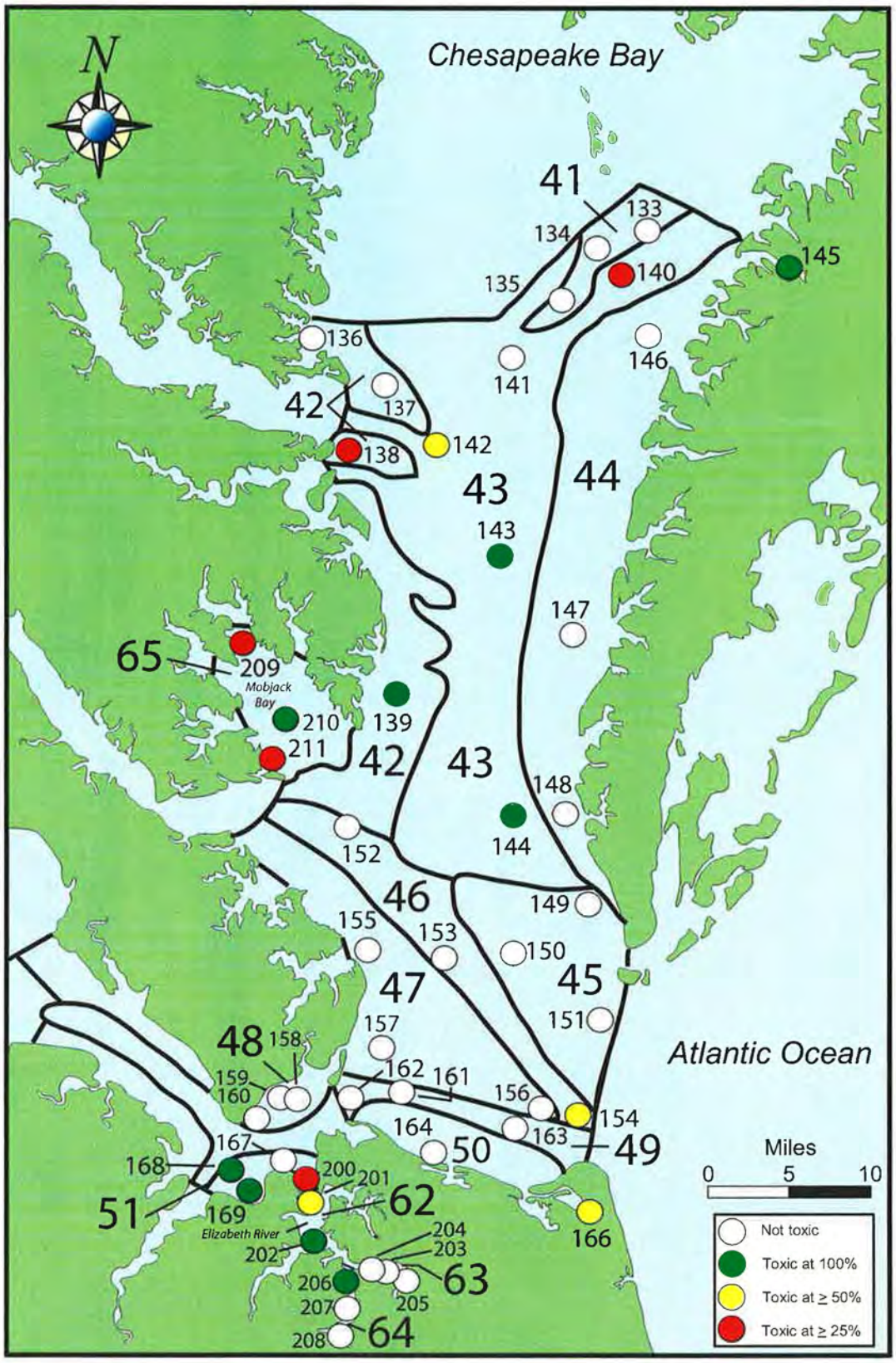

Figure 2. Sample stations in southern Chesapeake Bay and the Elizabeth River, strata 41-51 and 62-65. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's t-test, $\alpha < 0.05$ and detectable significance criteria applied).

