Final Report

on

Sediment Toxicity Test Results for the Puget Sound Assessment and Monitoring Program (PSAMP) and the Urban Waters Study 2009

submitted to

Washington State Department of Ecology
Puget Sound Assessment and Monitoring Program
300 Desmond Drive
Olympia, Washington 98504-7710

March 12, 2010

prepared by:

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Figure 1. Station locations of sediments collected for the 2009 PSAMP study in Puget Sound, Sinclair Inlet and Commencement Bay, Washington. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's *t*-test, a $\leq$ 0.05 and detectable significance criteria applied).

Figure 2. Station locations of sediments collected for the 2009 Urban Waters study in Liberty Bay, Dyes Inlet, Sinclair Inlet and surrounding waterways, Washington. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's *t*-test, a $\leq$ 0.05 and detectable significance criteria applied).

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APPENDICES

Appendix 1. Table of Sodium Dodecyl Sulfate reference toxicant control EC_{50}s (mg/L) from toxicity tests with *Strongylocentrotus purpuratus* from 1997-2009.

Appendix 2. Table of relevant sample dates of the PSAMP 2009 sediments.

Appendix 3. Table of relevant sample dates of the Urban Waters 2009 sediments.

Appendix 4. Chain of custody sheets from incoming samples arriving at the USGS Marine Ecotoxicology Research Station from June 2nd - 19th 2009.

INTRODUCTION

The Washington Department of Ecology annually determines the quality of recently deposited sediments in Puget Sound as a part of the Puget Sound Assessment and Monitoring Program (PSAMP) Sediment Component. The annual sediment quality studies use the Sediment Quality Triad (SQT) approach, thus relying upon measures of chemical contamination, toxicity, and benthic infaunal impacts. The area of study in 2009 included sediments throughout the Central Puget Sound sediment monitoring region, including samples from Elliot and Commencement Bay. As part of this multidisciplinary sediment quality survey the severity and spatial extent of the toxicity of surficial sediments collected from these sites was assessed using pore water in the sea urchin (*Strongylocentrotus purpuratus*) fertilization test.

In addition to the PSAMP samples, an additional 33 samples were collected in waterways surrounding Bainbridge Island including Liberty Bay, Dyes Inlet, and Sinclair Inlet and in the adjacent waterways. These samples were collected as part of Ecology's Urban Waters Initiative, and tested in an identical manner.

Sediment samples were collected by personnel from the Washington Department of Ecology, in June of 2009 and shipped to the U. S. Geological Survey (USGS) Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas, where the tests were performed. Sediment pore water was extracted with a pneumatic apparatus and was stored frozen. Just prior to testing, water quality parameters were measured and salinity adjusted, if necessary. A dilution series (100, 50 and 25%) test design was used to determine the toxicity of sediment porewater samples.

The specific objectives of this study were to:

- Extract sediment pore water from a total of 83 sediment samples (33 for the Urban Waters study) from the Puget Sound area within a day of receipt of the samples using a pneumatic extraction device.

- Measure water quality parameters (salinity, dissolved oxygen, pH, sulfide, and ammonia) of thawed porewater samples prior to testing and adjust salinity, temperature and dissolved oxygen, if necessary, to obtain optimal ranges for the test species.

- Conduct the fertilization toxicity test with pore water using sea urchin (*S. purpuratus*) gametes.

- Perform quality control assays with reference pore water, dilution blanks and a positive control dilution series with sodium dodecyl sulfate (SDS) in conjunction with each test.

- Make statistical comparisons between test and reference stations.

MATERIALS AND METHODS

Sediment Sample Receipt and Tracking

Surficial sediment samples were collected from 50 stations in areas throughout Puget Sound, including both Elliott and Commencement Bay. A separate set of 33 samples was collected in Port Madison, Liberty Bay, Dyes Inlet, Sinclair Inlet, and the passages that connect them. Samples were placed in pre-cleaned one-gallon high density polyethylene containers, chilled, and shipped in insulated coolers with blue ice. Samples were received by the USGS in Corpus Christi, Texas, the day following shipment. Shipments were accompanied by sample tracking sheets, and samples were logged into laboratory sample tracking systems. All porewater samples were extracted within 7 days from the time of field collection of sediment, and within 30 hours of arrival at the Corpus Christi laboratory.

Toxicity Testing

Sediment Porewater Extraction Procedure

Approximately 500 ml of pore water was extracted from each sediment sample using a pneumatic extraction device. The 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 (Carr and Chapman, 1992; 1995; Carr et al., 1996a; 1996b; USGS, 2002a; 2002b; 2002c; 2002d; 2003a; 2003b; 2005; 2007a; 2007b; 2008; 2009). The apparatus and extraction procedures are detailed in CERC SOP P.649 (Attachment 1). This pneumatic extraction method has been compared with other porewater extraction methods (e.g., centrifugation and vacuum) and shown to produce comparable samples (Carr and Chapman, 1995). After extraction, the porewater samples were centrifuged in polycarbonate bottles at 1200 x g for 20 minutes to remove any suspended particulate material; the supernatant was collected, divided into three pre-cleaned samples bottles, and frozen at -20ºC.

Two days before conducting a toxicity test, one bottle from each station was moved from the freezer to a refrigerator at 4ºC. One day prior to testing, samples were thawed and brought to room temperature in a tepid (20 ± 2ºC) water bath. Sample salinity was measured and adjusted to 30 ± 1$^{o}/_{oo}$, if necessary, using purified deionized water or concentrated brine (see CERC SOP P.651). Following water quality adjustments, the samples were stored overnight at 4ºC but were returned to 12 ± 1ºC (incubated in an environmental chamber) immediately before the start of the toxicity tests.

On the day of the test, subsamples were taken, acclimated to room temperature and water quality measurements (dissolved oxygen, pH and ammonia) were made (see CERC SOP P.646). Additional subsamples were preserved with SAOB II reagent for sulfide measurements and refrigerated to 4ºC until the following day when they were brought to room temperature and measured (see CERC SOP P.657). Dissolved oxygen (DO) was measured with a YSI® model 59

dissolved oxygen meter with a YSI model 5905 BOD probe. Salinity was measured with a Reichert® temperature compensated refractometer. Sulfide (as S^{-2}), pH and total ammonia (expressed as nitrogen; TAN) were measured with Orion® model 290 A meters and the Thermo–Orion model 9616 silver/sulfide probe, Thermo-Orion model 9107BN low maintenance triode, and the Thermo-Orion model 5912 ammonia probe, respectively. Room and environmental chamber temperature were measured using a Fisher brand 76mm Teflon encased mercury thermometer. Unionized ammonia (expressed as nitrogen) concentrations (UAN) were calculated for each sample using the respective salinity, temperature, pH, and TAN values (Bowers and Bidwell, 1978). Any samples containing less than 80% DO saturation were gently aerated by stirring the sample on a magnetic stir plate prior to testing.

Toxicity Testing with Sea Urchins

S. purpuratus urchins were obtained from Marinus Scientific Inc. For both series of samples (PSAMP and Urban Waters) each porewater sample was tested in a dilution series at 100, 50, and 25% of sample (after salinity adjustment) with 5 replicates per treatment (see CERC SOP P.645). A pretest was conducted which included the reference porewater, the dilution water and a limited reference toxicant dilution series to determine the optimum sperm dilution that would give acceptable fertilization rates in the reference pore waters but also acceptable fertilization in the dilution water and meet our laboratory standard in the reference toxicant. All pretests and tests were conducted at 12 ºC in a temperature controlled chamber with an exposure time of 20 minutes each for the sperm and the sperm plus eggs (Carr, 2007). At the end of the exposure, the tests were terminated by the addition of buffered formalin. The endpoint was determined by examining 100 eggs/replicate using a compound microscope to determine the presence or absence of a fertilization membrane. The percent fertilization was determined using the following formula.

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

Dilutions were made with 0.45 µm filtered seawater collected from the ship channel in Port Aransas, Texas. A reference porewater sample collected from Aransas Bay, Texas, which had been collected with a PVC corer, held refrigerated, and extracted 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 (USGS, 2002a, 2003a; 2003b; 2003c; 2003d; 2005; 2007a; 2007b; 2008; 2009).

In addition, a dilution water blank of filtered seawater was included in each test and a brine blank (control pore water diluted to the lowest salinity measured in the test samples and subsequently increased with brine) was included in those tests that contained samples that were adjusted with brine. Finally, a dilution series test with sodium dodecyl sulfate (SDS) was included in each assay as a positive control to evaluate overall test sensitivity. This positive control was mixed in,

and diluted with the same filtered seawater used to dilute the pore waters in the dilution series described above.

Sea Urchin Toxicity Testing Data Analysis

For the fertilization test, statistical comparisons among treatments were made using ANOVA and Dunnett's one-tailed t-test (which controls the experimentwise error rate) on the arcsine square root transformed data with the aid of SAS (SAS, 1989). The trimmed Spearman-Karber method (Hamilton et al., 1977) with Abbott's correction (Morgan, 1992) was used to calculate EC_{50} (50% effective concentration) values for the SDS dilution series.

Prior to statistical analysis, the transformed data sets were screened for equal variance using SAS/LAB® Software (SAS, 1992). The SAS/LAB Software performs a Levene's test for equal variance and when there was statistical evidence (based on performing a one way ANOVA on the absolute deviations of the observations from their respective group means) of unequal variances additional data transformations were performed and/or outliers removed. 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: $cv = t(df_{Error}, .05/(2 \times n))$. A second criterion was also used to compare test means to reference means. Detectable significance criteria (DSC) were developed to determine the 95% confidence value based on power analysis of similar tests performed by our lab (Carr and Biedenbach, 1999). This value is the percent minimum significant difference from the reference that is necessary to accurately detect a difference from the reference. The DSC value for the sea urchin fertilization assay at a = 0.05 is 15.5%. At a = 0.01, the DSC value is 19%. The DSC was developed using the sea urchin *Arbacia punctulata*, but was used to evaluate these data to aid in comparison to previous studies. A DSC value has not been determined for *S. purpuratus*.

RESULTS

Porewater Quality Measurements PSAMP 2009

Water quality measurements were conducted on sediment pore water from 50 stations. Table 1 reports the values for all the water quality measurements conducted.

No samples other than the reference station required salinity adjustment prior to testing. Salinities in test samples ranged from 29.5 to 31 ‰. Initial dissolved oxygen was > 80% in all the samples. Nine samples (006, 038, 058, 062, 094, 162, 258, 274, and 346) contained suspended sediment after thawing and water quality measurement and required centrifugation prior to testing to eliminate suspended particles which could interfere with fertilization. Total ammonia ranged from 0.92 to 41.70 mg/L while the unionized ammonia (the most toxic fraction)

ranged from 13.1 to 199.6 µg/L. Only one sample (# 274 at199.6 µg/L) exceeded the NOEC for unionized ammonia (170 µg/L) (Bailey et al., 1995). Sulfide concentrations ranged from less then detectable (< 0.01 mg/L) to 0.099 mg/L.

Sea Urchin Toxicity Testing PSAMP 2009

Two tests were conducted on December 16, 2009 with 25 samples in each test plus the references and controls. A dilution of eggs collected from a single female was subdivided into two aliquots and used in both tests so that animal variability would not be a factor between the two tests. Sperm from a single male was also used in both tests at the same dilution. Pretest results indicated an optimum sperm dilution of 1 part sperm to 1250 parts dilution water. Raw data and means from the fertilization tests are given in Tables 2 and 3.

There was one statistical outlying data point in the first test (Sample 094, 100% dilution, rep 4) (SAS 1992) and six in the second test (Rep 10, TXREF, 50%; Rep 2, 439, 100%; Rep 5, 446, 50%; Rep 5, 487, 50%; Rep 3 SDS, 2.5 mg/L; and Rep 4, SDS, 1.25 mg/L). The EC_{50} value for the SDS positive controls were calculated as 3.54 mg/L for both tests which compares to the historical mean for this species for our laboratory of 3.22 mg/L (95% CL 2.22-4.21; Appendix 1).

In test one, only the sample from station 162 was found to be significantly different from the reference pore water at both the 100% porewater concentration as well as at the 50% dilution and met the DSC (Figure 1). Sample 162 actually became more toxic upon dilution to the 50% concentration before becoming nontoxic at the 25% concentration; indicating that possible pH changes in the dilutions might have increased the toxicity of any toxicants present. An increase in pH is known to increase the unionized fractions of toxicants, including ammonia, sulfide, as well as other contaminants.

Several other samples (194, 202, 207, and 232) exhibited slight significance at the 50 and 25% dilutions but not at the 100% concentration (Table 2). However, none of these results met the DSC criteria and effects might have been caused by changing pH levels in the samples upon dilution. Unfortunately no water quality measurements were performed on the diluted fractions to confirm this.

