Final Report

on

TOXICITY TESTING OF SEDIMENTS FROM THE BEST/EMAP WESTERN COASTAL MONITORING STUDY YEAR II

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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

Attachment 4. (SOP F10.7) Sea Urchin Embryological Development Toxicity Test

INTRODUCTION

The Biomonitoring and Environmental Status and Trends Program (BEST) monitors, identifies, and assesses contaminants and their effects on biological resources, including biota and the habitats that support them. BEST is applying a suite of biomonitoring methods in large rivers as one approach for monitoring contaminants and their effects. This monitoring approach alone is not sufficient to address the wide array of issues concerning contaminant effects in the environment. Consequently, BEST is pursuing the development of additional monitoring options through collaborations with other national monitoring efforts to maximize its limited resources, avoid duplication, and capitalize on results (such as ecological condition) provided by other national monitoring efforts.

The Estuary Resource Group of the Environmental Monitoring and Assessment Program (EMAP) finalized plans this year to sample habitats along the Pacific coast. EMAP is a national program of the U.S. Environmental Protection Agency (EPA) that uses a probability-based sampling frame to periodically characterize ecological conditions at regional and national spatial scales. The core indicators of estuarine environments include measures of physiochemical attributes, water quality, sediment quality and habitat attributes as well as attributes of the fish and benthic communities. Many of these indicators are also BEST indicators. The addition of porewater toxicity testing as an additional indicator of sediment quality provides a more comprehensive picture of contaminant effects in estuarine habitats.

This study was undertaken to assess sediments from large estuaries from the states of Washington, Oregon and California using the sea urchin (*Arbacia punctulata*) fertilization and embryological development tests. Sediment samples were collected by various local field personnel and shipped to the United States Geological Survey (USGS) Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas where the tests were performed. A cooperative sampling effort was made in San Francisco Bay, California with the National Oceanic and Atmospheric Administrations (NOAA) Status and Trends project which allowed the number of samples to be expanded greatly in the estuary. In addition, testing was completed on archived samples of pore water stored at the MERS lab from previous NOAA samplings in the Puget Sound estuary. 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). 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. Additional subsamples of frozen pore water were analyzed for dissolved organic carbon.

The specific objectives of this study were to:

- Extract sediment pore water from all 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 and embryological development toxicity test with pore water using gametes of the sea urchin *Arbacia punctulata*. A control reference pore water and a dilution series with sodium dodecyl sulfate (SDS) as a quality assurance control was run in conjunction with each test.

- Retest archived NOAA samples with the sea urchin *A. puntulata* and compare results to previous test results performed with *Strongylocentrotus purpuratus*.

- Make statistical comparisons between test and reference stations for the pore water and calculate EC_{50} values where possible.

- Measure Dissolved Organic Carbon (DOC) in pore water from all stations.

MATERIALS AND METHODS

Sediment Sample Receipt and Tracking

Washington

Surficial sediment samples were collected from 19 stations from the Puget Sound estuary and sent to the USGS MERS in Corpus Christi, Texas between 6/15 and 8/16/2000 (Figure 1). Samples were collected by personnel of the Washington Department of Ecology. Table 1 lists the locations of sample stations as well as sample arrival temperatures and holding times for each sample prior to porewater extraction. Homogenized 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 MERS in Corpus Christi, Texas, the day following shipment in good condition with incoming temperatures ranging from 3 to 6 °C. Shipments were accompanied by sample tracking sheets, and samples were logged into a laboratory sample tracking system. Samples were processed for porewater extraction upon receipt. All porewater samples were extracted within 6 days of the time of field collection of sediment, and within 24 hours of arrival at the Corpus Christi laboratory and frozen at - 20°C. Sediment samples were held refrigerated at 4°C or were placed on blue ice and chilled while awaiting processing.

A subset of 40 porewater samples from sediments collected by NOAA in Puget Sound in 1997, 1998, and 1999 (Figure 1) were also thawed and tested with the sea urchin *A. punctulata*. Table 2 lists the locations of the sample stations as well as the sampling dates. Sediment samples were received at MERS from NOAA personell in 1997, 1998 and 1999 in similar precleaned one-gallon high denisty polyethylene containers and processed for porewater in the same manner as described below.

Initial testing of these samples for NOAA was with the pacific purple urchin *S. pupuratus*. Data collected by this lab in 1997 indicated that there was a similar toxicity response between the two urchin species (USGS 1997b). However, previous testing of the porewater sample was performed only with the sea urchin fertilization assay. It is not known how long samples can be stored frozen without changing the toxicolgical characteristics of the sample. As a result, both sea urchin assays were performed on the archived samples and the results compared back to previous test results with *S. purpuratus* to determine if any changes in sample toxicity during storage had occurred.

Oregon

Sediment samples were collected from 20 intertidal stations from the Columbia River estuary between 8/23 and 9/27/2000 by personnel of the Oregon Department of Environmental Quality (Figure 2). Table 3 lists the locations, arrival temperatures and holding times prior to extraction for each of the samples collected and shipped to MERS. All samples were processed and stored as described below.

California

A total of 99 sediment samples were collected from San Francisco Bay and shipped to MERS between 7/11 and 8/17/2000 (Figures 3 and 4). The majority of the samples were collected by NOAA personnel with the remainder being collected by personnel from the Moss Landing Marine Laboratory. Tables 4 and 5 lists geographical locations, sample arrival temperatures and holding times prior to extraction of all the samples. Eighteen NOAA samples arrived at temperatures exceeding 10°C. It is not known what effect elevated temperatures may have had on the toxicity of the pore water and this should be taken into considerattion when interpreting the results for these samples. Once the samples arrived at the Corpus Christi laboratory, all samples were either refrigerated at 4°C or were placed on blue ice and chilled while awaiting processing.

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). The apparatus and extraction procedures are detailed in SOP F10.9 (Attachment 1).

Sediment samples were held refrigerated (4°C) until the pore water was extracted. Pore water was extracted as soon as possible after receipt of the samples but in no event were the sediments held longer than 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, porewater samples were moved from the freezer to a refrigerator at 4°C. One day prior to testing, samples were completely thawed (if necessary) in a tepid water bath. Temperature of the samples was maintained at 20 ± 2°C. Sample salinity was measured and adjusted to 30 ± 1‰, 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 and the embryological development test with the sea urchin *Arbacia punctulata* following the procedures outlined in SOP F10.6 and F10.7 (Attachment 3 and 4). The sea urchins used in this study were obtained from Gulf Specimen Company, Inc. (Panacea, Florida). Each of the 178 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, 1993; NBS, 1993; 1994; 1995a; 1995b; USGS, 1997a; 1997b; 1998; 1999a; 1999b; 2000a; 2000b; 2000c), 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.

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). Further transformations was required with some data sets to adjust for response scaling inequalities. 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).

results may indicate some difference in sensitivity of the two species not seen previously or may indicate a problem with storing porewater in a frozen state for longer than 19 months. This descrepancy must be considered when interpreting the results of the embryological development test.

Toxicity test results of the embryological development test revealed that 17 of the 40 samples were toxic in the 100% adjusted porewater sample (Table 14). Furthermore, three samples were still toxic at the 50% dilution and one (243) was still very toxic at the 25% concentration. Table 15 summarizes the results of both tests with *A. punctulata* and Figures 5 and 6 illustrate the results on maps.

Oregon

Twenty samples were extracted and toxicity tested from the Columbia River estuary in Oregon. Salinity ranged from 0 to 28 ppt (Tables 16 and 17). Eight samples had salinities below 2 ppt. As with the Washington samples, the embryological development assay was repeated when the first assay failed to meet the acceptability criteria for the reference toxicant. As a result, two separate samples were used and water quality measurements are reported for both sea urchin assays (Tables 16 and 17). Dissolved oxygen was above the 80% saturation level in all samples except for sample OR2000-0049 in the embryological development assay which was inadvertantly not aerated. However, the low dissolved oxygen level was not found to add an effect in this sample in the embryological development assay. Sulfide was not detected in any of the samples for either test. Measurements of pH ranged from 7.82 to 8.33 in the samples used in the fertilization assay and ranged from 7.89 to 8.44 in the samples used in the embryological development assay. Total ammonia was near or below detection limits for most samples with the exception of OR2000-0034. Unionized ammonia did not exceed 22 µg/L which is well below the NOEC determined by our lab for both the sea urchin assasys.

Sea urchin fertilization assay results for the Oregon samples can be found in Tables 18 and 20. No samples tested were found to be toxic in this assay. Sample OR2000-0049 was determined to be statistically different than the reference but did not meet the detectable significance criteria (DSC). Likewise, no samples were found to be toxic in the embryological development assay. Raw and summarized results can be found in Tables 19 and 20. There were no EC_{50} values calculated for these samples as there was no toxic response.

California

BEST/EMAP samples

Thirteen samples were extracted and toxicity tested from San Francisco Bay for the BEST/EMAP program. Salinity of the samples ranged from 16 to 31 ppt (Table 21). Dissolved oxygen was in excess of the 80% saturation level required. Sample pH levels ranged from 6.45 to 8.00. Total ammonia ranged from 1.08 to 6.8 mg/L with the unionized fractions ranging from 3.3 to 166.6 µg/L.

Raw data for the sea urchin fertilization test can be found in Table 22. Data is also summarized with the embryological development data in Table 24 and graphically illustrated in

map format in Figure 7. Only 3 samples were found to be toxic at the 100% concentration. Sample CA0042 was found to be the most toxic with significant decreases in fertilization being exhibited in all three dilutions tested.

Sea urchin embryological development raw and summarized data can be found in Tables 23 and 24. Figure 7 also illustrates the results graphically. Nearly all of the samples (12 of 13) were found to be toxic in the sea urchin embryological development assay at the 100% adjusted porewater concentration. In addition, 8 samples were also toxic at the 50% concentration and one was toxic at the 25% concentration. Only three samples exceeded the LOEC for unionized ammonia (90 μg/L) and none of those samples had ammonia levels high enough to cause toxicity in the 50% dilution.

EC_{50} values were not calculable for samples in the fertilization test, but were calculated for 10 samples in the embryological development test (Table 25). EC_{50} values were greater than 100% concentration tested in the fertilization test samples with the exception of CA2000-0042 which had an EC_{50} value of < 25%. The remaining 3 samples in the embryological development test had EC_{50} values above the 100% concentration level. Quality controls used in the test resulted in acceptable values. EC_{50} values for the SDS positive controls were 5.19 and 3.90 mg/L for the fertilization and embryological development tests, respectively. These values fall within the control charts for the MERS laboratory.

NOAA samples

Eighty-six sediment samples were extracted and tested for toxicity from San Francisco Bay for the NOAA program. Salinity of the pore water ranged from 2 to 33 ppt (Table 26). Fourteen samples had salinities below 10 ppt. Initial dissolved oxygen of all samples exceeded 80% saturation and did not require aeration prior to testing. Sulfide measurements were below detection limits and pH ranged from 7.04-8.47. Total ammonia measured ranged from 0.1 to 25.1 mg/L with the unionized fraction ranging from 2.9 to 217.1 μg/L.

Four tests were required (two fertilization and two embryological development) to complete the toxicity testing for the NOAA and the BEST/EMAP samples. Toxicity testing of the 86 NOAA samples in the sea urchin fertilization test revealed 40 samples were toxic in the undiluted adjusted pore water when compared to the reference pore water at $\alpha \leq 0.05$. In addition 18 samples were toxic at the 50% dilution and two (38-3 and 40-2) were still toxic at the 25% porewater concentration. Tables 27, 28 and 31 give the raw and summarized data, respectively, for the fertilization test while Figures 8 through 12 illustrates the results in a graphical (map) format.

Sea urchin embryological development test results can be found in Tables 29 and 30, summarized in Table 31 and again graphically illustrated in Figures 8 through 12. The embryological development assay is often more sensitive than the fertilization assay as was the case with this set of samples. Seventy-five of the 86 samples (87%) were found to be toxic in the undiluted adjusted pore water. Fifty-seven samples were still toxic in the 50% dilution and 33 were toxic at the 25% conentration. Stations 38-3, 40-2 and 43-3 were the most toxic areas with no normal plutei development in each of the three dilutions. The sea urchin embryological development test is more sensitive to ammonia than the fertilization assay with the LOEC for unionized ammonia of 90 μg/L. Fourteen samples exceeded this level in the undiluted adjusted pore water. However, a 50% dilution would have dropped the level below the LOEC in all but 3

samples (33-5, 34-1 and 38-3). Toxicity persisted in many samples suggesting that other contaminants besides ammonia may be contributing to the toxicity observed in these samples.

EC_{50} values could be calculated for 13 stations in the fertilization test while it was calculable for 48 stations in the embryological development test (Table 32). In the remaining stations, EC_{50} values exceeded the 100% concentration or were less than the 25% concentration (at 11 stations in the embryological development test). Quality controls used in the test resulted in acceptable values. EC_{50} values for the SDS positive controls were 6.05 and 5.19 mg/L for the fertilization tests and 2.94 and 3.90 for the embryological development tests. These values fall within the control charts for the MERS laboratory.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) was measured in all porewater samples collected and frozen for the 2000 BEST/EMAP study and the 2000 NOAA study on San Francisco Bay. Results of the (DOC) analysis can be found in Tables 33-36. Samples were run in 9 batches with associated quality control standards. Tables 37 and 38 lists the results of the QA/QC controls run with each batch. No samples had to be reanalyzed. Samples were frozen at the time of extraction to preserve them for later analysis. DOC values reported reflect the concentrations in the porewaters at the time of the toxicity testing. 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 or acidified for preservation. DOC concentrations were the highest in San Francisco Bay samples with a range of 2.28 to 50.5 mg/L. DOC ranged from 5.25 to 22.89 in Washington and 1.80 to 17.83 mg/L in Oregon samples.

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

U.S. Geological Survey (USGS). 1998. Toxicity testing of sediments from Central Puget Sound, Washington and surrounding areas. Report submitted by the USGS to National Oceanic and Atmospheric Administration, Coastal Monitoring and Biological Assessment Division, Seattle, WA, 11pp. + 8 tables, 6 figures and 4 attachments.

U.S. Geological Survey (USGS). 1999a. Toxicity testing of sediments from Southern Puget Sound, Washington and surrounding areas. Report submitted by the USGS to National Oceanic and Atmospheric Administration, Coastal Monitoring and Biological Assessment Division, Seattle, WA, 11pp. + 7 tables, 5 figures and 4 attachments.

U.S. Geological Survey (USGS). 1999b. Toxicity testing of sediments from Chesapeake Bay and surrounding areas, Phase I. Report submitted by the USGS to National Oceanic and Atmospheric Administration, Office of Ocean Resources Conservation Assessment, Bioeffects Assessment Branch, Silver Springs, MD, 9 pp. + 9 tables, 3 figures and 4 attachments.

U.S. Geological Survey (USGS). 2000a. Toxicity testing of sediments from Chesapeake Bay and surrounding areas, Year II. Report submitted by the USGS to National Oceanic and Atmospheric Administration, Office of Ocean Resources Conservation Assessment, Bioeffects Assessment Branch, Silver Springs, MD, 9 pp. + 9 tables, 5 figures and 4 attachments.

U.S. Geological Survey (USGS). 2000b. Toxicity testing of sediments from Portsmouth Naval Shipyard, Kittery, Maine. Report submitted by the USGS to Tetra Tech NUS Inc., 600 Clark Avenue, Suite 3, King of Prussia, PA, 9 pp. + 8 tables, 1 figure and 5 attachments.

U.S. Geological Survey (USGS). 2000c. Toxicity testing of sediments from the BEST/EMAP Western Estuary Group Monitoring Stndy. Report submitted by the USGS to the U.S. Geological Survey, Biomonitoring of Environmental Status and Trends Program, 6006 Schroeder Road, Madison, WI, 10 pp. + 22 tables, 3 figures and 4 attachments.

TABLES 1-38

Table 1. Geographic locations, sample arrival temperatures and holding times of BEST/EMAP Puget Sound, Washington sampling sites.

Station ID	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature (°C)	Elapse Time: Collection-Process (Days)
E1	48° 56.293	123° 44.021	5-6	5
E4	48° 56.319	123° 12.074	5	4
E5	48° 57.330	123° 00.270	4.5	3
E6	48° 54.082	122° 55.518	4.5	5
E7	48° 48.278	123° 23.704	4.5	4
E8	48° 42.718	123° 31.703	4.5	4
E9	48° 49.434	122° 43.818	4.5	2
E13	48° 34.346	123° 20.102	5-6	4
E14	48° 37.483	122° 57.675	4	5
E15	48° 36.677	123° 59.988	4	5
E17	48° 36.643	122° 50.248	5	3
E19	48° 31.439	122° 50.857	5	3
E21	48° 29.224	122° 59.806	5-6	2
E22	48° 25.550	123° 17.289	5-6	4
E23	48° 23.287	122° 55.203	5-6	3
E24	48° 19.435	123° 03.251	5-6	3
E25	48° 18.960	122° 48.026	5	2
E29	48° 52.040	122° 25.190	5	2
E30	48° 35.403	122° 25.362	5	6

Table 3. Geographic locations, sample arrival temperatures and holding times of BEST/EMAP Columbia River, Oregon sampling sites.

Station ID	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature (°C)	Elapse Time: Collection-Process (Days)
OR2000-0031	46° 16.283	124° 02.689	7	2
OR2000-0032	46° 15.550	124° 01.280	7	2
OR2000-0033	46° 13.600	123° 58.681	5	3
OR2000-0034	46° 14.783	123° 51.873	6	3
OR2000-0035	46° 16.983	123° 47.568	6.5	6
OR2000-0036	46° 13.917	123° 56.357	6	2
OR2000-0037	46° 14.517	123° 51.510	6.5	4
OR2000-0038	46° 14.033	123° 52.804	5	2
OR2000-0039	46° 14.317	123° 04.738	6.5	5
OR2000-0040	46° 16.150	123° 42.762	6	2
OR2000-0041	46° 12.317	123° 52.892	6	2
OR2000-0042	46° 13.333	123° 47.829	6.5	5
OR2000-0043	46° 14.402	123° 43.942	6.5	6
OR2000-0044	46° 15.831	123° 39.502	6	2
OR2000-0045	46° 15.219	123° 33.731	5	3
OR2000-0046	46° 12.761	123° 46.853	7	3
OR2000-0047	46° 13.336	123° 39.908	5	4
OR2000-0048	46° 16.098	123° 30.110	5	4
OR2000-0049	46° 14.944	123° 26.423	5	5
OR2000-0050	46° 14.137	123° 25.620	5	3

Table 4. Geographic locations, sample arrival temperatures and holding times of BEST/EMAP San Francisco Bay, California sampling sites.

Station ID	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature	Elapse Time: Collection-Process (Days)
CA2000-0038	38° 12.327′	121° 34.388′	7	5
CA2000-0039	38° 10.074′	121° 32.057′	7	5
CA2000-0040	38° 10.718′	121° 18.203′	7	8
CA2000-0041	38° 7.112′	121° 30.581′	7	5
CA2000-0042	38° 8.777′	121° 18.815′	7	7
CA2000-0043	38° 5.599′	121° 15.536′	7	6
CA2000-0044	37° 57.885′	121° 28.426′	7	6
CA2000-0045	37° 46.063′	121° 13.711′	7	5
CA2000-0046	37° 30.318′	121° 10.624′	7	9
CA2000-0047	38° 2.317′	121° 28.304′	7	6
CA2000-0048	38° 1.657′	121° 29.399′	7	6
CA2000-0049	38° 40.486′	121° 22.130′	7	8
CA2000-0050	38° 41.065′	121° 12.562′	7	8

Table 5. Geographic locations, sample arrival temperatures and holding times of NOAA San Francisco Bay, California sampling sites.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature	Elapse Time: Collection-Process (Days)
1	2	38° 2.273′	121° 49.985′	14-15	6
2	2	38° 3.760′	121° 51.516′	14-15	6
3	1	38° 4.283′	121° 58.185′	14-15	6
3	3	38° 3.825′	121° 55.455′	14-15	5
4	2	38° 8.112′	122° 2.451′	6	5
4	BF21	38° 06.965′	122° 11.000′	6	5
5	1	38° 4.371′	122° 6.260′	6	5
5	2	38° 5.488′	122° 3.259′	6	5
5	5	38° 3.055′	121° 59.078′	14-15	5
6	1	38° 5.862′	121° 1.767′	14-15	6
6	4	38° 3.220′	121° 4.150′	14-15	5
7	1	38° 9.546′	122° 2.838′	6	5
7	4	38° 8.188′	122° 4.938′	6	5
7	6	38° 2.491′	121° 5.425′	14-15	5
8	1	38° 4.184′	122° 14.515′	6	4
8	3	38° 3.164′	122° 10.288′	6	5
9	2	38° 6.362′	122° 16.142′	6	5
10	1	38° 8.401′	122° 16.939′	6	4
10	3	38° 7.580′	122° 17.036′	6	4
11	1	38° 5.183′	122° 23.758′	4-5	2
11	3	38° 5.676′	122° 21.123′	4-5	2
11	6	38° 0.313′	122° 26.690′	4	6
11	BD22	38° 02.966′	122° 25.236′	4-5	3
12	1	38° 2.477′	122° 20.494′	6	3

Table 5. Continued.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature	Elapse Time: Collection-Process (Days)
13	1	38° 1.648′	122° 19.986′	6	3
14	1	38° 8.76′	122° 31.636′	4-5	3
15	1	37° 57.745′	122° 28.054′	4-5	2
15	3	37° 54.303′	122° 27.949′	4-5	1
16	1	37° 55.183′	122° 26.993′	6	2
17	1	37° 57.141′	122° 25.375′	4-6	5
17	2	37° 56.041′	122° 25.249′	6	2
18	1	37° 54.450′	122° 23.595′	4-5	4
19	2	37° 54.823′	122° 21.868′	4-6	4
19	3	37° 54.561′	122° 21.674′	4-6	4
20	1	37° 52.762′	122° 23.213′	4-6	5
20	5	37° 51.264′	122° 20.291′	4-6	5
20	6	37° 48.797′	122° 20.497′	4-6	6
21	1	37° 49.994′	122° 21.429′	4-6	3
21	3	37° 48.644′	122° 20.860′	26.5	6
22	1	37° 50.792′	122° 28.130′	4-6	4
22	3	37° 50.270′	122° 26.980′	4-6	4
22	6	37° 48.532′	122° 23.060′	4-6	4
23	2	37° 52.580′	122° 28.683′	4-5	1
24	2	37° 48.620′	122° 26.000′	4-6	4
25	1	37° 48.299′	122° 24.009′	4-6	2
25	3	37° 48.000′	122° 23.780′	26.5	8
26	1	37° 47.258′	122° 23.211′	4-6	3
26	2	37° 46.105′	122° 22.894′	4-6	2

Table 5. Continued.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature	Elapse Time: Collection-Process (Days)
27	1	37° 42.943′	122° 22.111′	4.5-9	5
28	1	37° 47.892′	122° 22.018′	4-6	2
28	4	37° 45.890′	122° 21.626′	26.5	8
28	5	37° 44.205′	122° 20.497′	4.5-9	5
29	2	37° 46.629′	122° 17.971′	4.5-9	5
30	1	37° 47.792′	122° 20.336′	26.5	7
30	3	37° 44.176′	122° 17.419′	4.5-9	8
30	BB70	37° 44.822′	122° 19.363′	4.5-9	5
31	2	37° 48.853′	122° 19.343′	26.5	6
31	4	37° 48.098′	122° 20.709′	12	6
31	6	37° 47.754′	122° 19.417′	12	6
32	2	37° 47.245′	122° 15.143′	4.5-9	5
32	3	37° 46.758′	122° 14.634′	4.5-9	5
32	6	37° 45.142′	122° 13.055′	4.5-9	5
33	5	37° 42.136′	122° 22.630′	4.5-9	5
34	1	37° 39.385′	122° 21.379′	12	5
34	3	37° 37.175′	122° 19.919′	12	5
35	2	37° 41.356′	122° 21.155′	12	5
35	3	37° 41.309′	122° 18.562′	4.5-9	8
36	1	37° 41.304′	122° 14.549′	4.5-9	8
36	2	37° 40.276′	122° 15.446′	4.5-9	8
36	3	37° 39.474′	122° 11.635′	4	5
38	1	37° 35.018′	122° 14.436′	4.5-9	7
38	3	37° 31.235′	122° 08.374′	4.5-9	7

Table 5. Continued.

Stratum	Site	Latitude (°N)	Longitude (°W)	Sample Arrival Temperature	Elapse Time: Collection-Process (Days)
39	1	37° 35.650′	122° 08.374′	4	5
40	2	37° 31.619′	122° 11.855′	4.5-9	7
40	3	37° 30.723′	122° 12.763′	4.5-9	7
42	1	37° 29.822′	122° 06.090′	4.5-9	4
42	3	37° 28.353′	122° 03.961′	4.5-9	4
42	BA21	37° 29.650′	122° 05.245′	4.5-9	4
43	3	37° 27.779′	122° 02.034′	4.5-9	4
44	1	37° 27.775′	122° 01.576′	4.5-9	4
44	2	37° 27.775′	122° 01.576′	4	5
46	1	37° 58.034′	122° 30.342′	4-5	2
46	3	38° 08.736′	122° 23.902′	4-5	3
46	4	37° 57.157′	122° 23.023′	4-6	5
47	3	37° 51.893′	122° 29.568′	4-6	7
47	4	37° 52.066′	122° 19.056′	4-6	6

Table 6. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples from BEST/EMAP stations in Puget Sound, Washington evaluated in the fertilization test.

Station ID	Salinity[1] (‰)	DO[2] (mg/L)	% DO[3]	pH	TAN[4] (mg/L)	UAN[5] (μg/L)	Sulfide[6] (mg/L)	% OUS[7]
REF-1[8]	40	6.25	85.3	8.43	0.481	38.5	<0.01	75
E1	30	6.42	88.1	7.15	2.40	10.9	<0.01	100
E4	32	6.03	82.5	7.54	1.11	12.3	<0.01	94
E5	30	6.58	90.1	7.52	<0.1	<1.1	<0.01	100
E6	32	6.56	89.6	7.71	1.30	21.2	<0.01	94
E7	32	6.23	85.3	7.5	1.30	13.2	<0.01	94
E8	32	6.01	81.8	7.52	1.20	12.7	<0.01	94
E9	31	5.98	80.0	7.52	1.13	12.0	<0.01	97
E13	30	5.92	80.9	7.46	2.09	19.3	<0.01	100
E14	31	6.25	85.8	7.47	1.31	12.4	<0.01	97
E15	31	6.49	89.3	7.59	1.76	21.9	<0.01	97
E17	30	6.59	82.3	7.66	1.11	16.2	<0.01	100
E19	31.5	5.81	80.0	7.39	1.11	8.75	<0.01	97
E21	32	5.82	80.1	7.44	1.35	11.9	<0.01	94
E22	32	6.65	88.2	7.70	1.74	27.8	<0.01	94
E23	34	6.29	86.7	7.73	0.712	12.2	<0.01	88
E24	32.5	5.96	82.0	7.82	1.25	26.2	<0.01	94
E25	34	5.82	80.3	7.82	0.37	7.74	<0.01	88
E29	28.5	6.24	85.9	7.73	<0.1	<1.7	<0.01	99
E30	32	6.31	87.1	7.58	1.34	16.3	<0.01	94

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

Table 7. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples from BEST/EMAP stations in Puget Sound, Washington evaluated in the embryological development test.

Station ID	Salinity[1] (‰)	DO[2] (mg/L)	% DO[3]	pH	TAN[4] (mg/L)	UAN[5] (μg/L)	Sulfide[6] (mg/L)	% OUS[7]
REF-1[8]	40	6.69	90.7	8.38	0.803	57.8	<0.01	75
E1	30	6.70	90.3	7.41	4.62	38.1	<0.01	100
E4	32	6.04	81.4	7.7	1.16	18.5	<0.01	94
E5	30	6.80	91.9	7.66	0.42	6.1	<0.01	100
E6	32	6.28	84.9	7.75	1.59	28.4	<0.01	94
E7	32	6.44	87.4	7.72	1.57	26.2	<0.01	94
E8	32	6.39	86.9	7.62	1.28	17.0	<0.01	94
E9	31	6.36	86.5	7.62	1.21	16.1	<0.01	97
E13	30	6.37	86.2	7.4	3.96	31.9	<0.01	100
E14	31	6.39	86.4	7.39	1.73	13.6	<0.01	97
E15	31	6.53	88.2	7.56	3.11	36.1	<0.01	97
E17	30	6.55	88.7	7.66	1.25	18.2	<0.01	100
E19	31.5	6.56	88.8	7.61	1.24	16.10	<0.01	97
E21	32	6.37	86.6	7.73	1.89	32.3	<0.01	94
E22	32	6.17	83.7	7.61	2.99	38.9	<0.01	94
E23	34	6.14	83.4	7.68	1.02	15.6	<0.01	88
E24	32.5	6.51	88.8	7.81	1.73	35.4	<0.01	94
E25	34	6.29	85.6	7.46	0.964	8.91	<0.01	88
E29	28.5	6.51	88.5	7.71	0.484	7.90	<0.01	99
E30	32	5.95	81.2	7.66	2.12	30.9	<0.01	94

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

Table 8. Sea urchin (*Arbacia punctulata*) fertilization test raw data and means for sediment porewater samples from Puget Sound, Washington. Asterisks indicate 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	100	100	100	98	100	99.6 ± 0.7	100
		99	100	99	100	100		
REF [3]	50	100	99	99	100	100	99.8 ± 0.4	100
		100	100	100	100	100		
REF [3]	25	99	100	100	100	99	99.8 ± 0.4	100
		100	100	100	100	100		
E1	100	59	46	43	49	66	52.6 ± 9.6 **	66
E1	50	97	100	100	98	99	98.8 ± 1.3 +	99
E1	25	100	100	100	100	100	100.0 ± 0.0	100
E4	100	100	100	100	100	100	100.0 ± 0.0	100
E4	50	100	100	100	99	100	99.8 ± 0.4	100
E4	25	100	100	100	100	100	100.0 ± 0.0	100
E5	100	100	100	100	100	100	100.0 ± 0.0	100
E5	50	100	100	100	100	100	100.0 ± 0.0	100
E5	25	100	100	100	100	100	100.0 ± 0.0	100
E6	100	100	100	100	100	100	100.0 ± 0.0	100
E6	50	100	100	100	100	100	100.0 ± 0.0	100
E6	25	100	100	100	99	100	99.8 ± 0.4	100
E7	100	100	100	100	100	100	100.0 ± 0.0	100
E7	50	100	100	100	100	100	100.0 ± 0.0	100
E7	25	98	100	100	100	100	99.6 ± 0.9	100

Table 8. Continued.

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
E8	100	100	100	100	100	100	100.0 ± 0.0	100
E8	50	100	100	100	99	100	99.8 ± 0.4	100
E8	25	100	100	100	100	100	100.0 ± 0.0	100
E9	100	100	100	100	100	100	100.0 ± 0.0	100
E9	50	100	100	100	100	100	100.0 ± 0.0	100
E9	25	100	99	100	100	100	99.8 ± 0.4	100
E13	100	100	100	100	100	100	100.0 ± 0.0	100
E13	50	100	100	100	100	100	100.0 ± 0.0	100
E13	25	100	99	100	100	100	99.8 ± 0.4	100
E14	100	100	100	100	100	100	100.0 ± 0.0	100
E14	50	99	100	100	99	100	99.6 ± 0.6	100
E14	25	100	100	100	100	100	100.0 ± 0.0	100
E15	100	100	100	100	100	99	99.8 ± 0.4	99
E15	50	99	100	99	100	100	99.6 ± 0.6	100
E15	25	100	100	99	100	100	99.8 ± 0.4	100
E17	100	100	100	100	100	100	100.0 ± 0.0	100
E17	50	99	100	100	100	99	99.6 ± 0.6	99
E17	25	100	100	100	100	100	100.0 ± 0.0	100
E19	100	99	100	100	100	100	99.8 ± 0.4	100
E19	50	100	100	99	100	100	99.8 ± 0.4	100
E19	25	100	100	100	100	100	100.0 ± 0.0	100
E21	100	100	100	99	100	100	99.8 ± 0.4	100
E21	50	100	100	100	100	100	100.0 ± 0.0	100
E21	25	100	100	98	100	100	99.6 ± 0.9	100

Table 8. Continued.

Station ID	% WQAS[1]	% Fertilized					Mean±SD	% of REF[2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
E22	100	99	99	100	99	100	99.4 ± 0.6	100
E22	50	100	100	100	100	100	100.0 ± 0.0	100
E22	25	100	100	100	100	100	100.0 ± 0.0	100
E23	100	100	100	100	100	100	100.0 ± 0.0	100
E23	50	100	100	99	100	100	99.8 ± 0.4	100
E23	25	100	100	100	100	100	100.0 ± 0.0	100
E24	100	99	100	97	100	100	99.2 ± 1.3	100
E24	50	100	100	100	100	100	100.0 ± 0.0	100
E24	25	100	99	100	98	100	99.4 ± 0.9	100
E25	100	100	100	100	100	100	100.0 ± 0.0	100
E25	50	100	100	100	100	100	100.0 ± 0.0	100
E25	25	100	99	100	100	100	99.8 ± 0.4	100
E29	100	100	100	100	100	100	100.0 ± 0.0	100
E29	50	100	100	100	100	100	100.0 ± 0.0	100
E29	25	100	100	99	100	99	99.6 ± 0.6	99
E30	100	100	99	100	100	99	99.6 ± 0.6	99
E30	50	99	100	99	100	99	99.4 ± 0.6	99
E30	25	100	100	100	100	100	100.0 ± 0.0	100

[1] Percent of water quality adjusted porewater sample.

[2] Station mean devided by Reference mean x 100.

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

Table 9. Sea urchin (*Arbacia punctulata*) embryological development test raw data and means for sediment porewater samples from Puget Sound, Washington. Asterisks indicate statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* α ≤0.05, ** α ≤0.01). Plus signs denote only statistical differences (Dunnett's *t*-test, + α ≤0.05, ++ α ≤0.01).