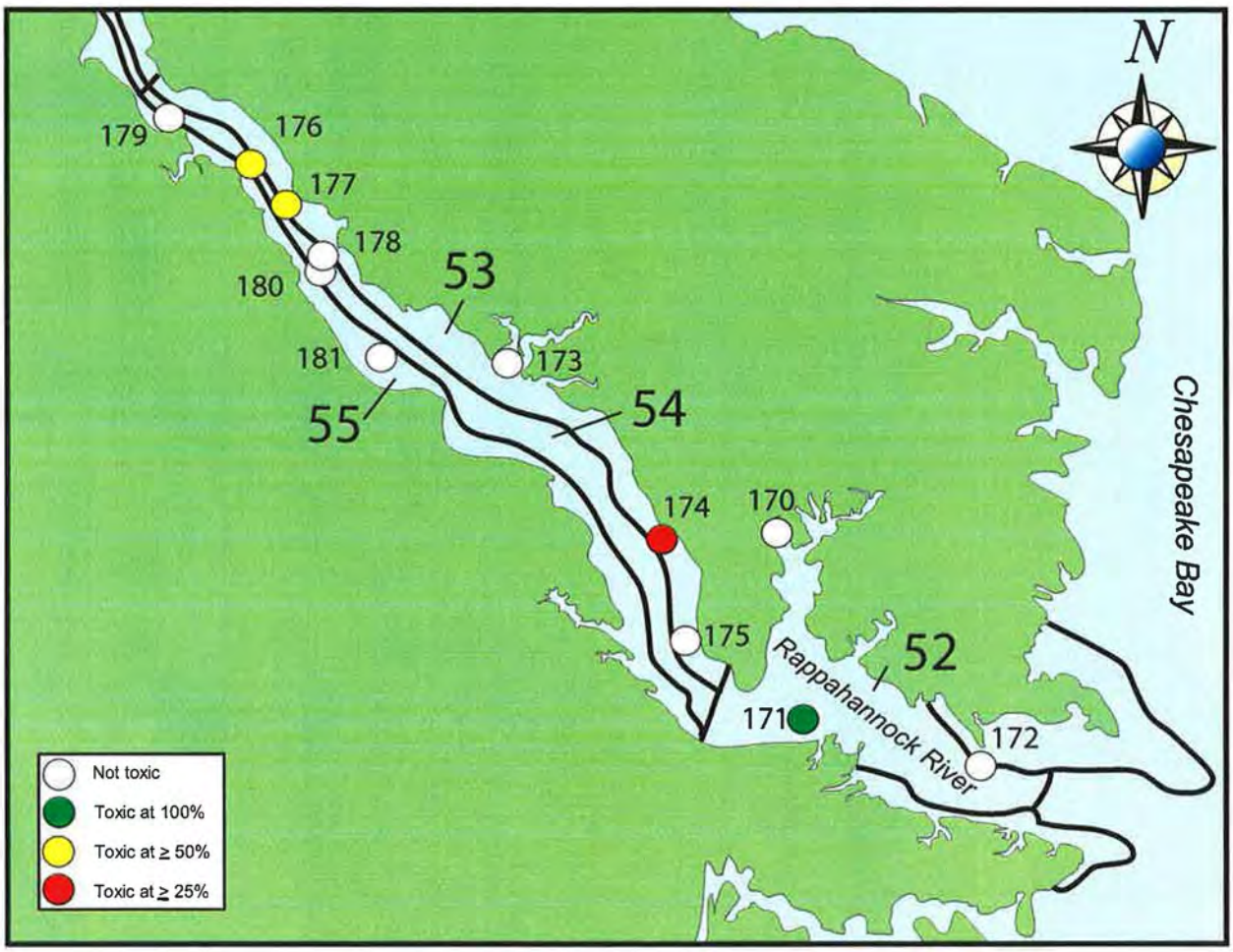

Figure 3. Sample stations in the Rappahannock River, strata 52-55. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's t-test, $\alpha < 0.05$ and detectable significance criteria applied).