Three stations in test two were found to be significantly different from the reference and met the DSC criteria (Table 3). Sample 274 was toxic at the 100% concentration while samples 306 and 322 were toxic at all three concentrations. Similar to test one, these latter two samples became more toxic upon dilution indicating a possible pH influence upon the toxicity results. Notably, samples 306 and 322 had the highest sulfide concentrations of the entire group of samples (Table 1).

Water Quality Measurements Urban Waters 2009

The sea urchin fertilization tests were performed with sediment pore waters from 33 stations collected in Liberty Bay, Dyes Inlet, Sinclair Inlet and the surrounding waterways. To satisfy the test salinity requirement of 30 ± 1.0 ‰, only one sample (sample 160) and the reference required salinity adjustment. Salinities ranged from 19 to 31 ‰. Table 4 reports the values for all the water quality measurements conducted. Initial dissolved oxygen was > 80% in all the samples. Total ammonia ranged from 1.26 to 6.43 mg/L while the unionized ammonia (the toxic fraction) ranged from 14.2 to 143.0 µg/L. No sample exceeded the NOEC for unionized ammonia (170 µg/L) (Bailey et al. 1995). Sulfide concentrations ranged from less then detectable (< 0.01 mg/L) to 0.081 mg/L.

Sea Urchin Toxicity Testing Urban Waters 2009

One fertilization test was conducted on December 9, 2009 with 33 samples plus the reference and controls. Pretest results indicated an optimum sperm dilution to be 1 part sperm to 625 parts of dilution water. This more concentrated sperm dilution may be the result of the animals not being as fertile earlier in the season. Raw data and means from the fertilization tests are given in Table 5. One data point was determined to be an outlier (144, 100%, rep 5) (SAS 1992). The EC_{50} value for the SDS positive control was 3.08 mg/L which compares favorably to the historical mean for this species for our laboratory (3.21 mg/L; Appendix 1). Eleven samples were found to be toxic when compared to the Texas Reference pore water (Figure 2). Samples 142, 143, 144, 145, 146, 147, 148, 161, and 170 were significantly different from the reference and these met the DSC at $\alpha = 0.01$ for all three concentrations. The majority of these samples were taken in Liberty Bay. All had measurable sulfide concentrations and many had elevated unionized ammonia concentrations. The NOEC value for total sulfides for *S. purpuratus* is 0.10 mg/L (Knezovich et al., 1996) In addition, sample 160 was toxic at both the 100% and 50% concentrations while sample 168 was toxic only at the 100% concentration.

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TABLES 1-5

Table 1. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples collected for the 2009 PSAMP study.

Station	Salinity[1] ‰	DO[2] (mg/L)	% DO[3]	pH	TAN[4] (mg/L)	UAN[5] (ug/L)	Sulfide[6] (mg/L)	% OUS[7]
TXREF [8]	34	6.97	93.2	7.571	0.38	4.5	< 0.01	88.3
MFS [9]	36	6.97	93.0	8.054	< 0.1	< 3.5	< 0.01	83.3
002	30	7.14	95.7	7.494	1.52	15.2	< 0.01	100.0
006	31	6.92	92.3	7.370	2.46	18.5	< 0.01	100.0
038	30	7.46	98.1	7.561	2.48	28.9	< 0.01	100.0
058	30	7.06	93.1	7.385	3.79	29.5	< 0.01	100.0
062	30	7.30	96.1	7.503	3.28	33.5	< 0.01	100.0
063	31	7.44	99.8	7.697	3.86	61.2	< 0.01	100.0
070	31	7.55	101.1	7.629	2.31	31.4	< 0.01	100.0
094	30	7.22	96.3	7.385	2.73	21.3	< 0.01	100.0
095	30.5	7.39	98.8	7.748	1.44	25.6	< 0.01	100.0
126	31	7.55	100.7	7.530	2.31	25.1	< 0.01	100.0
127	31	7.32	97.6	7.572	2.38	28.4	< 0.01	100.0
159	30	7.55	100.7	7.624	1.55	20.8	< 0.01	100.0
162	30.5	6.75	90.0	7.227	23.00	124.8	0.051	100.0
168	31	7.34	98.1	7.593	3.58	44.8	0.011	100.0
178	31	7.40	98.5	7.688	2.55	39.6	< 0.01	100.0
186	30	7.34	97.8	7.601	1.86	23.7	< 0.01	100.0
191	30.5	7.37	98.3	7.748	2.32	41.3	< 0.01	100.0
194	30.5	7.24	98.0	7.784	3.47	67.0	< 0.01	100.0
202	30	7.36	99.0	7.751	6.91	123.8	0.030	100.0
207	31	7.35	98.5	7.754	3.28	59.2	0.015	100.0
210	30	7.49	100.1	7.617	6.11	80.8	0.029	100.0
218	31	7.56	100.9	7.670	1.68	25.0	< 0.01	100.0
226	31	7.23	96.5	7.673	1.57	23.6	< 0.01	100.0
232	30	7.31	97.4	7.724	4.89	82.4	0.039	100.0
234	31	7.48	99.7	7.676	2.18	32.9	< 0.01	100.0
239	31	7.44	99.0	7.688	1.57	24.4	0.012	100.0
258	29.5	6.33	85.2	7.157	2.83	13.1	< 0.01	100.0
264	30	6.78	90.8	7.629	1.81	24.6	< 0.01	100.0
271	30	7.08	94.5	7.683	3.45	53.0	0.026	100.0
274	30	6.50	86.4	7.172	41.70	199.6	0.044	100.0
290	31	7.09	94.6	7.702	3.33	53.4	0.013	100.0

Table 1. Continued.

Station	Salinity[1] ‰	DO[2] (mg/L)	% DO[3]	pH	TAN[4] (mg/L)	UAN[5] (ug/L)	Sulfide[6] (mg/L)	% OUS[7]
295	31	6.90	92.2	7.726	1.05	17.8	< 0.01	100.0
298	31	7.09	94.5	7.869	1.82	42.5	< 0.01	100.0
304	31	7.30	97.4	7.763	1.86	34.2	< 0.01	100.0
306	30	7.18	95.8	7.795	4.93	97.5	0.072	100.0
316	30	7.22	97.8	7.588	6.08	75.2	0.023	100.0
322	29.5	7.38	99.1	7.848	2.94	65.5	0.099	100.0
327	31	7.56	101.0	8.097	2.34	91.0	< 0.01	100.0
330	31	7.59	101.3	7.697	2.98	47.2	< 0.01	100.0
346	29	6.26	83.5	7.065	6.18	23.1	0.018	100.0
370	31	7.55	100.6	7.727	1.49	25.3	< 0.01	100.0
375	30	7.65	101.8	7.652	1.19	17.0	< 0.01	100.0
426	31	7.26	96.6	7.758	1.50	27.3	< 0.01	100.0
439	31	7.44	98.9	7.707	1.92	31.1	< 0.01	100.0
446	31	7.21	95.7	7.714	2.48	40.9	< 0.01	100.0
455	30.5	7.20	95.7	7.719	2.21	36.8	0.015	100.0
458	31	7.26	96.6	7.719	2.19	36.5	< 0.01	100.0
480	31	7.42	98.8	7.649	1.57	22.3	< 0.01	100.0
487	31	7.64	101.5	7.659	0.92	13.3	< 0.01	100.0
498	30	7.25	96.5	7.739	2.86	49.9	< 0.01	100.0

[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 as nitrogen

[6] Measured as S^{-2}

[7] Percent of original sample after salinity adjustment

[8] Reference pore water extracted from sediment collected in Aransas Bay, Texas

[9] Millipore filtered seawater diluent

Table 2. Sea urchin fertilization test raw data and means for sediment porewater samples in test one of the PSAMP 2009 study. Asterisks denote statistically significant differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* a ≤ 0.05, ** a ≤ 0.01). Plus signs denote onlystatistically significant differences (Dunnett's t-test, + a < 0.05, ++ a < 0.01).

Station	% WQAS [1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
TXREF [4]	100	100	100	99	99	98	99.3	0.67		100.0
		99	99	100	100	99				
	50	98	100	100	98	100	97.8	2.78		100.0
		94	92	100	97	99				
	25	99	98	97	100	99	96.3	3.13		100.0
		97	92	92	97	92				
002	100	100	98	100	100	100	99.6	0.89		100.3
	50	100	100	100	99	100	99.8	0.45		102.0
	25	99	100	100	100	100	99.8	0.45		103.6
006	100	100	100	100	99	100	99.8	0.45		100.5
	50	100	100	100	100	100	100.0	0.00		102.2
	25	99	100	100	100	100	99.8	0.45		103.6
038	100	100	99	100	100	100	99.8	0.45		100.5
	50	100	100	100	100	100	100.0	0.00		102.2
	25	100	99	99	100	100	99.6	0.55		103.4
058	100	100	100	99	100	100	99.8	0.45		100.5
	50	100	100	100	100	100	100.0	0.00		102.2
	25	100	100	99	100	100	99.8	0.45		103.6
062	100	100	100	100	100	100	100.0	0.00		100.7
	50	99	100	100	100	99	99.6	0.55		101.8
	25	100	100	100	100	99	99.8	0.50		103.6
063	100	100	100	99	100	100	99.8	0.45		100.5
	50	100	100	100	99	100	99.8	0.45		102.0
	25	100	100	100	100	100	100.0	0.00		103.8
070	100	100	100	100	100	100	100.0	0.00		100.7
	50	100	99	100	100	99	99.6	0.55		101.8
	25	100	100	99	100	100	99.8	0.45		103.6
094	100	100	100	99	0 [5]	97	99.0	1.41		99.7
	50	100	99	100	100	99	99.6	0.55		101.8
	25	100	100	99	100	100	99.8	0.45		103.6

Table 2. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
095	100	100	100	99	100	100	99.8	0.45		100.5
	50	100	99	100	100	100	99.8	0.45		102.0
	25	100	100	100	100	100	100.0	0.00		103.8
126	100	100	100	100	100	100	100.0	0.00		100.7
	50	100	99	100	100	100	99.8	0.45		102.0
	25	100	100	100	100	100	100.0	0.00		103.8
127	100	100	100	100	100	100	100.0	0.00		100.7
	50	100	99	100	100	100	99.8	0.45		102.0
	25	100	99	99	100	100	99.6	0.55		103.4
159	100	100	100	100	98	100	99.6	0.89		100.3
	50	99	100	100	100	100	99.8	0.45		102.0
	25	97	100	100	99	100	99.2	1.30		103.0
162	100	54	72	65	58	49	59.6	9.07	**	60.0
	50	17	10	10	6	21	12.8	6.06	**	13.1
	25	98	99	100	98	100	99.0	1.00		102.8
168	100	100	100	100	99	100	99.8	0.45		100.5
	50	100	100	100	100	100	100.0	0.00		102.2
	25	100	100	99	99	100	99.6	0.55		103.4
178	100	100	100	99	100	100	99.8	0.45		100.5
	50	100	100	99	100	100	99.8	0.45		102.0
	25	100	100	100	100	100	100.0	0.00		103.8
186	100	100	99	100	100	100	99.8	0.45		100.5
	50	99	100	100	100	99	99.6	0.55		101.8
	25	100	100	100	100	100	100.0	0.00		103.8
191	100	100	99	99	100	100	99.6	0.55		100.3
	50	100	99	100	100	100	99.8	0.45		102.0
	25	100	100	100	99	100	99.8	0.45		103.6
194	100	100	99	100	100	99	99.6	0.55		100.3
	50	92	88	90	92	94	91.2	2.28	++	93.3
	25	92	100	98	97	89	95.2	4.55		98.9
202	100	99	98	99	100	100	99.2	0.84		99.9
	50	88	89	93	91	90	90.2	1.92	++	92.2
	25	90	91	85	90	92	89.6	2.70	++	93.0

Table 2. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
207	100	100	99	99	99	100	99.4	0.55		100.1
	50	91	91	88	89	90	89.8	1.30	++	91.8
	25	94	99	99	99	100	99.3	0.50		103.1
210	100	100	99	100	100	100	99.8	0.45		100.5
	50	98	100	97	100	96	98.2	1.79		100.4
	25	98	100	99	100	99	99.2	0.84		103.0
218	100	98	100	98	99	99	98.8	0.84		99.5
	50	100	100	100	100	100	100.0	0.00		102.2
	25	99	100	100	100	100	99.8	0.45		103.6
226	100	100	100	99	99	100	99.6	0.55		100.3
	50	99	100	100	100	98	99.4	0.89		101.6
	25	100	100	100	100	98	99.6	0.89		103.4
232	100	100	100	100	100	100	100.0	0.00		100.7
	50	95	95	96	92	91	93.8	2.17	++	95.9
	25	98	97	99	97	96	97.4	1.14		101.1
234	100	100	100	100	99	100	99.8	0.45		100.5
	50	100	99	100	100	100	99.8	0.45		102.0
	25	100	98	100	100	100	99.6	0.89		103.4
MFS [6]	100	99	98	98	99	99	98.5	0.53		99.2
		98	99	99	98	98				
SDS [7]	10	1	0	0	0	0	0.2	0.45	**	0.2
	5	0	0	2	0	0	0.4	0.89	**	0.4
	2.5	100	99	100	99	100	99.6	0.55		100.3
	1.25	99	100	99	100	100	99.6	0.55		100.3

[1] Percent of water quality adjusted porewater sampled

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

[3] Percent of TXREF control

[4] Reference pore water extracted from sediment collected in Aransas Bay, Texas

[5] Statistical outlier removed from analysis.