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	88	98	93	96	97	96.1 ± 3.4	100
		96	99	99	98	97		
REF [3]	50	93	97	97	98	100	97.9 ± 2.1	100
		99	98	97	100	100		
REF [3]	25	98	97	100	100	98	99.0 ± 1.0	100
		99	99	100	100	99		
E1	100	0	0	0	0	0	0.0 ± 0.0 **	0
E1	50	20	76	53	51	75	55.0 ± 22.8 **	56
E1	25	96	99	98	99	97	97.8 ± 1.3	99
E4	100	96	94	98	93	97	95.6 ± 2.1	99
E4	50	96	98	96	97	96	96.6 ± 0.9	99
E4	25	98	99	99	98	97	98.2 ± 0.8	99
E5	100	94	98	96	91	94	94.6 ± 2.6	98
E5	50	95	98	97	98	95	96.6 ± 1.5	99
E5	25	98	100	99	97	97	98.2 ± 1.3	99
E6	100	90	61	89	92	91	84.6 ± 13.2 ++	88
E6	50	93	99	96	97	99	96.8 ± 2.5	99
E6	25	96	98	99	100	99	98.4 ± 1.5	99
E7	100	90	92	96	92	92	92.4 ± 2.2	96
E7	50	94	97	96	97	98	96.4 ± 1.5	98
E7	25	97	99	100	100	99	99.0 ± 1.2	100

Table 9. Continued.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
E8	100	100	99	99	97	96	98.2 ± 1.6	102
E8	50	100	100	98	97	98	98.6 ± 1.3	101
E8	25	100	97	98	99	100	98.8 ± 1.3	100
E9	100	97	98	96	95	98	96.8 ± 1.3	101
E9	50	95	96	97	95	96	95.8 ± 0.8	98
E9	25	100	99	100	99	99	99.4 ± 0.6	100
E13	100	0	0	0	0	0	0.0 ± 0.0 **	0
E13	50	96	89	96	54 [4]	94	93.8 ± 3.3 +	96
E13	25	98	99	99	97	98	98.2 ± 0.8	99
E14	100	98	99	81	95	95	93.6 ± 7.3	97
E14	50	98	97	99	98	99	98.2 ± 0.8	100
E14	25	100	99	98	99	99	99.0 ± 0.7	100
E15	100	0	13	14	10	0	7.4 ± 6.9 **	8
E15	50	94	96	97	97	93	95.4 ± 1.8	97
E15	25	96	98	99	100	100	98.6 ± 1.7	100
E17	100	97	95	97	96	96	96.2 ± 0.8	100
E17	50	97	99	98	98	99	98.2 ± 0.8	100
E17	25	100	98	99	100	100	99.4 ± 0.9	100
E19	100	98	98	97	97	97	97.4 ± 0.6	101
E19	50	100	100	96	99	100	99.0 ± 1.7	101
E19	25	100	98	99	100	100	99.4 ± 0.9	100
E21	100	83	86	88	84	84	85.0 ± 2.0 ++	88
E21	50	96	97	100	99	98	98.0 ± 1.6	100
E21	25	100	98	99	98	100	99.0 ± 1.0	100

Table 9. Continued.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
E22	100	0	0	0	0	0	0.0 ± 0.0 **	0
E22	50	92	84	89	90	87	88.4 ± 3.0 ++	90
E22	25	97	98	92	100	96	96.6 ± 3.0	98
E23	100	98	94	98	94	96	96.0 ± 2.0	100
E23	50	100	99	98	97	98	98.4 ± 1.1	101
E23	25	99	96	98	100	100	98.6 ± 1.7	100
E24	100	90	88	89	90	87	88.8 ± 1.3 ++	92
E24	50	96	97	92	95	95	95.0 ± 1.9	97
E24	25	96	99	99	99	100	98.6 ± 1.5	100
E25	100	95	97	95	92	94	94.6 ± 1.8	98
E25	50	96	99	99	97	100	98.2 ± 1.6	100
E25	25	100	99	97	99	98	98.6 ± 1.1	100
E29	100	100	98	98	97	95	97.6 ± 1.8	102
E29	50	94	96	100	99	98	97.4 ± 2.4	99
E29	25	98	99	100	97	100	98.8 ± 1.3	100
E30	100	66	49	72	83	86	71.2 ± 14.8 **	74
E30	50	97	91	100	100	99	97.4 ± 3.8	99
E30	25	100	99	99	99	97	98.8 ± 1.1	100

[1] Percent of water quality adjusted porewater sample.

[2] Station mean devided by Reference mean x 100.

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

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

Table 10. Summary of station means and statistical significance for the sea urchin fertilization and embryological development test from 19 BEST/EMAP stations from Puget Sound, Washington. 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig.[4]	Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig. [4]
E1	100	52.6	9.6	53	99.6	0.7	**	0.0	0.0	0	96.1	3.4	**
	50	98.8	1.3	99	99.8	0.4	+	55.0	22.8	56	97.9	2.1	**
	25	100.0	0.0	100	99.8	0.4		97.8	1.3	99	99.0	1.0	
E4	100	100.0	0.0	100	99.6	0.7		95.6	2.1	99	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		96.6	0.9	99	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		98.2	0.8	99	99.0	1.0	
E5	100	100.0	0.0	100	99.6	0.7		94.6	2.6	98	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		96.6	1.5	99	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		98.2	1.3	99	99.0	1.0	
E6	100	100.0	0.0	100	99.6	0.7		84.6	13.2	88	96.1	3.4	+
	50	100.0	0.0	100	99.8	0.4		96.8	2.5	99	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		98.4	1.5	99	99.0	1.0	
E7	100	100.0	0.0	100	99.6	0.7		92.4	2.2	96	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		96.4	1.5	98	97.9	2.1	
	25	99.6	0.9	100	99.8	0.4		99.0	1.2	100	99.0	1.0	

Table 10. Continued.

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig.[4]	Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig. [4]
E8	100	100.0	0.0	100	99.6	0.7		98.2	1.6	102	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		98.6	1.3	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		98.8	1.3	100	99.0	1.0	
E9	100	100.0	0.0	100	99.6	0.7		96.8	1.3	101	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		95.8	0.8	98	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		99.4	0.6	100	99.0	1.0	
E13	100	100.0	0.0	100	99.6	0.7		0.0	0.0	0	96.1	3.4	**
	50	100.0	0.0	100	99.8	0.4		93.8	3.3	96	97.9	2.1	+
	25	99.8	0.4	100	99.8	0.4		98.2	0.8	99	99.0	1.0	
E14	100	100.0	0.0	100	99.6	07		93.6	7.3	97	96.1	3.4	
	50	99.6	0.6	100	99.8	0.4		98.2	0.8	100	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.0	0.7	100	99.0	1.0	
E15	100	99.8	0.4	100	99.6	0.7		7.4	6.9	8	96.1	3.4	**
	50	99.6	0.6	100	99.8	0.4		95.4	1.8	97	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		98.6	1.7	100	99.0	1.0	
E17	100	100.0	0.0	100	99.6	0.7		96.2	0.8	100	96.1	3.4	
	50	99.6	0.6	100	99.8	0.4		998.2	0.8	100	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.4	0.9	100	99.0	1.0	

Table 10. Continued.

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig.[4]	Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig. [4]
E19	100	99.8	0.4	100	99.6	0.7		97.4	0.6	101	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		99.0	1.7	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.4	0.9	100	99.0	1.0	
E21	100	99.8	0.4	100	99.6	0.7		85.0	2.0	88	96.1	3.4	++
	50	100.0	0.0	100	99.8	0.4		98.0	1.6	100	97.9	2.1	
	25	99.6	0.9	100	99.8	0.4		99.0	1.0	100	99.0	1.0	
E22	100	99.4	0.6	100	99.6	0.7		0.0	0.0	0	96.1	3.4	**
	50	100.0	0.0	100	99.8	0.4		88.4	3.0	90	97.9	2.1	++
	25	100.0	0.0	100	99.8	0.4		96.6	3.0	98	99.0	1.0	
E23	100	100.0	0.0	100	99.6	0.7		96.0	2.0	100	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		98.4	1.1	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		98.6	1.7	100	99.0	1.0	
E24	100	99.2	1.3	100	99.6	0.7		88.8	1.3	92	96.1	3.4	++
	50	100.0	0.0	100	99.8	0.4		95.0	1.9	97	97.9	2.1	
	25	99.4	0.9	100	99.8	0.4		98.6	1.5	100	99.0	1.0	
E25	100	100.0	0.0	100	99.6	0.7		94.6	1.8	98	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.2	1.6	100	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		98.6	1.1	100	99.0	1.0	

Table 10. Continued.

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig.[4]	Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig. [4]
E29	100	100.0	0.0	100	99.6	0.7		97.6	1.8	102	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		97.4	2.4	99	97.9	2.1	
	25	99.6	6.0	100	99.8	0.4		98.8	1.3	100	99.0	1.0	
E30	100	99.6	0.6	100	99.6	0.7		71.2	14.8	74	96.1	3.4	**
	50	99.4	0.6	100	99.8	0.4		97.4	3.8	99	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		98.8	1.1	100	99.0	1.0	

[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.

Table 11. Water quality parameters after salinity adjustment and original salinity of previously archived NOAA porewater samples collected from Puget Sound, Washington, 1997-1999 and tested in 2001.

Station ID[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]	40	6.9	85.1	8.43	0.546	43.7	<0.01	75
7	26	7.38	93.3	7.76	3.84	70.2	<0.01	95
12	30	7.41	93.3	8.16	1.8	80.0	<0.01	100
17	30	7.18	89.1	8.21	1.82	90.8	<0.01	100
29	30	7.19	89.8	7.86	2.54	58.2	<0.01	100
38	32	7.09	88.2	8.4	1.16	87.2	<0.01	94
42	30	7.32	92	7.87	4.42	103.5	<0.01	100
50	30	6.98	87.2	7.89	0.895	21.9	<0.01	100
51	30.5	7.42	92.6	8.32	3.5	221.6	<0.01	100
66	30	7.42	92.3	8.22	0.69	35.4	<0.01	100
69	26	7.19	90.2	8.23	4.51	234.9	<0.01	95
71	30	7.88	98.5	8.25	1.04	56.6	<0.01	100
76	30	7.64	95.2	8.09	0.647	24.8	<0.01	100
85	30	7.27	90.5	7.99	0.43	13.2	<0.01	100
107	32	7.38	92.3	8.02	0.843	27.6	<0.01	94
112	32	7.28	90.5	8	1.77	55.5	<0.01	94
116	30	7.53	93.7	7.97	0.731	21.4	<0.01	100
119	32	7.27	90.3	7.45	1.22	11.0	<0.01	94
126	31	7.02	87.3	7.85	0.879	19.7	<0.01	100
145	30	7.26	90.4	7.72	1.76	29.4	<0.01	100
179	30	7.38	93.6	7.65	0.60	8.6	<0.01	100
200	30	7.33	93.3	7.55	1.22	13.8	<0.01	100
207	30	6.96	87.7	7.47	1.79	16.9	<0.01	100
209	32	7.44	93.2	7.63	1.7	23.1	<0.01	94
214	30	7.83	98.2	7.76	2.8	51.2	<0.01	100
219	32	7.22	90.4	7.65	1.59	22.7	<0.01	94

Table 11. Continued.

Station ID[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
220	32	7.21	90.7	7.55	2.36	26.8	<0.01	94
221	31.5	7.3	92.5	7.6	0.583	7.4	<0.01	97
225	30	7.27	91.8	7.56	0.962	11.2	<0.01	100
226	31	7.83	98.4	7.57	0.34	4.0	<0.01	100
229	27	7.15	90	7.54	1.74	19.3	<0.01	96
237	30	7.84	98.6	7.61	1.28	16.7	<0.01	100
243	25	7.42	92.8	7.9	18.3	458.6	12.40	94
252	30	8.00	101.4	7.61	2.24	29.1	<0.01	100
260	30	7.61	96.3	7.62	1.89	25.2	<0.01	100
268	30	7.92	99.9	7.49	0.462	4.6	<0.01	100
269	30	7.56	95.7	7.4	3.88	31.3	<0.01	100
273	31	7.84	99.2	7.69	1.16	18.1	<0.01	100
274	31	7.98	101.7	7.63	0.91	12.4	<0.01	100
286	31	7.68	97.3	7.71	0.44	7.2	<0.01	100
304	30	7.88	99.8	7.69	2.90	45.2	<0.01	100

[1] NOAA station designation without original associated strata designations.
[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] Unionized 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 12. Sea urchin (*Arbacia punctulata*) fertilization test raw data and means for archived NOAA porewater samples collected from Puget Sound, Washington, Asterisks indicate 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).**

Station [1] ID	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	100	99	100	100	99	99.2 ± 1.0	100
		98	100	97	100	99		
REF [3]	50	98	98	99	98	99	98.7 ± 0.8	100
		100	98	100	98	99		
REF [3]	25	100	99	100	97	100	99.0 ± 1.0	100
		100	99	98	98	99		
7	100	98	96	100	100	99	98.6 ± 1.7	100
7	50	99	99	98	100	98	98.8 ± 0.8	99
7	25	97	97	98	98	99	97.8 ± 0.8	100
12	100	100	99	98	100	99	99.2 ± 0.8	100
12	50	97	99	100	100	100	99.2 ± 1.3	101
12	25	98	100	100	97	99	98.8 ± 1.3	100
17	100	100	99	100	99	100	99.6 ± 0.6	101
17	50	100	99	98	99	99	99.0 ± 0.7	100
17	25	97	100	99	100	100	99.2 ± 1.3	101
29	100	99	98	98	99	100	98.8 ± 0.8	101
29	50	97	100	99	98	100	98.8 ± 1.3	101
29	25	99	100	96	100	97	98.4 ± 1.8	98
38	100	99	97	100	100	99	99.0 ± 1.2	100
38	50	100	98	98	99	99	98.8 ± 0.8	100
38	25	98	100	99	99	100	99.2 ± 0.8	101

Table 12. Continued.

Station [1] ID	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
42	100	100	98	98	100	97	98.6 ± 1.3	98
42	50	99	100	97	100	100	99.2 ± 1.3	101
42	25	98	98	100	99	100	99.0 ± 1.0	101
50	100	100	97	97	98	100	98.4 ± 1.5	101
50	50	98	97	97	99	96	97.4 ± 1.1	97
50	25	100	99	100	100	99	99.6 ± 0.6	100
51	100	93	96	84	88	94	91.0 ± 4.9 ++	95
51	50	97	97	98	100	99	98.2 ± 1.3	100
51	25	98	99	98	98	97	98.0 ± 0.7	98
66	100	97	96	99	98	94	96.8 ± 1.9 +	95
66	50	98	100	98	98	100	98.8 ± 1.1	101
66	25	97	100	96	99	99	98.2 ± 1.6	100
69	100	97	96	95	92	95	95.0 ± 1.9 ++	96
69	50	97	100	94	99	96	97.2 ± 2.4	97
69	25	98	93	98	98	98	97.0 ± 2.2	99
71	100	100	98	98	100	100	99.2 ± 1.1	101
71	50	98	100	100	98	99	99.0 ± 1.0	100
71	25	98	98	97	99	100	98.4 ± 1.1	101
76	100	100	98	100	99	100	99.4 ± 0.9	101
76	50	98	99	100	98	99	98.8 ± 0.8	100
76	25	100	98	99	99	98	98.8 ± 0.8	99
85	100	99	100	98	97	100	98.8 ± 1.3	101
85	50	97	100	99	100	99	99.0 ± 1.2	100
85	25	99	99	98	98	100	98.8 ± 0.8	101

Table 12. Continued.

Station [1] ID	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
107	100	98	97	98	95	99	97.4 ± 1.5	100
107	50	98	99	99	98	98	98.4 ± 0.6	99
107	25	100	98	96	95	95	96.8 ± 2.2	96
112	100	45	24	31	35	28	32.6 ± 8.0 **	28
112	50	95	88	94	96	89	92.4 ± 3.6 ++	90
112	25	96	96	98	98	100	97.6 ± 1.7	101
116	100	95	99	98	99	99	98.0 ± 1.7	100
116	50	99	99	98	99	100	99.0 ± 0.7	101
116	25	99	97	97	100	96	97.8 ± 1.6	97
119	100	98	99	98	99	99	98.6 ± 0.6	100
119	50	97	99	98	98	100	98.4 ± 1.1	101
119	25	97	98	100	99	99	98.6 ± 1.1	100
126	100	98	100	98	99	99	98.8 ± 0.8	100
126	50	100	100	98	99	100	99.4 ± 0.9	101
126	25	98	98	99	99	100	98.8 ± 0.8	101
145	100	100	100	96	99	97	98.4 ± 1.8	98
145	50	100	100	100	98	98	99.2 ± 1.1	99
145	25	99	98	99	100	99	99.0 ± 0.7	100
179	100	96	100	100	100	98	98.8 ± 1.8	99
179	50	99	98	100	100	99	99.2 ± 0.8	100
179	25	99	100	97	99	99	98.8 ± 1.1	100
200	100	100	100	100	100	99	99.8 ± 0.4	100
200	50	100	100	99	99	97	99.0 ± 1.2	98
200	25	96	100	98	97	100	98.2 ± 1.8	101

Table 12. Continued.

Station [1] ID	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
207	100	98	99	97	99	98	98.2 ± 0.8	99
207	50	99	99	100	99	98	99.0 ± 0.7	99
207	25	97	100	98	99	99	98.6 ± 1.1	100
209	100	100	99	98	99	99	99.0 ± 0.7	100
209	50	100	99	99	100	100	99.6 ± 0.6	101
209	25	98	99	98	91	99	97.0 ± 3.4	100
214	100	78	82	71	81	77	77.8 ± 4.3 **	78
214	50	96	97	99	96	95	96.6 ± 1.5	96
214	25	98	98	100	98	99	98.6 ± 0.9	100
219	100	12	16	21	15	20	16.8 ± 3.7 **	20
219	50	57	59	43	70	71	60.0 ± 11.4 **	72
219	25	81	77	88	89	97	86.4 ± 7.7 ++	98
220	100	9	12	13	16	35 [4]	12.5 ± 2.9 **	13
220	50	69	47	66	70	60	62.4 ± 9.4 **	61
220	25	97	95	93	93	93	94.2 ± 1.8 ++	94
221	100	96	99	98	98	99	98.0 ± 1.2	100
221	50	100	98	100	98	96	98.4 ± 1.7	97
221	25	94	99	99	98	98	97.6 ± 2.1	99
225	100	99	99	99	99	97	98.6 ± 0.9	98
225	50	100	99	100	100	100	99.8 ± 0.4	101
225	25	100	100	97	99	100	99.2 ± 1.3	101
226	100	98	100	100	99	99	99.2 ± 0.8	100
226	50	100	100	100	99	100	99.8 ± 0.4	101
226	25	100	98	98	99	100	99.0 ± 1.0	101

Table 12. Continued.

Station [1] ID	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
229	100	100	100	100	97	97	98.8 ± 1.6	98
229	50	100	100	99	100	99	99.6 ± 0.6	100
229	25	100	99	97	100	99	99.0 ± 1.2	100
237	100	98	98	100	99	99	98.8 ± 0.8	100
237	50	100	97	100	99	99	99.0 ± 1.2	100
237	25	98	100	100	98	99	99.0 ± 1.0	100
243	100	2	0	0	0	0	0.4 ± 0.9 **	0
243	50	0	0	0	0	0	0.0 ± 0.0 **	0
243	25	0	1	0	0	0	0.2 ± 0.4 **	0
252	100	85	79	82	74	75	79.0 ± 4.6 **	76
252	50	99	99	100	97	97	98.4 ± 1.3	98
252	25	94	99	100	99	100	98.4 ± 2.5	101
260	100	91	85	88	85	94	88.6 ± 3.9 ++	95
260	50	99	98	98	99	99	98.6 ± 0.6	100
260	25	98	99	99	100	100	99.2 ± 0.8	101
268	100	97	98	99	98	98	98.0 ± 0.7	99
268	50	97	100	100	100	99	99.2 ± 1.3	100
268	25	98	99	100	97	100	98.8 ± 1.3	101
269	100	98	99	99	99	99	98.8 ± 0.4	100
269	50	99	100	100	100	98	99.4 ± 0.9	99
269	25	100	99	100	100	100	99.8 ± 0.4	101
273	100	98	98	99	98	100	98.6 ± 0.9	101
273	50	100	100	100	99	99	99.6 ± 0.6	100
273	25	99	97	100	100	98	98.8 ± 1.3	99

Table 12. Continued.

Station [1] ID	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
274	100	98	100	100	100	100	99.6 ± 0.9	101
274	50	100	100	99	98	98	99.0 ± 1.0	99
274	25	97	98	99	100	99	98.6 ± 1.1	100
286	100	100	100	100	99	99	99.6 ± 0.6	100
286	50	98	98	100	100	100	99.2 ± 1.1	101
286	25	97	100	100	99	99	99.0 ± 1.2	100
304	100	87	91	89	93	81	88.2 ± 4.6 ++	82
304	50	99	100	98	98	100	99.0 ± 1.0	101
304	25	99	100	99	94	100	98.4 ± 2.5	101

[1] NOAA station designation without original associated strata designations.

[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 13. Comparison of toxicity test results and statistical significance for the *Arbacia punctulata* fertilization test from 40 archived samples originally tested with *Strogylocentrotus purpuratus* fertilization test from 1997 to 1999. 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
7	42	100	98.6	1.7	99	99.2	1.0		98.8	0.4	117	84.6	3.1	
		50	98.8	0.8	100	98.7	0.8		98.8	1.3	103	95.8	3.2	
		25	97.8	0.8	99	99.0	1.0		99.0	1.4	101	98.2	1.3	
12	42	100	99.2	0.8	100	99.2	1.0		98.4	2.1	116	84.6	3.1	
		50	99.2	1.3	101	98.7	0.8		99.8	0.4	104	95.8	3.2	
		25	98.8	1.3	100	99.0	1.0		99.2	1.3	101	98.2	1.3	
17	42	100	99.6	0.6	100	99.2	1.0		97.0	2.0	115	84.6	3.1	
		50	99.0	0.7	100	98.7	0.8		97.2	1.3	101	95.8	3.2	
		25	99.2	1.3	100	99.0	1.0		95.4	1.5	97	98.2	1.3	
29	42.5	100	98.8	0.8	100	99.2	1.0		96.8	1.5	120	80.6	5.4	
		50	98.8	1.3	100	98.7	0.8		96.8	1.6	104	93.2	1.9	
		25	98.4	1.8	99	99.0	1.0		97.0	2.4	100	97.4	2.3	
38	43	100	99.0	1.2	100	99.2	1.0		93.4	3.6	116	80.6	5.4	
		50	98.8	0.8	100	98.7	0.8		94.2	2.4	101	93.2	1.9	
		25	99.2	0.8	100	99.0	1.0		90.6	2.7	93	97.4	2.3	++

Table 13. Continued.

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
42	43	100	98.6	1.3	99	99.2	1.0		90.0	3.2	112	80.6	5.4	
		50	99.2	1.3	101	98.7	0.8		90.4	5.5	97	93.2	1.9	
		25	99.0	1.0	100	99.0	1.0		93.0	2.4	95	97.4	2.3	+
50	43	100	98.4	1.5	99	99.2	1.0		92.6	3.0	115	80.6	5.4	
		50	97.4	1.1	99	98.7	0.8		92.6	2.7	99	93.2	1.9	
		25	99.6	0.6	101	99.0	1.0		88.4	4.3	91	97.4	2.3	++
51	43	100	91.0	4.9	92	99.2	1.0	++	41.4	11.2	51	80.6	5.4	**
		50	98.2	1.3	99	98.7	0.8		80.6	5.6	86	93.2	1.9	++
		25	98.0	0.7	99	99.0	1.0		89.4	7.2	92	97.4	2.3	++
66	42	100	96.8	1.9	98	99.2	1.0	+	84.0	3.7	88	95.2	4.0	++
		50	98.8	1.1	100	98.7	0.8		887.6	6.6	944	94.0	6.5	
		25	98.2	1.6	99	99.0	1.0		87.8	5.4	98	89.7	6.2	
69	42	100	95.0	1.9	96	99.2	1.0	++	97.8	2.2	103	95.2	4.0	
		50	97.2	2.4	98	98.7	0.8		96.2	3.6	102	94.0	6.5	
		25	97.0	2.2	98	99.0	1.0		96.0	4.7	107	89.7	6.2	
71	42	100	99.2	1.1	100	99.2	1.0		98.8	1.1	104	95.2	4.0	
		50	99.0	1.0	100	98.7	0.8		97.6	2.4	104	94.0	6.5	
		25	98.4	1.1	99	99.0	1.0		98.0	1.9	109	89.7	6.2	

Table 13. Continued.

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
76	30	100	99.4	0.9	100	99.2	1.0		89.6	4.6	94	95.2	4.0	
		50	98.8	0.8	100	98.7	0.8		93.2	3.5	99	94.0	6.5	
		25	98.8	0.8	100	99.0	1.0		93.4	3.0	104	89.7	6.2	
85	30	100	98.8	1.3	100	99.2	1.0		96.2	2.6	119	80.6	7.7	
		50	99.0	1.2	100	98.7	0.8		97.0	2.1	104	93.2	1.9	
		25	98.8	0.8	100	99.0	1.0		94.4	2.4	97	97.4	2.3	
107	30	100	97.4	1.5	98	99.2	1.0		98.4	0.6	117	84.1	11.8	
		50	98.4	0.6	100	98.7	0.8		99.0	1.1	101	99.1	1.0	
		25	96.8	2.2	98	99.0	1.0		99.4	0.6	118	98.6	1.6	
112	30	100	32.6	8.0	33	99.2	1.0	**	94.2	4.8	112	84.1	11.8	
		50	92.4	3.6	94	98.7	0.8	++	96.4	5.9	97	99.1	1.0	
		25	97.6	1.7	99	99.0	1.0		99.2	0.4	101	98.6	1.6	
116	30	100	98.0	1.7	99	99.2	1.0		99.6	0.6	118	84.1	11.8	
		50	99.0	0.7	100	98.7	0.8		99.2	0.4	100	99.1	1.0	
		25	97.8	1.6	99	99.0	1.0		99.0	1.2	100	98.6	1.6	
119	30	100	98.6	0.6	99	99.2	1.0		99.0	1.7	118	84.1	11.8	
		50	98.4	1.1	100	98.7	0.8		98.6	0.9	99	99.1	1.0	
		25	98.6	1.1	100	99.0	1.0		98.8	1.8	100	98.6	1.6	

Table 13. Continued.

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
126	30.5	100	98.8	0.8	100	99.2	1.0		98.4	2.5	117	84.1	11.8	
		50	99.4	0.9	101	98.7	0.8		99.0	1.4	100	99.1	1.0	
		25	98.8	0.8	100	99.0	1.0		99.2	0.8	101	98.6	1.6	
145	30.5	100	98.4	1.8	99	99.2	1.0		99.4	0.9	106	93.7	4.2	
		50	99.2	1.1	101	98.7	0.8		99.4	0.9	101	98.8	1.9	
		25	99.0	0.7	100	99.0	1.0		99.0	0.7	101	98.2	1.6	
179	30	100	98.8	1.8	100	99.2	1.0		65.4	3.8	81	80.4	5.8	*
		50	99.2	0.8	101	98.7	0.8		77.8	6.3	88	88.7	3.6	++
		25	98.8	1.1	100	99.0	1.0		73.8	5.7	84	87.7	3.5	*
200	30	100	99.8	0.4	101	99.2	1.0		54.8	14.5	68	80.4	5.8	**
		50	99.0	1.2	100	98.7	0.8		85.2	6.2	96	88.7	3.6	
		25	98.2	1.8	99	99.0	1.0		90.6	4.7	103	87.7	3.5	
207	18	100	98.2	0.8	99	99.2	1.0		99.0	1.0	107	92.9	4.9	
		50	99.0	0.7	100	98.7	0.8		96.6	1.8	98	98.2	1.2	
		25	98.6	1.1	100	99.0	1.0		97.2	1.3	100	97.3	2.2	
209	18	100	99.0	0.7	100	99.2	1.0		98.0	2.6	105	92.9	4.9	
		50	99.6	0.6	101	98.7	0.8		98.8	0.8	101	98.2	1.2	
		25	97.0	3.4	98	99.0	1.0		99.0	1.2	102	97.3	2.2	

Table 13. Continued.

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
214	18	100	77.8	4.3	78	99.2	1.0	**	66.4	10.4	71	92.9	4.9	**
		50	96.6	1.5	98	98.7	0.8		99.0	1.0	101	98.2	1.2	
		25	98.6	0.9	100	99.0	1.0		98.0	1.7	101	97.3	2.2	
219	18	100	16.8	3.7	17	99.2	1.0	**	38.0	10.2	41	92.9	4.9	**
		50	60.0	11.4	61	98.7	0.8	**	82.6	6.3	84	98.2	1.2	*
		25	86.4	7.7	87	99.0	1.0	++	96.4	1.5	99	97.3	2.2	
220	18	100	12.5	2.9	13	99.2	1.0	**	42.2	4.4	45	92.9	4.9	**
		50	62.4	9.4	63	98.7	0.8	**	90.4	2.7	92	98.2	1.2	++
		25	94.2	1.8	95	99.0	1.0	++	99.0	0.8	102	97.3	2.2	
221	18	100	98.0	1.2	99	99.2	1.0		99.0	1.0	107	92.9	4.9	
		50	98.4	1.7	100	98.7	0.8		98.0	2.4	100	98.2	1.2	
		25	97.6	2.1	99	99.0	1.0		97.6	1.3	100	97.3	2.2	
225	18	100	98.6	0.9	99	99.2	1.0		98.8	1.8	106	92.9	4.9	
		50	99.8	0.4	101	98.7	0.8		98.8	1.1	101	98.2	1.2	
		25	99.2	1.3	100	99.0	1.0		98.8	1.3	102	97.3	2.2	
226	18	100	99.2	0.8	100	99.2	1.0		95.8	1.9	103	92.9	4.9	
		50	99.8	0.4	101	98.7	0.8		97.4	1.5	99	98.2	1.2	
		25	99.0	1.0	100	99.0	1.0		96.8	1.8	99	97.3	2.2	

Table 13. Continued.

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
229	18.5	100	98.8	1.6	100	99.2	1.0		92.2	4.6	99	92.9	4.9	
		50	99.6	0.6	101	98.7	0.8		93.4	2.7	95	98.2	1.2	++
		25	99.0	1.2	100	99.0	1.0		88.8	8.6	91	97.3	2.2	++
237	18.5	100	98.8	0.8	100	99.2	1.0		96.2	2.5	104	92.9	4.9	
		50	99.0	1.2	100	98.7	0.8		98.6	0.9	100	98.2	1.2	
		25	99.0	1.0	100	99.0	1.0		97.6	1.5	100	97.3	2.2	
243	18.5	100	0.4	0.9	0	99.2	1.0	**	0.0	0.0	0	92.9	4.9	**
		50	0.0	0.0	0	98.7	0.8	**	0.4	0.6	0	98.2	1.2	**
		25	0.2	0.4	0	99.0	1.0	**	3.8	4.7	4	97.3	2.2	**
252	19	100	79.0	4.6	80	99.2	1.0	**	78.2	4.9	84	92.9	4.9	**
		50	98.4	1.3	100	98.7	0.8		96.2	1.3	98	98.2	1.2	
		25	98.4	2.5	99	99.0	1.0		97.6	0.9	100	97.3	2.2	
260	19	100	88.6	3.9	89	99.2	1.0	++	99.4	0.9	101	98.7	1.7	
		50	98.6	0.6	100	98.7	0.8		98.4	1.1	100	98.8	1.1	
		25	99.2	0.8	100	99.0	1.0		98.8	0.8	100	98.6	1.1	
268	19	100	98.0	0.7	99	99.2	1.0		98.6	1.1	100	98.7	1.7	
		50	99.2	1.3	101	98.7	0.8		98.8	1.1	100	98.8	1.1	
		25	98.8	1.3	100	99.0	1.0		99.4	0.6	101	98.6	1.1	

Table 13. Continued.

Station[1] ID	Storage Time (months)	WQAS [2]	*A. punctulata* Fertilization Test (% fertilized, 2001)						*S. purpuratus* Fertilization Test (% fertilized, 1997-1999)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig.[5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
269	18	100	98.8	0.4	100	99.2	1.0		99.6	0.6	101	98.7	1.7	
		50	99.4	0.9	101	98.7	0.8		99.0	0.7	100	98.8	1.1	
		25	99.8	0.4	101	99.0	1.0		99.2	0.4	101	98.6	1.1	
273	18	100	98.6	0.9	99	99.2	1.0		99.0	1.0	100	98.7	1.7	
		50	99.6	0.6	101	98.7	0.8		98.0	1.9	99	98.8	1.1	
		25	98.8	1.3	100	99.0	1.0		99.0	1.0	100	98.6	1.1	
274	18	100	99.6	0.9	100	99.2	1.0		99.2	1.3	101	98.7	1.7	
		50	99.0	1.0	100	98.7	0.8		99.2	0.4	100	98.8	1.1	
		25	98.6	1.1	100	99.0	1.0		98.8	0.8	100	98.6	1.1	
286	18	100	99.6	0.6	100	99.2	1.0		99.4	0.9	101	98.7	1.7	
		50	99.2	1.1	101	98.7	0.8		99.8	0.4	101	98.8	1.1	
		25	99.0	1.2	100	99.0	1.0		99.6	0.6	101	98.6	1.1	
304	18	100	88.2	4.6	89	99.2	1.0	++	98.4	0.9	100	98.7	1.7	
		50	99.0	1.0	100	98.7	0.8		99.2	1.3	100	98.8	1.1	
		25	98.4	2.5	99	99.0	1.0		97.8	1.6	99	98.6	1.1	

[1] NOAA station designation without original associated strata designations.

[2] Percent of water quality adjusted sample.

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

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

[5] Significant difference from reference.

Table 14. **Sea urchin (*Arbacia punctulata*) embryological development test raw data and means for archived NOAA porewater samples collected from Puget Sound, Washington, 1997-1999. Asterisks indicate statistical differences (Dunnett's *t*-test) anddetectable 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).**

Station [1] ID	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	95	99	97	93	98	97.6 ± 2.1	100
		98	99	99	99	99		
REF [3]	50	97	96	95	95	99	96.9 ± 1.4	100
		97	97	97	97	99		
REF [3]	25	97	95	98	94	97	96.3 ± 1.2	100
		95	97	97	96	97		
7	100	0	1	0	0	0	0.2 ± 0.4 **	0
7	50	95	97	97	97	96	96.4 ± 0.9	99
7	25	100	99	98	98	100	99.0 ± 1.0	104
12	100	97	93	94	96	91	94.2 ± 2.4	93
12	50	98	98	97	91	97	96.2 ± 3.0	100
12	25	97	99	97	100	98	98.2 ± 1.3	102
17	100	100	98	97	97	98	98.0 ± 1.2	100
17	50	97	96	99	94	98	96.8 ± 1.9	101
17	25	98	98	100	97	98	98.2 ± 1.1	102
29	100	80	66	67	74	69	71.2 ± 5.8 **	71
29	50	93	95	95	95	96	94.8 ± 1.1	99
29	25	97	96	98	97	98	97.2 ± 0.8	102
38	100	99	98	96	97	97	97.4 ± 1.1	99
38	50	100	98	98	100	99	99.0 ± 1.0	102
38	25	99	98	97	97	99	98.0 ± 1.0	103

Table 14. Continued.