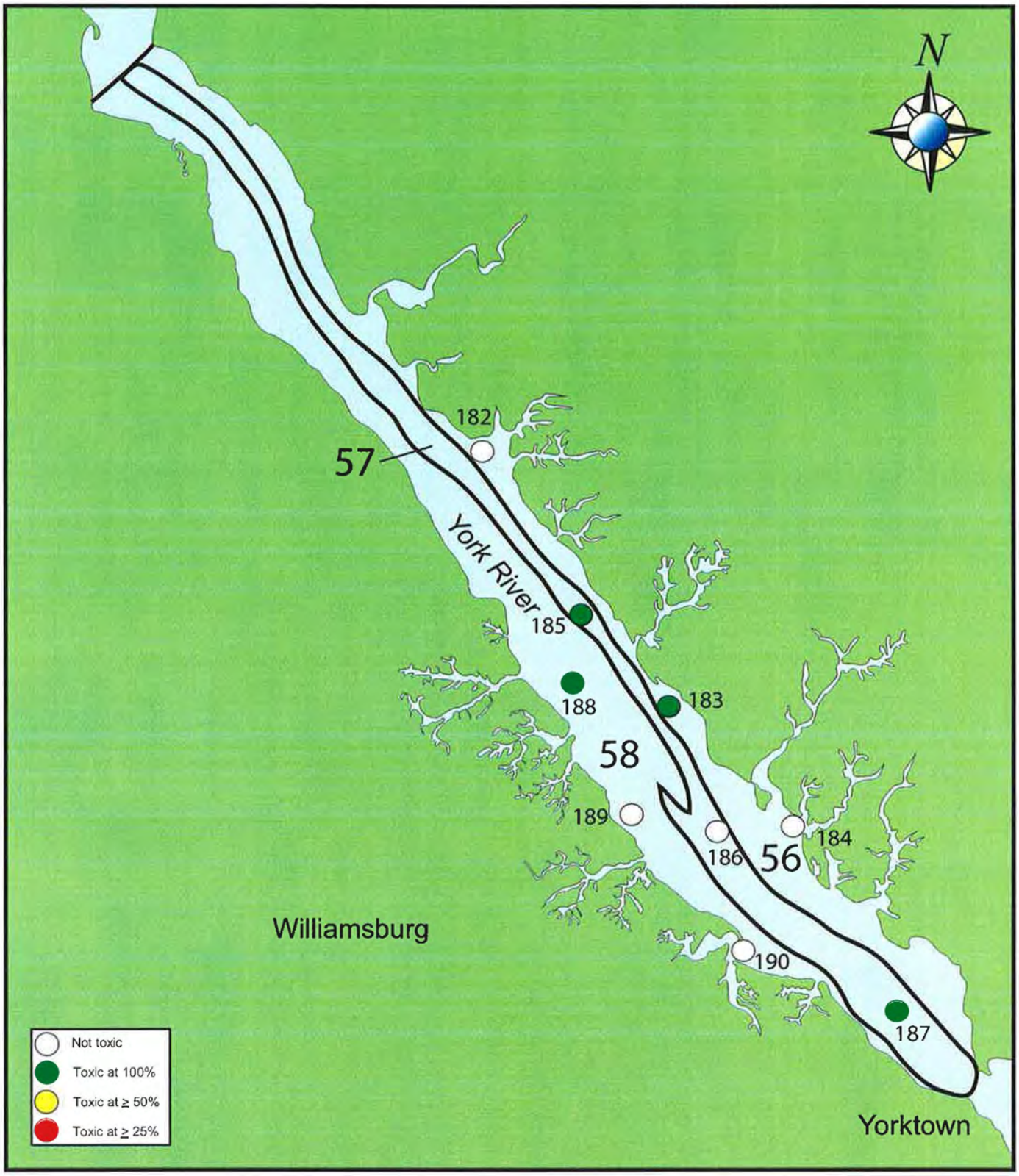

Figure 4. Sample stations in the York River, strata 56-58. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's t-test, $\alpha < 0.05$ and detectable significance criteria applied).