[6] Millipore filtered seawater diluent

[7] Sodium Dodecyl Sulfate positive control (in mg/L)

Table 3. Sea urchin fertilization test raw data and means for sediment porewater samples in test two of the PSAMP 2009 study. Asterisks denote statistically significant differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* a ≤ 0.05, ** a ≤ 0.01).

Station	% WQAS [1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
TXREF [4]	100	99	100	99	100	99	99.4	0.70		100.0
		100	98	100	100	99				
	50	100	100	100	99	99	99.4	0.73		100.0
		99	100	100	98	59 [5]				
	25	100	98	100	99	99	99.2	0.79		100.0
		98	100	99	100	99				
239	100	100	100	100	99	100	99.8	0.45		100.4
	50	100	99	100	100	100	99.8	0.45		100.4
	25	100	100	100	100	100	100.0	0.00		100.8
258	100	100	99	99	100	100	99.6	0.55		100.2
	50	99	98	100	99	100	99.2	0.84		99.8
	25	100	100	100	99	100	99.8	0.45		100.6
264	100	100	99	100	99	100	99.6	0.55		100.2
	50	100	100	100	99	100	99.8	0.45		100.4
	25	100	100	100	100	100	100.0	0.00		100.8
271	100	99	100	100	100	100	99.8	0.45		100.4
	50	99	100	100	98	99	99.2	0.84		99.8
	25	100	98	100	100	100	99.6	0.89		100.4
274	100	79	84	79	89	82	82.6	4.16	*	83.1
	50	99	99	99	100	100	99.4	0.55		100.0
	25	99	100	98	99	99	99.3	0.50		100.1
290	100	100	100	99	100	100	99.8	0.45		100.4
	50	99	100	100	100	100	99.8	0.45		100.4
	25	99	100	100	99	100	99.6	0.55		100.4
295	100	99	100	100	100	100	99.8	0.45		100.4
	50	100	100	100	100	100	100.0	0.00		100.6
	25	100	99	100	100	100	99.8	0.45		100.6
298	100	99	99	100	100	100	99.6	0.55		100.2
	50	96	100	99	100	100	99.0	1.73		99.6
	25	100	99	100	99	100	99.6	0.55		100.4

Table 3. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
304	100	100	100	100	100	100	100.0	0.00		100.6
	50	100	100	100	100	100	100.0	0.00		100.6
	25	100	100	99	100	100	99.8	0.45		100.6
306	100	8	4	2	2	12	5.6	4.34	**	5.6
	50	1	0	0	0	0	0.2	0.45	**	0.2
	25	0	0	0	0	0	0.0	0.00	**	0.0
316	100	99	96	99	100	97	98.2	1.64		98.8
	50	99	97	100	98	99	98.6	1.14		99.2
	25	100	100	99	99	100	99.6	0.55		100.4
322	100	23	20	21	25	19	21.6	2.41	**	21.7
	50	0	0	0	0	0	0.0	0.00	**	0.0
	25	4	7	14	5	4	6.8	4.21	**	6.9
327	100	100	100	98	100	99	99.4	0.89		100.0
	50	100	99	100	100	100	99.8	0.45		100.4
	25	100	100	100	100	100	100.0	0.00		100.8
330	100	100	100	100	100	100	100.0	0.00		100.6
	50	100	100	99	100	100	99.8	0.45		100.4
	25	100	100	100	100	99	99.8	0.45		100.6
346	100	98	100	100	99	99	99.2	0.84		99.8
	50	100	99	98	100	98	99.0	1.00		99.6
	25	99	97	98	99	99	98.4	0.89		99.2
370	100	100	99	99	100	100	99.6	0.55		100.2
	50	100	100	100	100	100	100.0	0.00		100.6
	25	99	100	100	99	100	99.6	0.55		100.4
375	100	99	100	100	99	100	99.6	0.55		100.2
	50	100	100	100	99	100	99.8	0.45		100.4
	25	100	99	100	100	98	99.4	0.89		100.2
426	100	99	100	100	100	100	99.8	0.45		100.4
	50	99	100	100	100	99	99.6	0.55		100.2
	25	100	96	100	100	100	99.2	1.79		100.0
439	100	99	74 [5]	100	100	100	99.8	0.50		100.4
	50	100	100	100	100	96	99.2	1.79		99.8
	25	100	100	98	100	100	99.6	0.89		100.4

Table 3. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
446	100	99	99	99	100	97	98.8	1.10		99.4
	50	100	100	100	100	78 [5]	100.0	0.00		100.6
	25	100	100	98	100	100	99.5	1.00		100.3
455	100	100	100	100	100	100	100.0	0.00		100.6
	50	100	100	100	100	99	99.8	0.45		100.4
	25	99	99	100	99	100	99.4	0.55		100.2
458	100	98	100	99	100	100	99.4	0.89		100.0
	50	99	99	96	100	97	98.2	1.64		98.8
	25	99	99	99	100	100	99.4	0.55		100.2
480	100	100	99	100	100	99	99.6	0.55		100.2
	50	100	100	99	100	100	99.8	0.45		100.4
	25	98	100	100	100	100	99.6	0.89		100.4
487	100	100	100	100	99	100	99.8	0.45		100.4
	50	100	100	100	100	82 [5]	100.0	0.00		100.6
	25	99	99	100	100	100	99.6	0.55		100.4
498	100	100	100	99	100	100	99.8	0.45		100.4
	50	100	100	100	100	100	100.0	0.00		100.6
	25	100	100	99	100	100	99.8	0.45		100.6
MFS [6]	100	99	98	100	98	98	99.0	0.94		99.6
		100	100	99	98	100				
SDS [7]	10	1	0	0	0	0	0.2	0.45	**	0.2
	5	2	0	0	0	0	0.4	0.89	**	0.4
	2.5	99	100	24 [5]	99	100	99.5	0.58		100.1
	1.25	100	100	99	52 [5]	100	99.8	0.50		100.4

[1] Percent of water quality adjusted porewater sampled

[2] Significant difference from reference denoted as asterisks

[3] Percent of TXREF control

[4] Reference pore water extracted from sediment collected in Aransas Bay, Texas

[5] Statistical outlier removed from analysis.

[6] Millipore filtered seawater diluent

[7] Sodium Dodecyl Sulfate positive control (in mg/L)

Table 4. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples collected for the 2009 Urban Waters study.

Station	Salinity[1] ‰	DO[2] (mg/L)	% DO[3]	pH	TAN[4] (mg/L)	UAN[5] (ug/L)	Sulfide[6] (mg/L)	% OUS[7]
TXREF [8]	34	7.46	102.2	7.631	0.35	4.7	< 0.01	88.2
MFS [9]	36	6.56	90.5	8.076	< 0.1	< 3.7	< 0.01	83.9
Brine Blnk[10]	30	7.62	105.2	7.897	0.14	3.5	< 0.01	55.5
124	30	7.55	102.9	7.624	4.28	57.5	< 0.01	100.0
125	30	7.65	104.7	7.671	3.40	50.8	< 0.01	100.0
126	30	7.73	106.3	7.645	5.09	71.7	< 0.01	100.0
142	30	7.60	104.7	7.795	3.06	60.5	0.019	100.0
143	30	7.36	100.9	7.863	4.09	94.3	0.070	100.0
144	30	7.92	108.5	7.903	2.94	74.2	0.025	100.0
145	30	7.38	101.5	7.791	4.33	84.9	0.071	100.0
146	30	7.07	97.3	7.906	4.35	110.5	0.058	100.0
147	30	7.14	98.0	7.955	4.65	131.8	0.081	100.0
148	30	7.18	98.6	7.885	5.40	130.8	0.064	100.0
149	30	7.29	99.9	7.772	4.28	80.4	0.033	100.0
150	30	7.33	100.6	7.601	1.75	22.3	< 0.01	100.0
151	30	7.62	104.6	7.641	2.27	31.7	< 0.01	100.0
152	30	7.41	101.7	7.793	1.97	38.8	< 0.01	100.0
153	30	7.68	105.5	7.736	3.36	58.2	< 0.01	100.0
154	30	7.16	98.2	7.707	3.77	61.1	0.044	100.0
155	30.5	7.59	104.0	7.624	3.95	53.1	0.041	100.0
156	30	7.50	102.5	7.814	3.95	81.6	0.020	100.0
157	30	7.61	104.2	7.814	3.10	64.0	0.014	100.0
158	30	7.54	103.3	7.922	4.23	111.4	0.016	100.0
159	30	7.26	99.7	7.847	6.43	143.0	0.029	100.0
160	19	7.68	105.7	8.010	3.64	116.7	0.030	86.7
161	30	6.62	91.1	7.812	5.46	112.2	0.048	100.0
162	30	7.49	103.2	7.648	1.58	22.4	< 0.01	100.0

Table 4. Continued.

Station	Salinity[1] ‰	DO[2] (mg/L)	% DO[3]	pH	TAN[4] (mg/L)	UAN[5] (ug/L)	Sulfide[6] (mg/L)	% OUS[7]
163	30	7.41	102.0	7.777	1.71	32.5	< 0.01	100.0
164	30	7.16	98.5	7.547	1.26	14.2	< 0.01	100.0
165	30	6.76	93.1	7.624	1.53	20.6	< 0.01	100.0
166	30	6.79	93.6	7.639	5.57	77.4	< 0.01	100.0
167	30	6.71	92.5	7.685	4.21	64.9	< 0.01	100.0
168	30	6.98	96.7	7.901	5.64	141.7	0.024	100.0
169	30	6.46	89.2	7.641	6.35	88.6	0.018	100.0
170	30	6.48	89.4	7.779	4.85	92.5	0.052	100.0
171	30	7.33	101.2	7.841	2.50	54.9	< 0.01	100.0

[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 as nitrogen

[6] Measured as S^{-2}

[7] Percent of original sample after salinity adjustment

[8] Reference pore water extracted from sediment collected in Aransas Bay, Texas

[9] Millipore filtered seawater diluent

[10] Brine blank of TXREF diluted to 19 ‰ with Milli-Q purified water and subsequently increased with brine to 30 ‰ (concentrated brine at 103 ‰)

Table 5. Sea urchin fertilization test raw data and means for sediment porewater samples in the Urban Waters 2009 study. Asterisks denote statistically significant differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (* a ≤ 0.05, ** a ≤ 0.01).

Station	% WQAS [1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
TXREF [4]	100	74	80	76	78	81	82.0	5.25		100.0
		84	85	89	83	90				
	50	88	82	79	81	90	88.1	6.01		100.0
		98	91	87	94	91				
	25	88	87	90	83	90	91.1	4.28		100.0
		95	95	95	92	96				
124	100	87	90	93	93	91	90.8	2.49		110.7
	50	94	88	93	90	92	91.4	2.41		103.7
	25	90	84	85	91	87	87.4	3.05		95.9
125	100	91	86	84	89	86	87.2	2.77		106.3
	50	86	78	89	88	83	84.8	4.44		96.3
	25	90	87	75	74	91	83.4	8.26		91.5
126	100	81	91	84	92	92	88.0	5.15		107.3
	50	90	92	85	90	90	89.4	2.61		101.5
	25	82	81	93	87	94	87.4	6.02		95.9
142	100	1	3	27	26	4	12.2	13.10	**	14.9
	50	51	15	44	44	8	32.4	19.45	**	36.8
	25	41	33	59	15	64	38.3	20.32	**	42.0
143	100	0	0	0	0	0	0.0	0.00	**	0.0
	50	0	0	0	0	0	0.0	0.00	**	0.0
	25	0	0	0	0	6	1.2	2.68	**	1.3
144	100	14	0	5	7	35 [5]	6.5	5.80	**	7.9
	50	25	27	25	27	19	24.6	3.29	**	27.9
	25	62	37	67	62	43	54.2	13.29	**	59.5
145	100	7	16	3	29	8	12.6	10.31	**	15.4
	50	5	13	4	1	34	11.4	13.39	**	12.9
	25	53	7	0	29	15	20.8	20.98	**	22.8
146	100	0	1	3	0	0	0.8	1.30	**	1.0
	50	0	0	2	0	0	0.4	0.89	**	0.5
	25	2	17	32	1	32	16.8	15.25	**	18.4