Station [1] ID	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
207	100	64	70	64	72	73	68.6 ± 4.3 **	75
207	50	97	98	99	99	99	98.4 ± 0.9	102
207	25	99	100	97	99	99	98.8 ± 1.1	103
209	100	91	90	86	90	88	89.0 ± 2.0 +	90
209	50	98	97	97	99	98	97.8 ± 0.8	101
209	25	99	100	99	99	100	99.4 ± 0.6	104
214	100	0	0	0	0	0	0.0 ± 0.0 **	0
214	50	96	97	96	99	96	96.8 ± 1.3	99
214	25	100	99	99	99	100	99.4 ± 0.6	104
219	100	74	79	71	63	76	72.6 ± 6.1 **	78
219	50	90	95	95	93	94	93.4 ± 2.1	97
219	25	96	96	100	99	99	98.0 ± 1.9	103
220	100	0	0	0	0	0	0.0 ± 0.0 **	0
220	50	89	88	90	93	82	88.4 ± 4.0 ++	85
220	25	98	92	96	94	93	94.6 ± 2.4	97
221	100	90	84	89	85	77	85.0 ± 5.2 ++	79
221	50	92	93	93	91	91	92.0 ± 1.0 ++	94
221	25	99	100	97	100	98	98.8 ± 1.3	102
225	100	98	96	95	99	99	97.4 ± 1.8	101
225	50	98	97	98	99	97	97.8 ± 0.8	100
225	25	98	97	98	100	98	98.2 ± 1.1	102
226	100	97	95	98	98	98	97.2 ± 1.3	100
226	50	97	97	99	97	96	97.2 ± 1.1	99
226	25	98	97	98	98	99	98.0 ± 0.7	103

Table 14. Continued.

Station [1] ID	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
229	100	2	95	4	91	1	38.6 ± 49.69 **	1
229	50	97	96	98	98	98	97.4 ± 0.9	101
229	25	98	96	100	99	99	98.4 ± 1.5	103
237	100	95	95	93	91	89	92.6 ± 2.6	91
237	50	96	95	94	94	97	95.2 ± 1.3	100
237	25	99	95	99	97	97	97.4 ± 1.7	101
243	100	0	0	0	0	0	0.0 ± 0.0 **	0
243	50	0	0	0	0	0	0.0 ± 0.0 **	0
243	25	0	0	0	0	0	0.0 ± 0.0 **	0
252	100	5	8	9	5	9	7.2 ± 2.0 **	9
252	50	96	93	94	97	92	94.4 ± 2.1	95
252	25	97	99	93	96	97	96.4 ± 2.2	101
260	100	18	19	15	19	12	16.6 ± 3.0	12
260	50	93	93	95	96	90	93.4 ± 2.3	93
260	25	98	98	98	96	95	97.0 ± 1.4	99
268	100	94	96	99	98	98	97.0 ± 2.0	100
268	50	99	99	97	99	99	98.6 ± 0.9	102
268	25	100	100	100	99	98	99.4 ± 0.9	102
269	100	0	0	0	0	0	0.0 ± 0.0 **	0
269	50	36	40	30	37	38	36.2 ± 3.8 **	39
269	25	94	95	96	96	96	95.4 ± 0.9	100
273	100	95	95	92	91	95	93.6 ± 2.0	97
273	50	100	99	100	99	97	99.0 ± 1.2	100
273	25	100	100	98	100	100	99.6 ± 0.9	104

Table 14. Continued.

Station [1] ID	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
274	100	97	93	98	95	95	95.6 ± 2.0	97
274	50	98	97	96	95	98	96.8 ± 1.3	101
274	25	99	98	94	96	97	96.8 ± 1.9	101
286	100	98	96	96	95	92	95.4 ± 2.2	94
286	50	98	97	96	97	na [4]	97.0 ± 0.8	4
286	25	98	99	99	98	98	98.4 ± 0.6	102
304	100	0	0	0	0	0	0.0 ± 0.0 **	0
304	50	94	88	90	93	92	91.4 ± 2.4 ++	95
304	25	98	95	94	96	98	96.2 ± 1.8	102

[1] NOAA station designation without original associated strata designations.

[2] Percent of water quality adjusted porewater sample.

[3] Station mean devided by the Reference Mean X 100, Reference pore water extracted from sediment collected in Redfish Bay, Texas.

[4] Missing data due to technical error.

Table 15. Summary of station means and statistical significance for the sea urchin fertilization and embryological development test from 40 archived NOAA porewater samples collected from Puget Sound, Washington. 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
7	42	100	98.6	1.67	99	99.2	1.0		0.2	0.45	0	97.6	2.1	**
		50	98.8	0.84	100	98.7	0.8		96.4	0.89	99	96.9	1.4	
		25	97.8	0.84	99	99.0	1.0		99	1.00	103	96.3	1.2	
12	42	100	99.2	0.84	100	99.2	1.0		94.2	2.39	97	97.6	2.1	
		50	99.2	1.30	101	98.7	0.8		96.2	2.95	99	96.9	1.4	
		25	98.8	1.30	100	99.0	1.0		98.2	1.30	102	96.3	1.2	
17	42	100	99.6	0.55	100	99.2	1.0		98	1.22	100	97.6	2.1	
		50	99	0.71	100	98.7	0.8		96.8	1.92	100	96.9	1.4	
		25	99.2	1.30	100	99.0	1.0		98.2	1.10	102	96.3	1.2	
29	42.5	100	98.8	0.84	100	99.2	1.0		71.2	5.81	73	97.6	2.1	**
		50	98.8	1.30	100	98.7	0.8		94.8	1.10	98	96.9	1.4	
		25	98.4	1.82	99	99.0	1.0		97.2	0.84	101	96.3	1.2	
38	43	100	99	1.22	100	99.2	1.0		97.4	1.14	100	97.6	2.1	
		50	98.8	0.84	100	98.7	0.8		99	1.00	102	96.9	1.4	
		25	99.2	0.84	100	99.0	1.0		98	1.00	102	96.3	1.2	

Table 15. Continued.

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
42	43	100	98.6	1.34	99	99.2	1.0		0.4	0.55	0	97.6	2.1	**
		50	99.2	1.30	101	98.7	0.8		90.6	2.07	93	96.9	1.4	++
		25	99	1.00	100	99.0	1.0		96.4	1.52	100	96.3	1.2	
50	43	100	98.4	1.52	99	99.2	1.0		95.8	2.17	98	97.6	2.1	
		50	97.4	1.14	99	98.7	0.8		97.2	1.30	100	96.9	1.4	
		25	99.6	0.55	101	99.0	1.0		95.8	1.64	99	96.3	1.2	
51	43	100	91	4.90	92	99.2	1.0	++	0	0.00	0	97.6	2.1	**
		50	98.2	1.30	99	98.7	0.8		94.6	3.13	98	96.9	1.4	
		25	98	0.71	99	99.0	1.0		96.2	1.10	100	96.3	1.2	
66	42	100	96.8	1.92	98	99.2	1.0	+	96.4	1.34	99	97.6	2.1	
		50	98.8	1.10	100	98.7	0.8		97.8	0.45	101	96.9	1.4	
		25	98.2	1.64	99	99.0	1.0		97.6	1.14	101	96.3	1.2	
69	42	100	95	1.87	96	99.2	1.0	++	0	0.00	0	97.6	2.1	**
		50	97.2	2.39	98	98.7	0.8		76.2	19.87	79	96.9	1.4	**
		25	97	2.24	98	99.0	1.0		97.2	1.64	101	96.3	1.2	
71	42	100	99.2	1.10	100	99.2	1.0		97.2	0.84	100	97.6	2.1	
		50	99	1.00	100	98.7	0.8		97.2	0.84	100	96.9	1.4	
		25	98.4	1.14	99	99.0	1.0		96.2	1.30	100	96.3	1.2	

Table 15. Continued.

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
76	30	100	99.4	0.89	100	99.2	1.0		95.4	1.67	98	97.6	2.1	
		50	98.8	0.84	100	98.7	0.8		98.6	0.89	102	96.9	1.4	
		25	98.8	0.84	100	99.0	1.0		96.6	1.34	100	96.3	1.2	
85	30	100	98.8	1.30	100	99.2	1.0		96.2	2.49	99	97.6	2.1	
		50	99	1.22	100	98.7	0.8		98	1.22	101	96.9	1.4	
		25	98.8	0.84	100	99.0	1.0		98.4	1.14	102	96.3	1.2	
107	30	100	97.4	1.52	98	99.2	1.0		93.8	1.48	96	97.6	2.1	
		50	98.4	0.55	100	98.7	0.8		99	0.71	102	96.9	1.4	
		25	96.8	2.17	98	99.0	1.0		98.8	1.10	103	96.3	1.2	
112	30	100	32.6	8.02	33	99.2	1.0	**	0	0.00	0	97.6	2.1	**
		50	92.4	3.65	94	98.7	0.8	++	90.4	1.14	93	96.9	1.4	++
		25	97.6	1.67	99	99.0	1.0		96	1.22	100	96.3	1.2	
116	30	100	98	1.73	99	99.2	1.0		97.4	0.55	100	97.6	2.1	
		50	99	0.71	100	98.7	0.8		96.8	1.30	100	96.9	1.4	
		25	97.8	1.64	99	99.0	1.0		97.6	1.14	101	96.3	1.2	
119	30	100	98.6	0.55	99	99.2	1.0		36	5.39	37	97.6	2.1	**
		50	98.4	1.14	100	98.7	0.8		96.8	0.84	100	96.9	1.4	
		25	98.6	1.14	100	99.0	1.0		96.6	1.14	100	96.3	1.2	

Table 15. Continued.

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
126	30.5	100	98.8	0.84	100	99.2	1.0		95.4	1.14	98	97.6	2.1	
		50	99.4	0.89	101	98.7	0.8		97.4	0.55	101	96.9	1.4	
		25	98.8	0.84	100	99.0	1.0		99.6	0.55	103	96.3	1.2	
145	30.5	100	98.4	1.82	99	99.2	1.0		3.2	0.84	3	97.6	2.1	**
		50	99.2	1.10	101	98.7	0.8		98.2	0.84	101	96.9	1.4	
		25	99	0.71	100	99.0	1.0		97.8	0.45	102	96.3	1.2	
179	30	100	98.8	1.79	100	99.2	1.0		98.2	1.92	101	97.6	2.1	
		50	99.2	0.84	101	98.7	0.8		98.6	1.14	102	96.9	1.4	
		25	98.8	1.10	100	99.0	1.0		98.4	1.14	102	96.3	1.2	
200	30	100	99.8	0.45	101	99.2	1.0		96	0.71	98	97.6	2.1	
		50	99	1.22	100	98.7	0.8		98.2	0.45	101	96.9	1.4	
		25	98.2	1.79	99	99.0	1.0		99.4	0.89	103	96.3	1.2	
207	18	100	98.2	0.84	99	99.2	1.0		68.6	4.34	70	97.6	2.1	**
		50	99	0.71	100	98.7	0.8		98.4	0.89	102	96.9	1.4	
		25	98.6	1.14	100	99.0	1.0		98.8	1.10	103	96.3	1.2	
209	18	100	99	0.71	100	99.2	1.0		89	2.00	91	97.6	2.1	+
		50	99.6	0.55	101	98.7	0.8		97.8	0.84	101	96.9	1.4	
		25	97	3.39	98	99.0	1.0		99.4	0.55	103	96.3	1.2	

Table 15. Continued.

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
214	18	100	77.8	4.32	78	99.2	1.0	**	0	0.00	0	97.6	2.1	**
		50	96.6	1.52	98	98.7	0.8		96.8	1.30	100	96.9	1.4	
		25	98.6	0.89	100	99.0	1.0		99.4	0.55	103	96.3	1.2	
219	18	100	16.8	3.70	17	99.2	1.0	**	72.6	6.11	74	97.6	2.1	**
		50	60	11.40	61	98.7	0.8	**	93.4	2.07	96	96.9	1.4	
		25	86.4	7.73	87	99.0	1.0	++	98	1.87	102	96.3	1.2	
220	18	100	12.5	2.89	13	99.2	1.0	**	0	0.00	0	97.6	2.1	**
		50	62.4	9.45	63	98.7	0.8	**	88.4	4.04	91	96.9	1.4	++
		25	94.2	1.79	95	99.0	1.0	++	94.6	2.41	98	96.3	1.2	
221	18	100	98	1.22	99	99.2	1.0		85	5.15	87	97.6	2.1	++
		50	98.4	1.67	100	98.7	0.8		92	1.00	95	96.9	1.4	++
		25	97.6	2.07	99	99.0	1.0		98.8	1.30	103	96.3	1.2	
225	18	100	98.6	0.89	99	99.2	1.0		97.4	1.82	100	97.6	2.1	
		50	99.8	0.45	101	98.7	0.8		97.8	0.84	101	96.9	1.4	
		25	99.2	1.30	100	99.0	1.0		98.2	1.10	102	96.3	1.2	
226	18	100	99.2	0.84	100	99.2	1.0		97.2	1.30	100	97.6	2.1	
		50	99.8	0.45	101	98.7	0.8		97.2	1.10	100	96.9	1.4	
		25	99	1.00	100	99.0	1.0		98	0.71	102	96.3	1.2	

Table 15. Continued.

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
229	18.5	100	98.8	1.64	100	99.2	1.0		38.6	49.69	40	97.6	2.1	**
		50	99.6	0.55	101	98.7	0.8		97.4	0.89	101	96.9	1.4	
		25	99	1.22	100	99.0	1.0		98.4	1.52	102	96.3	1.2	
237	18.5	100	98.8	0.84	100	99.2	1.0		92.6	2.61	95	97.6	2.1	
		50	99	1.22	100	98.7	0.8		95.2	1.30	98	96.9	1.4	
		25	99	1.00	100	99.0	1.0		97.4	1.67	101	96.3	1.2	
243	18.5	100	0.4	0.89	0	99.2	1.0	**	0	0.00	0	97.6	2.1	**
		50	0	0.00	0	98.7	0.8	**	0	0.00	0	96.9	1.4	**
		25	0.2	0.45	0	99.0	1.0	**	0	0.00	0	96.3	1.2	**
252	19	100	79	4.64	80	99.2	1.0	**	7.2	2.05	7	97.6	2.1	**
		50	98.4	1.34	100	98.7	0.8		94.4	2.07	97	96.9	1.4	
		25	98.4	2.51	99	99.0	1.0		96.4	2.19	100	96.3	1.2	
260	19	100	88.6	3.91	89	99.2	1.0	++	16.6	3.05	17	97.6	2.1	**
		50	98.6	0.55	100	98.7	0.8		93.4	2.30	96	96.9	1.4	
		25	99.2	0.84	100	99.0	1.0		97	1.41	101	96.3	1.2	
268	19	100	98	0.71	99	99.2	1.0		97	2.00	99	97.6	2.1	
		50	99.2	1.30	101	98.7	0.8		98.6	0.89	102	96.9	1.4	
		25	98.8	1.30	100	99.0	1.0		99.4	0.89	103	96.3	1.2	

Table 15. Continued.

Station[1] ID	Storage Time (Months)	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
			Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
269	18	100	98.8	0.45	100	99.2	1.0		0	0.00	0	97.6	2.1	**
		50	99.4	0.89	101	98.7	0.8		36.2	3.77	37	96.9	1.4	**
		25	99.8	0.45	101	99.0	1.0		95.4	0.89	99	96.3	1.2	
273	18	100	98.6	0.89	99	99.2	1.0		93.6	1.95	96	97.6	2.1	
		50	99.6	0.55	101	98.7	0.8		99	1.22	102	96.9	1.4	
		25	98.8	1.30	100	99.0	1.0		99.6	0.89	103	96.3	1.2	
274	18	100	99.6	0.89	100	99.2	1.0		95.6	1.95	98	97.6	2.1	
		50	99	1.00	100	98.7	0.8		96.8	1.30	100	96.9	1.4	
		25	98.6	1.14	100	99.0	1.0		96.8	1.92	101	96.3	1.2	
286	18	100	99.6	0.55	100	99.2	1.0		95.4	2.19	98	97.6	2.1	
		50	99.2	1.10	101	98.7	0.8		97	0.82	100	96.9	1.4	
		25	99	1.22	100	99.0	1.0		98.4	0.55	102	96.3	1.2	
304	18	100	88.2	4.60	89	99.2	1.0	++	0	0.00	0	97.6	2.1	**
		50	99	1.00	100	98.7	0.8		91.4	2.41	94	96.9	1.4	++
		25	98.4	2.51	99	99.0	1.0		96.2	1.79	100	96.3	1.2	

[1] NOAA station designation without original associated strata designations.

[2] Percent of water quality adjusted sample.

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

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

[5] Significant difference from reference.

Table 16. Water quality parameters after salinity adjustment and original salinity of BEST/EMAP sediment porewater samples from stations in the Columbia River, Oregon evaluated in the fertilization test.

Station ID	Salinity [1] (‰)	DO [2] (mg/L)	% DO [3]	pH	TAN [4] (mg/L)	UAN [5] (µg/L)	Sulfide [6] (mg/L)	% OUS[7]
REF-1[8]	40	6.25	85.3	8.43	0.48	38.5	< 0.01	75
OR2000-0031	28	6.65	91.6	7.82	<0.1	<2.1	< 0.01	98
OR2000-0032	27.5	6.66	92.0	7.83	<0.1	<2.1	< 0.01	97
OR2000-0033	18.5	6.82	94.2	8.09	<0.1	<3.8	< 0.01	88
OR2000-0034	18.5	6.40	88.5	8.03	0.27	9.0	< 0.01	88
OR2000-0035	4	6.24	86.5	7.93	<0.1	<2.7	< 0.01	77
OR2000-0036	6	6.500	90.2	8.20	<0.1	<4.9	< 0.01	78
OR2000-0037	12	6.79	93.9	8.07	<0.1	<3.7	< 0.01	82
OR2000-0038	24	6.57	90.9	8.01	<0.1	<3.2	< 0.01	94
OR2000-0039	4	6.44	89.1	8.18	<0.1	<4.7	< 0.01	77
OR2000-0040	1	5.74	80.1	8.29	<0.1	<5.9	< 0.01	75
OR2000-0041	6	6.46	89.9	8.08	<0.1	<3.7	< 0.01	78
OR2000-0042	5	6.26	87.1	8.38	<0.1	<7.2	< 0.01	77
OR2000-0043	1	6.01	83.6	8.24	<0.1	<5.3	< 0.01	75
OR2000-0044	1	5.78	80.0	8.26	<0.1	<5.6	< 0.01	75
OR2000-0045	1	6.10	85.3	8.23	<0.1	<5.2	< 0.01	75
OR2000-0046	4	6.79	92.8	8.24	<0.1	<5.3	< 0.01	77
OR2000-0047	0	6.18	86.4	8.08	<0.1	<3.7	< 0.01	74
OR2000-0048	1	6.01	84.2	8.24	<0.1	<5.3	< 0.01	75
OR2000-0049	0	6.04	83.7	8.22	<0.1	<6.5	< 0.01	74
OR2000-0050	1	6.13	83.4	8.33	<0.1	<6.9	< 0.01	76

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

Table 17. Water quality parameters after salinity adjustment and original salinity of BEST/EMAP sediment porewater samples from stations in the Columbia River, Oregon evaluated in the embryological development test.

Station ID	Salinity [1] (‰)	DO [2] (mg/L)	% DO [3]	pH	TAN [4] (mg/L)	UAN [5] (μg/L)	Sulfide[6] (mg/L)	% OUS[7]
REF-1[8]	40	6.69	90.7	8.38	0.803	57.8	<0.01	75
OR2000-0031	28	6.42	87.7	8.09	<0.1	<3.8	<0.01	98
OR2000-0032	27.5	6.33	86.4	7.91	<0.1	<2.6	<0.01	97
OR2000-0033	18.5	6.54	88.9	8.15	<0.1	<4.4	<0.01	88
OR2000-0034	18.5	6.47	87.8	7.89	0.915	22.4	<0.01	88
OR2000-0035	4	6.48	88.5	7.96	0.333	9.5	<0.01	77
OR2000-0036	6	6.910	94.7	8.26	<0.1	<5.6	<0.01	78
OR2000-0037	12	6.73	91.9	8.19	<0.1	<4.8	<0.01	82
OR2000-0038	24	6.49	88.6	8.08	<0.1	<3.7	<0.01	94
OR2000-0039	4	6.37	87.2	8.09	<0.1	<3.8	<0.01	77
OR2000-0040	1	7.21	99	8.22	0.1	5.1	<0.01	75
OR2000-0041	6	6.7	92.2	8.11	0.127	5.1	<0.01	78
OR2000-0042	5	6.79	93.2	7.81	<0.1	<2.0	<0.01	77
OR2000-0043	1	6.77	93.2	8.23	<0.1	<5.2	<0.01	75
OR2000-0044	1	6.18	85.1	8.31	<0.1	<6.2	<0.01	75
OR2000-0045	1	6.47	88.8	8.35	<0.1	<6.8	<0.01	75
OR2000-0046	4	7.13	98.1	8.44	<0.1	<8.2	<0.01	77
OR2000-0047	0	7.06	97.3	8.35	0.1	6.8	<0.01	74
OR2000-0048	1	6.68	92.1	8.3	<0.1	<6.1	<0.01	75
OR2000-0049	0	5.01	69.8	8.15	<0.1	<4.4	<0.01	74
OR2000-0050	1	7.24	100	8.32	<0.1	<6.3	<0.01	76

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

Table 18. Sea urchin (*Arbacia punctulata*) fertilization test raw data and means for BEST/EMAP sediment porewater samples from the Columbia River, Oregon. No statistical comparisons met the detectable significance criteria. Plus signs denote only statistical differences (Dunnett's *t*-test, + α ≤0.05, ++ α ≤ 0.01)

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	100	100	100	98	100	99.6 ± 0.7	100
		99	100	99	100	100		
REF [3]	50	100	99	99	100	100	99.8 ± 0.4	100
		100	100	100	100	100		
REF [3]	25	99	100	100	100	99	99.8 ± 0.4	100
		100	100	100	100	100		
OR2000-0031	100	99	100	100	99	100	99.6 ± 0.6	100
OR2000-0031	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0031	25	100	92 [4]	100	100	100	100.0 ± 0.0	100
OR2000-0032	100	100	100	99	100	100	99.8 ± 0.4	100
OR2000-0032	50	100	100	100	99	100	99.8 ± 0.4	100
OR2000-0032	25	100	99	100	96	100	99.0 ± 1.7	99
OR2000-0033	100	100	100	100	100	99	99.8 ± 0.4	100
OR2000-0033	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0033	25	100	99	99	100	100	99.6 ± 0.6	100
OR2000-0034	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0034	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0034	25	100	100	100	99	100	99.8 ± 0.4	100
OR2000-0035	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0035	50	100	99	99	99	99	99.2 ± 0.4 +	99
OR2000-0035	25	100	100	100	100	100	100.0 ± 0.0	100

Table 18. Continued.

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
OR2000-0036	100	100	100	100	99	100	99.8 ± 0.4	100
OR2000-0036	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0036	25	100	100	100	100	99	99.8 ± 0.4	100
OR2000-0037	100	100	100	100	100	98	99.6 ± 0.9	100
OR2000-0037	50	100	100	99	100	100	99.8 ± 0.4	100
OR2000-0037	25	95	100	95	100	100	98.0 ± 2.7	98
OR2000-0038	100	99	100	99	100	99	99.4 ± 0.6	100
OR2000-0038	50	100	100	99	100	100	99.8 ± 0.4	100
OR2000-0038	25	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0039	100	99	100	100	100	100	99.8 ± 0.4	100
OR2000-0039	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0039	25	100	100	99	100	98	99.4 ± 0.9	100
OR2000-0040	100	99	100	100	100	100	99.8 ± 0.4	100
OR2000-0040	50	100	100	100	100	99	99.8 ± 0.4	100
OR2000-0040	25	100	100	100	99	100	99.8 ± 0.4	100
OR2000-0041	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0041	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0041	25	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0042	100	99	100	100	100	100	99.8 ± 0.4	100
OR2000-0042	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0042	25	99	100	100	100	99	99.6 ± 0.6	100
OR2000-0043	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0043	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0043	25	99	100	100	100	100	99.8 ± 0.4	100

Table 18. Continued.

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
OR2000-0044	100	100	99	100	100	100	99.8 ± 0.4	100
OR2000-0044	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0044	25	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0045	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0045	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0045	25	100	100	99	100	100	99.8 ± 0.4	100
OR2000-0046	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0046	50	99	99	100	100	100	99.6 ± 0.6	100
OR2000-0046	25	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0047	100	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0047	50	100	100	100	99	100	99.8 ± 0.4	100
OR2000-0047	25	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0048	100	99	100	100	100	100	99.8 ± 0.4	100
OR2000-0048	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0048	25	100	100	99	100	100	99.8 ± 0.4	100
OR2000-0049	100	98	99	98	99	99	98.6 ± 0.6 ++	99
OR2000-0049	50	100	100	100	100	100	100.0 ± 0.0	100
OR2000-0049	25	99	100	100	100	100	99.8 ± 0.4	100
OR2000-0050	100	99	98	99	100	100	99.2 ± 0.8	100
OR2000-0050	50	99	99	100	100	100	99.6 ± 0.6	100
OR2000-0050	25	99	100	100	98	100	99.4 ± 0.9	100

[1] Percent of water quality adjusted porewater sample.

[2] Percent of reference at the same dilution.

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

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

Table 19. Sea urchin (*Arbacia punctulata*) embryological development test raw data and means for BEST/EMAP sediment porewater samples from the Columbia River, Oregon. No significant differences were observed between reference and test stations.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	88	98	93	96	97	96.1 ± 3.4	100
		96	99	99	98	97		
REF [3]	50	93	97	97	98	100	97.9 ± 2.1	100
		99	98	97	100	100		
REF [3]	25	98	97	100	100	98	99.0 ± 1.0	100
		99	99	100	100	99		
OR2000-0031	100	98	97	98	99	100	98.4 ± 1.1	104
OR2000-0031	50	99	97	97	99	97	97.8 ± 1.1	99
OR2000-0031	25	99	98	100	100	100	99.4 ± 0.9	101
OR2000-0032	100	99	100	99	97	99	98.8 ± 1.1	103
OR2000-0032	50	98	100	100	100	99	99.4 ± 0.9	101
OR2000-0032	25	100	100	97	99	100	99.2 ± 1.3	101
OR2000-0033	100	98	98	99	96	98	97.8 ± 1.1	102
OR2000-0033	50	98	98	97	99	100	98.4 ± 1.1	102
OR2000-0033	25	100	99	100	99	97	99.0 ± 1.2	98
OR2000-0034	100	100	94	95	95	95	95.8 ± 2.4	99
OR2000-0034	50	99	97	100	99	99	98.8 ± 1.1	101
OR2000-0034	25	99	100	100	99	98	99.2 ± 0.8	99
OR2000-0035	100	100	100	100	99	99	99.6 ± 0.6	103
OR2000-0035	50	99	100	97	99	99	98.8 ± 1.1	101
OR2000-0035	25	100	98	99	100	100	99.4 ± 0.9	101

Table 19. Continued.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
OR2000-0036	100	100	100	100	100	98	99.6 ± 0.9	102
OR2000-0036	50	95	100	99	99	100	98.6 ± 2.1	102
OR2000-0036	25	97	99	100	100	99	99.0 ± 1.2	100
OR2000-0037	100	100	99	100	98	99	99.2 ± 0.8	103
OR2000-0037	50	98	97	98	100	99	98.4 ± 1.1	101
OR2000-0037	25	99	98	99	100	99	99.0 ± 0.7	100
OR2000-0038	100	100	97	100	100	100	99.4 ± 1.3	104
OR2000-0038	50	100	99	100	97	99	99.0 ± 1.2	101
OR2000-0038	25	100	99	100	100	100	99.8 ± 0.4	101
OR2000-0039	100	94	100	97	97	99	97.4 ± 2.3	103
OR2000-0039	50	98	100	99	100	100	99.4 ± 0.9	102
OR2000-0039	25	100	100	100	99	99	99.6 ± 0.6	100
OR2000-0040	100	94	98	97	96	92	95.4 ± 2.4	96
OR2000-0040	50	95	98	96	97	96	96.4 ± 1.1	98
OR2000-0040	25	100	99	100	100	98	99.4 ± 0.9	99
OR2000-0041	100	96	96	95	92	98	95.4 ± 2.2	102
OR2000-0041	50	100	99	96	98	99	98.4 ± 1.5	101
OR2000-0041	25	99	99	100	98	100	99.2 ± 0.8	101
OR2000-0042	100	94	92	97	98	99	96.0 ± 2.9	103
OR2000-0042	50	98	98	99	98	97	98.0 ± 0.7	99
OR2000-0042	25	98	100	99	98	100	99.0 ± 1.0	101
OR2000-0043	100	100	100	99	99	97	99.0 ± 1.2	101
OR2000-0043	50	100	99	99	96	97	98.2 ± 1.6	99
OR2000-0043	25	95	99	98	100	100	98.4 ± 2.1	101

Table 19. Continued.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
OR2000-0044	100	100	97	99	97	100	98.6 ± 1.5	104
OR2000-0044	50	100	98	99	99	96	98.4 ± 1.5	98
OR2000-0044	25	99	100	100	99	99	99.4 ± 0.6	100
OR2000-0045	100	96	94	98	99	95	96.4 ± 2.1	99
OR2000-0045	50	100	99	99	98	100	99.2 ± 0.8	102
OR2000-0045	25	100	98	97	97	98	98.0 ± 1.2	99
OR2000-0046	100	100	98	100	100	99	99.4 ± 0.9	103
OR2000-0046	50	100	99	100	98	99	99.2 ± 0.8	101
OR2000-0046	25	100	100	100	97	98	99.0 ± 1.4	99
OR2000-0047	100	95	97	98	98	97	97.0 ± 1.2	101
OR2000-0047	50	100	99	100	100	98	99.4 ± 0.9	100
OR2000-0047	25	98	100	100	98	100	99.2 ± 1.1	101
OR2000-0048	100	100	97	100	96	98	98.2 ± 1.8	102
OR2000-0048	50	98	99	100	98	98	98.6 ± 0.9	100
OR2000-0048	25	100	99	99	100	99	99.4 ± 0.6	100
OR2000-0049	100	99	97	95	92	98	96.2 ± 2.8	102
OR2000-0049	50	98	97	99	98	100	98.4 ± 1.1	102
OR2000-0049	25	100	100	99	100	99	99.6 ± 0.6	100
OR2000-0050	100	99	98	97	96	98	97.6 ± 1.1	102
OR2000-0050	50	100	98	100	100	97	99.0 ± 1.4	99
OR2000-0050	25	100	100	100	98	100	99.6 ± 0.9	101

[1] Percent of water quality adjusted porewater sample.

[2] Percent of reference at the same dilution.

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

Table 20. Summary of station means and statistical significance for the sea urchin fertilization and embryological development test from 20 BEST/EMAP stations from the Columbia River, Oregon. No statistical comparisons met the detectable significance criteria for toxicity. Plus signs denote only statistical differences (Dunnett's *t*-test, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig.[4]	Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
OR2000-0031	100	99.6	0.6	100	99.6	0.7		98.4	1.1	102	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		97.8	1.1	100	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.4	0.9	100	99.0	1.0	
OR2000-0032	100	99.8	0.4	100	99.6	0.7		98.8	1.1	103	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		99.4	0.9	102	97.9	2.1	
	25	99.0	1.7	99	99.8	0.4		99.2	1.3	100	99.0	1.0	
OR2000-0033	100	99.8	0.4	100	99.6	0.7		97.8	1.1	102	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.4	1.1	101	97.9	2.1	
	25	99.6	0.6	100	99.8	0.4		99.0	1.2	100	99.0	1.0	
OR2000-0034	100	100.0	0.0	100	99.6	0.7		95.8	2.4	100	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.8	1.1	101	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		99.2	0.8	100	99.0	1.0	
OR2000-0035	100	100.0	0.0	100	99.6	0.7		99.6	0.6	104	96.1	3.4	
	50	99.2	0.4	99	99.8	0.4	+	98.8	1.1	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.4	0.9	100	99.0	1.0	

Table 20. Continued.

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig.[4]	Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
OR2000-0036	100	99.8	0.4	100	99.6	0.7		99.6	0.9	104	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.6	2.1	101	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		99.0	1.2	100	99.0	1.0	
OR2000-0037	100	99.6	0.9	100	99.6	0.7		99.2	0.8	103	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		98.4	1.1	101	97.9	2.1	
	25	98.0	2.7	98	99.8	0.4		99.0	0.7	100	99.0	1.0	
OR2000-0038	100	99.4	0.6	100	99.6	0.7		99.4	1.3	103	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		99.0	1.2	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.8	0.4	101	99.0	1.0	
OR2000-0039	100	99.8	0.4	100	99.6	0.7		97.4	2.3	101	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		99.4	0.9	102	97.9	2.1	
	25	99.4	0.9	100	99.8	0.4		99.6	0.6	101	99.0	1.0	
OR2000-0040	100	99.8	0.4	100	99.6	0.7		95.4	2.4	99	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		96.4	1.1	98	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		99.4	0.9	100	99.0	1.0	
OR2000-0041	100	100.0	0.0	100	99.6	0.7		95.4	2.2	99	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.4	1.5	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.2	0.8	100	99.0	1.0	

Table 20. Continued.