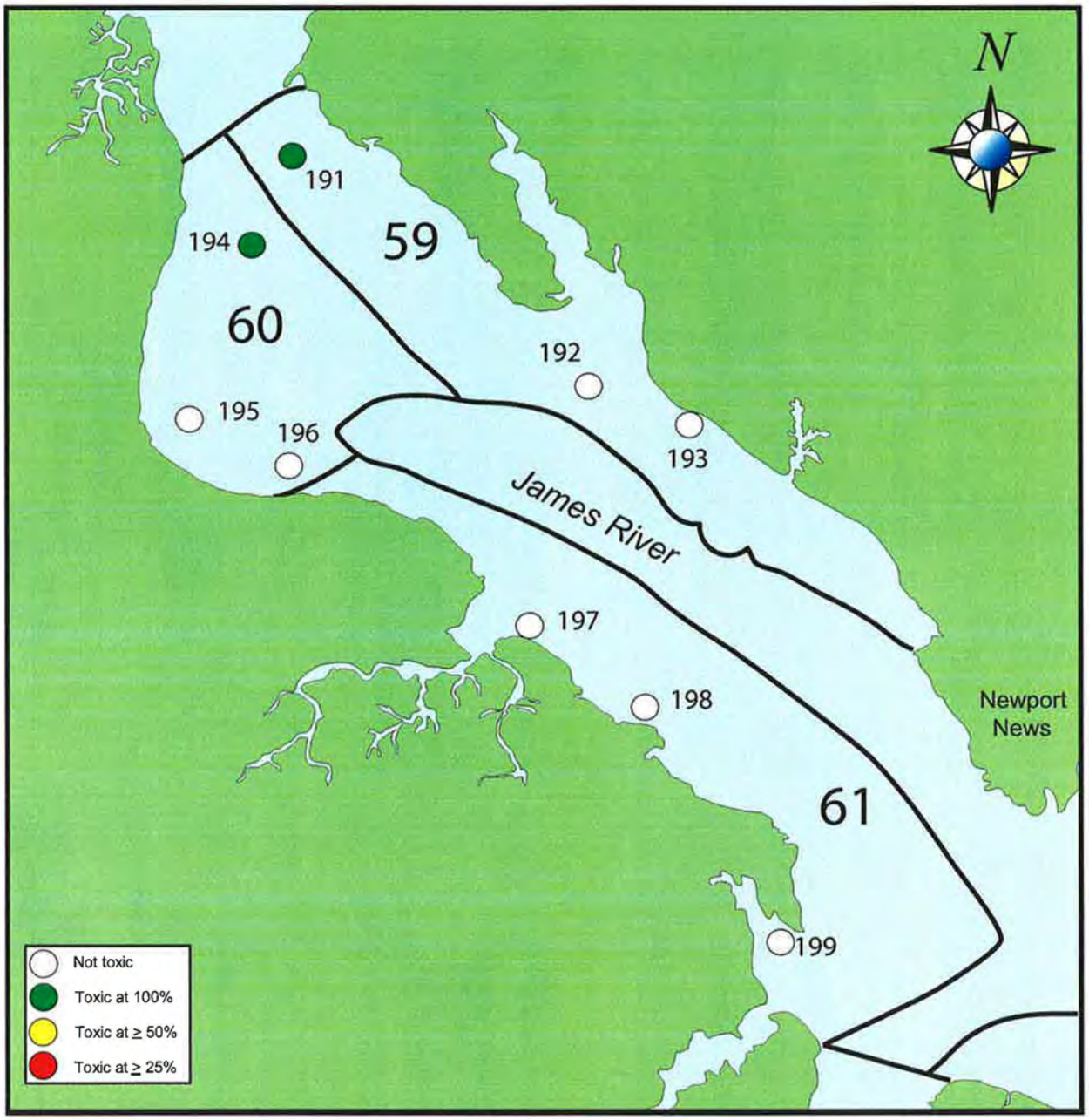

Figure 5. Sample stations in the James River, strata 59-61. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay (Dunnett's t-test, $\alpha < 0.05$ and detectable significance criteria applied).