Table 5. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
147	100	0	6	1	0	1	1.6	2.51	**	2.0
	50	2	1	0	1	17	4.2	7.19	**	4.8
	25	0	0	2	0	0	0.4	0.89	**	0.4
148	100	0	0	1	0	0	0.2	0.45	**	0.2
	50	1	0	0	2	0	0.6	0.89	**	0.7
	25	5	1	46	1	1	10.8	19.75	**	11.9
149	100	89	77	77	76	85	80.8	5.85		98.5
	50	93	92	99	98	92	94.8	3.42		107.6
	25	95	94	97	91	91	93.6	2.61		102.7
150	100	79	92	94	92	86	88.6	6.15		108.0
	50	82	89	90	73	87	84.2	6.98		95.6
	25	81	83	90	79	87	84.0	4.47		92.2
151	100	89	87	88	95	88	89.4	3.21		109.0
	50	87	92	74	94	90	87.4	7.92		99.2
	25	97	94	97	88	84	92.0	5.79		101.0
152	100	90	91	91	98	87	91.4	4.04		111.5
	50	94	85	93	94	95	92.2	4.09		104.7
	25	82	91	93	94	90	90.0	4.74		98.8
153	100	87	85	77	84	80	82.6	4.04		100.7
	50	90	92	83	95	88	89.6	4.51		101.7
	25	91	79	93	93	91	89.4	5.90		98.1
154	100	96	90	90	94	89	91.8	3.03		112.0
	50	97	97	99	96	98	97.4	1.14		110.6
	25	97	97	98	99	98	97.8	0.84		107.4
155	100	70	79	69	69	62	69.8	6.06		85.1
	50	91	97	91	94	91	92.8	2.68		105.3
	25	96	93	99	95	89	94.4	3.71		103.6
156	100	97	92	94	93	94	94.0	1.87		114.6
	50	100	100	100	98	99	99.4	0.89		112.8
	25	99	99	100	99	97	98.8	1.10		108.5
157	100	99	96	97	92	91	95.0	3.39		115.9
	50	99	98	99	94	99	97.8	2.17		111.0
	25	93	97	95	96	95	95.2	1.48		104.5

Table 5. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
158	100	95	97	97	98	95	96.4	1.34		117.6
	50	99	98	100	100	100	99.4	0.89		112.8
	25	96	97	99	97	98	97.4	1.14		106.9
159	100	53	83	82	72	61	70.2	13.10		85.6
	50	91	97	99	95	97	95.8	3.03		108.7
	25	94	97	96	97	98	96.4	1.52		105.8
160	100	45	54	47	51	61	51.6	6.31	**	62.9
	50	58	66	58	78	84	68.8	11.80	**	78.1
	25	96	93	91	94	94	93.6	1.82		102.7
161	100	12	2	6	6	0	5.2	4.60	**	6.3
	50	42	11	52	40	47	38.4	16.01	**	43.6
	25	57	60	63	67	71	63.6	5.55	**	69.8
162	100	75	92	73	78	93	82.2	9.58		100.2
	50	89	92	66	95	80	84.4	11.72		95.8
	25	59	95	92	95	96	87.4	15.95		95.9
163	100	87	90	89	92	91	89.8	1.92		109.5
	50	97	91	87	94	87	91.2	4.38		103.5
	25	80	82	80	75	93	82.0	6.67		90.0
164	100	60	77	89	87	87	80.0	12.12		97.6
	50	89	75	63	87	92	81.2	12.05		92.2
	25	94	69	75	82	73	78.6	9.81		86.3
165	100	82	77	92	76	81	81.6	6.35		99.5
	50	89	90	80	95	88	88.4	5.41		100.3
	25	83	85	91	70	94	84.6	9.29		92.9
166	100	86	89	89	90	93	89.4	2.51		109.0
	50	99	96	97	95	93	96.0	2.24		109.0
	25	95	92	87	95	89	91.6	3.58		100.5
167	100	94	97	86	88	88	90.6	4.67		110.5
	50	99	95	99	95	93	96.2	2.68		109.2
	25	88	46	92	100	96	84.4	21.93		92.6
168	100	69	41	56	59	51	55.2	10.31	**	67.3
	50	86	82	77	81	81	81.4	3.21		92.4
	25	82	93	92	94	95	91.2	5.26		100.1

Table 5. Continued.

Station	% WQAS[1]	% Fertilized					Mean	SD	Sig.[2]	% of Control [3]
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
169	100	97	95	88	94	97	94.2	3.70		114.9
	50	94	96	90	88	89	91.4	3.44		103.7
	25	96	93	95	93	95	94.4	1.34		103.6
170	100	44	38	16	36	22	31.2	11.71	**	38.0
	50	56	50	54	60	58	55.6	3.85	**	63.1
	25	71	62	83	62	66	68.8	8.76	**	75.5
171	100	89	96	95	92	94	93.2	2.77		113.7
	50	94	94	91	92	94	93.0	1.41		105.6
	25	95	94	92	93	93	93.4	1.14		102.5
MFS [6]	100	92	84	74	83	73	86.7	9.08		113.7
		93	96	80	96	96				
Brine Blnk [7]	100	82	86	79	89	89	85.0	4.42		103.7
SDS [8]	10	0	0	0	0	0	0.0	0.00	**	0.0
	5	0	0	0	0	0	0.0	0.00	**	0.0
	2.5	73	64	74	72	69	70.4	4.04		85.9
	1.25	80	87	40 [5]	92	88	86.8	4.99		105.8

[1] Percent of water quality adjusted porewater sampled

[2] Significant difference from reference denoted as asterisks.

[3] Percent of TXREF control

[4] Reference pore water extracted from sediment collected in Aransas Bay, Texas

[5] Statistical outlier removed from analysis.

[6] Millipore filtered seawater diluent

[7] Brine blank consisting of TXREF reference porewater diluted to 19 ‰ and supsequently increased with brine to 30 ‰ with concentrated brine.

[8] Sodium Dodecyl Sulfate positive control (in mg/L)

FIGURES

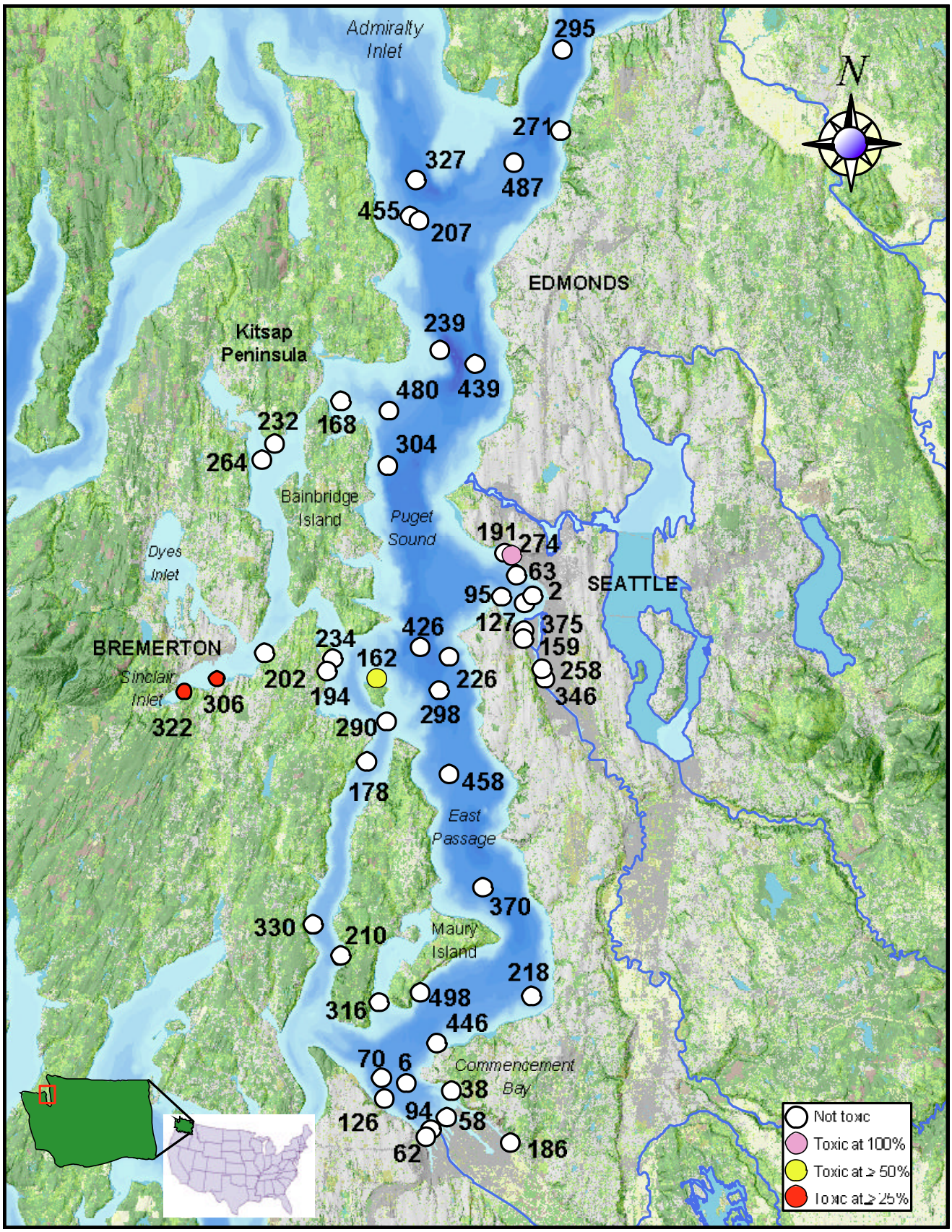

Figure 1. Station locations of sediments collected for the 2009 PSAMP study in Puget Sound, Sinclair Inlet, and Commencement Bay, Washington. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's *t*-test, ≤ 0.05 and detectable significance criteria applied).

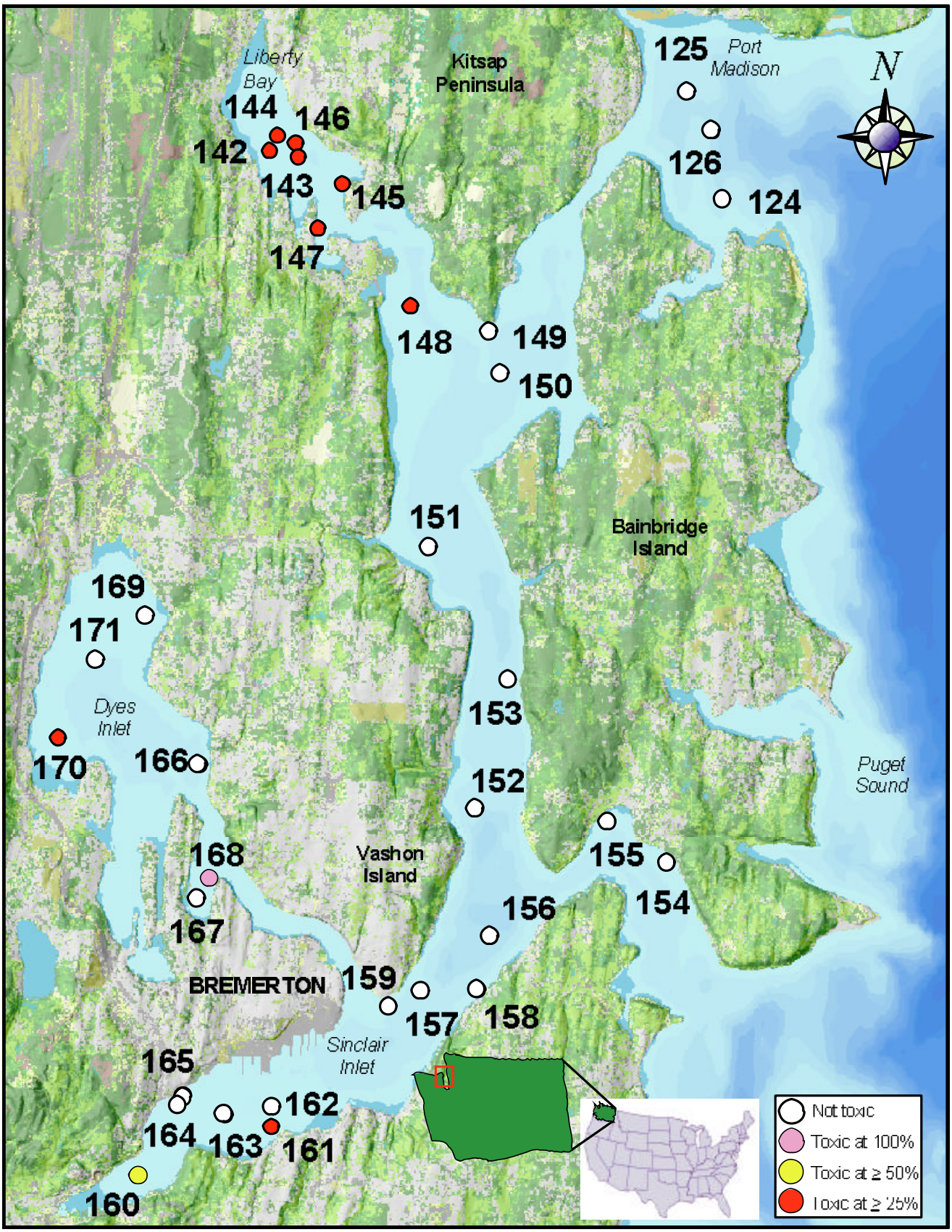

Figure 2. Station locations of sediments collected for the 2009 Urban Waters study in Liberty Bay, Dyes Inlet, Sinclair Inlet and surrounding waterways, Washington. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's *t*-test, < 0.05 and detectable significance criteria applied).