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]	Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
OR2000-0042	100	99.8	0.4	100	99.6	0.7		96.0	2.9	100	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.0	0.7	100	97.9	2.1	
	25	99.6	0.6	100	99.8	0.4		99.0	1.0	100	99.0	1.0	
OR2000-0043	100	100.0	0.0	100	99.6	0.7		99.0	1.2	103	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.2	1.6	100	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		98.4	2.1	99	99.0	1.0	
OR2000-0044	100	99.8	0.4	100	99.6	0.7		98.6	1.5	103	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.4	1.5	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.4	0.6	100	99.0	1.0	
OR2000-0045	100	100.0	0.0	100	99.6	0.7		96.4	2.1	100	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		99.2	0.8	101	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		98.0	1.2	99	99.0	1.0	
OR2000-0046	100	100.0	0.0	100	99.6	0.7		99.4	0.9	103	96.1	3.4	
	50	99.6	0.6	100	99.8	0.4		99.2	0.8	101	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.0	1.4	100	99.0	1.0	
OR2000-0047	100	100.0	0.0	100	99.6	0.7		97.0	1.2	101	96.1	3.4	
	50	99.8	0.4	100	99.8	0.4		99.4	0.9	102	97.9	2.1	
	25	100.0	0.0	100	99.8	0.4		99.2	1.1	100	99.0	1.0	

Table 20. Continued.

Station ID	WQAS [1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig.[4]	Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
OR2000-0048	100	99.8	0.4	100	99.6	0.7		98.2	1.8	102	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.6	0.9	101	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		99.4	0.6	100	99.0	1.0	
OR2000-0049	100	98.6	0.6	99	99.6	0.7	++	96.2	2.8	100	96.1	3.4	
	50	100.0	0.0	100	99.8	0.4		98.4	1.1	101	97.9	2.1	
	25	99.8	0.4	100	99.8	0.4		99.6	0.6	101	99.0	1.0	
OR2000-0050	100	99.2	0.8	100	99.6	0.7		97.6	1.1	102	96.1	3.4	
	50	99.6	0.6	100	99.8	0.4		99.0	1.4	101	97.9	2.1	
	25	99.4	0.9	100	99.8	0.4		99.6	0.9	101	99.0	1.0	

[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.

Table 21. Water quality parameters after salinity adjustment and original salinity of BEST/EMAP sediment porewater samples from San Francisco Bay, California.

Station ID	Salinity [1] (‰)	DO [2] (mg/L)	% DO [3]	pH	TAN [4] (mg/L)	UAN [5] (μg/L)	Sulfide [6] (mg/L)	% OUS [7]
REF-1[8]	40	8.36	100.3	8.38	2.72[9]	196.0	<0.01	75
CA2000-0038	24	8.03	97.8	7.74	3.96	69.2	< 0.01	94
CA2000-0039	23	8.31	101.3	7.48	4.43	42.9	< 0.01	93
CA2000-0040	18	8.09	99.1	7.47	1.08	10.2	< 0.01	88
CA2000-0041	24	8.06	98.9	7.81	2.20	45.0	< 0.01	94
CA2000-0042	16	7.42	90.9	6.45	3.66	3.3	< 0.01	86
CA2000-0043	18	8.22	101.1	7.56	3.89	45.2	< 0.01	88
CA2000-0044	26.5	7.98	98.2	7.62	2.48	33.0	< 0.01	96
CA2000-0045	30	7.99	98.5	7.94	3.36	92.1	< 0.01	100
CA2000-0046	28	8.02	98.2	7.91	3.22	82.5	< 0.01	98
CA2000-0047	23	7.96	97.3	7.88	2.07	49.6	< 0.01	93
CA2000-0048	24	8.14	101.5	7.89	1.90	46.6	< 0.01	94
CA2000-0049	31	7.74	96.1	7.89	6.8	166.6	< 0.01	100
CA2000-0050	30	8.06	99.3	8.0	4.61	0.0	< 0.01	100

[1] Salinity of sample prior to adjustment. Sample adjusted to 30±1‰.
[2] Dissolved oxygen
[3] Percent saturation of dissolved oxygen
[4] Total ammonia as nitrogen
[5] Unionized ammonia
[6] Measured as S^{-2}
[7] Percent of original sample after salinity adjustment
[8] Reference pore water extracted from sediment collected in Redfish Bay, Texas.
[9] Total and unionized ammonia concentration suspect due to probable technical error.

Table 22. Sea urchin fertilization test raw data and means for BEST/EMAP sediment porewater samples from San Francisco Bay, California. 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [2]	100	100	100	98	100	100	99.0 ± 1.0	100
		99	99	98	97	99		
REF [2]	50	100	99	100	100	100	99.5 ± 0.5	100
		99	99	99	100	99		
REF [2]	25	100	99	100	100	99	99.0 ± 1.0	100
		100	99	98	97	98		
CA2000-0038	100	49	97 [3]	65	71	70	63.8 ± 10.1 **	64
CA2000-0038	50	100	100	97	99	99	99.0 ± 1.2	99
CA2000-0038	25	100	100	98	100	100	99.6 ± 0.9	101
CA2000-0039	100	100	96	100	90	100	97.2 ± 4.4	98
CA2000-0039	50	100	96	99	100	99	98.8 ± 1.6	99
CA2000-0039	25	99	100	100	100	100	99.8 ± 0.4	101
CA2000-0040	100	92	98	99	93	100	96.4 ± 3.6	97
CA2000-0040	50	100	100	100	100	100	100.0 ± 0.0	101
CA2000-0040	25	98	100	99	100	98	99.0 ± 1.0	100
CA2000-0041	100	96	96	100	98	97	97.4 ± 1.7	98
CA2000-0041	50	98	99	98	100	100	99.0 ± 1.0	99
CA2000-0041	25	100	100	100	99	100	99.8 ± 0.4	101
CA2000-0042	100	4	0	0	0	0	0.8 ± 1.8 **	1
CA2000-0042	50	1	2	3	2	1	1.8 ± 0.8 **	2
CA2000-0042	25	1	1	0	1	0	0.6 ± 0.6 **	1

Table 22. Continued.

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
CA2000-0043	100	78	85	82	88	86	83.8 ± 3.9 ++	85
CA2000-0043	50	100	100	100	99	100	99.8 ± 0.4	100
CA2000-0043	25	100	100	97	98	99	98.8 ± 1.3	100
CA2000-0044	100	99	98	100	98	98	98.6 ± 0.9	100
CA2000-0044	50	99	100	100	97	98	98.8 ± 1.3	99
CA2000-0044	25	99	99	100	98	100	99.2 ± 0.8	100
CA2000-0045	100	100	96	99	99	100	98.8 ± 1.6	100
CA2000-0045	50	100	100	100	100	98	99.6 ± 0.9	100
CA2000-0045	25	100	100	100	100	100	100.0 ± 0.0	101
CA2000-0046	100	89	92	90	92	93	91.2 ± 1.6 ++	9
CA2000-0046	50	100	100	98	99	100	99.4 ± 0.9	100
CA2000-0046	25	100	100	99	100	100	99.8 ± 0.4	101
CA2000-0047	100	100	98	99	97	100	98.8 ± 1.3	100
CA2000-0047	50	99	100	99	99	100	99.4 ± 0.6	100
CA2000-0047	25	98	100	99	100	100	99.4 ± 0.9	100
CA2000-0048	100	97	98	99	100	99	98.6 ± 1.1	100
CA2000-0048	50	100	99	99	97	100	99.0 ± 1.2	99
CA2000-0048	25	100	100	100	100	100	100.0 ± 0.0	101
CA2000-0049	100	68	72	59	55	55	61.8 ± 7.8 **	62
CA2000-0049	50	96	86 [3]	98	100	99	98.2 ± 1.7	99
CA2000-0049	25	99	100	100	100	99	99.6 ± 0.6	101

Table 22. Continued.

Station ID	% WQAS [1]	% Fertilized					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
CA2000-0050	100	83	92	93	90	89	89.4 ± 3.9 ++	90
CA2000-0050	50	97	99	99	100	98	98.6 ± 1.1	99
CA2000-0050	25	98	100	100	100	98	99.2 ± 1.1	100

[1] Percent of water quality adjusted porewater sample.

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

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

Table 23. Sea urchin (*Arbacia punctulata*) embryological development test raw data and means for BEST/EMAP sediment porewater samples from San Francisco Bay, California. 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [2]	100	93	95	91	93	90	91.8 ± 2.9	100
		97	89	87	91	92		
REF [2]	50	92	99	94	92	98	94.3 ± 2.6	100
		92	96	94	94	92		
REF [2]	25	98	99	98	95	97	96.7 ± 1.8	100
		98	94	97	97	94		
CA2000-0038	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0038	50	74	52	60	66	58	62.0 ± 8.4 **	66
CA2000-0038	25	90	95	92	93	91	92.2 ± 1.9 ++	95
CA2000-0039	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0039	50	51	50	45	48	54	49.6 ± 3.4 **	53
CA2000-0039	25	87	84	86	90	94	88.2 ± 3.9 ++	91
CA2000-0040	100	49	62	na [3]	77	66	63.5 ± 11.6 **	69
CA2000-0040	50	89	81	84	83	81	83.6 ± 3.3 ++	89
CA2000-0040	25	91	90	94	95	97	93.4 ± 2.9 +	97
CA2000-0041	100	49	41	35	44	42	42.2 ± 5.1 **	46
CA2000-0041	50	92	84	86	84	67	82.6 ± 9.3 ++	88
CA2000-0041	25	91	93	94	89	90	91.4 ± 2.1 ++	95
CA2000-0042	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0042	50	59	61	60	61	66	61.4 ± 2.7 **	65
CA2000-0042	25	88	89	86	84	84	86.2 ± 2.3 ++	89

Table 23. Continued.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
CA2000-0043	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0043	50	58	49	52	54	51	52.8 ± 3.4 **	56
CA2000-0043	25	96	89	91	88	92	91.2 ± 3.1 ++	94
CA2000-0044	100	27	38	44	32	29	34.0 ± 7.0 **	37
CA2000-0044	50	88	86	88	89	91	88.4 ± 1.8 +	94
CA2000-0044	25	93	91	93	90	87	90.8 ± 2.5 ++	94
CA2000-0045	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0045	50	77	69	71	67	68	70.4 ± 4.0 **	75
CA2000-0045	25	87	84	90	91	90	88.4 ± 2.9 ++	91
CA2000-0046	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0046	50	65	66	63	68	72	66.8 ± 3.4 **	71
CA2000-0046	25	85	82	86	85	84	84.4 ± 1.5 ++	87
CA2000-0047	100	82	81	84	80	89	83.2 ± 3.6 ++	91
CA2000-0047	50	88	86	89	98	85	89.2 ± 5.2	95
CA2000-0047	25	91	91	92	94	91	91.8 ± 1.3++	95
CA2000-0048	100	76	75	73	69	66	71.8 ± 4.2 **	78
CA2000-0048	50	83	86	85	87	88	85.8 ± 1.9 ++	91
CA2000-0048	25	89	94	92	95	86	91.2 ± 3.7++	94
CA2000-0049	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0049	50	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0049	25	48	50	51	47	53	49.8 ± 2.4 **	51

Table 23. Continued.

Station ID	% WQAS [1]	% Normal Development					Mean±SD	% of REF [2]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
CA2000-0050	100	0	0	0	0	0	0.0 ± 0.0 **	0
CA2000-0050	50	20	21	19	20	23	20.6 ± 1.5 **	22
CA2000-0050	25	83	89	83	87	84	85.2 ± 2.7 ++	88

[1] Percent of water quality adjusted porewater sample.

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

[3] Missing data due to technical error.

Table 24. Summary of station means and statistical significance for the sea urchin fertilization and embryological development test from 13 BEST/EMAP stations in San Francisco Bay, California. 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, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Station ID	WQAS[1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig.[4]	Mean	SD	% of REF[2]	REF[3] Mean	SD	Sig.[4]
CA2000-0038	100	63.8	10.1	64	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	99.0	1.2	99	99.5	0.5		62.0	8.4	66	94.3	2.6	**
	25	99.6	0.9	101	99.0	1.0		92.2	1.9	95	96.7	1.8	++
CA2000-0039	100	97.2	4.4	98	99.0	1.0		0.0	0.0	0	91.8	2.9	**
	50	98.8	1.6	99	99.5	0.5		49.6	3.4	53	94.3	2.6	**
	25	99.8	0.4	101	99.0	1.0		88.2	3.9	91	96.7	1.8	++
CA2000-0040	100	96.4	3.6	97	99.0	1.0		63.5	11.6	69	91.8	2.9	**
	50	100.0	0.0	101	99.5	0.5		83.6	3.3	89	94.3	2.6	++
	25	99.0	1.0	100	99.0	1.0		93.4	2.9	97	96.7	1.8	+
CA2000-0041	100	97.4	1.7	98	99.0	1.0		42.2	5.1	46	91.8	2.9	**
	50	99.0	1.0	99	99.5	0.5		82.6	9.3	88	94.3	2.6	++
	25	99.8	0.4	101	99.0	1.0		91.4	2.1	95	96.7	1.8	++
CA2000-0042	100	0.8	1.8	1	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	1.8	0.8	2	99.5	0.5	**	61.4	2.7	65	94.3	2.6	**
	25	0.6	0.6	1	99.0	1.0	**	86.2	2.3	89	96.7	1.8	++

Table 24. Continued.

Station ID	WQAS[1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]	Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
CA2000-0043	100	83.8	3.9	85	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	99.8	0.4	100	99.5	0.5		52.8	3.4	56	94.3	2.6	**
	25	98.8	1.3	100	99.0	1.0		91.2	3.1	94	96.7	1.8	++
CA2000-0044	100	98.6	0.9	100	99.0	1.0		34.0	7.0	37	91.8	2.9	**
	50	98.8	1.3	99	99.5	0.5		88.4	1.8	94	94.3	2.6	+
	25	99.2	0.8	100	99.0	1.0		90.8	2.5	94	96.7	1.8	++
CA2000-0045	100	98.8	1.6	100	99.0	1.0		0.0	0.0	0	91.8	2.9	**
	50	99.6	0.9	100	99.5	0.5		70.4	4.0	75	94.3	2.6	**
	25	100.0	0.0	101	99.0	1.0		88.4	2.9	91	96.7	1.8	++
CA2000-0046	100	91.2	1.6	92	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	99.4	0.9	100	99.5	0.5		66.8	3.4	71	94.3	2.6	**
	25	99.8	0.4	101	99.0	1.0		84.4	1.5	87	96.7	1.8	++
CA2000-0047	100	98.8	1.3	100	99.0	1.0		83.2	3.6	91	91.8	2.9	++
	50	99.4	0.6	100	99.5	0.5		89.2	5.2	95	94.3	2.6	
	25	99.4	0.9	100	99.0	1.0		91.8	1.3	95	96.7	1.8	++
CA2000-0048	100	98.6	1.1	100	99.0	1.0		71.8	4.2	78	91.8	2.9	**
	50	99.0	1.2	99	99.5	0.5		85.8	1.9	91	94.3	2.6	++
	25	100.0	0.0	101	99.0	1.0		91.2	3.7	94	96.7	1.8	++

Table 24. Continued.

Station ID	WQAS[1]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]	Mean	SD	% of REF [2]	REF [3] Mean	SD	Sig. [4]
CA2000-0049	100	61.8	7.8	62	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	98.2	1.7	99	99.5	0.5		0.0	0.0	0	94.3	2.6	**
	25	99.6	0.6	101	99.0	1.0		49.8	2.4	51	96.7	1.8	**
CA2000-0050	100	89.4	3.9	90	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	98.6	1.1	99	99.5	0.5		20.6	1.5	22	94.3	2.6	**
	25	99.2	1.1	100	99.0	1.0		85.2	2.7	88	96.7	1.8	++

[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.

Table 25. EC_{50} values of sediment porewater samples from the BEST/EMAP San Francisco Bay, California study, assayed in the sea urchin fertilization and embryological development tests.

Station ID	Fertilization Test		Embryological Development Test	
	EC_{50}[1]	95% Confidence Limits	EC_{50}[1]	95% Confidence Limits
CA2000-0038	>100	-	56.19	52.56-60.08
CA2000-0039	>100	-	50.67	46.86-54.80
CA2000-0040	>100	-	>100	-
CA2000-0041	>100	-	92.59	80.76-106.14
CA2000-0042	<25	-	55.47	51.27-60.01
CA2000-0043	>100	-	52.23	48.59-56.13
CA2000-0044	>100	-	85.61	78.22-93.70
CA2000-0045	>100	-	60.00	56.07-64.20
CA2000-0046	>100	-	58.25	53.82-63.04
CA2000-0047	>100	-	>100	-
CA2000-0048	>100	-	>100	-
CA2000-0049	>100	-	26.32	nr[2]
CA2000-0050	>100	-	39.16	36.55-41.96

[1] Percent of water quality adjusted porewater sample.

[2] 95% confidence limits not reliable.

Table 26. Water quality parameters after salinity adjustment and original salinity of NOAA sediment porewater samples San Francisco Bay, California.

Designation[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
REF-1[9]	40	6.97	84.8	8.40	0.89	66.7	<0.01	76
REF-2[9]	40	8.36	100.3	8.38	2.72[10]	195.9[10]	<0.01	75
1-2	2	7.83	93.4	8.47	0.518	45.2	< 0.01	76
2-2	2	7.47	84.3	8.23	0.10	2.9	< 0.01	76
3-1	6	8.04	94.9	7.99	3.51	107.6	< 0.01	79
3-3	4	7.65	91.3	8.01	0.71	22.8	< 0.01	77
4-2	6	7.53	89.2	7.76	1.65	30.2	< 0.01	79
5-1	8	7.93	94.2	7.44	3.84	33.9	< 0.01	80
5-2	9	7.74	93.3	7.91	1.27	32.5	< 0.01	81
5-5	4	7.68	94.5	7.68	2.71	41.3	< 0.01	77
6-1	6	7.94	98.1	7.59	4.70	58.4	< 0.01	79
6-4	9	7.69	95.6	7.20	2.92	14.9	< 0.01	81
7-1	5	7.80	96.4	7.54	2.75	30.5	< 0.01	78
7-4	6	7.76	98.7	7.55	3.28	37.2	< 0.01	79
7-6	8.5	7.81	98.0	7.62	6.70	89.2	< 0.01	81
8-1	20	7.78	96.7	8.02	1.53	50.1	< 0.01	91
8-3	16	7.64	95.2	8.21	1.02	50.9	< 0.01	87
9-2	15	7.27	89.8	7.62	2.43	32.3	< 0.01	91
10-1	14	7.84	97.5	7.52	5.18	54.9	< 0.01	85
10-3	14	7.66	95.3	7.44	4.91	43.4	< 0.01	85
11-1	22	8.05	100.7	8.04	1.08	37.0	< 0.01	93
11-3	22	7.83	98.1	7.96	0.96	27.5	< 0.01	93
12-1	26	7.80	97.8	7.60	2.32	29.5	< 0.01	96

Table 26. Continued.

Designation[1]	Salinity[2] (‰)	DO[3] (mg/L)	% DO[4]	pH	TAN[5] (mg/L)	UAN[6] (μg/L)	Sulfide[7] (mg/L)	% OUS[8]
13-1	24	7.95	99.3	7.63	2.76	37.6	< 0.01	95
14-1	22	7.99	98.8	7.58	3.75	45.6	< 0.01	92
15-1	28	7.80	97.0	8.04	2.99	102.5	< 0.01	98
15-3	30	8.22	99.9	8.00	0.60	18.9	< 0.01	100
16-1	30	7.66	93.0	7.79	0.57	11.2	< 0.01	100
17-1	30	7.94	96.4	7.56	5.14	59.7	< 0.01	100
17-2	30	7.69	93.2	7.57	1.78	21.1	< 0.01	100
18-1	31	7.42	90.1	7.71	1.84	30.0	< 0.01	100
19-2	32	7.88	96.4	7.65	1.97	28.1	< 0.01	94
19-3	31	8.32	101.8	7.75	7.07	126.4	< 0.01	100
20-1	32	7.75	95.6	7.65	9.69	138.1	< 0.01	94
20-5	31	7.63	93.5	7.79	3.33	65.1	< 0.01	100
20-6	31	7.53	92.2	7.77	2.80	52.4	< 0.01	100
21-1	32	7.61	93.0	7.79	2.34	45.8	< 0.01	94
21-3	32	7.85	95.8	7.43	9.69	83.7	< 0.01	94
22-1	32.5	7.98	97.6	7.87	2.41	56.5	< 0.01	92
22-3	32	7.86	95.8	7.88	1.50	35.9	< 0.01	94
22-6	32	7.94	96.8	7.84	0.77	16.8	< 0.01	94
23-2	32	7.37	94.0	7.77	0.88	16.4	< 0.01	94
24-2	33	7.67	94.1	7.84	2.23	48.8	< 0.01	91
25-1	32	7.66	94.2	7.75	3.00	53.6	< 0.01	94
25-3	32	7.81	96.4	7.41	5.77	47.6	< 0.01	94
26-1	32	8.01	99.0	7.77	3.02	56.5	< 0.01	94
26-2	32	7.57	93.9	7.73	2.34	40.0	< 0.01	94
27-1	31	7.49	92.3	7.79	3.92	76.7	< 0.01	100

Table 26. 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]
28-1	31	7.51	92.2	7.92	0.33	8.7	< 0.01	100
28-4	32	7.65	94.1	7.87	6.44	150.9	< 0.01	94
28-5	32	7.48	95.6	7.99	1.73	53.0	< 0.01	94
29-2	30	7.15	88.2	7.80	1.45	29.0	< 0.01	100
30-1	30.5	8.10	96.8	7.49	6.21	61.5	< 0.01	100
30-3	29	8.27	98.6	7.85	1.6	36.1	< 0.01	100
BB70[11]	30.5	7.78	95.0	7.36	5.4	39.7	< 0.01	100
31-2	31	8.3	98.9	7.67	7.03	104.8	< 0.01	100
31-4	30	8.35	100.3	7.78	5.08	97.2	< 0.01	100
31-6	30	7.5	89.8	7.33	12.9	88.6	< 0.01	100
32-2	30	7.87	94.3	7.66	4.87	71.0	< 0.01	100
32-3	30	7.56	90.2	7.82	4.87	101.9	< 0.01	100
32-6	30	7.57	89.9	7.87	2.7	63.3	< 0.01	100
33-5	30	7.96	95.4	7.86	9.48	217.1	< 0.01	100
34-1	31	8.17	96.7	7.90	8.49	212.8	< 0.01	100
34-3	30.5	8.35	98.9	7.47	4.43	41.9	< 0.01	100
35-2	30.5	8.24	98.7	7.54	6.77	75.1	< 0.01	100
35-3	30	7.76	92.9	7.85	3.86	86.4	< 0.01	100
36-1	29	7.74	92.6	7.8	3.08	61.6	< 0.01	100
36-2	30	8.01	95.6	8.0	2.32	0.0	< 0.01	100
36-3	30	7.97	96.6	7.75	5.14	91.9	<0.01	100
38-1	28	7.95	95.2	7.86	2.88	66.0	< 0.01	98
38-3	26	8.04	96.7	7.35	25.1	180.5	< 0.01	96
39-1	30	8.14	99.4	7.96	4.14	118.7	<0.01	100
40-2	26	8.17	98.0	7.04	18.1	64.0	< 0.01	96

Table 26. 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]
40-3	27	8.22	99.0	7.71	2.95	48.2	< 0.01	97
42-1	26	8.16	98.5	7.39	6.34	50.0	< 0.01	96
42-3	24	7.54	91.1	7.30	6.89	44.2	< 0.01	94
BA21[11]	26	8.2	99.9	7.07	9.29	35.2	< 0.01	96
43-3	20	7.92	95.5	6.8	19.9	40.5	< 0.01	90
44-1	23	8.09	97.7	7.24	3.46	19.3	< 0.01	93
44-2	16	8.06	98.0	8.01	2.61	83.7	<0.01	86
46-1	28	8.11	97.8	7.65	2.54	36.2	< 0.01	98
46-3	23	8.14	99.0	7.45	4.50	40.7	< 0.01	93
46-4	25	7.71	93.9	7.48	3.57	34.5	< 0.01	95
47-3	32	7.81	95.0	7.76	5.23	95.6	< 0.01	94
47-4	30	7.99	97.1	7.77	3.18	59.5	< 0.01	100
BF21[11]	6	7.99	97.1	7.7	2.78	44.4	< 0.01	79
11-6	26	8.15	100.0	7.44	2.52	22.3	<0.01	96
BD22[11]	24	7.74	94.6	8.09	2.35	90.0	< 0.01	94

[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. Reference 1 was used in a test of San Francisco samples from Strata 1 through 29 (except BF21 and BD22); Reference 2 was used in a test of San Francisco samples from Strata 30 through 47 and samples BF21 and BD22).
[10] Total and unionized ammonia concentration suspect due to probable technical error.
[11] Stations BF21, BD22, BB70 and BA21 were located in strata 4, 11, 30, and 42, respectively.

Table 27. Sea urchin (*Arbacia puntulata*) fertilization test one raw data and means for NOAA sediment porewater samples from San Francisco Bay, California. Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$). Plus signs denote only statistical differences (Dunnett's *t*-test, + $\alpha \leq 0.05$, ++ $\alpha \leq 0.01$).

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	95	93	90	91	94	92.9 ± 2.1	100
		96	91	91	93	95		
REF [3]	50	94	95	93	96	91	93.9 ± 1.8	100
		96	95	92	92	95		
REF [3]	25	95	93	95	93	90	94.3 ± 2.4	100
		97	95	97	94	97		
1-2	100	94	93	94	94	95	94.0 ± 0.7	101
1-2	50	93	94	93	95	91	93.2 ± 1.5	99
1-2	25	89	92	93	93	91	91.6 ± 1.7	97
2-2	100	93	88	90	94	94	91.8 ± 2.7	99
2-2	50	95	93	96	97	94	95.0 ± 1.6	101
2-2	25	97	93	95	93	93	94.2 ± 1.8	100
3-1	100	92	84	86	82	87	86.2 ± 3.8 ++	93
3-1	50	96	93	92	93	94	93.6 ± 1.5	100
3-1	25	94	95	93	94	95	94.2 ± 0.8	100
3-3	100	91	94	94	89	92	92.0 ± 2.1	99
3-3	50	95	91	96	94	94	94.0 ± 1.9	100
3-3	25	95	94	97	95	96	95.4 ± 1.1	101
4-2	100	96	90	95	92	94	93.4 ± 2.4	101
4-2	50	94	95	94	96	93	94.4 ± 1.1	101
4-2	25	93	94	98	93	92	94.0 ± 2.4	100

Table 27. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
5-1	100	60	55	54	61	69	59.8 ± 6.0 **	64
5-1	50	75	69	67	75	58	68.8 ± 7.0 **	73
5-1	25	93	90	81	88	88	88.0 ± 4.4 ++	93
5-2	100	87	91	94	93	95	92.0 ± 3.2	99
5-2	50	94	93	90	94	94	93.0 ± 1.7	99
5-2	25	96	94	91	98	95	94.8 ± 2.6	101
5-5	100	83	62	77	66	73	72.2 ± 8.4 **	78
5-5	50	93	94	91	96	91	93.0 ± 2.1	99
5-5	25	94	95	87	94	95	93.0 ± 3.4	99
6-1	100	63	45	61	58	69	59.2 ± 8.9 **	64
6-1	50	95	86	89	84	86	88.0 ± 4.3 ++	94
6-1	25	98	82	92	94	94	92.0 ± 6.0	98
6-4	100	91	80	84	81	86	84.4 ± 4.4 ++	91
6-4	50	92	94	92	96	91	93.0 ± 2.0	99
6-4	25	93	92	92	90	95	92.4 ± 1.8	98
7-1	100	63	61	52	56	56	57.6 ± 4.4 **	62
7-1	50	82	76	78	62	74	74.4 ± 7.5 **	79
7-1	25	96	91	91	91	94	92.6 ± 2.3	98
7-4	100	27	20	22	26	24	23.8 ± 2.9 **	26
7-4	50	56	62	45	69	74	61.2 ± 11.3 **	65
7-4	25	89	84	80	82	89	84.8 ± 4.1 ++	90
7-6	100	84	81	79	85	77	81.2 ± 3.4 ++	87
7-6	50	93	90	94	90	88	91.0 ± 2.4	97
7-6	25	87	94	95	91	95	92.4 ± 3.4	98

Table 27. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
8-1	100	91	86	84	91	83	87.0 ± 3.8 +	94
8-1	50	94	93	92	92	98	93.8 ± 2.5	100
8-1	25	95	90	96	96	95	94.4 ± 2.5	100
8-3	100	85	88	82	87	82	84.8 ± 2.8 ++	91
8-3	50	93	90	90	94	93	92.0 ± 1.9	98
8-3	25	90	90	94	93	94	92.2 ± 2.0	98
9-2	100	74	64	65	78	59	68.0 ± 7.8 **	73
9-2	50	80	84	75	72	89	80.0 ± 6.8 ++	85
9-2	25	94	96	90	87	89	91.2 ± 3.7	97
10-1	100	45	40	41	47	48	44.2 ± 3.6 **	48
10-1	50	80	60	74	58	59	66.2 ± 10.1 **	71
10-1	25	93	91	93	91	90	91.6 ± 1.3	97
10-3	100	41	41	45	45	40	42.4 ± 2.4 **	46
10-3	50	69	67	58	63	60	63.4 ± 4.6 **	68
10-3	25	97	90	92	88	90	91.4 ± 3.4	97
11-1	100	89	82	90	82	87	86.0 ± 3.8 ++	93
11-1	50	91	91	92	93	94	92.2 ± 1.3	98
11-1	25	95	89	92	90	90	91.2 ± 2.4	97
11-3	100	86	87	88	92	93	89.2 ± 3.1	96
11-3	50	92	90	93	94	88	91.4 ± 2.4	97
11-3	25	86	94	97	95	89	92.2 ± 4.6	98
12-1	100	63	61	71	68	61	64.8 ± 4.5 **	70
12-1	50	91	85	90	94	93	90.6 ± 3.5	96
12-1	25	95	90	87	90	90	90.4 ± 2.9	96

Table 27. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
13-1	100	68	77	71	72	63	70.2 ± 5.2 **	76
13-1	50	88	88	92	86	91	89.0 ± 2.4 +	95
13-1	25	90	92	94	92	90	91.6 ± 1.7	97
14-1	100	88	84	94	83	90	87.8 ± 4.5	95
14-1	50	94	95	95	94	95	94.6 ± 0.6	101
14-1	25	90	96	95	93	95	93.8 ± 2.4	99
15-1	100	89	92	91	93	91	91.2 ± 1.5	98
15-1	50	87	92	89	87	91	89.2 ± 2.3 +	95
15-1	25	97	89	96	93	90	93.0 ± 3.5	99
15-3	100	90	87	90	94	91	90.4 ± 2.5	97
15-3	50	87	93	92	92	92	91.2 ± 2.4	97
15-3	25	93	89	90	91	93	91.2 ± 1.8	97
16-1	100	91	88	95	88	91	90.6 ± 2.9	98
16-1	50	92	94	90	97	91	92.8 ± 2.8	99
16-1	25	92	91	92	89	95	91.8 ± 2.2	97
17-1	100	63	75	63	60	71	66.4 ± 6.3 **	71
17-1	50	91	91	90	94	89	91.0 ± 1.9	97
17-1	25	89	91	92	86	88	89.2 ± 2.4 +	95
17-2	100	93	91	97	88	95	92.8 ± 3.5	100
17-2	50	88	93	88	88	91	89.6 ± 2.3 +	95
17-2	25	90	91	94	92	93	92.0 ± 1.6	98
18-1	100	87	88	86	93	89	88.6 ± 2.7	95
18-1	50	86	89	90	88	89	88.4 ± 1.5 ++	94
18-1	25	95	87	95	93	92	92.4 ± 3.3	98

Table 27. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
19-2	100	87	85	85	87	88	86.4 ± 1.3 ++	93
19-2	50	97	94	93	92	95	94.2 ± 1.9	100
19-2	25	95	94	96	95	95	95.0 ± 0.7	101
19-3	100	38	32	34	31	44	35.8 ± 5.3 **	39
19-3	50	62	60	61	59	61	60.6 ± 1.1 **	65
19-3	25	91	95	97	94	92	93.8 ± 2.4	99
20-1	100	60 [4]	30	23	27	36	29.0 ± 5.5 **	31
20-1	50	49	51	54	54	58	53.2 ± 3.4 **	57
20-1	25	96	92	94	94	95	94.2 ± 1.5	100
20-5	100	94	93	90	91	89	91.4 ± 2.1	98
20-5	50	89	91	96	89	92	91.4 ± 2.9	97
20-5	25	86	94	92	93	95	92.0 ± 3.5	98
20-6	100	93	92	91	89	94	91.8 ± 1.9	99
20-6	50	96	96	94	91	93	94.0 ± 2.1	100
20-6	25	94	92	92	92	93	92.6 ± 0.9	98
21-1	100	87	88	84	86	86	86.2 ± 1.5 ++	93
21-1	50	91	95	95	93	91	93.0 ± 2.0	99
21-1	25	90	94	93	92	91	92.0 ± 1.6	98
21-3	100	56	66	79	66	64	66.2 ± 8.2 **	71
21-3	50	89	74	79	72	78	78.4 ± 6.6 *	83
21-3	25	92	95	94	88	90	91.8 ± 2.9	97
22-1	100	89	81	82	80	84	83.2 ± 3.6 ++	90
22-1	50	95	89	90	85	87	89.2 ± 3.8 +	95
22-1	25	97	94	92	97	92	94.4 ± 2.5	100

Table 27. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
22-3	100	96	94	92	88	92	92.4 ± 3.0	99
22-3	50	96	93	95	96	92	94.4 ± 1.8	101
22-3	25	89	93	94	94	93	92.6 ± 2.1	98
22-6	100	96	96	96	93	96	95.4 ± 1.3	103
22-6	50	93	96	93	94	97	94.6 ± 1.8	101
22-6	25	94	92	96	94	97	94.6 ± 2.0	100
23-2	100	94	88	95	95	90	92.4 ± 3.2	99
23-2	50	94	93	94	93	95	93.8 ± 0.8	100
23-2	25	95	90	93	95	98	94.2 ± 3.0	100
24-2	100	87	82	83	88	87	85.4 ± 2.7 ++	92
24-2	50	94	84	94	92	93	91.4 ± 4.2	97
24-2	25	94	91	92	94	92	92.6 ± 1.3	98
25-1	100	94	89	76	86	94	87.8 ± 7.4	94
25-1	50	91	93	94	95	95	93.6 ± 1.7	100
25-1	25	95	93	92	91	92	92.6 ± 1.5	98
25-3	100	62	54	59	47	62	56.8 ± 6.4 **	61
25-3	50	70	64	67	69	76	69.2 ± 4.4 **	74
25-3	25	95	84	91	90	87	89.4 ± 4.2 +	95
26-1	100	93	89	76	76	74	81.6 ± 8.7 ++	88
26-1	50	94	84	90	88	89	89.0 ± 3.6 +	95
26-1	25	98	91	94	95	93	94.2 ± 2.6	100
26-2	100	90	89	92	86	92	89.8 ± 2.5	97
26-2	50	91	95	96	92	94	93.6 ± 2.1	100
26-2	25	93	97	97	94	93	94.8 ± 2.0	101

Table 27. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
27-1	100	58	57	54	37	58	52.8 ± 9.0 **	57
27-1	50	75	67	60	72	71	69.0 ± 5.8 **	73
27-1	25	93	95	90	92	90	92.0 ± 2.1	98
28-1	100	91	92	92	92	91	91.6 ± 0.6	99
28-1	50	90	95	92	93	94	92.8 ± 1.9	99
28-1	25	89	92	95	94	93	92.6 ± 2.3	98
28-4	100	13	13	16	14	na [5]	14.0 ± 1.4 **	15
28-4	50	41	68 [4]	31	39	34	36.2 ± 4.6 **	39
28-4	25	93	95	90	88	90	91.2 ± 2.8	97
28-5	100	93	89	87	86	90	89.0 ± 2.7	96
28-5	50	93	95	91	88	86	90.6 ± 3.6	96
28-5	25	91	95	95	91	90	92.4 ± 2.4	98
29-2	100	75	39 [4]	76	74	75	75.0 ± 0.8 **	81
29-2	50	92	88	93	89	87	89.8 ± 2.6	96
29-2	25	94	94	96	92	95	94.2 ± 1.5	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.