ATTACHMENTS 1-3

This is NOT A CITABLE DOCUMENT
and is intended for reference only.

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.

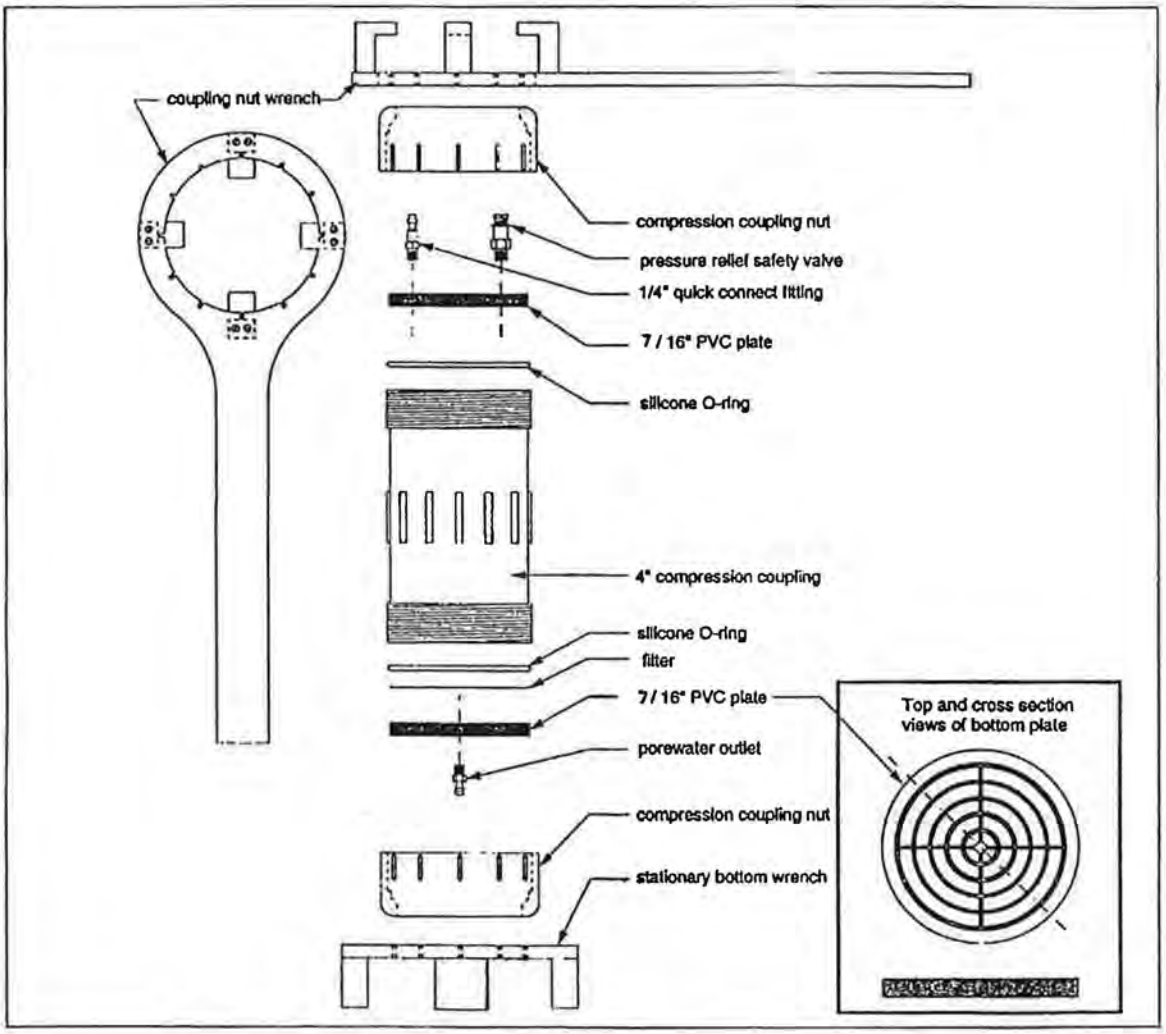

Figure 1. Sediment pore water squeeze extraction device.