ATTACHMENTS 1-5

Date Prepared: May 5, 1990

Date Revised: July 18, 2007

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, et al., 1996a, 1996b, 1996c, 2000, 2001).

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.

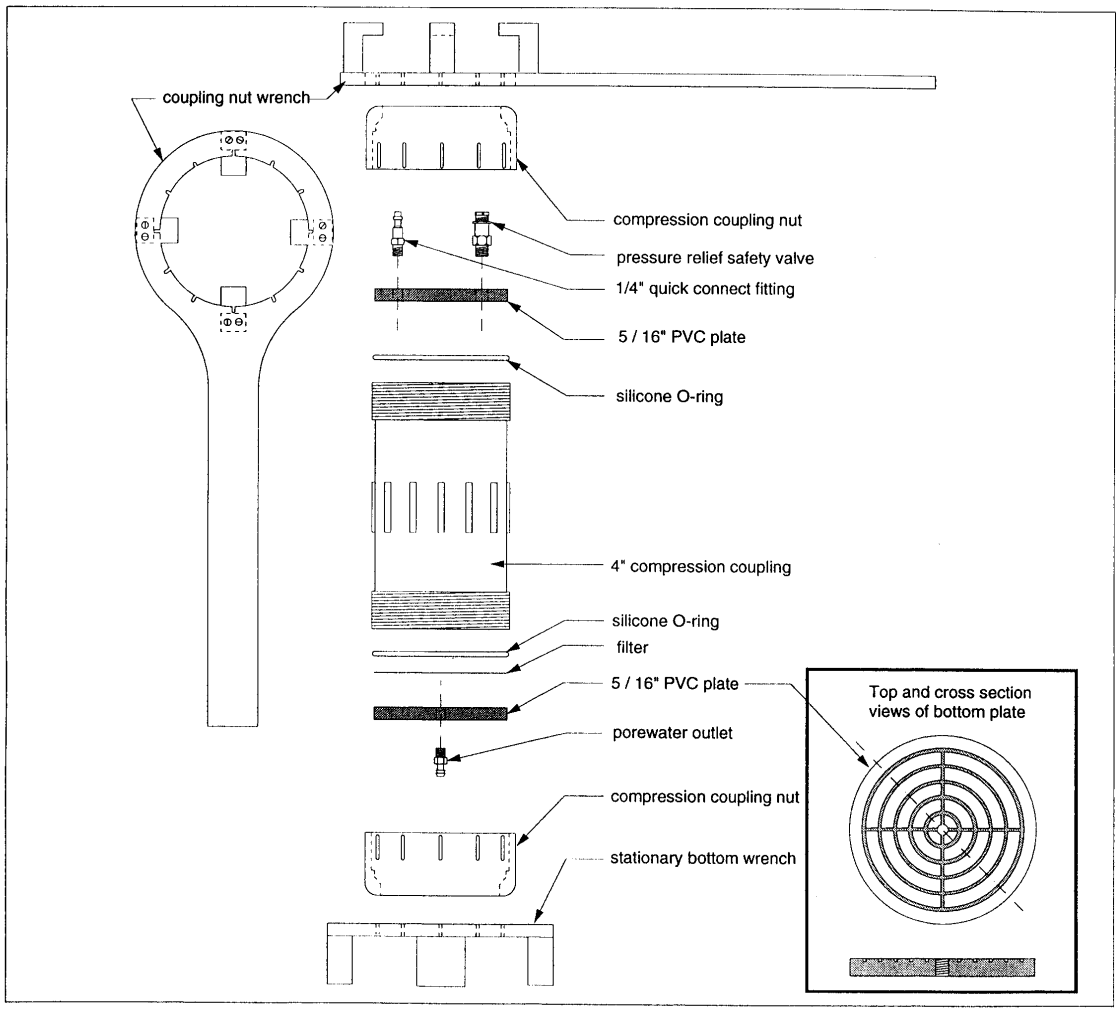

Figure 1. Sediment pore water squeeze extraction device.

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.

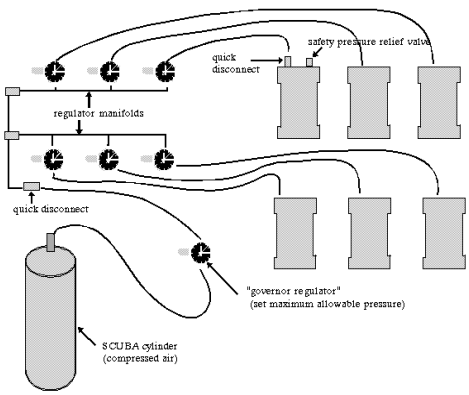

Figure 2. Schematic of sediment porewater pressure extraction system.

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 coarse 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 polyethylene containers or Zip-Lock® bags), labeled, 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 position an appropriately labeled porewater sample container (usually an I-Chem® amber 250 mL or 125 mL glass jar cleaned to EPA standards, with Teflon® lid liner) underneath the outlet.

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

3. Transfer sediment from the sample container/bag to the extractor by pouring and/or using a clean Teflon® or plastic spatula or spoon. If necessary, particularly when extracting pore water from sandy or shelly sediments, the spatula may be used to compress the sample in the cylinder to eliminate channelization. The amount of sediment to be transferred will depend on the texture of the sample. The cylinder may be filled nearly full with a sandy sediment. However, when extracting pore water from a clay sediment, a relatively impermeable layer of compressed clay will eventually form on the filter, so that extraction of a large volume of clay sediment at once would take an extremely long time. When extracting pore water from extremely fine grained sediments, the cylinder should be less than one-third filled. If additional pore water is needed, this process can be repeated by removing the sediment including 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 labeled 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 (CERC SOP P.651) 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.

7.0 ATTACHMENTS

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

8.0 REFERENCES

Carr, R.S., D.C. Chapman, C.L. Howard, and J. Biedenbach. 1996a. Sediment Quality Triad assessment survey in the Galveston Bay Texas system. *Ecotoxicology* 5:341-361.

Carr, R.S., D.C. Chapman, B.J. Presley, J.M. Biedenbach, L. Robertson, P. Boothe, R. Kilada, T. Wade and P. Montagna. 1996b. Sediment porewater toxicity assessment studies in the vicinity of offshore oil and gas production platforms in the Gulf of Mexico. *Can . J. Fish. Aq. Sci.* 53:2618-2628.

Carr, R.S., E.R. Long., D.C. Chapman, G. Thursby, J.M. Biedenbach, H. Windom, G. Sloane and D.A. Wolfe. 1996c. Toxicity assessment studies of contaminated sediments in Tampa Bay, Florida. *Environ. Toxicol. Chem.* 15:1218-1231.

Carr, R.S., P.A. Montagna, J.M. Biedenbach, R. Kalke, M.C. Kennicutt, R. Hooten, and G. Cripe. 2000. Impact of storm water outfalls on sediment quality in Corpus Christi Bay, Texas. *Environ. Toxicol. Chem.* 19:561-574.

Carr, R.S., J.M. Biedenbach, and R. Hooten. 2001. Sediment porewater toxicity test survey and phase I sediment toxicity identification evaluation studies in Lavaca Bay, Texas - an estuarine Superfund site. *Environ. Toxicol.* 16:20-30.

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Quality Assurance Officer

Attachment 1

REQUIRED EQUIPMENT AND MATERIALS

To construct a sediment pore water extraction device:

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

To use a pore water extraction device:

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

Other required supplies/equipment:

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

Attachment 2

SAMPLE HISTORY DATA FORM

Sample Designation: _____ Study Protocol: _____ Initials: _____

Date of acquisition: _____ Sample type: _____

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

_____

_____

_____

Initials	Date	Action Taken

Date Prepared: March 14, 1991

Date Revised: July 17, 2007

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 *Schizopera knabeni*, 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 35-40EC) and gently aerating filtered natural seawater (1 µm) to concentrate the salts by evaporation. For samples higher in salinity than target salinity, Millipore Milli-Q® ultrapure water is added to decrease salinity.

3.0 PROCEDURES

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

3.1 Preparation for Salinity Adjustment

1. Although fresh samples are routinely tested at the Corpus Christi field station, most of the samples tested are stored frozen in amber I-Chem® jars. If frozen, remove samples from freezer and allow them to thaw at room temperature or immerse them in a tepid water bath to thaw, ensuring that sample temperature does not exceed 25EC. The samples may be thawed the day of water quality adjustment (WQA) or may be transferred from the freezer to a refrigerator (4EC) 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"1EC, 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 $^{o}/_{oo}$) H sample vol. in mL = A
(ii) sample vol. ! A = B
(iii) sample vol.) A = C
(iv) B H 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 $^{o}/_{oo}$) H 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 P.652). 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 P.658) and record on the Water Quality Adjustment Data Form.

2. Measure and record ammonia concentration (SOP P.646).

3. Measure and record sulfide concentration if required (SOP P.657).

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. Multiple sinks are present in each of the labs, and an eye flushing station and emergency shower is present near the entrance door of each lab. 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 by donning the appropriate safety clothing and gloves.

8.0 ATTACHMENTS

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

Prepared by:

_____
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Biologist

Approved by:

_____
R. Scott Carr
Field Station Leader

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Laverne Cleveland
Chief, Field Stations Research Branch

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Paul Heine
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)
Milli-Q® ultrapure water
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 ($^{o}/_{oo}$)	_____
Vol. Milli-Q® water added (mL)	_____
Vol. ____$^{o}/_{oo}$ brine added (mL)	_____
% of original sample	_____
(initial vol./final vol. x 100)	

B. Character of Sample (after salinity adjustment):

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

COMMENTS_____

_____

_____

_____

_____

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Date Prepared: July 21, 1989

Date Revised: July 24, 2007

MEASURING DISSOLVED AMMONIA WITH THE THERMO ORION® MODEL 95-12 AMMONIA PROBE

1.0 APPLICATION

The ammonia electrode is used to measure the concentration of ammonia in aqueous media. The detectable concentration range is from 0.01 to 14,000 mg/L ammonia nitrogen, although in our laboratory concentrations measured usually fall within the range of 0.1 to 100 mg/L.

2.0 OPERATION

2.1 Required Equipment and Solutions

1. Thermo Orion® model 95-12 ammonia probe
2. Thermo Orion® model 290A pH/ISE meter
3. Magnetic stirrer
4. Bonded membrane caps (Thermo Orion® #951205) or loose membranes (Thermo Orion® #951204)
5. Electrode internal filling solution (Thermo Orion® #951202)
6. Standardizing Solution, NH_4Cl, 1000 mg/L as nitrogen (Thermo Orion® #951007) or prepare as described below.
7. 10 M NaOH

2.2 Electrode Preparation and Assembly

2.2.1 Electrode Setup

When the electrode is first received or after it has been stored dry, soak the inner body in internal filling solution for at least two hours before assembling the electrode. For best results, soak the inner body overnight. Then, follow these steps:

1. Unscrew top cap and remove glass electrode inner body, and drain old filling solution. Set cap with inner body aside carefully.

2. Remove bottom cap from electrode outer body. If using bonded membrane caps, screw cap in end of electrode until finger-tight, and proceed to step 5. If using loose membranes, proceed with instructions in step 3.

3. Using tweezers, carefully grasp a white membrane from between paper separators. Hold membrane at the edge with the tweezer. Holding electrode outer body in free hand, loosely stretch the membrane across opening in bottom of probe, holding the ends against the threads with your thumb and forefinger. Avoid excessive handling of the membrane since this may affect its hydrophobic properties and reduce its life.

4. Replace cap onto probe and screw until finger-tight. The membrane should be smooth with no wrinkles.

5. Add 2.5 ml internal filling solution into the electrode outer body. If measuring low ammonia concentrations (eg. <0.06 mg/L ammonia nitrogen), the filling solution can be diluted by 1/10 to increase response time.

6. Replace inner body into outer body and screw on upper cap.

7. Shake fully assembled electrode as if it were a clinical thermometer to remove bubbles.

.

8. Record date of membrane and internal filling solution change in instrument record/equipment log.

9. Soak the assembled probe in 10 mg/L NH_4Cl standard for at least 2 hours before making measurements.

2.2.2 Checking Electrode Operation

Obtaining the slope value (the change in mV observed with every tenfold change in concentration) provides the best means for checking electrode operation. If using the model 290A meter and problems have not been encountered, proceed to step 6.