[5] Missing data, technical error.

Table 28. Sea urchin (*Arbacia puntulata*) fertilization test two raw data and means for NOAA sediment porewater samples from San Francisco Bay, California. 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	100	100	98	100	100	99.0 ± 1.0	100
		99	99	98	97	99		
REF [3]	50	100	99	100	100	100	99.5 ± 0.5	100
		99	99	99	100	99		
REF [3]	25	100	99	100	100	99	99.0 ± 1.0	100
		100	99	98	97	98		
30-1	100	52	44	56	43	58	50.6 ± 6.8 **	51
30-1	50	78	88	87	79	96	85.6 ± 7.4 ++	86
30-1	25	99	98	96	98	98	97.8 ± 1.1	99
30-3	100	89	87	91	95	97	91.8 ± 4.2 ++	93
30-3	50	98	91	98	97	99	96.6 ± 3.2	97
30-3	25	98	99	96	100	99	98.4 ± 1.5	99
BB70 [5]	100	42	55	55	44	57	50.6 ± 7.0 **	51
BB70 [5]	50	90	91	92	92	97	92.4 ± 2.7 ++	93
BB70 [5]	25	98	98	95	97	96	96.8 ± 1.3	98
31-2	100	18	13	29	21	19	20.0 ± 5.8 **	20
31-2	50	79	55	63	60	64	64.2 ± 9.0 **	65
31-2	25	99	97	92	86	94	93.6 ± 5.0 ++	95
31-4	100	86	56	81	77	64	72.8 ± 12.4 **	74
31-4	50	96	96	96	96	98	96.4 ± 0.9 +	97
31-4	25	97	88	92	99	99	95.0 ± 4.8 +	96

Table 28. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
31-6	100	25	33	35	23	22	27.6 ± 6.0 **	28
31-6	50	96	94	88	88	64	86.0 ± 12.8 ++	87
31-6	25	92	95	90	84	87	89.6 ± 4.3 ++	91
32-2	100	80	86	82	84	85	83.4 ± 2.4 *	84
32-2	50	98	97	99	100	99	98.6 ± 1.1	99
32-2	25	99	100	100	100	99	99.6 ± 0.6	101
32-3	100	55	49	65	57	72	59.6 ± 9.0 **	60
32-3	50	71	88	91	87	83	84.0 ± 7.8 *	84
32-3	25	100	100	100	100	100	100.0 ± 0.0	101
32-6	100	77	80	80	80	84	80.2 ± 2.5 *	81
32-6	50	98	100	100	98	100	99.2 ± 1.1	100
32-6	25	99	99	98	98	98	98.4 ± 0.6	99
33-5	100	49	48	57	33	54	48.2 ± 9.3 **	49
33-5	50	86	62	65	88	75	75.2 ± 11.8 **	76
33-5	25	92	88	98	100	98	95.2 ± 5.0	96
34-1	100	73	70	43	54	65	61.0 ± 12.4 **	62
34-1	50	87	77	78	81	91	82.8 ± 6.0 *	83
34-1	25	98	99	99	99	100	99.0 ± 0.7	100
34-3	100	81	93	86	94	96	90.0 ± 6.3 ++	91
34-3	50	98	98	99	99	98	98.4 ± 0.6	99
34-3	25	100	99	99	98	99	99.0 ± 0.7	100
35-2	100	46	41	50	47	58	48.4 ± 6.3 **	49
35-2	50	92	91	83	98	98	92.4 ± 6.2 ++	93
35-2	25	97	98	100	99	96	98.0 ± 1.6	99

Table 28. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
35-3	100	94	89	92	84	96	91.0 ± 4.7 ++	92
35-3	50	100	99	100	97	99	99.0 ± 1.2	99
35-3	25	99	100	99	100	99	99.4 ± 0.6	100
36-1	100	75	95	84	100	96	90.0 ± 10.3 ++	91
36-1	50	96	100	98	100	98	98.4 ± 1.7	99
36-1	25	98	98	99	97	99	98.2 ± 0.8	99
36-2	100	98	90	90	93	93	92.8 ± 3.3 ++	94
36-2	50	99	98	99	100	99	99.0 ± 0.7	99
36-2	25	100	100	99	100	99	99.6 ± 0.6	101
36-3	100	34	51	31	46	50	42.4 ± 9.3 **	43
36-3	50	87	87	90	85	74	84.6 ± 6.2 ++	85
36-3	25	99	98	98	100	99	98.8 ± 0.8	100
38-1	100	89	96	95	99	97	95.2 ± 3.8	96
38-1	50	99	99	99	100	99	99.2 ± 0.4	100
38-1	25	100	99	98	100	100	99.4 ± 0.9	100
38-3	100	34	41	38	27	29	33.8 ± 5.9 **	34
38-3	50	75	68	57	51	52	60.6 ± 10.5 **	61
38-3	25	82	83	80	74	68	77.4 ± 6.3 **	78
39-1	100	85	84	90	92	93	88.8 ± 4.1 ++	90
39-1	50	97	96	100	97	98	97.6 ± 1.5	98
39-1	25	98	98	99	99	98	98.4 ± 0.6	99
40-2	100	9	7	6	12	11	9.0 ± 2.6 **	9
40-2	50	35	31	31	32	56	37.0 ± 10.7 **	37
40-2	25	47	68	97 [4]	65	75	63.8 ± 11.9 **	64

Table 28. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
40-3	100	90	97	85	87	99	91.6 ± 6.2 ++	93
40-3	50	98	97	99	100	98	98.4 ± 1.1	99
40-3	25	99	99	100	96	100	98.8 ± 1.6	100
42-1	100	47	39	78	50	53	53.4 ± 14.7 **	54
42-1	50	86	90	99	100	97	94.4 ± 6.1 +	95
42-1	25	97	100	95	100	99	98.2 ± 2.2	99
42-3	100	72	53	42	46	63	55.2 ± 12.3 **	56
42-3	50	95	82	83	88	95	88.6 ± 6.3 ++	89
42-3	25	97	98	96	100	100	98.2 ± 1.8	99
BA21 [5]	100	80	82	87	68	75	78.4 ± 7.2 **	79
BA21 [5]	50	92	93	95	94	87	92.2 ± 3.1 ++	93
BA21 [5]	25	100	100	99	98	100	99.4 ± 0.9	100
43-3	100	11	21	22	8	25	17.4 ± 7.4 **	18
43-3	50	31	50	49	37	48	43.0 ± 8.5 **	43
43-3	25	83	84	92	86	94	87.8 ± 4.9 ++	89
44-1	100	82	57	71	73	93	75.2 ± 13.4 **	76
44-1	50	93	98	100	82	76	89.8 ± 10.4 ++	90
44-1	25	100	100	100	100	100	100.0 ± 0.0	101
44-2	100	65	70	87	8	87	78.0 ± 10.0 **	79
44-2	50	98	95	95	100	98	97.2 ± 2.2	98
44-2	25	97	99	98	99	99	98.4 ± 0.9	99

Table 28. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
46-1	100	98	98	99	99	95	97.8 ± 1.6	99
46-1	50	99	100	97	99	100	99.0 ± 1.2	99
46-1	25	100	96	97	98	99	98.0 ± 1.6	99
46-3	100	88	89	83	80	93	86.6 ± 5.1 ++	87
46-3	50	100	98	97	100	98	98.6 ± 1.3	99
46-3	25	97	98	93	98	99	97.0 ± 2.4	98
46-4	100	82	65	83	87	85	80.4 ± 8.8 *	81
46-4	50	97	98	97	99	99	98.0 ± 1.0	98
46-4	25	97	100	98	99	96	98.0 ± 1.6	99
47-3	100	85	75	78	84	75	79.4 ± 4.8 **	80
47-3	50	100	98	97	97	99	98.2 ± 1.3	99
47-3	25	99	97	98	100	100	98.8 ± 1.3	100
47-4	100	98	97	98	97	95	97.0 ± 1.2	98
47-4	50	98	98	99	98	97	98.0 ± 0.7	98
47-4	25	100	100	99	100	98	99.4 ± 0.9	100
BF21 [5]	100	89	96	85	70	90	86.0 ± 9.8 ++	87
BF21 [5]	50	98	98	100	93	97	97.2 ± 2.6	98
BF21 [5]	25	98	99	97	98	100	98.4 ± 1.1	99

Table 28. Continued.

Designation [1]	% WQAS [2]	% Fertilized					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
11-6	100	100	98	99	98	99	98.8 ± 0.8	100
11-6	50	100	100	100	100	99	99.8 ± 0.4	100
11-6	25	100	99	99	100	99	99.4 ± 0.6	100
BD22 [5]	100	98	93	99	92	97	95.8 ± 3.1	97
BD22 [5]	50	99	98	97	98	98	98.0 ± 0.7	98
BD22 [5]	25	99	97	98	98	100	98.4 ± 1.1	99

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

[2] Percent of water quality adjusted porewater sample.

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

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

[5] Stations BF21, BD22, BB70, and BA21, located in strata 4, 11, 30, and 42, respectively.

Table 29. Sea urchin (*Arbacia puntulata*) embryological development test one raw data and means for NOAA sediment porewater samples from San Francisco Bay, California. 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]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	78	81	84	80	81	76.0 ± 6.0	100
		74	65	71	76	70		
REF [3]	50	86	88	84	88	81	85.3 ± 2.6	100
		88	85	86	86	81		
REF [3]	25	85	90	88	90	92	86.4 ± 3.6	100
		81	87	83	84	84		
1-2	100	80	82	84	85	75	81.2 ± 4.0	107
1-2	50	84	91	92	82	85	86.8 ± 4.4	102
1-2	25	85	89	90	89	87	88.0 ± 2.0	102
2-2	100	70	71	68	79	79	73.4 ± 5.2	97
2-2	50	75	74	79	77	76	76.2 ± 1.9 ++	89
2-2	25	73	81	90	84	92	84.0 ± 7.6	97
3-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
3-1	50	45	45	59	56	45	50.0 ± 6.9 **	59
3-1	25	76	69	67	57	77	69.2 ± 8.1 *	80
3-3	100	62	71	74	75	78	72.0 ± 6.1	95
3-3	50	84	76	70	78	80	77.6 ± 5.2 ++	91
3-3	25	86	83	75	71	83	79.6 ± 6.3 +	92
4-2	100	74	53	66	65	62	64.0 ± 7.6 ++	84
4-2	50	78	60	64	70	73	69.0 ± 7.1 *	81
4-2	25	78	77	78	82	76	78.2 ± 2.3 ++	91

Table 29. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
5-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
5-1	50	36	39	39	20	34	33.6 ± 7.9 **	39
5-1	25	68	74	72	69	71	70.8 ± 2.4 *	82
5-2	100	58	55	58	57	60	57.6 ± 1.8 **	76
5-2	50	67	64	68	62	70	66.2 ± 3.2 **	78
5-2	25	65	72	73	70	73	70.6 ± 3.4 *	82
5-5	100	0	0	0	0	0	0.0 ± 0.0 **	0
5-5	50	48	47	44	41	47	45.4 ± 2.9 **	53
5-5	25	71	69	70	71	65	69.2 ± 2.5 *	80
6-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
6-1	50	28	27	34	26	30	29.0 ± 3.2 **	34
6-1	25	55	54	56	60	57	56.4 ± 2.3 **	65
6-4	100	29	50	55	52	54	48.0 ± 10.8 **	63
6-4	50	58	63	62	60	58	60.2 ± 2.3 **	71
6-4	25	66	65	65	68	68	66.4 ± 1.5 **	77
7-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
7-1	50	54	54	48	50	52	51.6 ± 2.6 **	60
7-1	25	67	70	71	67	66	68.2 ± 2.2 **	79
7-4	100	0	0	0	0	0	0.0 ± 0.0 **	0
7-4	50	40	42	38	42	44	41.2 ± 2.3 **	48
7-4	25	63	65	60	67	64	63.8 ± 2.6 **	74
7-6	100	40	39	41	38	37	39.0 ± 1.6 **	51
7-6	50	60	62	60	58	53	58.6 ± 3.4 **	69
7-6	25	65	70	68	68	67	67.6 ± 1.8 **	78

Table 29. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
8-1	100	30	31	14	31	24	26.0 ± 7.3 **	34
8-1	50	73	75	68	71	68	71.0 ± 3.1 *	83
8-1	25	80	66	74	64	70	70.8 ± 6.4 *	82
8-3	100	56	61	63	51	43	54.8 ± 8.1 **	72
8-3	50	68	67	63	68	66	66.4 ± 2.1 **	78
8-3	25	84	76	78	74	68	76.0 ± 5.8 ++	88
9-2	100	0	0	0	0	0	0.0 ± 0.0 **	0
9-2	50	55	56	49	58	54	54.4 ± 3.4 **	64
9-2	25	72	70	70	69	71	70.4 ± 1.1 *	81
10-1	100	1	0	0	0	0	0.2 ± 0.4 **	0
10-1	50	0	0	0	0	0	0.0 ± 0.0 **	0
10-1	25	53	57	58	62	54	56.8 ± 3.6 **	66
10-3	100	0	1	0	0	0	0.2 ± 0.4 **	0
10-3	50	0	2	0	0	1	0.6 ± 0.9 **	1
10-3	25	64	56	58	60	60	59.6 ± 3.0 **	69
11-1	100	70	70	66	72	62	68.0 ± 4.0 +	89
11-1	50	75	76	70	74	75	74.0 ± 2.4 ++	87
11-1	25	76	79	76	72	68	74.2 ± 4.3 ++	86
11-3	100	51	50	47	49	50	49.4 ± 1.5 **	65
11-3	50	70	72	71	68	72	70.6 ± 1.7 *	83
11-3	25	74	80	78	77	76	77.0 ± 2.2 ++	89
12-1	100	13	9	14	11	10	11.4 ± 2.1 **	15
12-1	50	61	52	60	62	57	58.4 ± 4.0 **	68
12-1	25	72	71	70	69	56	67.6 ± 6.6 **	78

Table 29. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
13-1	100	2	0	1	1	0	0.8 ± 0.8 **	1
13-1	50	51	46	48	50	43	47.6 ± 3.2 **	56
13-1	25	58	59	60	56	62	59.0 ± 2.2 **	68
14-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
14-1	50	29	30	27	38	12	27.2 ± 9.5 **	32
14-1	25	74	68	67	64	68	68.2 ± 3.6 **	79
15-1	100	56	67	63	60	60	61.2 ± 4.1 *	81
15-1	50	69	56	76	78	61	68.0 ± 9.5 *	80
15-1	25	77	72	72	75	78	74.8 ± 2.8 ++	87
15-3	100	47	62	67	66	60	60.4 ± 8.0 *	79
15-3	50	83	69	76	67	64	71.8 ± 7.7 ++	84
15-3	25	73	71	80	76	76	75.2 ± 3.4 ++	87
16-1	100	70	74	73	71	73	72.2 ± 1.6	95
16-1	50	80	78	82	81	73	78.8 ± 3.6 ++	92
16-1	25	83	73	75	82	72	77.0 ± 5.2 ++	89
17-1	100	1	1	0	0	0	0.4 ± 0.6 **	1
17-1	50	19	3	4	12	8	9.2 ± 6.5 **	11
17-1	25	52	48	47	50	51	49.6 ± 2.1 **	57
17-2	100	33	29	33	37	35	33.4 ± 3.0 **	44
17-2	50	67	64	61	66	62	64.0 ± 2.6 **	75
17-2	25	76	78	72	71	67	72.8 ± 4.3 ++	84
18-1	100	46	43	47	48	44	45.6 ± 2.1 **	60
18-1	50	65	67	66	72	68	67.6 ± 2.7 **	79
18-1	25	75	78	82	80	77	78.4 ± 2.7 ++	91

Table 29. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
19-2	100	22	12	28	25	35	24.4 ± 8.4 **	32
19-2	50	62	71	77	74	81	73.0 ± 7.2 ++	86
19-2	25	66	79	74	82	78	75.8 ± 6.2 ++	88
19-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
19-3	50	0	0	0	0	0	0.0 ± 0.0 **	0
19-3	25	9	12	24 [4]	10	6	9.2 ± 2.5 **	11
20-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
20-1	50	0	0	0	0	0	0.0 ± 0.0 **	0
20-1	25	20	39	25	35	39	31.6 ± 8.6 **	37
20-5	100	47	34	21	24	18	28.8 ± 11.8 **	38
20-5	50	69	66	66	65	68	66.8 ± 1.6 **	78
20-5	25	67	68	67	72	62	67.2 ± 3.6 **	78
20-6	100	64	64	65	66	61	64.0 ± 1.9 ++	84
20-6	50	77	79	77	82	80	79.0 ± 2.1 ++	93
20-6	25	84	81	79	80	76	80.0 ± 2.9 +	93
21-1	100	60	47	61	57	59	56.8 ± 5.7 **	75
21-1	50	81	83	78	81	77	80.0 ± 2.4 +	94
21-1	25	84	86	82	87	85	84.8 ± 1.9	98
21-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
21-3	50	0	0	0	0	0	0.0 ± 0.0 **	0
21-3	25	24	26	23	29	27	25.8 ± 2.4 **	30
22-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
22-1	50	76	72	78	74	80	76.0 ± 3.2 ++	89
22-1	25	84	86	86	83	76	83.0 ± 4.1	96

Table 29. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
22-3	100	72	61	60	32 [4]	52	61.2 ± 8.2 *	80
22-3	50	79	79	78	76	84	79.2 ± 3.0 +	93
22-3	25	88	86	83	87	87	86.2 ± 1.9	100
22-6	100	81	80	84	79	64	77.6 ± 7.8	102
22-6	50	89	92	84	89	80	86.8 ± 4.8	102
22-6	25	80	88	87	83	81	83.8 ± 3.6	97
23-2	100	71	78	66	71	70	71.2 ± 4.3	94
23-2	50	80	83	79	84	78	80.8 ± 2.6	95
23-2	25	80	86	84	88	86	84.8 ± 3.0	98
24-2	100	0	0	0	0	0	0.0 ± 0.0 **	0
24-2	50	62	68	74	65	71	68.0 ± 4.7 *	80
24-2	25	82	80	82	79	76	79.8 ± 2.5 +	92
25-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
25-1	50	64	62	60	58	61	61.0 ± 2.2 **	72
25-1	25	75	77	74	75	77	75.6 ± 1.3 ++	88
25-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
25-3	50	0	0	0	0	0	0.0 ± 0.0 **	0
25-3	25	45	51	51	48	58	50.6 ± 4.8 **	59
26-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
26-1	50	66	60	67	66	65	64.8 ± 2.8 **	76
26-1	25	75	76	68	78	69	73.2 ± 4.4 ++	85
26-2	100	16	17	19	17	14	16.6 ± 1.8 **	22
26-2	50	73	74	73	73	77	74.0 ± 1.7 ++	87
26-2	25	72	80	81	78	87	79.6 ± 5.4 +	92

Table 29. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
27-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
27-1	50	10	16	29	21	28	20.8 ± 8.0 **	24
27-1	25	77	81	79	72	66	75.0 ± 6.0 ++	87
28-1	100	81	78	81	78	80	79.6 ± 1.5	105
28-1	50	77	87	84	81	86	83.0 ± 4.1	97
28-1	25	86	81	88	85	84	84.8 ± 2.6	98
28-4	100	0	0	0	0	0	0.0 ± 0.0 **	0
28-4	50	0	0	0	0	0	0.0 ± 0.0 **	0
28-4	25	9	7	7	6	9	7.6 ± 1.3 **	9
28-5	100	52	50	50	51	46	49.8 ± 2.3 **	66
28-5	50	78	74	76	81	76	77.0 ± 2.6 ++	90
28-5	25	79	86	84	82	80	82.2 ± 2.9	95
29-2	100	37	34	30	32	36	33.8 ± 2.9 **	44
29-2	50	78	76	72	79	80	77.0 ± 3.2 ++	90
29-2	25	71	71	78	81	73	74.8 ± 4.5 ++	87

[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 30. Sea urchin (*Arbacia puntulata*) embryological development test two raw data and means for NOAA sediment porewater samples from San Francisco Bay, California. 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]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
REF [3]	100	93	95	91	93	90	91.8 ± 2.9	100
		97	89	87	91	92		
REF [3]	50	92	99	94	92	98	94.3 ± 2.6	100
		92	96	94	94	92		
REF [3]	25	98	99	98	95	97	96.7 ± 1.8	100
		98	94	97	97	94		
30-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
30-1	50	0	0	0	0	0	0.0 ± 0.0 **	0
30-1	25	66	78	72	63	72	70.2 ± 5.8 **	73
30-3	100	66	71	69	65	70	68.2 ± 2.6 **	74
30-3	50	88	82	86	84	89	85.8 ± 2.9 ++	91
30-3	25	90	93	89	93	91	91.2 ± 1.8 ++	94
BB70 [5]	100	0	0	0	0	0	0.0 ± 0.0 **	0
BB70 [5]	50	6	12	21	17	19	15.0 ± 6.0 **	16
BB70 [5]	25	88	93	94	91	96	92.4 ± 3.0 ++	96
31-2	100	0	0	0	0	0	0.0 ± 0.0 **	0
31-2	50	0	0	0	0	0	0.0 ± 0.0 **	0
31-2	25	33	35	25	34	51	35.6 ± 9.5 **	37
31-4	100	0	0	0	0	0	0.0 ± 0.0 **	0
31-4	50	79	16 [4]	70	76	80	76.2 ± 4.5 *	81
31-4	25	84	78	83	92	89	85.2 ± 5.4 ++	88

Table 30. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
31-6	100	0	0	0	0	0	0.0 ± 0.0 **	0
31-6	50	0	0	0	0	0	0.0 ± 0.0 **	0
31-6	25	0	31	26	0	0	11.4 ± 15.7 **	12
32-2	100	0	0	0	0	0	0.0 ± 0.0 **	0
32-2	50	34	15	26	21	50	29.2 ± 13.6 **	31
32-2	25	86	88	82	86	85	85.4 ± 2.2 ++	88
32-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
32-3	50	6	9	4	10	65 [4]	7.2 ± 2.8 **	8
32-3	25	88	86	88	80	82	84.8 ± 3.6 ++	88
32-6	100	0	0	0	0	0	0.0 ± 0.0 **	0
32-6	50	87	86	87	89	87	87.2 ± 1.1 ++	92
32-6	25	95	89	91	95	92	92.4 ± 2.6 ++	96
33-5	100	0	0	0	0	0	0.0 ± 0.0 **	0
33-5	50	0	0	0	0	0	0.0 ± 0.0 **	0
33-5	25	40	19	24	71 [4]	90 [4]	27.7 ± 11.0 **	29
34-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
34-1	50	0	0	0	0	0	0.0 ± 0.0 **	0
34-1	25	5	18	11	63 [4]	28	15.5 ± 9.9 **	16
34-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
34-3	50	8	15	17	11	33	16.8 ± 9.7 **	18
34-3	25	80	87	84	85	87	84.6 ± 2.9 ++	87
35-2	100	0	0	0	0	0	0.0 ± 0.0 **	0
35-2	50	0	0	0	0	0	0.0 ± 0.0 **	0
35-2	25	84	75	73	82	74	77.6 ± 5.0 *	80

Table 30. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
35-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
35-3	50	54	57	69	59	55	58.8 ± 6.0 **	62
35-3	25	88	88	88	87	88	87.8 ± 0.4 ++	91
36-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
36-1	50	78	77	81	79	77	78.4 ± 1.7 *	83
36-1	25	79	84	87	90	91	86.2 ± 4.9 ++	89
36-2	100	44	13	38	22	29	29.2 ± 12.4 **	32
36-2	50	89	87	84	85	83	85.6 ± 2.4 ++	91
36-2	25	92	91	91	93	94	92.2 ± 1.3 ++	95
36-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
36-3	50	24	17	8	10	21	16.0 ± 6.9 **	17
36-3	25	21 [4]	83	87	86	84	85.0 ± 1.8 ++	88
38-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
38-1	50	86	79	81	86	88	84.0 ± 3.8 ++	89
38-1	25	91	93	94	94	89	92.2 ± 2.2 ++	95
38-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
38-3	50	0	0	0	0	0	0.0 ± 0.0 **	0
38-3	25	0	0	0	0	0	0.0 ± 0.0 **	0
39-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
39-1	50	84	87	86	84	82	84.6 ± 2.0 ++	90
39-1	25	93	92	95	94	91	93.0 ± 1.6 +	96
40-2	100	0	0	0	0	0	0.0 ± 0.0 **	0
40-2	50	0	0	0	0	0	0.0 ± 0.0 **	0
40-2	25	0	0	0	0	0	0.0 ± 0.0 **	0

Table 30. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
40-3	100	30	54	61	70	27	48.4 ± 19.1 **	53
40-3	50	88	83	81	86	87	85.0 ± 2.9 ++	90
40-3	25	95	89	91	93	92	92.0 ± 2.2 ++	95
42-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
42-1	50	27	83 [4]	20	66 [4]	18	21.7 ± 4.7 **	23
42-1	25	89	90	90	89	91	89.8 ± 0.8 ++	93
42-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
42-3	50	0	0	0	0	0	0.0 ± 0.0 **	0
42-3	25	54	71	73	77	71	69.2 ± 8.8 **	72
BA21 [5]	100	0	0	0	0	0	0.0 ± 0.0 **	0
BA21 [5]	50	0	0	0	0	0	0.0 ± 0.0 **	0
BA21 [5]	25	54 [4]	83	91	95	93	90.5 ± 5.3 ++	94
43-3	100	0	0.	0	0	0	0.0 ± 0.0 **	0
43-3	50	0	0	0	0	0	0.0 ± 0.0 **	0
43-3	25	0	0	0	0	0	0.0 ± 0.0 **	0
44-1	100	0	0	0	0	0	0.0 ± 0.0 **	0
44-1	50	87	85	81	82	78	82.6 ± 3.5 ++	88
44-1	25	87	88	95	97	96	92.6 ± 4.7 +	96
44-2	100	73	70	58	60	70	66.2 ± 6.7 **	72
44-2	50	82	87	86	84	86	85.0 ± 2.0 ++	90
44-2	25	94	94	92	91	89	92.0 ± 2.1 ++	95

Table 30. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
46-1	100	38	15	48	58	51	42.0 ± 16.7 **	46
46-1	50	78	63	76	74	82	74.6 ± 7.1 **	79
46-1	25	87	93	90	93	91	90.8 ± 2.5 ++	94
46-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
46-3	50	84 [4]	68	48	57	47	55.0 ± 9.8 **	58
46-3	25	88	85	87	87	91	87.6 ± 2.2 ++	91
46-4	100	0	0	0	0	0	0.0 ± 0.0 **	0
46-4	50	59	61	60	66	67	62.6 ± 3.6 **	66
46-4	25	93	90	95	94	92	92.8 ± 1.9 ++	96
47-3	100	0	0	0	0	0	0.0 ± 0.0 **	0
47-3	50	50	43	58	66	14 [4]	54.2 ± 10.0 **	58
47-3	25	93	88	91	90	89	90.2 ± 1.9 ++	93
47-4	100	29	39	46	46	74	46.8 ± 16.7 **	51
47-4	50	90	88	90	85	89	88.4 ± 2.1 ++	94
47-4	25	87	89	92	81	92	88.2 ± 4.6 ++	91
BF21 [5]	100	8	6	18	31	0	12.6 ± 12.2 **	14
BF21 [5]	50	86	85	86	87	87	86.2 ± 0.8 ++	91
BF21 [5]	25	89	91	90	97	97	92.8 ± 3.9 +	96

Table 30. Continued.

Designation [1]	% WQAS [2]	% Normal Development					Mean±SD	% of REF [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
11-6	100	0	0	0	0	0	0.0 ± 0.0 **	0
11-6	50	62	33	47	51	36	45.8 ± 11.7 **	49
11-6	25	83	97	91	94	92	91.4 ± 5.2 ++	95
BD22 [5]	100	86	93	89	87	93	89.6 ± 3.3	98
BD22 [5]	50	92	91	90	90	95	91.6 ± 2.1 +	97
BD22 [5]	25	96	95	96	94	96	95.4 ± 0.9	99

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

[2] Percent of water quality adjusted porewater sample.