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.

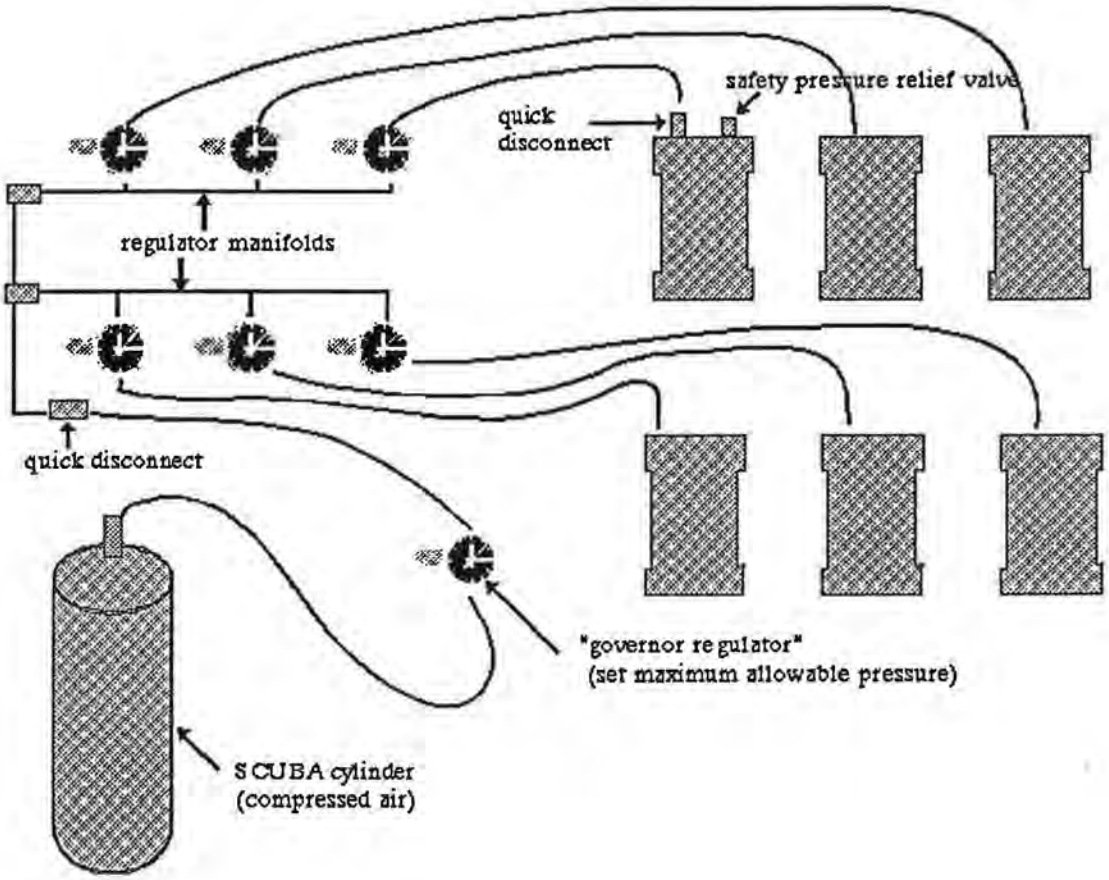

Figure 2. Schematic of sediment porewater pressure extraction system.

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.

Prepared by:

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Joseph B. Hunn 6-28-94
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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$^{o}/_{oo}$. 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$^{o}/_{oo}$ 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$^{o}/_{oo}$) 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. Prior to use, a 10% addition of reference pore water is added to the brine to replace lost trace elements. For samples higher in salinity than target salinity, Milli-Q, HPLC grade ultrapure water 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 (‰)	_____
Vol. Baker® HPLC water added (mL)	_____
Vol. ____‰ brine added (mL)	_____
% of original sample (initial vol./final vol. x 100)	_____

B. Character of Sample (after salinity adjustment):

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

COMMENTS_____

_____

_____

_____

_____

_____

_____

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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 <u>bottom</u> 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 1. 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

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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

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

COMMENTS _____