1. Place 100 ml DI water into a 150-mL beaker. Add 1 mL 10 M NaOH and stir thoroughly. (The NaOH addition raises the pH above pH 11where all of the ammonia present is in the unionized form.) Set the function switch of the meter to the mV mode.

2. Rinse electrode with DI water and place in the solution prepared in step 1.
3. Pipet 1 mL of the 1000 mg/L ammonium chloride (NH_4Cl) standard into the beaker and stir thoroughly. Record the electrode potential in mV.

4. Pipet 10 mL of the same 1000 mg/L NH_4Cl standard into the same beaker and stir thoroughly. Again, record the electrode potential.

5. The difference between the first and second potential reading is the slope of the electrode. The electrode slope should fall within the range of -54 to -60 mV when the temperature of the solution is 20-25EC. Note the electrode slope in the instrument records/equipment log. If the slope is not within this range, check meter operation, ensure that the electrode is properly prepared and assembled, or refer to section 4.3 Troubleshooting.

6. Alternatively, the slope can be viewed after performing the normal calibration procedures undertaken before making measurements (Section 2.4 Perform Calibration Using Standard Solutions). Using the Model 290A meter (Orion®), the slope value is flashed on the LCD display after the "measure" button on the keypad is first depressed. Also, the slope can be reviewed at any time, following calibration, in the "setup" menu under category 2-1.

2.3 Preparation of Standards

1. A 1000 mg/L ammonia as nitrogen stock solution can be purchased (Themo Orion® #951007) or prepared. Prepare by adding 3.82 g reagent-grade NH_4Cl to 500 mL DI water in a 1000 mL volumetric flask, stir to dissolve and dilute to volume with DI water. Make or purchase again before shelf life expires (~6 months).

2. Prepare 100, 10, 1.0, and 0.1 mg/L standard solutions by serial dilution from the 1000 mg/L stock solution using 0.45 µm filtered seawater (SW). These can be made in scintillation vials:

   1 ml 1000 mg/L standard + 9 ml SW = 100 mg/L
   1 ml 100 mg/L standard + 9 ml SW = 10 mg/L
   1 ml 10 mg/L standard + 9 ml SW = 1 mg/L
   1 ml 1 mg/L standard + 9 ml SW = 0.1 mg/L

2.4 Perform Calibration Using Standard Solutions

1. Ensure that the mode is set to "concentration". For detailed instruction on how to operate the model 290A meter, see SOP P.658 (Measuring pH with the Thermo Orion® Model 290A pH/ISE/Temperature/mV Meter).

2. Run calibration using three standard solutions (0.1, 1.0 and 10 mg/L) beginning with the lowest concentration. Add 2 drops 10 M NaOH to 10 mL of each respective standard solution just prior to measuring. Ensure that each sample is fully stirred during measurement.

3. Check the slope (see item 6 in section 2.22 Checking Electrode Operation) and record in the comments section of the Water Quality Adjustment Data Form (Attachment 1)

and the equipment log.

4. If concentrations <0.1 mg/L are to be measured, another series of standard solutions for a lower range of values should be prepared, and another calibration should be performed.

5. Reproducibility of measurements, according to the instruction manual should be "2% with calibration performed every hour. Alternatively, the calibration can be verified every 1-2 hours by measuring a standard solution or internal reference solution. If the value is similar to that expected (within "2%), recalibration is not necessary at that time. When verification and recalibration are undertaken, the data should be noted on the Water Quality Checksheet.

2.5 Measurements

Following calibration, measure the concentration of at least one standard solution for verification. If the value is not within 2%, repeat the calibration procedures. Additionally, an internal reference sample (standard NH_4Cl solutions previously prepared and frozen) may be measured as further verification. Once verification is complete, measure the various sample concentrations and record the data on the Water Quality Adjustment Data Form for each sample. As done with the standard solutions, 2 drops of 10 M NaOH should be added to 10 mL of each test sample just prior to measurement or 3 drops if the sample volume is 15 mL.

2.6 Measuring Hints

1. The electrode should be rinsed with DI water between measurements.

2. The electrode should be checked for bubbles on the membrane. If present, they can be removed by shaking the electrode.

3. All samples should be magenetically stirred during measurement. Magnetic stirrers may generate some heat, and a layer of styrofoam can be placed below the sample to help limit any change in temperature.

4. It is critical that the temperature be consistent for all samples and standards. This ion selective electrode is not temperature compensated. A 1EC difference will introduce a 2% measurement error. Water quality measurements should generally be made at the same temperature that the test will be carried out. At the Marine Ecotoxicology Research Station (MERS), most testing is done at 20EC. Consequently, room temperature while performing water quality measurements and the various toxicity tests should be maintained at 20EC.

5. If electrode response is slow, the membrane may contain a surface layer of

contaminants. To restore performance in this case, soak the electrode in DI water for -5 minutes and then soak in a standard solution for -1 hour before use or replace the membrane and soak in a 10 mg/L NH_4Cl standard for -2 hours. For large numbers of samples, more then one probe may be assembled and utilized to reduce or eliminate the waiting time for probe equilibration.

6. If samples can not be measured on the same day they can be acidified with 1 M HCl to a pH of less then 2 and held refrigerated until the following day.

3.0 MAINTENANCE

3.1 Electrode Storage

Between measurements, do not allow the electrode tip to dry. For overnight or week-long storage, the electrode tip should be immersed in a 1000 mg/L NaOH-free ammonia standard. For storage over one week or if the electrode is stored indefinitely, disassemble completely and rinse the inner body, outer body, and bottom cap with DI water. Allow the parts to dry, then reassemble the electrode without internal filling solution or a membrane.

3.2 Membrane Life

According to the instruction manual, membranes will last from 1 week to several months depending on usage. At MERS, membranes may last less than one day when measuring samples with contaminants or high ammonia levels. Membrane failure is characterized by a shift in electrode potential, drift, and poor response. Membrane failure may be apparent on visual inspection as dark spots or discoloration of the membrane or carbonate deposits on the membrane and end cap. Follow the procedures given in section 2.21 Electrode Setup to replace the membrane and add internal filling solution.

3.3 Troubleshooting

Generally, if the meter is operating properly, and if the electrode has been prepared and assembled properly and has passed the electrode checkout procedures, the electrode slope should fall within the range of -54 to -60 mV when the temperature of the solution is 20-25EC. When the temperature of the solution is 20EC, the electrode slope should be approximately -58.2 mV. For problems encountered that this SOP does not address, refer to the Model 290A meter instruction manual (Thermo Orion®, Inc. 1991) and the Model 95-12 ammonia electrode instruction manual (Thermo Orion®, Inc. 2002).

4.0 TRAINING

Personnel who will perform this task should first read this protocol and then operate under supervision until proper technique and accuracy of measurements is ensured.

5.0 SAFETY

10 M NaOH is a highly caustic liquid. Care should be taken to avoid its contact with skin or clothing. Should such contact occur, quickly flush affected area with water. Sinks are present along 2 walls of the wet lab, and an eye flushing station and shower is present near the entrance door of all labs. 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 by donning gloves, lab coats and other necessary safety equipment.

6.0 ATTACHMENTS

Attachment 1. Water Quality Adjustment Data Form

7.0 REFERENCES

Thermo Orion®, Inc. 1991. Portable pH/ISE Meters Instruction Manual (Models 230A, 250A, and 290A). Thermo Electron Corporation, Beverly, Massachusetts. 60 pages.

Thermo Orion®, Inc. 2002. Model 95-12 Ammonia Electrode Instruction Manual. Thermo Electron Corporation, Beverly, Massachusetts. 42 pages.

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Attachment 1

WATER QUALITY ADJUSTMENT DATA FORM

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ DATE_____

A. Salinity Adjustment:

Initial volume (mL) _____

Initial salinity ($^{o}/_{oo}$) _____

Vol. Milli-Q® water added (mL) _____

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

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

B. Character of Sample (after salinity adjustment):

Volume (mL) _____

Salinity ($^{o}/_{oo}$) _____

pH _____

Dissolved oxygen (mg/L) _____

DO saturation (%) _____

Total ammonia (mg/L) _____

Sulfide (mg/L) _____

COMMENTS_____

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Date Prepared: October 13, 1993

Date Revised: July 26, 2007

MEASURING SULFIDE WITH THE THEMO ORION® MODEL 9616 SUREFLOW COMBINATION SILVER/SULFIDE PROBE

1.0 APPLICATION

The silver/sulfide probe is used to measure the concentration of silver or sulfide ion in solution by measuring an electrode potential which develops across the sensing element. This potential, which depends on the level of free silver or sulfide ion in solution, is measured against a constant reference potential with a digital pH/mV or specific ion meter.

2.0 OPERATION

2.1 Required Equipment and Solutions

1. Thermo Orion® model 9616 Sureflow Combination silver/sulfide electrode
2. Thermo Orion® "A" Optimum Results™ Reference Electrode Filling Solution (# 900061)
3. Thermo Orion® model 290A pH/mV/specific ion meter or comparable meter
4. Sodium Sulfide stock solution (see 2.1.1)
5. Weekly Sulfide standard (see 2.1.2)
6. Standard sulfide solutions: 10, 1, .1, 0.5, 0.25, 0.125, .01, .009, 0.008, .007, .006, 005 mg/L (see 2.1.3)
7. Magnetic stir bars and stirrer
8. SAOB II solution (see 2.1.4 below)
9. Deaerated DI water and deaerated filtered seawater

2.1.1 Sodium sulfide stock solution

Prepare a stock solution of saturated sodium sulfide by dissolving approximately 100 g reagent grade $Na_2S \bullet 9H_2O$ in 100 mL deionized, deaerated water. Deionized water (DI) may be deaerated by passing through a .45 F filter under vacuum suction. Shake the solution and let stand capped overnight in a fume hood.

2.1.2 Weekly sufide standard

Prepare a weekly sulfide standard by pipetting 1 mL of the stock solution into a 100 mL volumetric flask. Add 50 mL of SAOB II solution and dilute to volume with deionized, deaerated water.

Determine the exact concentration, by titrating 10 mL of the standard with

0.1 M Lead perchlorate, using the electrode pair as the end point indicator. Additions of Lead perchlorate are best accomplished using a 1 CC syringe with a fine needle. The end point is the point at which the mV reading drops rapidly with the addition of one more drop of Lead perchlorate. In other words, if the mV readings were being graphed versus volume of Lead perchlorate added, then the electrode pair would be the point on the graph with the greatest absolute slope.

Calculate the concentration (**C**) of the weekly standard using the equation:

$$\mathbf{C = 3206\ (V_t/V_s)}$$ where:

C = concentration as ppm sulfide
V_t = volume of Lead perchlorate at end point
V_s = volume of standard (10 mL)

2.1.3 Standard sulfide solutions

Prepare other standards daily by serial dilution of the weekly standard with equal volumes of 0.45 F filtered, deareated seawater (FDS) and SAOB II. For example, to do a tenfold dilution, pipet 1 mL of the standard into a small beaker and add 4.5 mL of SAOB II and 4.5 mL of FDS. It is recommended that the first dilution from the weekly stock solution be 100 mg/L followed by serial dilutions to achieve the desired working range (eg..005 to 10 mg/L). Remember: all dilutions must be made with both SAOB II and FDS.

2.1.4 SAOB II

SAOB II solution is commercially available from Thermo Orion® (# 941609) or can be prepared by dissolving 40 g of Sodium Hydroxide and 29.2 g of EDTA in deaerated deionized water in a 500 mL volumetric flask. Prior to use this solution is "activated" by the addition of 18 g of ascorbic acid. Once activated this solution will last up to 1 month if stored in a dark bottle.

2.2 Instrument Setup

1. Remove the rubber cap covering the electrode tip.
2. Fill the electrode with "A" Optimum Results™ reference electrode filling solution.
3. Connect the electrodes to the meter soak the probe in reference electrode filling solution for 10 minutes before calibrating the probe

2.3 Calibration

Measure 5 mL of the most dilute standard (0.005 mg/L) into a small beaker. Add 5 mLs of SAOB II solution and a stir bar and place on the automatic stirrer. Rinse electrodes, blot dry, and place in beaker. Wait for a stable millivolt reading and plot the reading (linear axis) against concentration (log axis) on standard 4-cycle semilog paper. Repeat this process with the progressively higher concentration standards. Calculate the slope of the linear portion of the graph. The linear portion of the graft is that portion above 0.5 mg/L. The slope of the curve should be between -25 and -30 mv/decade. If the slope is out of range, perform the electrode slope check described in the probe instruction manual and/or consult the troubleshooting section of the manual.

2.4 Sample Measurement

Measure 5 mLs of sample into a small beaker. Add 5 mL of SAOB II solution and a stir bar and place on the automatic stirrer. Place probes in the solution and press the measure button on the meter. When the reading stabilizes, record the reading and read concentration from the calibration curve. Remove the probes, rinse with DI and blot. Continue with any additional samples.