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

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

[5] Stations BF21, BD22, BB70, and BA21, located in strata 4, 11, 30, and 42, respectively

Table 31. Summary of station means and statistical significance for the sea urchiu fertilization and embryological development test from 86 NOAA stations in San Francisco Bay, California. Asterisks denote statistical differences (Dunnett's *t*-test) and detectable significance criteria between test aud 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]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
1-2	100	94.0	0.7	101	92.9	2.1		81.2	4.0	107	76.0	6.0	
	50	93.2	1.5	99	93.9	1.8		86.8	4.4	102	85.3	2.6	
	25	91.6	1.7	97	94.3	2.4		88.0	2.0	102	86.4	3.6	
2-2	100	91.8	2.7	99	92.9	2.1		73.4	5.2	97	76.0	6.0	
	50	95.0	1.6	101	93.9	1.8		76.2	1.9	89	85.3	2.6	++
	25	94.2	1.8	100	94.3	2.4		84.0	7.6	97	86.4	3.6	
3-1	100	86.2	3.8	93	92.9	2.1	++	0.0	0.0	0	76.0	6.0	**
	50	93.6	1.5	100	93.9	1.8		50.0	6.9	59	85.3	2.6	**
	25	94.2	0.8	100	94.3	2.4		69.2	8.1	80	86.4	3.6	*
3-3	100	92.0	2.1	99	92.9	2.1		72.0	6.1	95	76.0	6.0	
	50	94.0	1.9	100	93.9	1.8		77.6	5.2	91	85.3	2.6	++
	25	95.4	1.1	101	94.3	2.4		79.6	6.3	92	86.4	3.6	+
4-2	100	93.4	2.4	101	92.9	2.1		64.0	7.6	84	76.0	6.0	++
	50	94.4	1.1	101	93.9	1.8		69.0	7.1	81	85.3	2.6	*
	25	94.0	2.4	100	94.3	2.4		78.2	2.3	91	86.4	3.6	++

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
5-1	100	59.8	6.0	64	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	68.8	7.0	73	93.9	1.8	**	33.6	7.9	39	85.3	2.6	**
	25	88.0	4.4	93	94.3	2.4	++	70.8	2.4	82	86.4	3.6	*
5-2	100	92.0	3.2	99	92.9	2.1		57.6	1.8	76	76.0	6.0	**
	50	93.0	1.7	99	93.9	1.8		66.2	3.2	78	85.3	2.6	**
	25	94.8	2.6	101	94.3	2.4		70.6	3.4	82	86.4	3.6	*
5-5	100	72.2	8.4	78	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	93.0	2.1	99	93.9	1.8		45.4	2.9	53	85.3	2.6	**
	25	93.0	3.4	99	94.3	2.4		69.2	2.5	80	86.4	3.6	*
6-1	100	59.2	8.9	64	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	88.0	4.3	94	93.9	1.8	++	29.0	3.2	34	85.3	2.6	**
	25	92.0	6.0	98	94.3	2.4		56.4	2.3	65	86.4	3.6	**
6-4	100	84.4	4.4	91	92.9	2.1	++	48.0	10.8	63	76.0	6.0	**
	50	93.0	2.0	99	93.9	1.8		60.2	2.3	71	85.3	2.6	**
	25	92.4	1.8	98	94.3	2.4		66.4	1.5	77	86.4	3.6	**
7-1	100	57.6	4.4	62	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	74.4	7.5	79	93.9	1.8	**	51.6	2.6	60	85.3	2.6	**
	25	92.6	2.3	98	94.3	2.4		68.2	2.2	79	86.4	3.6	**

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
7-4	100	23.8	2.9	26	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	61.2	11.3	65	93.9	1.8	**	41.2	2.3	48	85.3	2.6	**
	25	84.8	4.1	90	94.3	2.4	++	63.8	2.6	74	86.4	3.6	**
7-6	100	81.2	3.4	87	92.9	2.1	++	39.0	1.6	51	76.0	6.0	**
	50	91.0	2.4	97	93.9	1.8		58.6	3.4	69	85.3	2.6	**
	25	92.4	3.4	98	94.3	2.4		67.6	1.8	78	86.4	3.6	**
8-1	100	87.0	3.8	94	92.9	2.1	+	26.0	7.3	34	76.0	6.0	**
	50	93.8	2.5	100	93.9	1.8		71..0	3.1	83	85.3	2.6	*
	25	94.4	2.5	100	94.3	2.4		70.8	6.4	82	86.4	3.6	*
8-3	100	84.8	2.8	91	92.9	2.1	++	54.8	8.1	72	76.0	6.0	**
	50	92.0	1.9	98	93.9	1.8		66.4	2.1	78	85.3	2.6	**
	25	92.2	2.0	98	94.3	2.4		76.0	5.8	88	86.4	3.6	++
9-2	100	68.0	7.8	73	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	80.0	6.8	85	93.9	1.8	++	54.4	3.4	64	85.3	2.6	**
	25	91.2	3.7	97	94.3	2.4		70.4	1.1	81	86.4	3.6	*
10-1	100	44.2	3.6	48	92.9	2.1	**	0.2	0.4	0	76.0	6.0	**
	50	66.2	10.1	71	93.9	1.8	**	0.0	0.0	0	85.3	2.6	**
	25	91.6	1.3	97	94.3	2.4		56.8	3.6	66	86.4	3.6	**

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
10-3	100	42.4	2.4	46	92.9	2.1	**	0.2	0.4	0	76.0	6.0	**
	50	63.4	4.6	68	93.9	1.8	**	0.6	0.9	1	85.3	2.6	**
	25	91.4	3.4	97	94.3	2.4		59.6	3.0	69	86.4	3.6	**
11-1	100	86.0	3.8	93	92.9	2.1	++	68.0	4.0	89	76.0	6.0	+
	50	92.2	1.3	98	93.9	1.8		74.0	2.4	87	85.3	2.6	++
	25	91.2	2.4	97	94.3	2.4		74.2	4.3	86	86.4	3.6	++
11-3	100	89.2	3.1	96	92.9	2.1		49.4	1.5	65	76.0	6.0	**
	50	91.4	2.4	97	93.9	1.8		70.6	1.7	83	85.3	2.6	*
	25	92.2	4.6	98	94.3	2.4		77.0	2.2	89	86.4	3.6	++
12-1	100	64.8	4.5	70	92.9	2.1	**	11.4	2.1	15	76.0	6.0	**
	50	90.6	3.5	96	93.9	1.8		58.4	4.0	68	85.3	2.6	**
	25	90.4	2.9	96	94.3	2.4		67.6	6.6	78	86.4	3.6	**
13-1	100	70.2	5.2	76	92.9	2.1	**	0.8	0.8	1	76.0	6.0	**
	50	89.0	2.4	95	93.9	1.8	+	47.6	3.2	56	85.3	2.6	**
	25	91.6	1.7	97	94.3	2.4		59.0	2.2	68	86.4	3.6	**
14-1	100	87.8	4.5	95	92.9	2.1		0.0	0.0	0	76.0	6.0	**
	50	94.6	0.6	101	93.9	1.8		27.2	9.5	32	85.3	2.6	**
	25	93.8	2.4	99	94.3	2.4		68.2	3.6	79	86.4	3.6	**

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
15-1	100	91.2	1.5	98	92.9	2.1		61.2	4.1	81	76.0	6.0	*
	50	89.2	2.3	95	93.9	1.8	+	68.0	9.5	80	85.3	2.6	*
	25	93.0	3.5	99	94.3	2.4		74.8	2.8	87	86.4	3.6	++
15-3	100	90.4	2.5	97	92.9	2.1		60.4	8.0	79	76.0	6.0	*
	50	91.2	2.4	97	93.9	1.8		71.8	7.7	84	85.3	2.6	++
	25	91.2	1.8	97	94.3	2.4		75.2	3.4	87	86.4	3.6	++
16-1	100	90.6	2.9	98	92.9	2.1		72.2	1.6	95	76.0	6.0	
	50	92.8	2.8	99	93.9	1.8		78.8	3.6	92	85.3	2.6	++
	25	91.8	2.2	97	94.3	2.4		77.0	5.2	89	86.4	3.6	++
17-1	100	66.4	6.3	71	92.9	2.1	**	0.4	0.6	1	76.0	6.0	**
	50	91.0	1.9	97	93.9	1.8		9.2	6.5	11	85.3	2.6	**
	25	89.2	2.4	95	94.3	2.4	+	49.6	2.1	57	86.4	3.6	**
17-2	100	92.8	3.5	100	92.9	2.1		33.4	3.0	44	76.0	6.0	**
	50	89.6	2.3	95	93.9	1.8	+	64.0	2.6	75	85.3	2.6	**
	25	92.0	1.6	98	94.3	2.4		72.8	4.3	84	86.4	3.6	++
18-1	100	88.6	2.7	95	92.9	2.1		45.6	2.1	60	76.0	6.0	**
	50	88.4	1.5	94	93.9	1.8	++	67.6	2.7	79	85.3	2.6	**
	25	92.4	3.3	98	94.3	2.4		78.4	2.7	91	86.4	3.6	++

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
19-2	100	86.4	1.3	93	92.9	2.1	++	24.4	8.4	32	76.0	6.0	**
	50	94.2	1.9	100	93.9	1.8		73.0	7.2	86	85.3	2.6	++
	25	95.0	0.7	101	94.3	2.4		75.8	6.2	88	86.4	3.6	++
19-3	100	35.8	5.3	39	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	60.6	1.1	65	93.9	1.8	**	0.0	0.0	0	85.3	2.6	**
	25	93.8	2.4	99	94.3	2.4		9.2	2.5	11	86.4	3.6	**
20-1	100	29.0	5.5	31	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	53.2	3.4	57	93.9	1.8	**	0.0	0.0	0	85.3	2.6	**
	25	94.2	1.5	100	94.3	2.4		31.6	8.6	37	86.4	3.6	**
20-5	100	91.4	2.1	98	92.9	2.1		28.8	11.8	38	76.0	6.0	**
	50	91.4	2.9	97	93.9	1.8		66.8	1.6	78	85.3	2.6	**
	25	92.0	3.5	98	94.3	2.4		67.2	3.6	78	86.4	3.6	**
20-6	100	91.8	1.9	99	92.9	2.1		64.0	1.9	84	76.0	6.0	++
	50	94.0	2.1	100	93.9	1.8		79.0	2.1	93	85.3	2.6	++
	25	92.6	0.9	98	94.3	2.4		80.0	2.9	93	86.4	3.6	+
21-1	100	86.2	1.5	93	92.9	2.1	++	56.8	5.7	75	76.0	6.0	**
	50	93.0	2.0	99	93.9	1.8		80.0	2.4	94	85.3	2.6	+
	25	92.0	1.6	98	94.3	2.4		84.8	1.9	98	86.4	3.6	

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
21-3	100	66.2	8.2	71	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	78.4	6.6	83	93.9	1.8	*	0.0	0.0	0	85.3	2.6	**
	25	91.8	2.9	97	94.3	2.4		25.8	2.4	30	86.4	3.6	**
22-1	100	83.2	3.6	90	92.9	2.1	++	0.0	0.0	0	76.0	6.0	**
	50	89.2	3.8	95	93.9	1.8	+	76.0	3.2	89	85.3	2.6	++
	25	94.4	2.5	100	94.3	2.4		83.0	4.1	96	86.4	3.6	
22-3	100	92.4	3.0	99	92.9	2.1		61.2	8.2	80	76.0	6.0	*
	50	94.4	1.8	101	93.9	1.8		79.2	3.0	93	85.3	2.6	+
	25	92.6	2.1	98	94.3	2.4		86.2	1.9	100	86.4	3.6	
22-6	100	95.4	1.3	103	92.9	2.1		77.6	7.8	102	76.0	6.0	
	50	94.6	1.8	101	93.9	1.8		86.8	4.8	102	85.3	2.6	
	25	94.6	2.0	100	94.3	2.4		83.8	3.6	97	86.4	3.6	
23-2	100	92.4	3.2	99	92.9	2.1		71.2	4.3	94	76.0	6.0	
	50	93.8	0.8	100	93.9	1.8		80.8	2.6	95	85.3	2.6	
	25	94.2	3.0	100	94.3	2.4		84.8	3.0	98	86.4	3.6	
24-2	100	85.4	2.7	92	92.9	2.1	++	0.0	0.0	0	76.0	6.0	**
	50	91.4	4.2	97	93.9	1.8		68.0	4.7	80	85.3	2.6	*
	25	92.6	1.3	98	94.3	2.4		79.8	2.5	92	86.4	3.6	+

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
25-1	100	87.8	7.4	94	92.9	2.1		0.0	0.0	0	76.0	6.0	**
	50	93.6	1.7	100	93.9	1.8		61.0	2.2	72	85.3	2.6	**
	25	92.6	1.5	98	94.3	2.4		75.6	1.3	88	86.4	3.6	++
25-3	100	56.8	6.4	61	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	69.2	4.4	74	93.9	1.8	**	0.0	0.0	0	85.3	2.6	**
	25	89.4	4.2	95	94.3	2.4	+	50.6	4.8	59	86.4	3.6	**
26-1	100	81.6	8.7	88	92.9	2.1	++	0.0	0.0	0	76.0	6.0	**
	50	89.0	3.6	95	93.9	1.8	+	64.8	2.8	76	85.3	2.6	**
	25	94.2	2.6	100	94.3	2.4		73.2	4.4	85	86.4	3.6	++
26-2	100	89.8	2.5	97	92.9	2.1		16.6	1.8	22	76.0	6.0	**
	50	93.6	2.1	100	93.9	1.8		74.0	1.7	87	85.3	2.6	++
	25	94.8	2.0	101	94.3	2.4		79.6	5.4	92	86.4	3.6	+
27-1	100	52.8	9.0	57	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	69.0	5.8	73	93.9	1.8	**	20.8	8.0	24	85.3	2.6	**
	25	92.0	2.1	98	94.3	2.4		75.0	6.0	87	86.4	3.6	++
28-1	100	91.6	0.6	99	92.9	2.1		79.6	1.5	105	76.0	6.0	
	50	92.8	1.9	99	93.9	1.8		83.0	4.1	97	85.3	2.6	
	25	92.6	2.3	98	94.3	2.4		84.8	2.6	98	86.4	3.6	

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
28-4	100	14.0	1.4	15	92.9	2.1	**	0.0	0.0	0	76.0	6.0	**
	50	36.2	4.6	39	93.9	1.8	**	0.0	0.0	0	85.3	2.6	**
	25	91.2	2.8	97	94.3	2.4		7.6	1.3	9	86.4	3.6	**
28-5	100	89.0	2.7	96	92.9	2.1		49.8	2.3	66	76.0	6.0	**
	50	90.6	3.6	96	93.9	1.8		77.0	2.6	90	85.3	2.6	++
	25	92.4	2.4	98	94.3	2.4		82.2	2.9	95	86.4	3.6	
29-2	100	75.0	0.8	81	92.9	2.1	**	33.8	2.9	44	76.0	6.0	**
	50	89.8	2.6	96	93.9	1.8		77.0	3.2	90	85.3	2.6	++
	25	94.2	1.5	100	94.3	2.4		74.8	4.5	87	86.4	3.6	++
30-1	100	50.6	6.8	51	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	85.6	7.4	86	99.5	0.5	++	0.0	0.0	0	94.3	2.6	**
	25	97.8	1.1	99	99.0	1.0		70.2	5.8	73	96.7	1.8	**
30-3	100	91.8	4.2	93	99.0	1.0	++	68.2	2.6	74	91.8	2.9	**
	50	96.6	3.2	97	99.5	0.5		85.8	2.9	91	94.3	2.6	++
	25	98.4	1.5	99	99.0	1.0		91.2	1.8	94	96.7	1.8	++
BB70 [6]	100	50.6	7.0	51	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	92.4	2.7	93	99.5	0.5	++	15.0	6.0	16	94.3	2.6	**
	25	96.8	1.3	98	99.0	1.0		92.4	3.0	96	96.7	1.8	++

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
31-2	100	20.0	5.8	20	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	64.2	9.0	65	99.5	0.5	**	0.0	0.0	0	94.3	2.6	**
	25	93.6	5.0	95	99.0	1.0	++	35.6	9.5	37	96.7	1.8	**
31-4	100	72.8	12.4	74	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	96.4	0.9	97	99.5	0.5	+	76.2	4.5	81	94.3	2.6	*
	25	95.0	4.8	96	99.0	1.0	+	85.2	5.4	88	96.7	1.8	++
31-6	100	27.6	6.0	28	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	86.0	12.8	87	99.5	0.5	++	0.0	0.0	0	94.3	2.6	**
	25	89.6	4.3	91	99.0	1.0	++	11.4	15.7	12	96.7	1.8	**
32-2	100	83.4	2.4	84	99.0	1.0	*	0.0	0.0	0	91.8	2.9	**
	50	98.6	1.1	99	99.5	0.5		29.2	13.6	31	94.3	2.6	**
	25	99.6	0.6	101	99.0	1.0		85.4	2.2	88	96.7	1.8	++
32-3	100	59.6	9.0	60	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	84.0	7.8	84	99.5	0.5	*	7.2	2.8	8	94.3	2.6	**
	25	100.0	0.0	101	99.0	1.0		84.8	3.6	88	96.7	1.8	++
32-6	100	80.2	2.5	81	99.0	1.0	*	0.0	0.0	0	91.8	2.9	**
	50	99.2	1.1	100	99.5	0.5		87.2	1.1	92	94.3	2.6	++
	25	98.4	0.6	99	99.0	1.0		92.4	2.6	96	96.7	1.8	++

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
33-5	100	48.2	9.3	49	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	75.2	11.8	76	99.5	0.5	**	0.0	0.0	0	94.3	2.6	**
	25	95.2	5.0	96	99.0	1.0		27.7	11.0	29	96.7	1.8	**
34-1	100	61.0	12.4	62	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	82.8	6.0	83	99.5	0.5	*	0.0	0.0	0	94.3	2.6	**
	25	99.0	0.7	100	99.0	1.0		15.5	9.9	16	96.7	1.8	**
34-3	100	90.0	6.3	91	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	98.4	0.6	99	99.5	0.5		16.8	9.7	18	94.3	2.6	**
	25	99.0	0.7	100	99.0	1.0		84.6	2.9	87	96.7	1.8	++
35-2	100	48.4	6.3	49	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	92.4	6.2	93	99.5	0.5	++	0.0	0.0	0	94.3	2.6	**
	25	98.0	1.6	99	99.0	1.0		77.6	5.0	80	96.7	1.8	*
35-3	100	91.0	4.7	92	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	99.0	1.2	99	99.5	0.5		58.8	6.0	62	94.3	2.6	**
	25	99.4	0.6	100	99.0	1.0		87.8	0.4	91	96.7	1.8	++
36-1	100	90.0	10.3	91	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	98.4	1.7	99	99.5	0.5		78.4	1.7	83	94.3	2.6	*
	25	98.2	0.8	99	99.0	1.0		86.2	4.9	89	96.7	1.8	++

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
36-2	100	92.8	3.3	94	99.0	1.0	++	29.2	12.4	32	91.8	2.9	**
	50	99.0	0.7	99	99.5	0.5		85.6	2.4	91	94.3	2.6	++
	25	99.6	0.6	101	99.0	1.0		92.2	1.3	95	96.7	1.8	++
36-3	100	42.4	9.3	43	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	84.6	6.2	85	99.5	0.5	++	16.0	6.9	17	94.3	2.6	**
	25	98.8	0.8	100	99.0	1.0		85.0	1.8	88	96.7	1.8	++
38-1	100	95.2	3.8	96	99.0	1.0		0.0	0.0	0	91.8	2.9	**
	50	99.2	0.4	100	99.5	0.5		84.0	3.8	89	94.3	2.6	++
	25	99.4	0.9	100	99.0	1.0		92.2	2.2	95	96.7	1.8	++
38-3	100	33.8	5.9	34	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	60.6	10.5	61	99.5	0.5	**	0.0	0.0	0	94.3	2.6	**
	25	77.4	6.3	78	99.0	1.0	**	0.0	0.0	0	96.7	1.8	**
39-1	100	88.8	4.1	90	99.0	1.0	++	0.0	0.0	0	91.8	2.9	++
	50	97.6	1.5	98	99.5	0.5		84.6	2.0	90	94.3	2.6	++
	25	98.4	0.6	99	99.0	1.0		93.0	1.6	96	96.7	1.8	+
40-2	100	9.0	2.6	9	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	37.0	10.7	37	99.5	0.5	**	0.0	0.0	0	94.3	2.6	**
	25	63.8	11.9	64	99.0	1.0	**	0.0	0.0	0	96.7	1.8	**

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
40-3	100	91.6	6.2	93	99.0	1.0	++	48.4	19.1	53	91.8	2.9	**
	50	98.4	1.1	99	99.5	0.5		85.0	2.9	90	94.3	2.6	++
	25	98.8	1.6	100	99.0	1.0		92.0	2.2	95	96.7	1.8	++
42-1	100	53.4	14.7	54	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	94.4	6.1	95	99.5	0.5	+	21.7	4.7	23	94.3	2.6	**
	25	98.2	2.2	99	99.0	1.0		89.8	0.8	93	96.7	1.8	++
42-3	100	55.2	12.3	56	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	88.6	6.3	89	99.5	0.5	++	0.0	0.0	0	94.3	2.6	**
	25	98.2	1.8	99	99.0	1.0		69.2	8.8	72	96.7	1.8	**
BA21 [6]	100	78.4	7.2	79	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	92.2	3.1	93	99.5	0.5	++	0.0	0.0	0	94.3	2.6	**
	25	99.4	0.9	100	99.0	1.0		90.5	5.3	94	96.7	1.8	++
43-3	100	17.4	7.4	18	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	43.0	8.5	43	99.5	0.5	**	0.0	0.0	0	94.3	2.6	**
	25	87.8	4.9	89	99.0	1.0	++	0.0	0.0	0	96.7	1.8	**
44-1	100	75.2	13.4	76	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	89.8	10.4	90	99.5	0.5	++	82.6	3.5	88	94.3	2.6	++
	25	100.0	0.0	101	99.0	1.0		92.6	4.7	96	96.7	1.8	+

Table 31. Continued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
44-2	100	78.0	10.0	79	99.0	1.0	**	66.2	6.7	72	91.8	2.9	**
	50	97.2	2.2	98	99.5	0.5		85.0	2.0	90	94.3	2.6	++
	25	98.4	0.9	99	99.0	1.0		92.0	2.1	95	96.7	1.8	++
46-1	100	97.8	1.6	99	99.0	1.0		42.0	16.7	46	91.8	2.9	**
	50	99.0	1.2	99	99.5	0.5		74.6	7.1	79	94.3	2.6	**
	25	98.0	1.6	99	99.0	1.0		90.8	2.5	94	96.7	1.8	++
46-3	100	86.6	5.1	87	99.0	1.0	++	0.0	0.0	0	91.8	2.9	**
	50	98.6	1.3	99	99.5	0.5		55.0	9.8	58	94.3	2.6	**
	25	97.0	2.4	98	99.0	1.0		87.6	2.2	91	96.7	1.8	++
46-4	100	80.4	8.8	81	99.0	1.0	*	0.0	0.0	0	91.8	2.9	**
	50	98.0	1.0	98	99.5	0.5		62.6	3.6	66	94.3	2.6	**
	25	98.0	1.6	99	99.0	1.0		92.8	1.9	96	96.7	1.8	++
47-3	100	79.4	4.8	80	99.0	1.0	**	0.0	0.0	0	91.8	2.9	**
	50	98.2	1.3	99	99.5	0.5		54.2	10.0	58	94.3	2.6	**
	25	98.8	1.3	100	99.0	1.0		90.2	1.9	93	96.7	1.8	++
47-4	100	97.0	1.2	98	99.0	1.0		46.8	16.7	51	91.8	2.9	**
	50	98.0	0.7	98	99.5	0.5		88.4	2.1	94	94.3	2.6	++
	25	99.4	0.9	100	99.0	1.0		88.2	4.6	91	96.7	1.8	++

Table 31. Coutiuued.

Designation[1]	WQAS [2]	Fertilization Test (% Fertilized)						Embryological Development Test (% Normal Development)					
		Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]	Mean	SD	% of REF [3]	REF [4] Mean	SD	Sig. [5]
BF21 [6]	100	86.0	9.8	87	99.0	1.0	++	12.6	12.2	14	91.8	2.9	**
	50	97.2	2.6	98	99.5	0.5		86.2	0.8	91	94.3	2.6	++
	25	98.4	1.1	99	99.0	1.0		92.8	3.9	96	96.7	1.8	+
11-6	100	98.8	0.8	100	99.0	1.0		0.0	0.0	0	91.8	2.9	**
	50	99.8	0.4	100	99.5	0.5		45.8	11.7	49	94.3	2.6	**
	25	99.4	0.6	100	99.0	1.0		91.4	5.2	95	96.7	1.8	++
BD22 [6]	100	95.8	3.1	97	99.0	1.0		89.6	3.3	98	91.8	2.9	
	50	98.0	0.7	98	99.5	0.5		91.6	2.1	97	94.3	2.6	+
	25	98.4	1.1	99	99.0	1.0		95.4	0.9	99	96.7	1.8	

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

[2] Percent of water quality adjusted sample.

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

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

[5] Significant difference from reference.

[6] Stations BF21, BD22, BB70, and BA21, located in strata 3, 11, 30 and 42, respectively.

Table 32. EC_{50} values of sediment porewater samples from the NOAA San Francisco Bay, California study assayed in the sea urchin fertilization and embryological development tests.

Designation[1]	Fertilization Test		Embryological Development Test	
	EC_{50}[2]	95% Confidence Limits	EC_{50}[2]	95% Confidence Limits
1-2	>100	-	>100	-
2-2	>100	-	>100	-
3-1	>100	-	54.34	49.61-59.51
3-3	>100	-	>100	-
4-2	>100	-	>100	-
BF21[4]	>100	-	72.92	70.13-75.81
5-1	>100	-	46.11	42.33-50.23
5-2	>100	-	>100	-
5-5	>100	-	51.50	46.93-56.52
6-1	>100	-	38.61	33.92-43.95
6-4	>100	-	>100	-
7-1	>100	-	55.82	50.92-61.20
7-4	64.79	54.18-77.48	48.43	43.38-54.07
7-6	>100	-	100.00	nr[3]
8-1	>100	-	81.61	75.19-88.58
8-3	>100	-	>100	-
9-2	>100	-	56.80	52.26-61.75
10-1	91.7	71.10-118.26	31.10	nr[3]
10-3	85.42	66.43-109.84	31.98	30.42-33.62
11-1	>100	-	>100	-
11-3	>100	-	>100	-
BD22[4]	>100	-	>100	-
11-6	>100	-	49.65	46.17-53.41

Table 32. Continued.

Designation[1]	Fertilization Test		Embryological Development Test	
	EC_{50}[2]	95% Confidence Limits	EC_{50}[2]	95% Confidence Limits
12-1	>100	-	63.28	57.53-69.59
13-1	>100	-	53.33	46.68-60.92
14-1	>100	-	41.81	38.26-45.70
15-1	>100	-	>100	-
15-3	>100	-	>100	-
16-1	>100	-	>100	-
17-1	>100	-	30.13	27.36-33.18
17-2	>100	-	87.06	75.73-100.08
18-1	>100	-	>100	-
19-2	>100	-	81.14	75.44-87.26
19-3	74.58	62.01-89.71	<25	-
20-1	61.47	48.23-78.36	<25	-
20-5	>100	-	83.20	75.01-92.29
20-6	>100	-	>100	-
21-1	>100	-	>100	-
21-3	>100	-	<25	-
22-1	>100	-	69.26	67.64-70.91
22-3	>100	-	>100	-
22-6	>100	-	>100	-
23-2	>100	-	>100	-
24-2	>100	-	64.62	61.67-67.70
25-1	>100	-	60.82	57.14-64.73
25-3	>100	-	29.34	nr[3]
26-1	>100	-	63.44	59.63-67.49

Table 32. Continued.

Designation[1]	Fertilization Test		Embryological Development Test	
	EC_{50}[2]	95% Confidence Limits	EC_{50}[2]	95% Confidence Limits
26-2	>100	-	76.65	72.84-80.67
27-1	>100	-	41.51	38.79-44.42
28-1	>100	-	>100	-
28-4	47.00	42.30-52.23	<25	-
28-5	>100	-	>100	-
29-2	>100	-	92.72	82.93-103.65
30-1	>100	-	31.69	nr[3]
30-3	>100	-	>100	-
BB70[4]	>100	-	39.18	37.19-41.28
31-2	62.09	54.39-70.89	<25	-
31-4	>100	-	63.20	59.11-67.58
31-6	77.22	71.83-83.02	<25	-
32-2	>100	-	41.71	38.62-45.05
32-3	>100	-	35.36	34.25-36.49
32-6	>100	-	68.02	65.72-70.39
33-5	97.47	76.11-124.81	<25	-
34-1	>100	-	<25	-
34-3	>100	-	37.70	35.45-40.08
35-2	98.44	84.39-114.82	33.10	nr[3]
35-3	>100	-	54.71	50.73-59.01
36-1	>100	-	64.08	60.21-68.21
36-2	>100	-	80.86	75.11-87.06
36-3	89.33	78.06-102.23	37.42	35.26-39.72
38-1	>100	-	66.59	63.91-69.38

Table 32. Continued.

Designation[1]	Fertilization Test		Embryological Development Test	
	EC_{50}[2]	95% Confidence Limits	EC_{50}[2]	95% Confidence Limits
38-3	67.86	49.37-93.27	<25	-
39-1	>100	-	66.90	64.43-69.46
40-2	36.73	30.88-43.70	<25	-
40-3	>100	-	>100	-
42-1	>100	-	40.88	38.35-43.57
42-3	>100	-	31.50	nr[3]
BA21 [4]	>100	-	34.02	nr[3]
43-3	48.25	43.00-54.13	<25	-
44-1	>100	-	65.98	63.29-68.78
44-2	>100	-	>100	-
46-1	>100	-	90.82	76.91-107.25
46-3	>100	-	52.65	48.73-56.89
46-4	>100	-	56.64	53.10-60.43
47-3	>100	-	52.48	48.75-56.50
47-4	>100	-	>100	-

[1] Designation refers to the zone, strata, and sample site number, respectively.

[2] Percent of water quality adjusted porewater sample.

[3] 95% confidence limits not reliable.

[4] Stations BF21, BD22, BB70 and BA21, located in strata 4, 11, 30 and 42, respectively.

Table 33. Dissolved organic carbon (DOC) measurements (mg/L) of BEST/EMAP porewater samples collected from sediments from Puget Sound, Washington.

Sample ID	Date Analyzed	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
E1	12-Apr-01	1	2	16.47	16.25	15.23	15.99	4.05
E4	13-Apr-01	1	3	4.24	4.22	4.25	4.24	0.37
E5	12-Apr-01	1	3	7.43	7.06	6.89	7.13	3.67
E6	12-Apr-01	1	4	8.15	7.69	7.62	7.82	3.49
E7	12-Apr-01	1	5	8.29	7.56	7.08	7.64	7.65
E8	12-Apr-01	1	6	6.08	5.61	5.34	5.68	6.16
E9	12-Apr-01	1	7	6.04	5.71	5.63	5.79	3.47
E13	12-Apr-01	1	8	14.54	13.49	13.54	13.86	4.14
E14	12-Apr-01	1	9	5.51	5.16	5.07	5.25	4.17
E15	12-Apr-01	1	10	14.59	13.45	12.59	13.54	7.2
E17	12-Apr-01	1	11	6.07	5.83	5.74	5.88	2.71
E19	12-Apr-01	1	13	6.94	6.61	6.88	6.81	2.43
E21	13-Apr-01	1	14	10.67	10.82	10.92	10.80	1.11
E22	13-Apr-01	1	15	22.71	22.60	23.32	22.89	1.62

Table 33. Continued.

Sample ID	Date Analyzed	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
E23	13-Apr-01	1	16	7.84	8.17	7.98	8.00	1.93
E24	13-Apr-01	1	17	16.61	15.99	15.43	16.01	3.62
E25	13-Apr-01	1	19	6.45	5.75	5.64	5.95	6.95
E29	13-Apr-01	1	20	9.40	8.44	8.59	8.81	5.64
E30	13-Apr-01	1	21	6.78	6.13	6.19	6.36	5.3

Table 34. Dissolved organic carbon (DOC) measurements (mg/L) of BEST/EMAP porewater samples collected from sediments from the Columbia River, Oregon.

Sample Id	Date Analyzed	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
OR2000-0031	16-Oct-00	1	2	2.78	2.91	2.93	2.88	2.51
OR2000-0032	16-Oct-00	1	3	2.64	2.61	2.49	2.58	2.69
OR2000-0033	16-Oct-00	1	4	2.46	2.36	2.37	2.4	2.03
OR2000-0038	16-Oct-00	1	5	2.52	2.45	2.36	2.44	2.82
OR2000-0044	16-Oct-00	1	6	1.87	1.93	1.91	1.9	1.16
OR2000-0045	17-Oct-00	1	7	1.32	1.3	1.32	1.31	0.56
OR2000-0046	17-Oct-00	1	9	2.74	2.7	2.72	2.72	0.66
OR2000-0047	17-Oct-00	1	10	15.24	14.96	15.11	15.11	0.9
OR2000-0048	17-Oct-00	1	11	2.48	2.31	2.31	2.37	3.58
OR2000-0050	17-Oct-00	1	12	4.76	4.71	4.76	4.75	0.54
OR2000-0034	17-Oct-00	2	3	11.51	11.42	11.35	11.43	0.68
OR2000-0035	17-Oct-00	2	4	8.27	8.21	8.25	8.24	0.36
OR2000-0036	17-Oct-00	2	6	12.6	12.8	12.46	12.62	1.3
OR2000-0037	17-Oct-00	2	7	2.41	2.39	2.34	2.38	1.31

Table 34. Continued.

Sample Id	Date Analyzed	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
OR2000-0039	17-Oct-00	2	9	1.78	1.86	1.77	1.8	2.1
OR2000-0040	17-Oct-00	2	11	17.56	17.5	18.44	17.83	2.87
OR2000-0041	17-Oct-00	2	12	9.37	8.94	8.8	9.04	3.08
OR2000-0042	17-Oct-00	2	13	2.72	2.26	2.22	2.4	9.47
OR2000-0043	18-Oct-00	2	14	3.16	2.93	3.03	3.04	3.18

Table 35. Dissolved organic carbon (DOC) measurements (mg/L) of BEST/EMAP porewater samples collected from sediments from San Francisco Bay, California.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
CA2000-0038	6-Apr-01	1	6	30.77	30.16	29.49	30.14	2.11
CA2000-0039	6-Apr-01	1	7	11.87	11.65	11.62	11.71	1.12
CA2000-0040	6-Apr-01	1	8	8.68	8.78	8.9	8.79	1.19
CA2000-0041	6-Apr-01	1	9	6.57	6.57	6.67	6.6	0.85
CA2000-0042	6-Apr-01	1	10	6.36	6.28	6.23	6.29	1.02
CA2000-0043	6-Apr-01	1	11	6.26	6.09	6.22	6.19	1.37
CA2000-0044	6-Apr-01	1	13	6.48	6.3	6.41	6.4	1.3
CA2000-0045	6-Apr-01	1	14	9.81	9.64	9.8	9.75	0.94
CA2000-0046	6-Apr-01	1	16	7.5	7.54	7.35	7.46	1.31
CA2000-0047	6-Apr-01	1	17	5.44	5.51	5.36	5.44	1.29
CA2000-0048	6-Apr-01	1	18	5.66	5.67	5.64	5.66	0.24
CA2000-0049	6-Apr-01	1	19	8.36	8.27	8.35	8.33	0.58
CA2000-0050	6-Apr-01	1	20	18.93	18.96	18.94	18.94	0.06

Table 36. Dissolved organic carbon (DOC) measurements (mg/L) of NOAA porewater samples collected from sediments from San Francisco Bay, California.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
1-2	18-Jul-01	1	4	2.60	2.56	2.63	2.60	1.09
2-2	18-Jul-01	1	8	1.28	1.22	1.25	1.25	1.54
3-1	30-Mar-01	2	2	16.47	16.62	16.58	16.56	0.47
3-2	30-Mar-01	2	3	4.74	4.63	4.67	4.68	1.15
4-2	30-Mar-01	2	4	5.64	5.59	5.53	5.59	0.93
5-1	30-Mar-01	2	5	11.14	10.94	11.12	11.07	0.94
5-2	30-Mar-01	2	6	7.11	6.93	7.07	7.04	1.24
5-5	30-Mar-01	2	7	8.07	8.07	8.04	8.06	0.19
6-1	30-Mar-01	2	8	8.42	8.43	8.68	8.51	1.64
6-4	30-Mar-01	2	9	5.68	5.57	5.56	5.60	1.09
7-1	30-Mar-01	2	10	11.32	11.44	11.45	11.41	0.60
7-4	30-Mar-01	2	11	11.28	11.01	11.31	11.20	1.46
7-6	30-Mar-01	2	12	7.42	7.41	7.53	7.45	0.83
8-1	30-Mar-01	2	13	6.05	6.02	5.92	6.00	1.08

Table 36. Continued.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
8-3	30-Mar-01	2	15	6.00	6.038	6.05	6.03	0.36
9-2	30-Mar-01	2	16	8.44	8.24	8.32	8.33	1.15
10-1	31-Mar-01	2	18	11.26	11.12	11.38	11.25	1.09
10-3	31-Mar-01	2	19	12.88	12.57	12.65	12.70	1.20
11-1	31-Mar-01	2	20	6.10	6.08	6.06	6.08	0.37
11-3	31-Mar-01	2	21	5.82	5.74	5.76	5.77	0.69
11-6	6-Apr-01	1	5	5.74	5.74	5.86	5.78	1.16
12-1	31-Mar-01	2	22	5.91	5.86	5.90	5.89	0.45
13-1	31-Mar-01	2	23	6.46	6.25	6.45	6.38	1.79
14-1	31-Mar-01	2	24	9.42	9.27	9.26	9.32	0.93
15-1	31-Mar-01	2	25	8.46	8.26	8.42	8.38	1.18
15-3	31-Mar-01	2	27	6.54	6.35	6.48	6.46	1.41
16-1	31-Mar-01	2	28	8.19	8.05	8.11	8.12	0.82
17-1	31-Mar-01	2	29	27.87	27.46	28.24	27.86	1.37
17-2	31-Mar-01	2	30	6.54	5.84	5.94	6.11	5.89

Table 36. Continued.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
18-1	2-Apr-01	3	2	10.64	10.82	10.81	10.76	0.91
19-2	2-Apr-01	3	3	8.54	8.61	8.54	8.56	0.48
19-3	2-Apr-01	3	4	10.05	7.20	7.33	8.20	18.76
20-1	2-Apr-01	3	5	50.00	50.22	51.33	50.51	1.41
20-5	2-Apr-01	3	6	9.49	8.78	8.57	8.95	5.16
20-6	2-Apr-01	3	7	10.32	9.64	10.28	10.08	3.62
21-1	2-Apr-01	3	8	12.37	11.55	11.73	11.88	3.49
21-3	2-Apr-01	3	9	10.27	8.60	8.55	9.14	10.29
22-1	2-Apr-01	3	10	18.32	17.84	17.72	17.96	1.73
22-3	2-Apr-01	3	11	11.78	10.44	10.82	11.01	6.05
22-6	2-Apr-01	3	12	4.88	4.12	4.05	4.35	9.75
23-2	2-Apr-01	3	13	9.33	9.04	9.39	9.25	1.93
24-2	2-Apr-01	3	14	8.96	8.38	8.40	8.58	3.68
25-1	3-Apr-01	3	15	9.76	8.90	9.03	9.23	4.80
25-3	3-Apr-01	3	16	8.23	7.45	7.14	7.61	7.10

Table 36. Continued.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
26-1	3-Apr-01	3	18	9.80	8.81	9.06	9.22	5.39
26-2	3-Apr-01	3	19	13.60	12.08	13.18	12.95	5.89
27-1	4-Apr-01	4	2	19.42	19.60	19.83	19.62	1.05
28-1	3-Apr-01	3	21	2.64	2.15	2.07	2.29	11.63
28-4	4-Apr-01	4	3	8.98	8.83	9.08	8.94	1.35
28-5	4-Apr-01	4	4	10.22	10.30	10.35	10.29	0.60
29-2	4-Apr-01	4	5	7.82	7.81	7.76	7.80	0.36
30-1	3-Apr-01	3	26	7.04	6.01	6.19	6.41	8.13
30-3	4-Apr-01	4	6	6.18	6.11	6.20	6.16	0.69
31-2	3-Apr-01	3	28	7.06	7.02	7.06	7.05	0.36
31-4	3-Apr-01	3	30	6.34	6.32	6.66	6.44	2.80
31-6	3-Apr-01	3	31	9.26	9.06	9.18	9.17	1.07
32-2	4-Apr-01	4	7	8.53	8.29	8.52	8.45	1.54
32-3	4-Apr-01	4	8	8.12	8.14	8.31	8.19	1.20
32-6	4-Apr-01	4	9	6.21	5.70	5.89	5.93	4.08

Table 36. Continued.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
33-5	4-Apr-01	4	10	7.71	7.84	7.85	7.80	0.97
34-1	4-Apr-01	4	11	30.00	30.10	29.77	29.96	0.56
34-3	4-Apr-01	4	12	14.66	13.55	13.95	14.05	3.88
35-2	4-Apr-01	4	14	25.42	24.13	23.58	24.38	3.83
35-3	4-Apr-01	4	15	17.33	15.49	15.38	16.07	6.67
36-1	5-Apr-01	4	16	7.71	7.24	7.15	7.37	3.91
36-3	6-Apr-01	1	2	17.49	17.53	17.41	17.48	0.33
38-1	5-Apr-01	4	17	7.68	6.73	6.72	7.04	7.49
38-3	5-Apr-01	4	18	13.34	12.91	13.04	13.10	1.64
39-1	6-Apr-01	1	3	19.54	19.01	19.19	19.25	1.38
40-2	5-Apr-01	4	19	13.85	13.82	14.56	14.08	2.89
40-3	5-Apr-01	4	20	6.10	6.13	6.02	6.08	0.88
42-1	5-Apr-01	4	21	9.99	9.92	10.04	9.98	0.57
42-3	5-Apr-01	4	23	11.53	11.12	10.98	11.21	2.50
43-3	5-Apr-01	4	24	20.26	20.90	19.75	20.30	2.80

Table 36. Continued.

Sample ID	Date of Analysis	Batch #	Run #	Rep 1	Rep 2	Rep 3	Mean	Relative SD
44-1	5-Apr-01	4	25	8.10	8.17	8.36	8.21	1.58
44-2	6-Apr-01	1	4	5.51	5.40	5.37	5.42	1.35
BA-21	5-Apr-01	4	27	20.14	20.14	20.52	20.27	1.06
BB-70	5-Apr-01	4	28	36.95	36.72	37.06	36.91	0.46
46-1	5-Apr-01	5	2	7.54	7.34	7.43	7.44	1.28
46-3	5-Apr-01	5	3	6.87	6.63	6.79	6.76	1.71
46-4	5-Apr-01	5	4	13.77	13.44	13.50	13.57	1.25
47-3	5-Apr-01	5	5	9.32	9.16	9.15	9.21	1.03
47-4	5-Apr-01	5	6	7.50	7.33	7.42	7.42	1.09
BD-22	5-Apr-01	5	8	5.33	5.25	5.36	5.32	1.01
BE-21	5-Apr-01	5	9	7.55	7.41	7.67	7.54	1.69

Table 37. Dissolved organic carbon (DOC) concentrations (mg/L) of quality assurance samples run in conjunction with pore water samples from all the BEST/EMAP study sites.

Sample ID	Date of Analysis	Associated Location	Batch #	Rep 1	Rep 2	Rep 3	Mean	Relative SD	Evaluation Criteria
Procedure Blank -									
Blank	12-Apr-01	WA	1	0.21	0.22	0.22	0.22	0.92	-
Blank	16-Oct-00	OR	1	0.02	0.02	0.02	0.02	1.74	-
Blank	17-Oct-00	OR	2	0.03	0.03	0.02	0.03	3.84	-
Blank	6-Apr-01	CA	1	0.08	0.07	0.06	0.07	3.29	-
Duplicates-									**%Difference [1]**
E17	12-Apr-01	WA	1	5.87	5.83	5.75	5.82	1.01	1.05
E30	13-Apr-01	WA	1	6.20	6.23	6.42	6.28	1.77	1.28
OR2000-0050	17-Oct-00	OR	1	4.77	4.42	4.73	4.64	3.83	2.24
OR2000-0039	17-Oct-00	OR	2	1.79	1.80	1.72	1.77	2.05	1.80
CA2000-0043	6-Apr-01	CA	1	6.07	6.02	5.94	6.01	1.00	2.85
Spiked Duplicates-									**% Recovery**
E24	13-Apr-01	WA	1	20.37	20.09	19.89	20.11	1.17	95.8
OR2000-0035	17-Oct-00	OR	2	12.60	12.80	12.46	12.62	1.30	95.3

Table 37. Continued.

Sample ID	Date of Analysis	Associated Location	Batch #	Rep 1	Rep 2	Rep 3	Mean	Relative SD	Evaluation Criteria
CA2000-0045	6-Apr-01	CA	1	14.09	14.08	14.22	14.13	0.54	95.8
Control Standard-									**% Recovery**
5 ppm chk std	17-Oct-00	OR	2	4.97	5.11	5.10	5.06	1.43	101.3

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

Table 38. Dissolved organic carbon (DOC) concentrations (mg/L) of quality assurance samples run in conjunction with NOAA porewater samples from San Francisco Bay, California.

Sample ID	Date of Analysis	Batch #	Rep 1	Rep 2	Rep 3	Mean	Relative SD	Evaluation Criteria
Procedure Blank -								
Blank	18-Jul-00	1	0.02	0.11	0.08	0.07	13.28	-
Blank	30-Mar-01	2	0.06	0.14	0.13	0.11	8.19	-
Blank	2-Apr-01	3	0.14	0.10	0.12	0.12	3.69	-
Blank	4-Apr-01	4	0.12	0.15	0.11	0.13	4.46	-
Blank	5-Apr-01	5	0.29	0.16	0.21	0.22	11.73	-
Duplicates-								**%Difference [1]**
8-1	30-Mar-01	2	5.69	5.72	5.66	5.69	0.52	5.1
15-1	31-Mar-01	2	8.55	8.27	8.58	8.47	1.93	8.5
25-3	2-Apr-01	3	8.35	7.06	7.20	7.54	8.96	0.9
31-6	3-Apr-01	3	9.19	9.35	9.06	9.20	1.51	0.4
42-1	4-Apr-01	4	10.06	9.74	9.77	9.86	1.69	1.2
44-1	4-Apr-01	4	8.14	7.93	8.12	8.06	1.36	1.8
47-4	5-Apr-01	5	7.56	7.43	7.24	7.41	2.12	0.1

Table 38. Continued.

Sample ID	Date of Analysis	Batch #	Rep 1	Rep 2	Rep 3	Mean	Relative SD	Evaluation Criteria
Spiked Duplicates								**% Recovery**
9-2	30-Mar-01	2	12.65	12.78	12.86	12.76	0.81	95.7
31-2	3-Apr-01	3	12.41	11.80	11.01	11.74	5.76	97.4
34-3	4-Apr-01	4	18.88	18.68	18.21	18.59	1.81	97.6
Laboratory Control Samples-								**% Recovery**
check	18-Jul-00	1	5.04	4.89	5.03	4.99	1.56	99.8
check	3-Apr-01	3	9.35	9.73	9.68	9.59	2.14	95.9
check	5-Apr-01	5	23.48	24.36	24.57	24.14	2.36	96.5

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

FIGURES 1-12

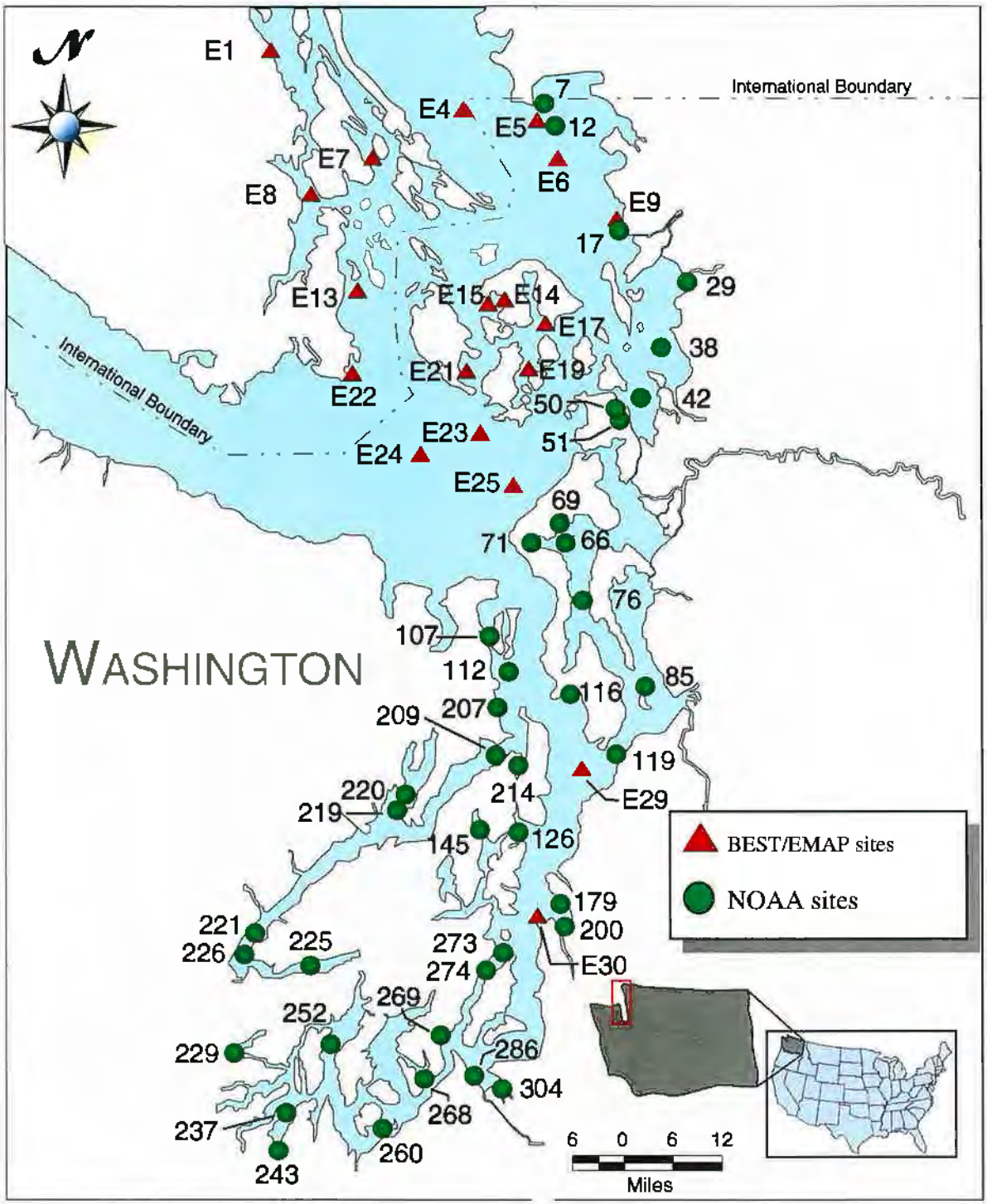

Figure 1. Location of BEST/EMAP stations and historically sampled NOAA sites in Puget Sound, Washington for sea urchin toxicity tests.

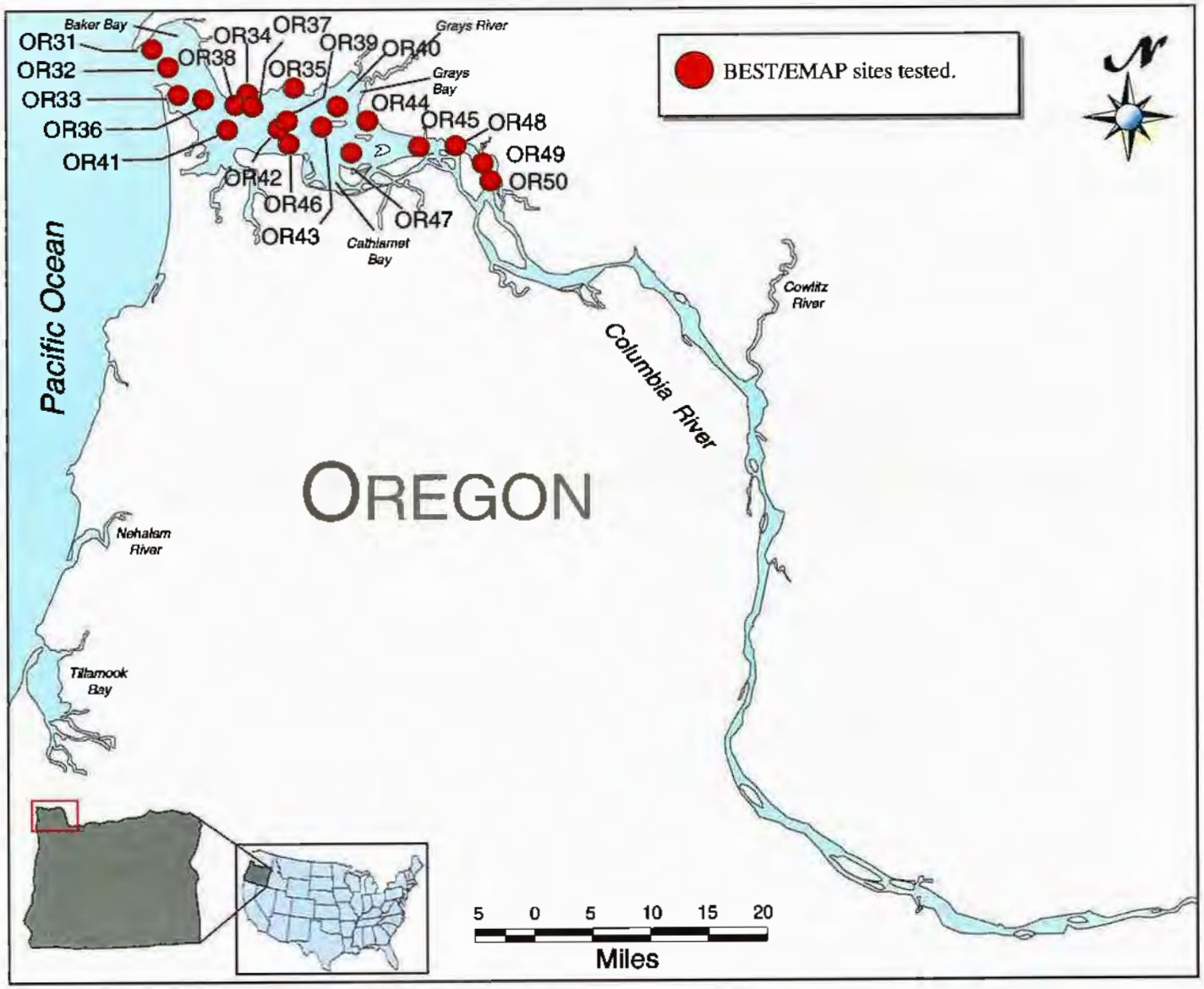

Figure 2. Location of BEST/EMAP stations sampled along the Columbia River estuary for sea urchin toxicity tests.

Figure 3. Location of BEST/EMAP stations sampled in San Francisco Bay, California, for sea urchin sediment porewater toxicity tests.

Figure 4. Location of NOAA stations sampled in San Francisco Bay, California for sea urchin porewater toxicity tests.

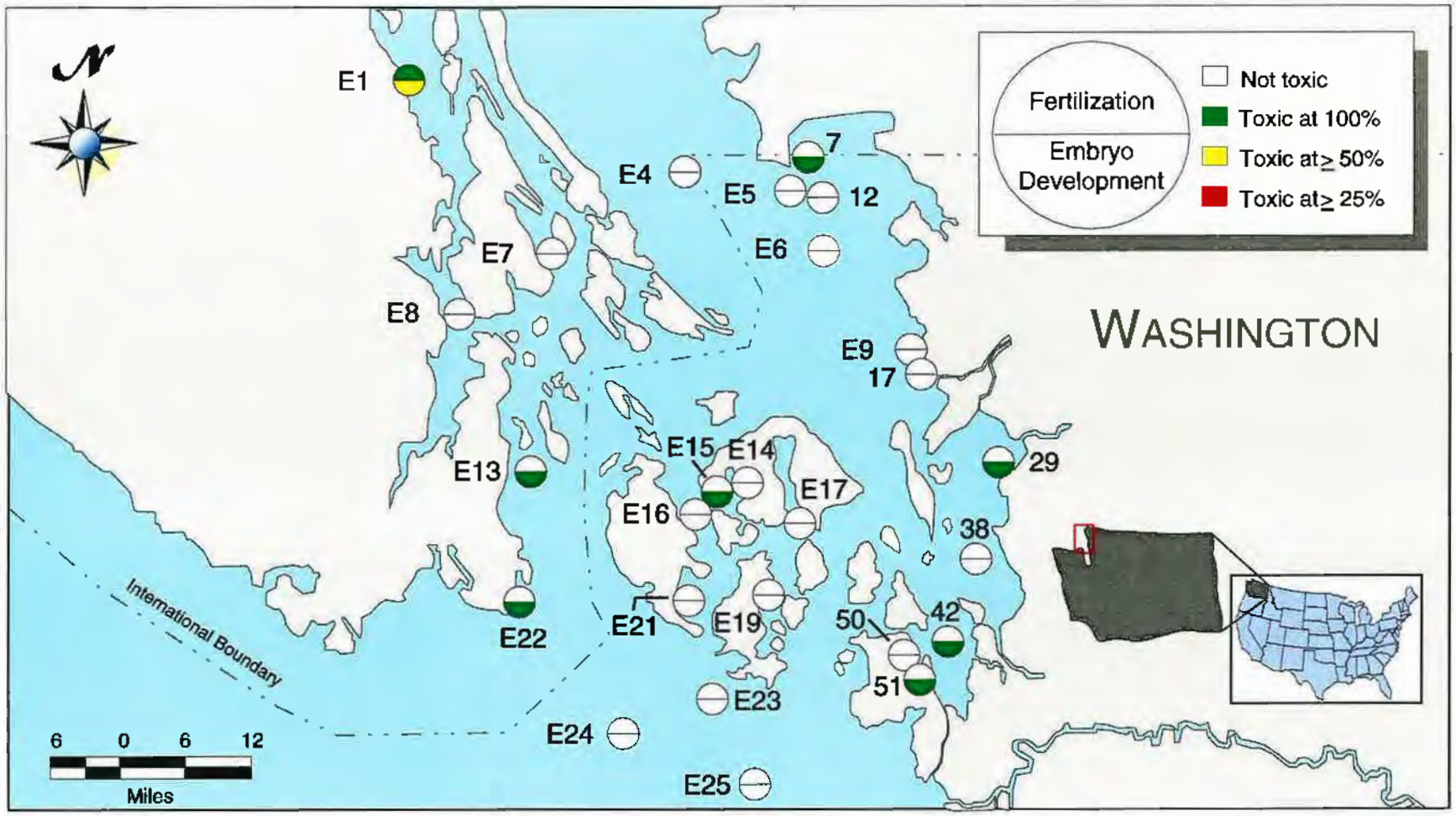

Figure 5. Sea urchin toxicity test results for BEST/EMAP and archived NOAA samples in northern Puget Sound, Washington. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization and embryological development assays (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

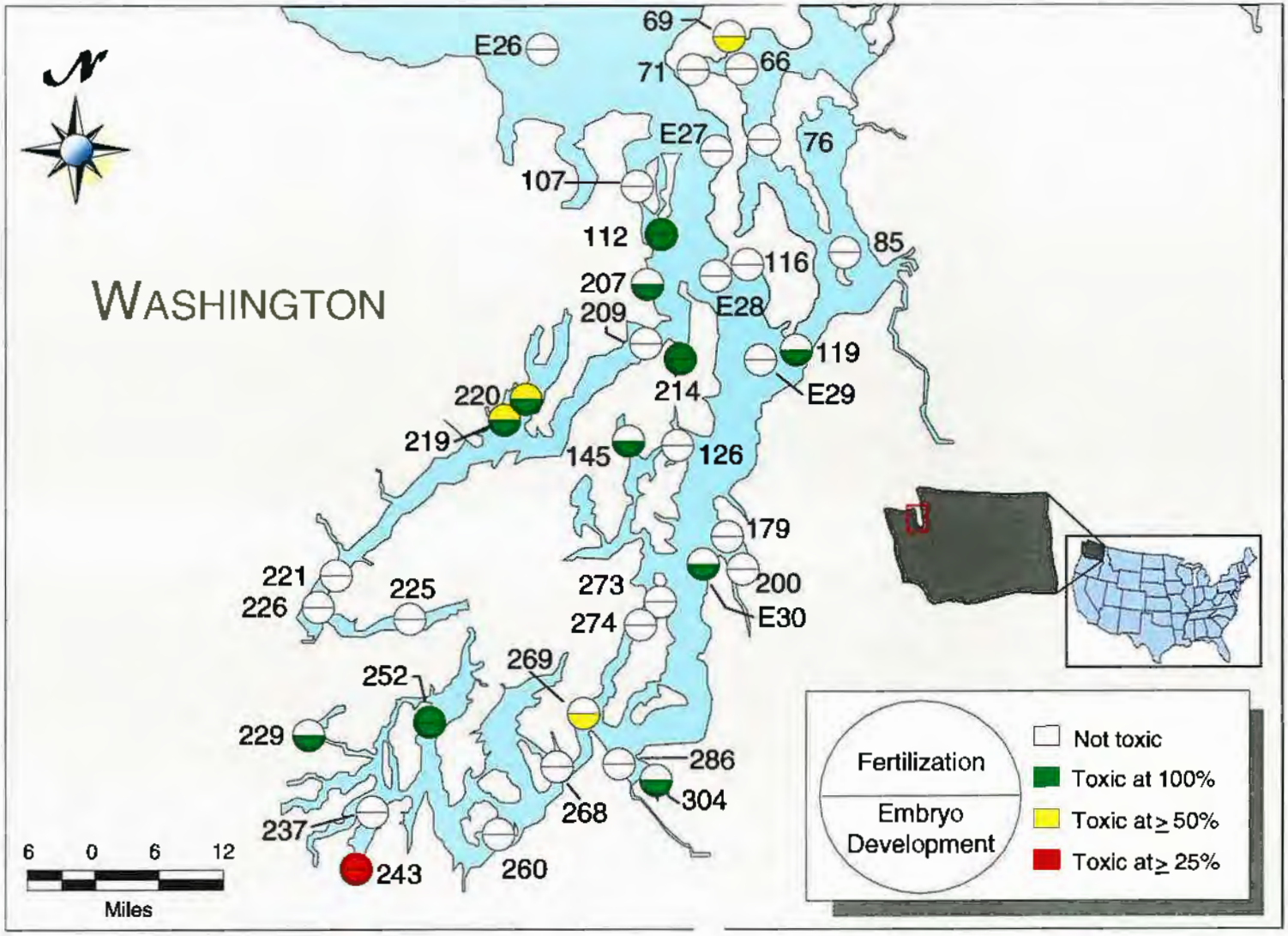

Figure 6. Toxicity test results from BEST/EMAP and archived NOAA samples collected in southern Puget Sound, Washington. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization and embryological development assays (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

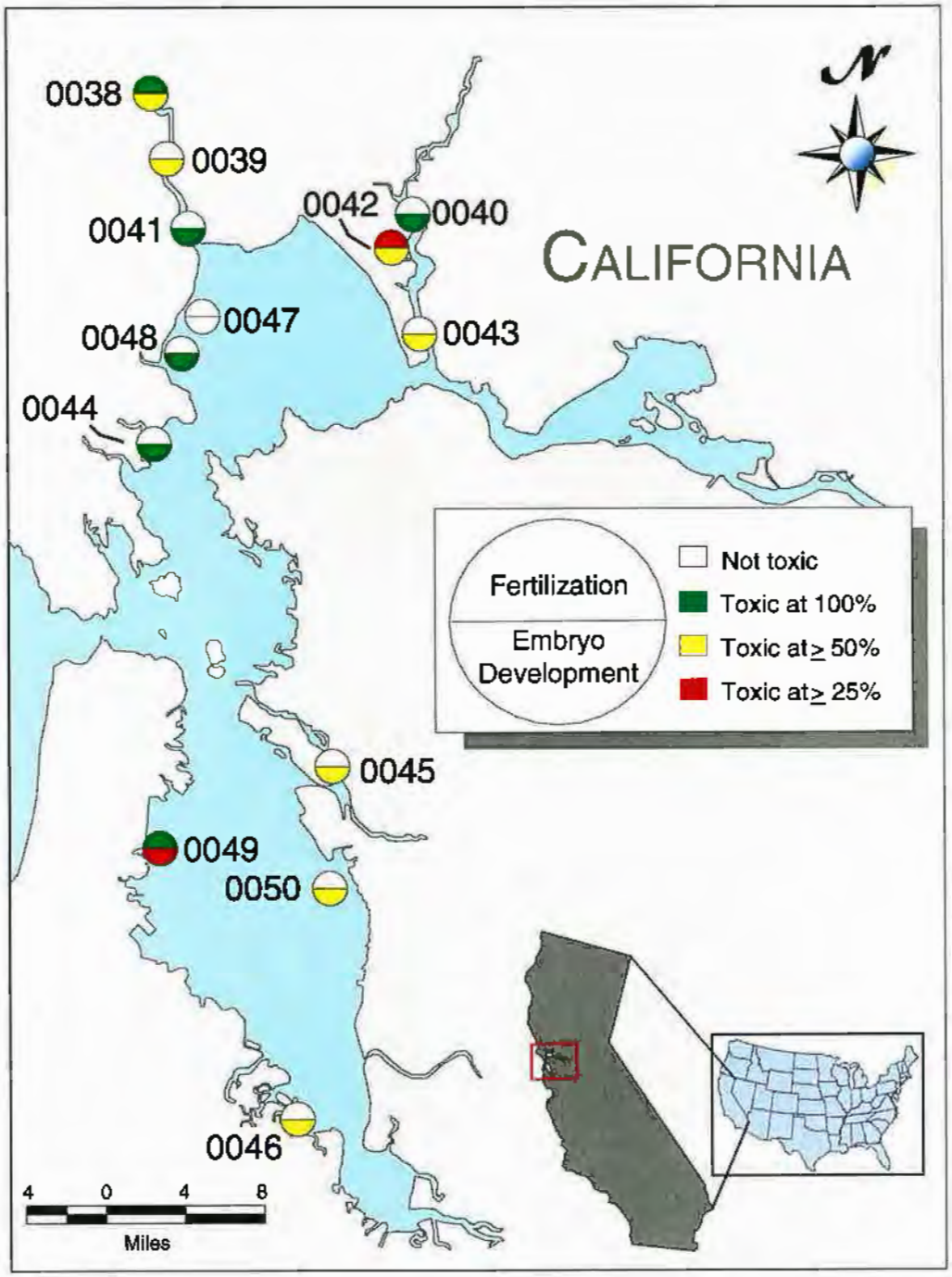

Figure 7. Sea urchin toxicity test results for BEST/EMAP sites in San Francisco Bay, California. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization and embryological development assays (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

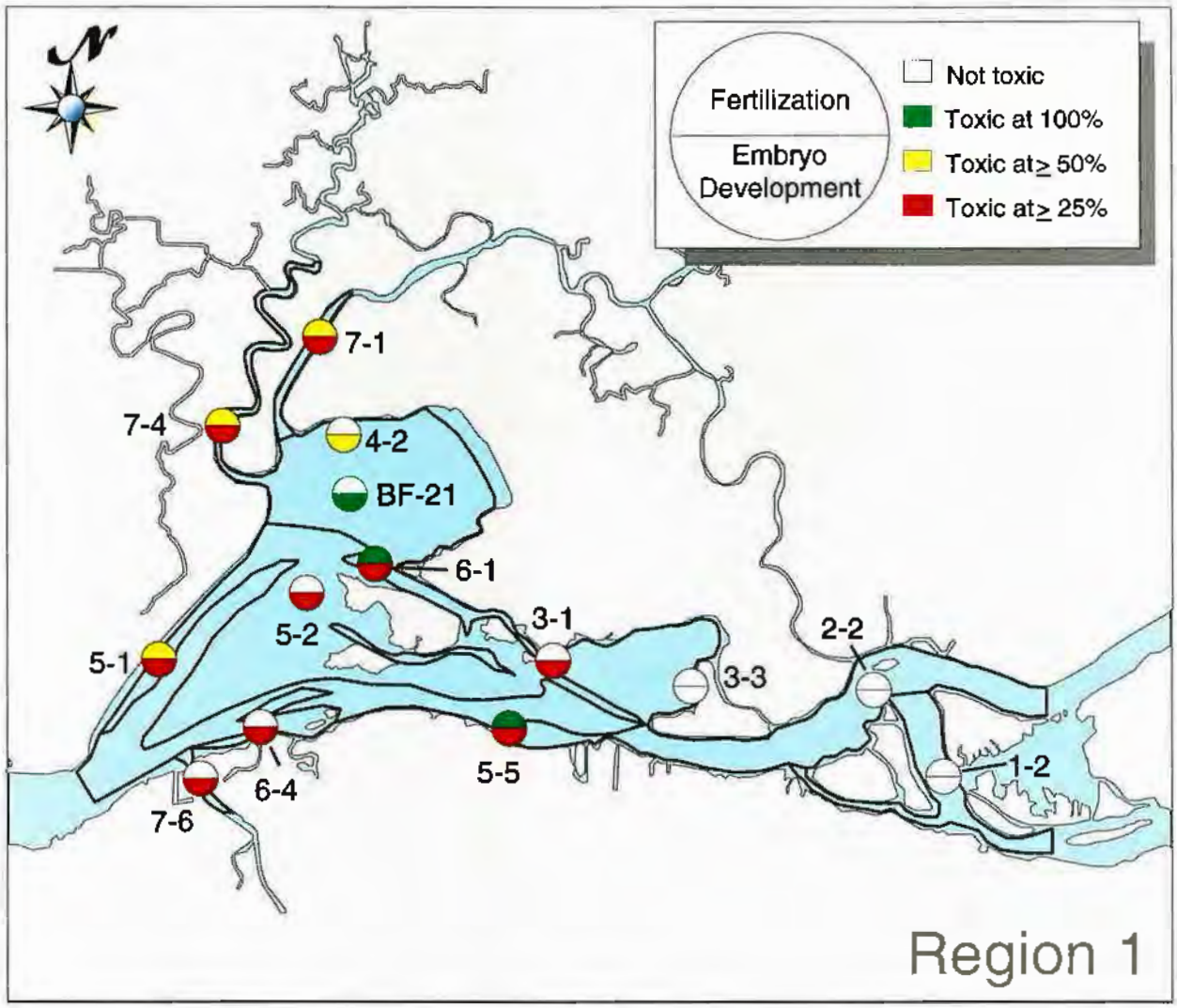

Figure 8. Sea urchin toxicity test results for NOAA sites in strata 1 through 7. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay and embryological development assay (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

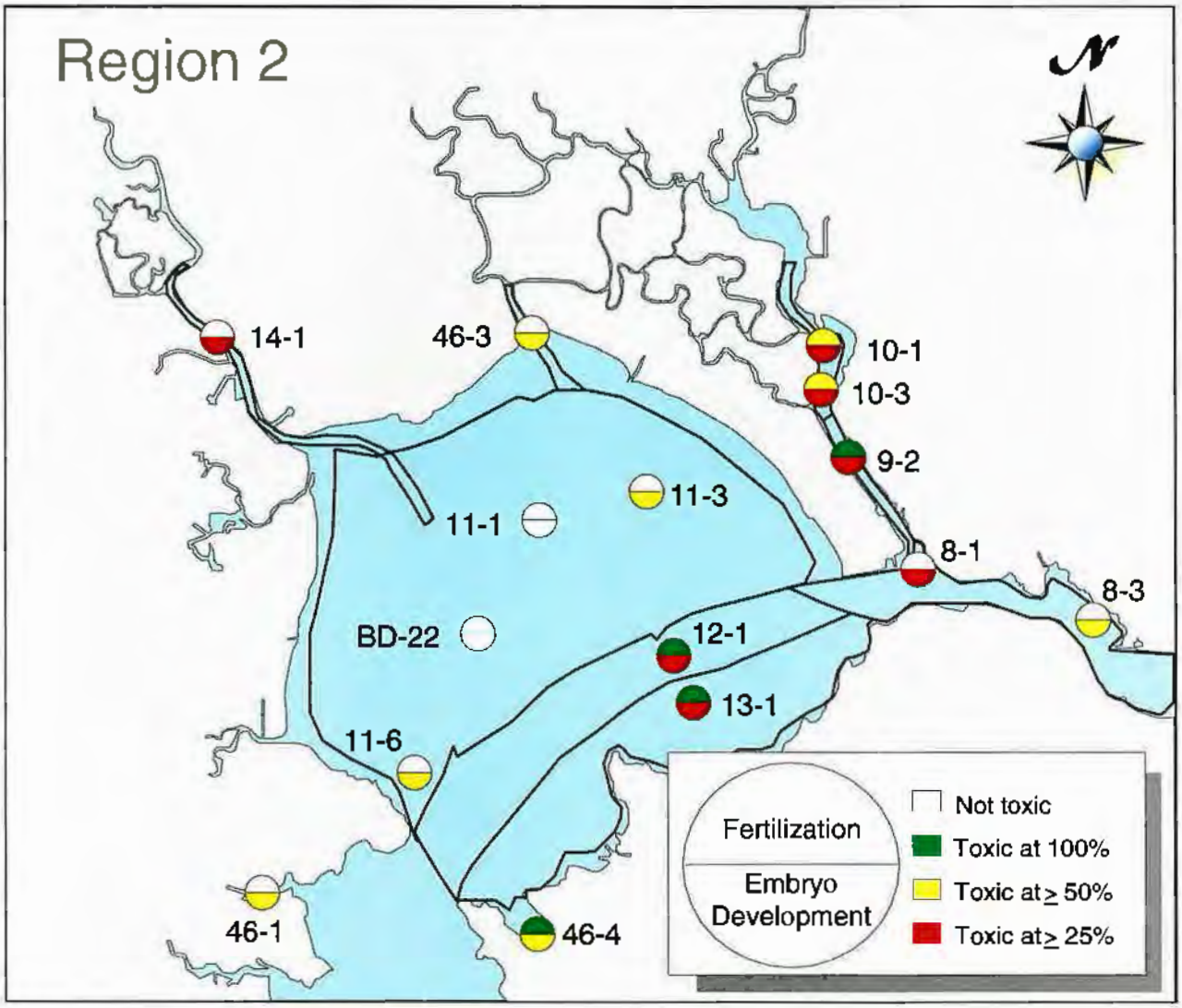

Figure 9. Sea urchin toxicity test results for NOAA sites in strata 8 through 14, and 46. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay and embryological development assay (Dunnett's *t*-test $\alpha \leq 0.05$ aud detectable significance criteria applied).