Recheck the accuracy of the measurements by measuring the concentration of a known standard periodically. If the reading deviates more than 5% from the known value, recalibrate the probe.

2.8 Sample requirements and measuring hints

1. Samples and standards should be at the same temperature. Temperature must be less than 100EC.

2. Sulfide samples must be buffered at pH >12 with SAOB II so that HS^- and H_2S are converted to $S^=$.

3. Always use fresh standards for calibration mixed with deareated water.

4. Rinse electrodes with DI water between measurements and blot electrode dry.

5. For high ionic strength samples, (e.g., seawater) prepare standards with composition similar to that of sample.

3.0 MAINTENANCE

1. If electrode response is slow, the sensing element may contain a surface layer of contaminants. Restore performance by polishing with polishing strips (included with the probe) and deionized water. Cut off 1 inch of the strip and polish the sensing element with a circular motion for 30 seconds. Rinse and soak in standard solution for 5 minutes before use.

2. The silver/sulfide probe should always be stored dry. The rubber cap should be replaced over the element during periods of long storage to protect it.

3. The reference electrode may be stored dry or in internal reference filling solution for short periods of time (less than a week). For longer storage times, the solutions in the probes should be emptied and the chambers rinsed with DI to prevent crystallization.

4.0 TRAINING

Personnel who will perform this task should first read this SOP and operate the probe the first time under supervision.

5.0 SAFETY

SAOB II is a caustic solution. Lead perchlorate and sodium sulfide are irritants and toxic. Care should be taken and proper safety gear worn to avoid chemical contact with skin or clothing, or prolonged breathing of vapors. Should contact occur, flush the affected area with water and seek medical attention if warranted. An eye flushing station is present in the corner of the lab nearest the door, should it be needed.

6.0 REFERENCES

Thermo Orion®, Inc. 1991. Portable pH/ISE Meters Instruction Manual (Models 230A, 250A, and 290A). Thermo Electron Corporation, Beverly, Massachusetts. 60 pages.

Thermo Orion®, Inc. 2001. Model 94-16 Silver/Sulfide Electrodes Instruction Manual. Thermo Electron Corporation, Beverly, Massachusetts. 67 pages.

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Date Prepared : September 19, 2006

Date Revised: May 10, 2007

SEA URCHIN FERTILIZATION TOXICITY TEST WITH *STRONGYLOCENTROTUS PURPURATUS*

1.0 OBJECTIVE

The purpose of the fertilization toxicity test with the sea urchin, *Strongylocentrotus purpuratus*, is to determine if a seawater, 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}).

2.0 TEST PREPARATION

2.1 Test Animals

Gametes from the sea urchin, *S. purpuratus* are used in the sea urchin fertilization toxicity test. Animals can be collected in the field or obtained from a commercial supplier. *S. purpuratus* is a species of urchin which are found along the entire west coast of the United States and are characterized by a dark purple test with short light and dark purple spines. This species also has numerous tube feet between the rows of spines on both the dorsal and ventral surfaces of the test and often is found with algae, rocks and debris being held to the body with the tube feet. Due to the sensitivity of this species to the pH of Gulf Coast waters it is recommended to receive urchins for use in testing on the day of or the day before the actual test is to take place. If urchins are to be held overnight, they should be acclimated slowly to the temperature controlled aquarium water. Temperature of the cultures will prolong the useful life of the urchins and should be maintained at 12 " 1EC when gametes are not required. 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 if the sex can be determined before use in testing.

2.2 Dilution Water

Milli-Q 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 refrigeration. On the day of the test, pH, ammonia, sulfide 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. Whenever possible, temperatures of wash, dilution and test waters should be maintained at test temperatures.

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. For larger tests, (> 20 samples) it may be desirable to include control samples including dilutions at the beginning and at the end of the test to account for any variability due to decreased sperm viability during the pipetting process and provide for more statistical robustness for comparisons with a large number of samples.

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>0.5 M KCl solution:</u>

3.73 g Potassium Chloride crystals
100 mL Milli-Q reagent grade water

<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 urchins and place inverted individually in shallow empty Carolina bowls. Shake free as much water as possible before placing the urchins in each bowl.

2. To stimulate release of gametes from gonopores, inject each urchin with 0.5 to 1.0 ml of a 0.5 M solution of KCl in several injections surrounding the soft membrane of the mouth opening. Agitate each urchin slightly after injecting. Gametes should appear from the gonopores within a few minutes. If not, inject with another 0.5 to 1 mL aliquot of KCL solution. Replace urchins that shed no gametes after two series of injections.

3. Sperm are white to off white in color and eggs are tan to orange in color. If eggs appear, fill the Carolina dish half full with MFS and allow the urchin to shed eggs into the dish while inverted. Collect a few eggs from the bottom of the dish using a 10 mL disposable syringe with a large gauge blunt-tipped needle attached. Place 2 to 5 drops of eggs onto a scintillation vial containing 10 mL of filtered seawater. Rinse syringe and repeat for each female. Additional injections of KCl may be required to collect a full complement of eggs.

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

5. If sperm is shed from the urchins, place males upright on a pad made up of several layers of paper towels. Dab each male with additional paper towels to remove as much water as possible from the surface of the urchins. It is important to collect the sperm without any water coming in contact with it.

6. If sperm is watery, reject the animal and choose another. Sperm should be the consistency of condensed milk. Collect sperm using a Pasteur 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 20-50 µL 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 inverted into a small Carolina dish and add enough seawater to cover one half of the urchin's test. Stimulate release of eggs as described above with KCl injections.

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, give one or two additional injections of KCL and agitate the urchin and replace inverted into the dish.

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.

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 KCl injected males on a pad made up of several layers of paper towels dab each male with additional paper towels to remove as much water as possible from the surface of the urchins. Once sperm has contacted water it becomes activated and has a limited life span. This species has a tendency to expel water from the anus during gamete collection so it is important to watch when this happens and discard the sperm on the surface of the urchin at that time, dab with a paper towel to remove water and wetted sperm and continue collecting only dry sperm. It may be necessary to repeatedly invert the urchin for short periods of time or provide additional injections of KCl to facilitate additional shedding of sperm. Collect about 1.0 mL of unwetted sperm from between spines using a pasteur pipette. Place sperm into a plastic microcentrifuge tube. Keep on ice until used. Do not allow the sperm to freeze. To prevent this, wrap each microcentrifuge tube in a small amount of paper towel before placing it on ice. 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 60-95%. 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 95% 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. Due to the sensitivity of *S. purpuratus* sperm to pHs > 8.1, a higher concentration of sperm is generally required in tests utilizing Gulf coast sea waters and pore waters. This effectively reduces the overall sensitivity of the test. Generally, a dilution of between 1:2500 and 1:750 will result in desirable fertilization rates, if the animals are in good condition.

For example, if a sperm dilution of 1:2500 is required (as determined from the pretest), add 20 µL sperm to 5 mL MFS. Mix thoroughly, then add 1 mL of this solution to 9 mL of MFS. Sperm should not be wetted until just before starting the test. Sperm wetted more than 10 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 10 minutes of wetting.

2. Incubate all test vials at 12 " 1°C for 20 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 20 minutes at 12 " 1°C. The timer may be used again at this point.

5. Using the dispenser, add 0.75 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.

4.0 DATA COLLECTION AND TABULATION

1. Transfer approximately 1 mL eggs and water from <u>bottom</u> of test vials to counting slide. Observe eggs using compound microscope under 100X magnification. Dark field viewing

is useful here in identifying fertilization membranes; however, *S. purpuratus* fertilization membranes are typically very easily discernable.

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 pore water, filtered sea water, 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 can be sharp and fragile and may puncture the skin and break off if handled roughly. First aid similar to treatment of wood splinters is effective in this case (removal of spine and treatment with antiseptic). Collection of sea urchins by snorkeling should not be done alone.

9.0 ATTACHMENTS

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

10.0 REFERENCES

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

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

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

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Attachment 1

EQUIPMENT LIST FOR FERTILIZATION TOXICITY TEST

Small Carolina dishes (at least 2)
20 mL Wheaton scintillation vials (These should be type shipped with caps with polyseal cone 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)
10 cc syringe with large diameter blunt ended needle (make by grinding sharp point off the needle with a grinding stone)
1 cc syringe with fine tip needle at least 2 cm in length.
0.5 M KCl solution
Paper towels
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
Milli -Q reagent grade water
Approximately 100 $^{o}/_{oo}$ concentrated brine
Biological Control Chamber or Incubator adjusted to 12°C

Attachment 2

PRETEST TO INSURE SELECTION OF QUALITY GAMETES

1. Using the procedure in section 2.4.1, select 1 to 4 females and at least 1 male urchin 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 5 pretest sperm concentrations, 20 vials (2 X 2 X 5) would be needed. Additional reference solutions may be added to the pretest to aid in selecting the proper sperm dilution. Typically, one rep each of three different reference toxicant concentrations (2.5, 5.0, and 10 mg/L) is added for each sperm concentration to ascertain if the sperm dilution selected will fall within the control range for that reference toxicant. Arrange and mark vials accordingly in a rack and incubate at 12°C until the test solutions acclimate to the test temperature.

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 60-95% 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: 1000 (1 ml of 1:250 and 3 ml MFS)
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)

Sperm must be used within 10 minutes of dilution and should be made up in MFS maintained at the test temperature. 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 20 minutes at approximately 12°C, and add 200 µL of the egg suspension. Incubate for another 20 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 ($^{o}/_{oo}$) _____

Vol. Milli-Q water added (mL) _____

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

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

B. Character of Sample (after salinity adjustment):

Volume (mL) _____

Salinity ($^{o}/_{oo}$) _____

pH _____

Dissolved oxygen (mg/L) _____

DO saturation (%) _____

Total ammonia (mg/L) _____

Sulfide (mg/L) _____

COMMENTS_____

_____

_____

_____

_____

_____

_____

Attachment 4

SEAURCHIN PRETEST DATA SHEET

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ 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

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ 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

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ 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

STUDY PROTOCOL_____ INITIALS_____

SAMPLE DESIGNATION_____ DATE_____

	PERCENT FERTILIZED Replicate						
Treatment	1	2	3	4	5	Mean"SD	Unfert.

Comments:_____

_____

_____

_____

_____

_____

_____

APPENDICIES

Appendix 1. Table of Sodium Dodecyl Sulfate reference toxicant control EC_{50}s (mg/L) from toxicity tests with *Strongylocentrotus purpuratus* from 1997-2009.

Year	Test	EC_{50}	EC_{50}	Method[1]	Stations included
1997	Puget Sound-1	2.41		30/30 exposure 15°C	26 - 58, 83 - 100
1997	Puget Sound-2A	3.23		30/30 exposure 15°C	59 - 82
1997	Puget Sound 4	3.51		30/30 exposure 15°C	1 - 25
1997	PS Ref Tox	2.91		30/30 exposure 15°C	
1998	Puget Sound II-2	2.32		30/30 exposure 15°C	157 -205, 114, 115
1998	Puget Sound II-3	5.36		30/30 exposure 15°C	106 - 132
1998	Puget Sound II-4	4.03		30/30 exposure 15°C	133-156
1999	Puget Sound III-2A	3.69		30/30 exposure 15°C	254-305
1999	Puget Sound III-2B	2.31		30/30 exposure 15°C	206-253
2002	PSAMP 2002	4.77		30/30 exposure 15°C	1 - 161
2002	PSAMP 2002	5.7		30/30 exposure 15°C	177 - 2123
2003	PSAMP 2003		2.49	20/20 exposure 12°C	297 - 523
2003	PSAMP 2003		2.76	20/20 exposure 12°C	527 - 1387
2005	PSAMP 2004	3.69		30/30 exposure 15°C	8 - 336
2005	PSAMP 2004		3.54	20/20 exposure 12°C	selected 24 - 336
2005	PSAMP 2003 Retest	5.7		30/30 exposure 15°C	297 - 1387
2006	PSAMP Methods comp		3.58	20/20 exposure 12°C	All
2006	PSAMP Methods comp	3.49		30/30 exposure 15°C	All
2006	PSAMP test 1		3.66	20/20 exposure 12°C	3- 165
2006	PSAMP test 2		3.87	20/20 exposure 12°C	189 - 667
2007	PSAMP Test 1		2.62	20/20 exposure 12°C	5 - 151
2007	PSAMP Test 2		3.54	20/20 exposure 12°C	155 - 371
2007	Urban Waters		2.42	20/20 exposure 12°C	All
2008	PSAMP		3.56	20/20 exposure 12°C	All
2008	Urban Waters		2.83	20/20 exposure 12°C	All
2009	PSAMP-Test 1		3.54	20/20 exposure 12°C	002-234
2009	PSAMP-Test 2		3.54	20/20 exposure 12°C	239-498
2009	Urban Waters		3.08	20/20 exposure 12°C	All
	Mean	**3.79429**	**3.21643**		
	Stdev	**1.18682**	**0.49512**		

[1] Refers to minutes of exposure of sperm and then egg with sperm at incubated temperature.