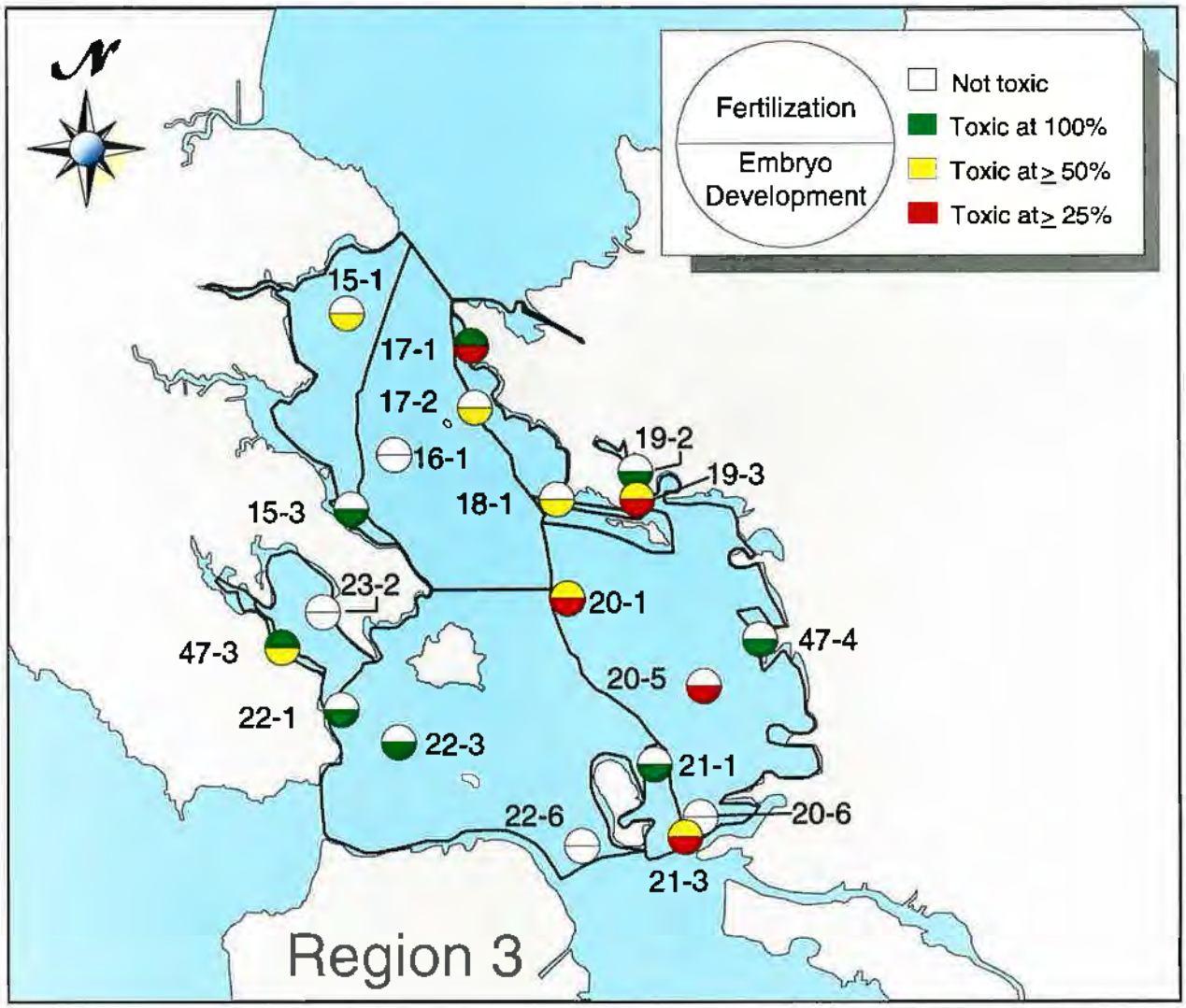

Figure 10. Sea urchin toxicity test results for NOAA sites in strata 15 through 23, and 47. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay and embryological development assay (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

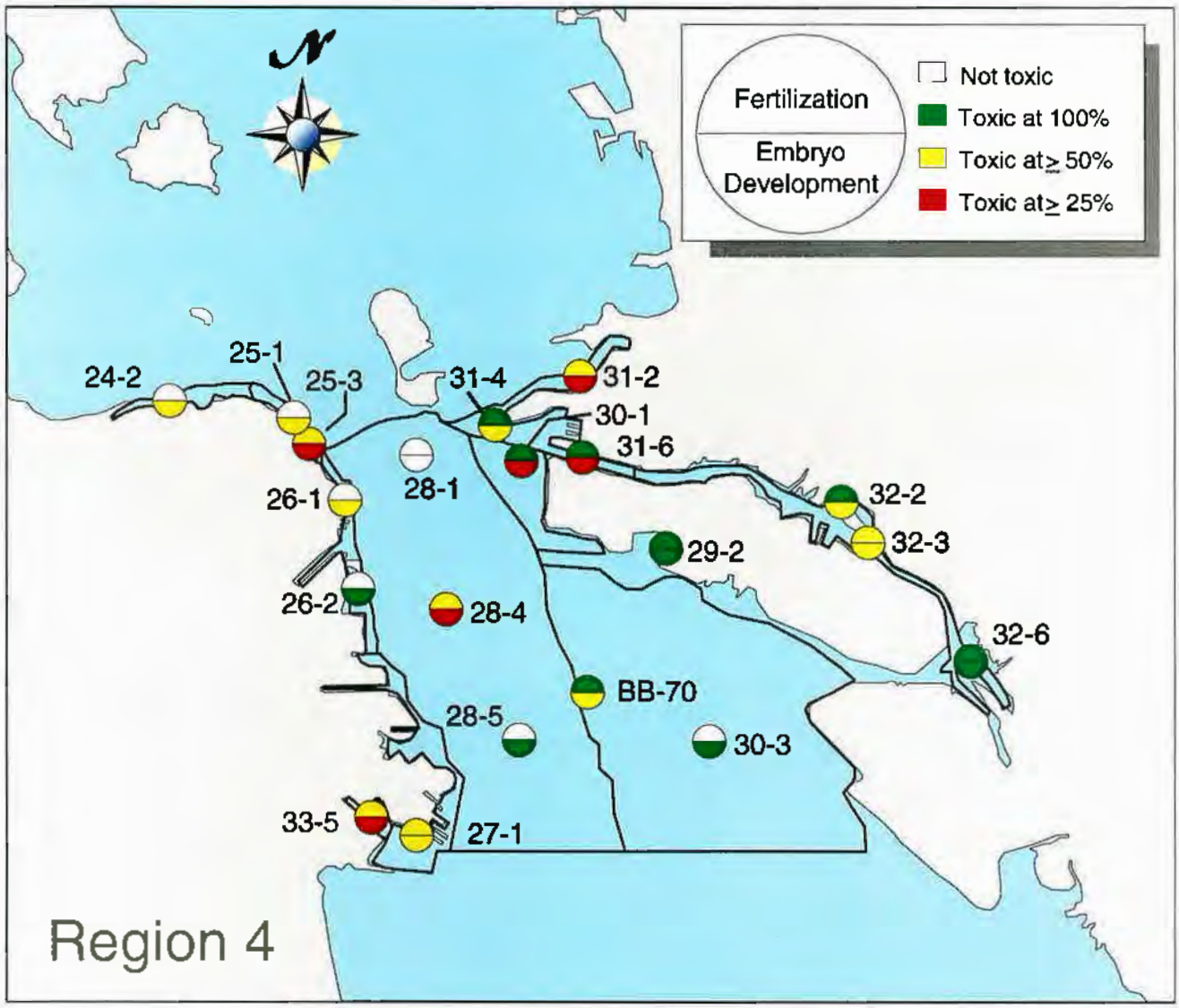

Figure 11. Sea urchin toxicity test results for NOAA sites in strata 24 through 33. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay and embryological development assay (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

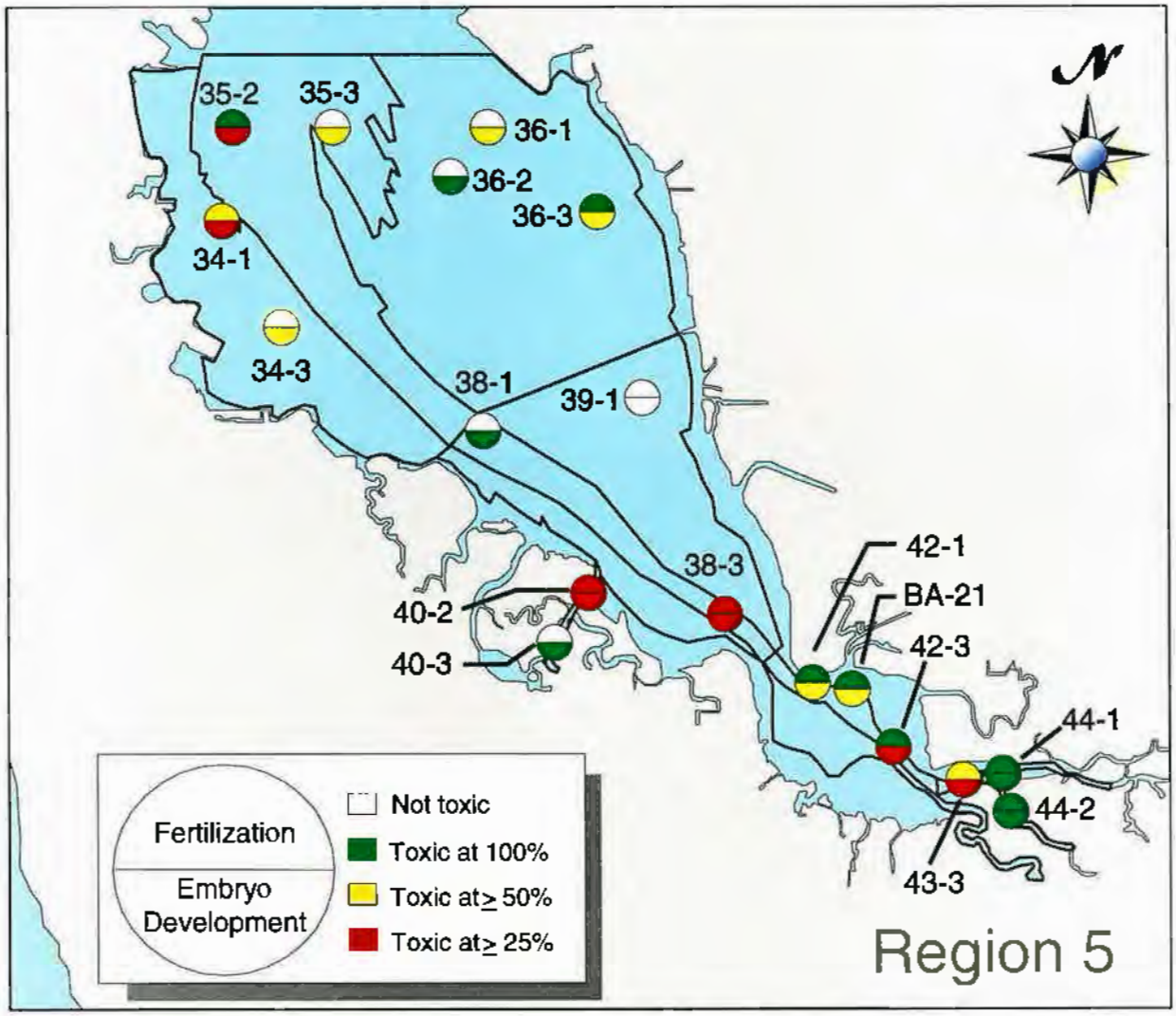

Figure 12. Sea urchin toxicity test results for NOAA sites in strata 34 through 44. Color differentiation of symbol indicates those stations that were significantly different than the reference in the sea urchin (*Arbacia punctulata*) fertilization assay and embryological development assay (Dunnett's *t*-test $\alpha \leq 0.05$ and detectable significance criteria applied).

ATTACHMENTS 1-4

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.

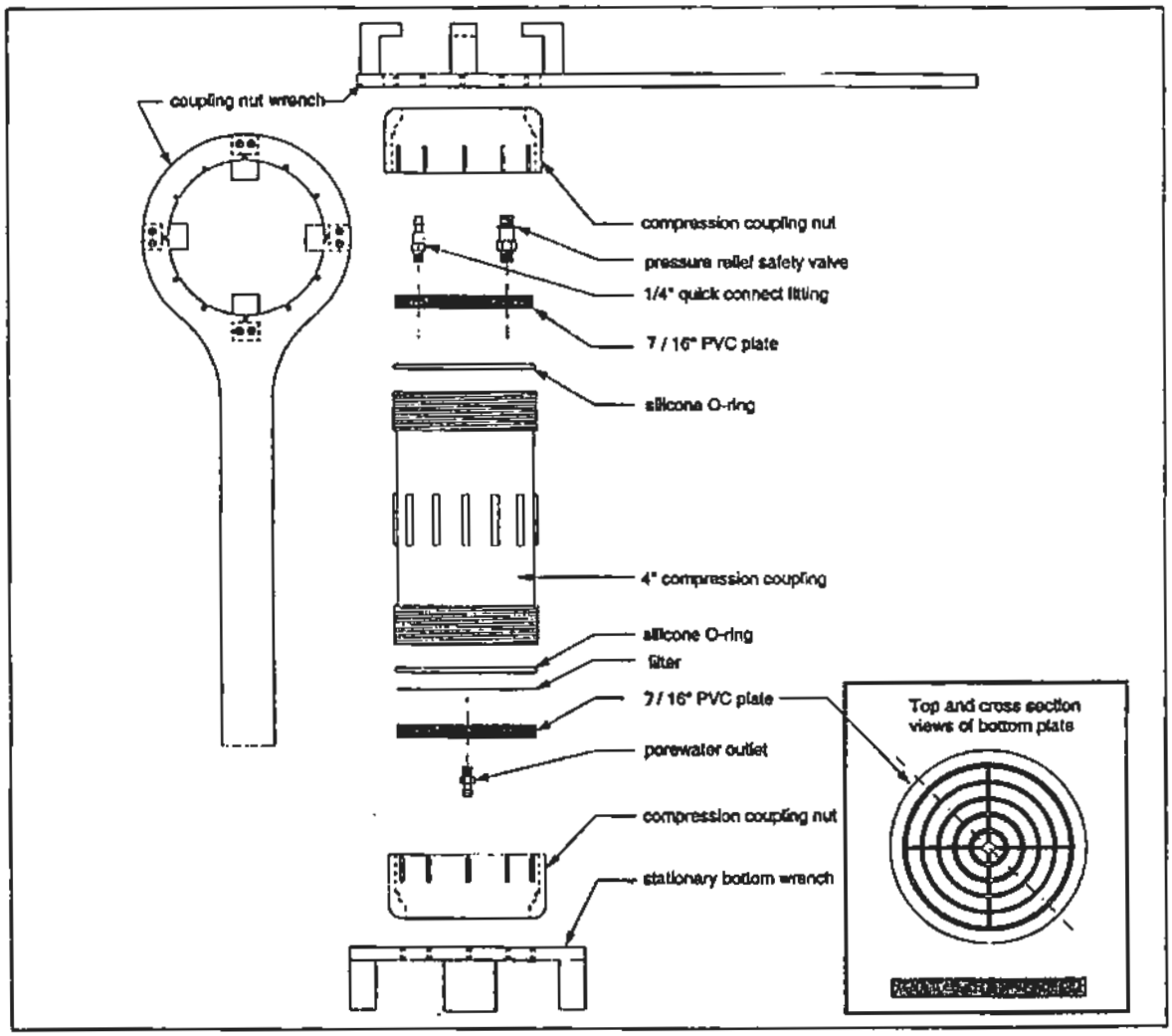

Figure 1. Sediment pore water squeeze extraction device.

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.

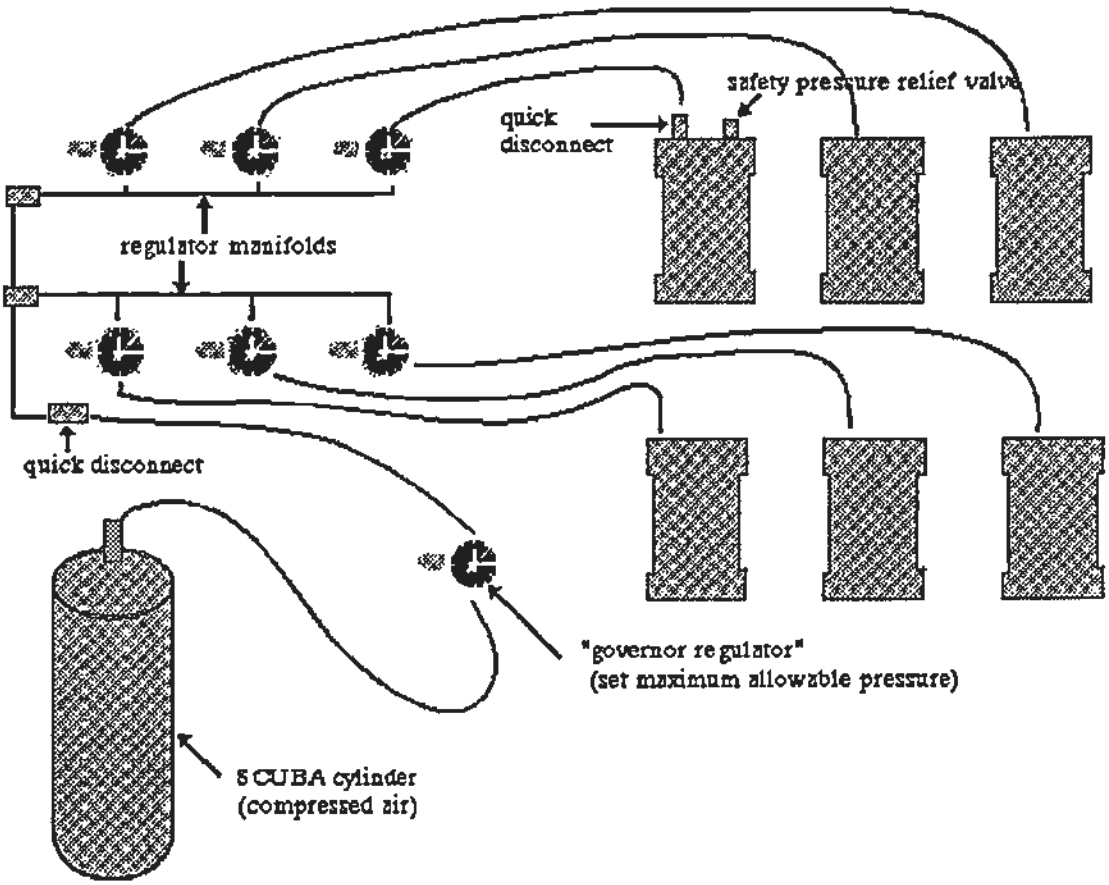

Figure 2. Schematic of sediment porewater pressure extraction system.

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 fllter, so that extraction of a large volume of clay sediment at once would take an extremely long time. When extracting pore water from extremely fine grained sediments, the cylinder should be less than one-third filled. If additional pore water is needed, this process can be repeated by removing the sediment including

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

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

3.3 Porewater Extraction

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

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

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

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

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

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

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

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

3.4 Centrifugation of Porewater Samples

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

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

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

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

3.5 Storage of Porewater Samples

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

4.0 QUALITY CONTROL

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

5.0 TRAINING

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

6.0 SAFETY

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

7.0 ATTACHMENTS

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

8.0 REFERENCES

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

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

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

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

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

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

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

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

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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.

3.1 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 $^0/_{00}$ ÷ sample $^0/_{00}$) × sample vol. in mL = A
(ii) sample vol. - A = B
(iii) sample vol. ÷ A = C
(iv) B × C = volume of HPLC water to add

3.0 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

Prepared by:

Duane C. Chapman
Fishery Biologist

Approved by:

R. Scott Carr
Field Station Leader

Anne E. Kinsinger
Chief, Field Research Division

Joseph B. Hunn 5-20-94
Quality Assurance Officer

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 (°/₀₀) _____

Vol. Baker® HPLC water added (mL) _____

Vol. ____°/₀₀ brine added (mL) _____

% of original sample _____

(initial vol./final vol. x 100)

B. Character of Sample (after salinity adjustment):

Final Volume (mL) _____

Final Salinity (°/₀₀) _____

pH _____

Dissolved oxygen (mg/L) _____

DO saturation (%) _____

Total ammonia (mg/L) _____

Sulfide (mg/L) _____

COMMENTS_____

_____

_____

_____

_____

_____

_____

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

Date Prepared : April 10, 1990

Date Revised: March 10, 1995

SEA URCHIN FERTILIZATION TOXICITY TEST

1.0 OBJECTIVE

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

2.0 TEST PREPARATION

2.1 Test Animals

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

2.2 Dilution Water

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

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

2.3 Test System: Equipment

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

2.3.1 Equipment

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

2.3.2 Solutions

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

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

1 mL needed for each replicate. Fill the dispenser.

2.4 Collection and Preparation of Gametes

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

2.4.1 Selection of Urchins to be Used in Toxicity Test.

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

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

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

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

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

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

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

2.4.2 Obtain Eggs

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

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

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

4. Repeat washing procedure.

2.4.3 Prepare Appropriate Egg Concentration

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

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

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

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

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

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

2.4.4 Obtain Sperm

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

2.4.5 Prepare Appropriate Sperm Dilution

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

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

3.0 TEST PROCEDURES

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

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

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

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

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

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

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

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

4.0 DATA COLLECTION AND TABULATION

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

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

3. Calculate fertilization percentage for each replicate test:

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

5.0 DATA ANALYSIS

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

6.0 QUALITY CONTROL

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

7.0 TRAINING

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

8.0 SAFETY

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

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

9.0 ATTACHMENTS

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

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

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1-13-93

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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 _____

Date Prepared : April 10, 1990

Date Revised: February 29, 2000

SEA URCHIN EMBRYOLOGICAL DEVELOPMENT TOXICITY TEST

1.0 OBJECTIVE

The purpose of the embryological development toxicity test with the sea urchin, *Arbacia punctulata*, is to determine if a sea water, pore water, sea surface microlayer, or other sample affects development of exposed embryos (development arrested at an early stage or a developmental abnormality) relative to that of embryos exposed to a reference sample. The test may also be used to determine the concentration of a test substance which affects development. Test results are reported as treatment (or concentration) which produces statistically significant developmental effect. This test can be performed concurrently with Sea Urchin Fertilization Toxicity Test (SOP 10.6) 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 embryological development 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 Embryological Development 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)

0.75 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 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 eggs of females may be combined 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 (mL)

   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.

5. Just before the eggs are to be used, add 2 mL of a penicillin-G stock solution (5000 units/mL) per 100 mL of eggs in the egg suspension. The addition of penicillin to the embryological development test has been shown to be beneficial in evalution of the stages of development by inhibiting bacterial growth which can cause the embryos to disintegrate before the test is terminated.

   The penicillin stock solution is prepare by diluting 296 mg of Penicillin-G sodium salt (1690 units/mg) in 100 mL of MFS and mixing until dissolved. The addition of 2 mL/100 mL of eggs will result in a final concentration of 4 units/mL in each replicate. The number of units of penicillin per mg of penicillin-G sodium salt is variable with each lot. Thus, the quantity added to the stock will change in order to keep the final concentration at 4 units/mL.

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

As in the Sea Urchin Fertilization Test, it is desirable for control fertilization to be 70-90%. Although controls outside these bounds do not automatically disqualify a test, particularly if a valuable dose response is generated, the chance of inducing polyspermy is increased with increased concentrations of sperm, and good dose responses may be difficult to obtain with less than 70% normal plutues 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 1:1250 and 1:7500 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. The quantity of

sperm to be added to the egg dilution is calculated by dividing the total volume of eggs by five and adding 50 µL of sperm dilution per that number. Sperm should be allowed to incubate with the eggs for 10 minutes to allow fertilization to take place. After 10 minutes, eggs should be evaluated under 100 X magnification for fertilization membranes. If 70-90% of the eggs are fertilized, the embryos can be pipetted into the test vials. If the percentage is lower than 70%, additional sperm may be added and/or more time allowed for fertilization. If the fertilization does not increase above 70% after 30 minutes, the embryos should be discarded and new gametes selected for use. Embryos should not be allowed to undergo division before pipetting them into the test vials.

3.0 TEST PROCEDURES

1. While gently swirling the embryo solution to maintain even mixing, use a 200 µL pipetter to add 200 µL diluted embryo suspension to each vial. Record time of embryo addition.

2. Incubate all test vials at 20 ± 1°C for 48 hours.

3. Using the dispenser, add 0.75 mL 10% buffered formalin to each vial.

4. Vials may now be capped and stored overnight or for several days until evaluated.

4.0 DATA COLLECTION AND TABULATION

1. Transfer approximately 1 mL embryos and water from bottom of test vials to counting slide. Observe embryos using a compound microscope under 100X magnification.

2. Count 100 embryos/sample using hand counter with multiple keys (such as a blood cell counter), using one key to indicate normally developed pluteus larvae and others to indicate unfertilized eggs, embryos arrested in earlier developmental stages, and other abnormalities or for more efficient data collection, stages other than pleuteus and abnormalities may be lumped together and counted on one key. Attachment 3 has a list of developmental stages and drawings of each.

3. Calculate the proportion of normal plutei for each replicate test:

$$\frac{\text{Number normal plutei X 100}}{\text{Total no. eggs/embryos}} = \text{Percent normal plutei}$$

5.0 DATA ANALYSIS

Data are recorded on standardized data sheets (See Attachments 4-9). Normally, percent normal development (normal plutei) 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 gametes 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, embryological stages and 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 embryological development 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 Embryological Development Toxicity Test
Attachment 2. Pretest to Insure Selection of Quality Gametes
Attachment 3. Development of Sea Urchin Eggs to Pluteus Larvae
Attachment 4. Water Quality Adjustment Data Form
Attachment 5. Sea Urchin Pretest Data Sheet
Attachment 6. Sea Urchin Pretest Continuation Data Sheet
Attachment 7. Sea Urchin Fertilization/Embryological Development Toxicity Test Gamete Data Sheet
Attachment 8. Sea Urchin Embryological Development Test Data Sheet
Attachment 9. Sea Urchin Embryological Development Test Abridged Data Sheet

10.0 REFERENCES

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

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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 EMBRYOLOGICAL DEVELOPMENT 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

DEVELOPMENT OF SEA URCHIN EGGS TO PLUTEUS LARVAE

The development of sea urchin eggs from fertilization to pluteus larvae normally occurs in approximately 48 hours. Although development is a continuous process of mitosis and cellular differentiation, developmental biology defines distinct stages of development by gross morphological characteristics. For the purpose of the Sea Urchin Embryological Development Test, six stages are defined and used in the characterization of embryos (Drawings on following page).

1. Unfertilized egg - single cell which appears dense and lacks a fertilization membrane.

2. Fertilized egg - egg with a distinct fertilization membrane which appears as a thin band lying slightly away from the central egg. The early stages of cell division are included in this group.

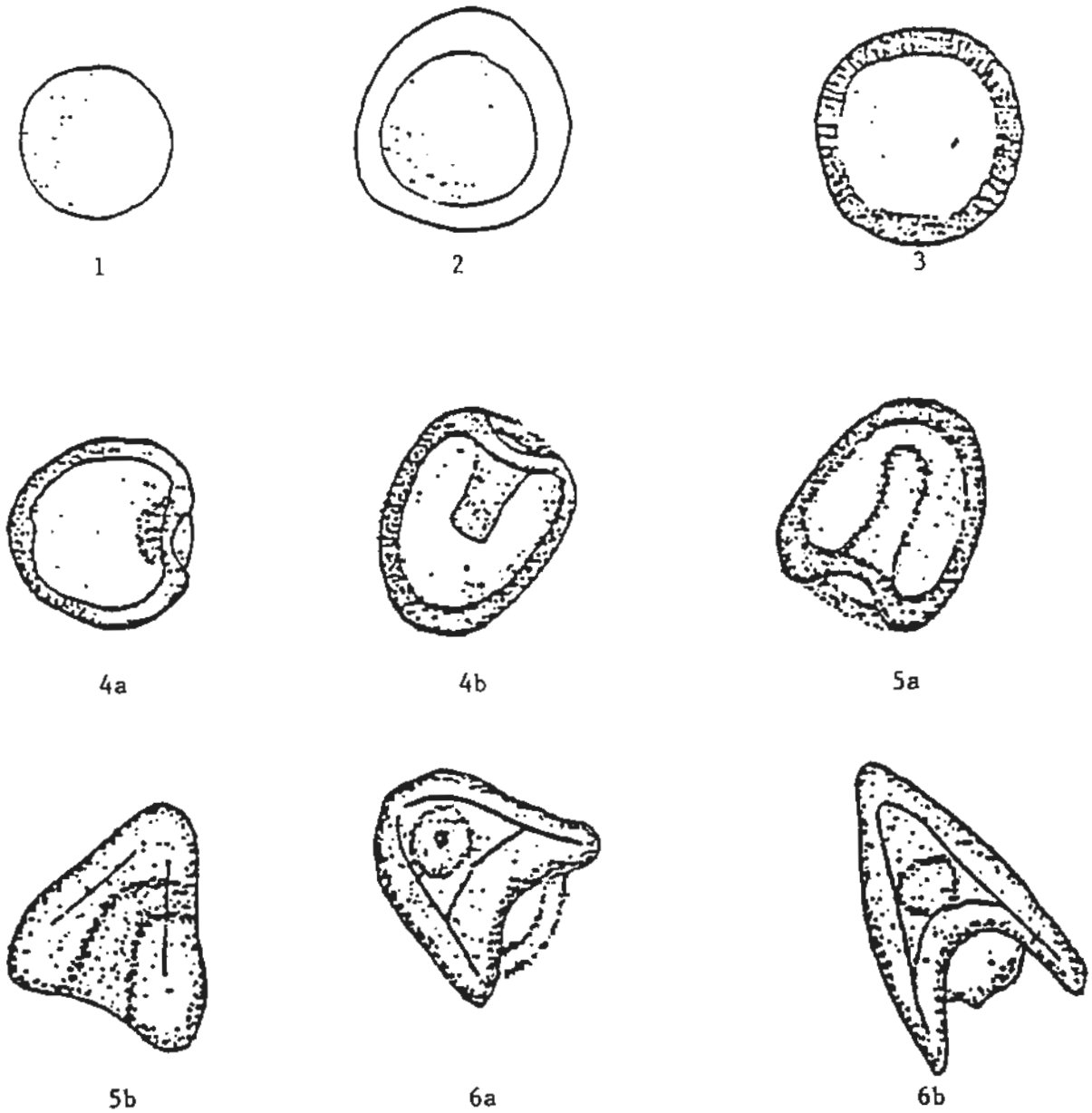
3. Blastula - spherical, "hollow-ball" stage which is ciliated and becomes free-swimming by breaking out of the fertilization membrane.

4. Early gastrula - beginnings of invagination of the blastula wall are evident. Cells move inward (invaginate) to form a central cavity (archenteron). Early gastrula includes embryos with the earliest stages of invagination and continues until the archenteron reaches approximately two-thirds of the diameter of the embryo.

5. Late gastrula - gastrula in which archenteron has developed in length to two-thirds of the embryo diameter and has begun to differentiate and bend towards and break through the embryo wall. Included are the later stages (prism) with primitive gut (complete digestive system), early skeletal rod development, and beginnings of deltoid shape formation.

6. Pluteus - deltoid-shaped larval stage with complete digestive system, skeletal rods, and growth of projecting arms.

Attachment 3 Continued



Stages in development of sea urchin, from unfertilized egg to pluteus larvae. Numbers relate to descriptions on previous page.

Attachment 4

WATER QUALITY ADJUSTMENT DATA FORM

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ DATE_____

A. Salinity Adjustment:

Initial volume (mL) _____

Initial salinity (°/$_{oo}$) _____

Vol. Milli-Q water added (mL) _____

Vol. ___°/$_{oo}$ brine added (mL) _____

% of original sample _____
(initial vol./final vol. x 100)

B. Character of Sample (after salinity adjustment):

Volume (mL) _____

Salinity (°/$_{oo}$) _____

pH _____

Dissolved oxygen (mg/L) _____

DO saturation (%) _____

Total ammonia (mg/L) _____

Sulfide (mg/L) _____

COMMENTS_____

_____

_____

_____

_____

Attachment 5

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 6

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 7

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 8

SEA URCHIN EMBRYOLOGICAL DEVELOPMENT TEST DATA SHEET

TEST ID_____ INITIALS_____

STUDY PROTOCOL_____ DATE_____

Treatment	Rep.	Eggs	Blastula	Early Gastrula	Late Gastrula	Pluteus	% Normal Development	%Non-Norm

Comments_____

Attachment 9

SEA URCHIN EMBRYOLOGICAL DEVELOPMENT TOXICITY TEST

ABRIDGED DATA SHEET

TEST ID_____ INITIALS_____

STUDY PROTOCOL_____ DATE_____

PERCENT NORMAL PLUTEI

Treatment	Replicate 1	2	3	4	5	Mean±SD	Comments