Appendix 2. Table of relevant sample dates for the PSAMP 2009 sediments.

Sample ID	Date [1] Collected	Date [2] shipped	Date [3] received	Date [4] extracted	Volume [5] extracted	Holding [6] time	Date [7] Thawed	Date [8] Tested
2	6/3/2009	6/8/2009	6/9/2009	6/9/2009	450	6	12/14/2009	12/16/2009
6	6/18/2009	6/22/2009	6/23/2009	6/25/2009	450	7	12/14/2009	12/16/2009
38	6/18/2009	6/22/2009	6/23/2009	6/25/2009	450	7	12/14/2009	12/16/2009
58	6/19/2009	6/22/2009	6/23/2009	6/25/2009	450	6	12/14/2009	12/16/2009
62	6/19/2009	6/22/2009	6/23/2009	6/25/2009	450	6	12/14/2009	12/16/2009
63	6/3/2009	6/8/2009	6/9/2009	6/9/2009	450	6	12/14/2009	12/16/2009
70	6/18/2009	6/22/2009	6/23/2009	6/25/2009	450	7	12/14/2009	12/16/2009
94	6/19/2009	6/22/2009	6/23/2009	6/25/2009	450	6	12/14/2009	12/16/2009
95	6/3/2009	6/8/2009	6/9/2009	6/9/2009	450	6	12/14/2009	12/16/2009
126	6/18/2009	6/22/2009	6/23/2009	6/25/2009	450	7	12/14/2009	12/16/2009
127	6/3/2009	6/8/2009	6/9/2009	6/9/2009	450	6	12/14/2009	12/16/2009
159	6/5/2009	6/8/2009	6/9/2009	6/10/2009	450	5	12/14/2009	12/16/2009
162	6/11/2009	6/15/2009	6/16/2009	6/16/2009	450	5	12/14/2009	12/16/2009
168	6/4/2009	6/8/2009	6/9/2009	6/10/2009	450	6	12/14/2009	12/16/2009
178	6/11/2009	6/15/2009	6/16/2009	6/16/2009	450	5	12/14/2009	12/16/2009
186	6/18/2009	6/22/2009	6/23/2009	6/25/2009	450	7	12/14/2009	12/16/2009
191	6/3/2009	6/8/2009	6/9/2009	6/9/2009	450	6	12/14/2009	12/16/2009
194	6/11/2009	6/15/2009	6/16/2009	6/16/2009	450	5	12/14/2009	12/16/2009
202	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/14/2009	12/16/2009
207	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
210	6/17/2009	6/22/2009	6/23/2009	6/24/2009	450	7	12/14/2009	12/16/2009
218	6/17/2009	6/22/2009	6/23/2009	6/24/2009	450	7	12/14/2009	12/16/2009
226	6/16/2009	6/17/2009	6/18/2009	6/18/2009	450	2	12/14/2009	12/16/2009
232	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/14/2009	12/16/2009
234	6/11/2009	6/15/2009	6/16/2009	6/16/2009	450	5	12/14/2009	12/16/2009
239	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
258	6/5/2009	6/8/2009	6/9/2009	6/10/2009	450	5	12/14/2009	12/16/2009
264	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/14/2009	12/16/2009
271	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009

Appendix 2. Continued.

Sample ID	Date [1] Collected	Date [2] shipped	Date [3] received	Date [4] extracted	Volume [5] extracted	Holding [6] time	Date [7] Thawed	Date [8] Tested
274	6/3/2009	6/8/2009	6/9/2009	6/9/2009	450	6	12/14/2009	12/16/2009
290	6/11/2009	6/15/2009	6/16/2009	6/16/2009	450	5	12/14/2009	12/16/2009
295	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
298	6/16/2009	6/17/2009	6/18/2009	6/18/2009	450	2	12/14/2009	12/16/2009
304	6/4/2009	6/8/2009	6/9/2009	6/10/2009	450	6	12/14/2009	12/16/2009
306	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/14/2009	12/16/2009
316	6/17/2009	6/22/2009	6/23/2009	6/24/2009	450	7	12/14/2009	12/16/2009
322	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/14/2009	12/16/2009
327	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
330	6/17/2009	6/22/2009	6/23/2009	6/24/2009	450	7	12/14/2009	12/16/2009
346	6/5/2009	6/8/2009	6/9/2009	6/10/2009	450	5	12/14/2009	12/16/2009
370	6/16/2009	6/17/2009	6/18/2009	6/18/2009	450	2	12/14/2009	12/16/2009
375	6/5/2009	6/8/2009	6/9/2009	6/10/2009	450	5	12/14/2009	12/16/2009
426	6/16/2009	6/17/2009	6/18/2009	6/18/2009	450	2	12/14/2009	12/16/2009
439	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
446	6/17/2009	6/22/2009	6/23/2009	6/24/2009	450	7	12/14/2009	12/16/2009
455	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
458	6/16/2009	6/17/2009	6/18/2009	6/18/2009	450	2	12/14/2009	12/16/2009
480	6/4/2009	6/8/2009	6/9/2009	6/10/2009	450	6	12/14/2009	12/16/2009
487	6/2/2009	6/3/2009	6/4/2009	6/4/2009	450	2	12/14/2009	12/16/2009
498	6/17/2009	6/22/2009	6/23/2009	6/24/2009	450	7	12/14/2009	12/16/2009

[1] Date sediment sample collected from the field
[2] Date sediment sample was shipped to MERS laboratory in Corpus Christi, Texas
[3] Date sediment sample was received at the MERS laboratory in Corpus Christi, Texas
[4] Date that pore water was extracted from the sediment and frozen.
[5] Volume of pore water extracted and frozen (ml).
[6] Number of days between sample collected in the field and porewater extraction and freezing.
[7] Date that the pore water was removed from the freezer to begin thawing for the salinity adjustment water quality measurement and testing.
[8] Date that salinity adjusted pore water was tested.

Appendix 3. Table of relevant sample dates for the Urban Waters 2009 sediments.

Sample ID	Date [1] Collected	Date [2] shipped	Date [3] received	Date [4] extracted	Volume [5] extracted	Holding [6] time	Date [7] Thawed	Date [8] Tested
124	6/4/2009	6/8/2009	6/9/2009	6/10/2009	450	6	12/7/2009	12/9/2009
125	6/4/2009	6/8/2009	6/9/2009	6/10/2009	450	6	12/7/2009	12/9/2009
126	6/4/2009	6/8/2009	6/9/2009	6/10/2009	450	6	12/7/2009	12/9/2009
142	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
143	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
144	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
145	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
146	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
147	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
148	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
149	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
150	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
151	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
152	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
153	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
154	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
155	6/9/2009	6/10/2009	6/11/2009	6/11/2009	450	2	12/7/2009	12/9/2009
166	6/10/2009	6/15/2009	6/16/2009	6/16/2009	450	6	12/7/2009	12/9/2009
167	6/10/2009	6/15/2009	6/16/2009	6/16/2009	450	6	12/7/2009	12/9/2009
168	6/10/2009	6/15/2009	6/16/2009	6/16/2009	450	6	12/7/2009	12/9/2009
169	6/10/2009	6/15/2009	6/16/2009	6/16/2009	450	6	12/7/2009	12/9/2009
170	6/10/2009	6/15/2009	6/16/2009	6/16/2009	450	6	12/7/2009	12/9/2009
171	6/10/2009	6/15/2009	6/16/2009	6/16/2009	450	6	12/7/2009	12/9/2009
156	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/7/2009	12/9/2009
157	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/7/2009	12/9/2009
158	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/7/2009	12/9/2009
159	6/12/2009	6/15/2009	6/16/2009	6/16/2009	450	4	12/7/2009	12/9/2009

Appendix 3. Continued.

Sample ID	Date [1] Collected	Date [2] shipped	Date [3] received	Date [4] extracted	Volume [5] extracted	Holding [6] time	Date [7] Thawed	Date [8] Tested
160	6/15/2009	6/17/2009	6/18/2009	6/18/2009	450	3	12/7/2009	12/9/2009
161	6/15/2009	6/17/2009	6/18/2009	6/18/2009	450	3	12/7/2009	12/9/2009
162	6/15/2009	6/17/2009	6/18/2009	6/18/2009	450	3	12/7/2009	12/9/2009
163	6/15/2009	6/17/2009	6/18/2009	6/18/2009	450	3	12/7/2009	12/9/2009
164	6/15/2009	6/17/2009	6/18/2009	6/18/2009	450	3	12/7/2009	12/9/2009
165	6/15/2009	6/17/2009	6/18/2009	6/18/2009	450	3	12/7/2009	12/9/2009

[1] Date sediment sample collected from the field

[2] Date sediment sample was shipped to MERS laboratory in Corpus Christi, Texas

[3] Date sediment sample was received at the MERS laboratory in Corpus Christi, Texas

[4] Date that pore water was extracted from the sediment and frozen.

[5] Volume of pore water extracted and frozen (ml).

[6] Number of days between sample collected in the field and porewater extraction and freezing.

[7] Date that the pore water was removed from the freezer to begin thawing for the salinity adjustment water quality measurement and testing.

[8] Date that salinity adjusted pore water was tested.

Appendix 4. Chain of custody sheets from incoming samples arriving at the USGS Marine Ecotoxicology Research Station from June 2nd – 19th 2009.

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/2/2009	207	OK	Urchin Fertilization Test	OK			OK 6°C
6/2/2009	439	OK	Urchin Fertilization Test	OK			OK
6/2/2009	239	OK	Urchin Fertilization Test	OK			OK 6°C
6/2/2009	487	OK	Urchin Fertilization Test	OK			#N/A
6/2/2009	295	OK	Urchin Fertilization Test	OK			OK
6/2/2009	~~217~~ 271	OK	Urchin Fertilization Test	OK			#N/A
6/2/2009	455	OK	Urchin Fertilization Test	OK			OK
6/2/2009	327	OK	Urchin Fertilization Test	OK			OK 6°C

Relinquished By	Date	Received By	Date	Comments
Maggie Dittly	6/2/09	QC walk in		
QC walk in	6/3/09	Sandra Weakland	6/3/09	
Sandra Weakland	6/3/09	Fed Ex	6/3/09	
[illegible]	6-4-09	[illegible]	6-4-09	Temp processed and Rover all on 6-4-09

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/3/2009	002	OK	Urchin Fertilization Test	OK			OK 4°C
6/3/2009	063	OK	Urchin Fertilization Test	OK			OK
6/3/2009	095	OK	Urchin Fertilization Test	OK			OK
6/3/2009	127	OK	Urchin Fertilization Test	OK			OK 5°C
6/3/2009	191	OK	Urchin Fertilization Test	OK			OK
6/3/2009	274	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
O. Pawlicki	6/3/09	OC walk-in cooler	6/3/09	
O.C walk-in cooler	6/8/09	HQ mailroom (FedEx)	6/8/09	
		[illegible]	6/9/09	processed 6-9-09
				[illegible]

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/4/2009	304	OK	Urchin Fertilization Test	OK			OK
6/4/2009	480	OK	Urchin Fertilization Test	OK			OK
6/4/2009	168	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/4/09	O.C. walk-in cooler	6/4/09	
O.C. walk-in cooler	6/8/09	HQ mailroom (FedEx)	6/8/09	processed 6-10-09
		[illegible]	6/9/09	[illegible]

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/5/2009	159	OK	Urchin Fertilization Test	OK			OK
6/5/2009	258	OK	Urchin Fertilization Test	OK			OK
6/5/2009	346	OK	Urchin Fertilization Test	OK			OK
6/5/2009	375	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
U. Partridge	6/5/09	O.C. walk-in cooler	6/5/09	
O.C. walk-in cooler	6/8/09	HQ mailroom (FedEx)	6/8/09	processed 6-10-09
			6/9/09	

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/9/2009	232	OK	Urchin Fertilization Test	OK			OK
6/9/2009	264	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
Sandra Weakland	6/9/09	OC walk in	6/9/09	
O.C walk in center	6/10/09	Sandra Weakland	6/10/09	
Sandra Weakland	6/10/09	Fed Ex Shipper	6/10/09	
		[illegible]	6/11-09	processed 6-11-09

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/11/2009	194	OK	Urchin Fertilization Test	OK			OK
6/11/2009	290	OK	Urchin Fertilization Test	OK			OK
6/11/2009	234	OK	Urchin Fertilization Test	OK			OK
6/11/2009	162	OK	Urchin Fertilization Test	OK			OK 5.5°C
6/11/2009	178	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/11/09	O.C. walk-in cooler	6/11/09	
O.C. walk-in cooler	6/15/09	HQ mailroom (FedEx)	6/15/09	
		[illegible]	6/16/09	

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/12/2009	202	OK	Urchin Fertilization Test	OK			OK 5°C
6/12/2009	306	OK	Urchin Fertilization Test	OK			OK
6/12/2009	322	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/12/09	D.C. walk-in cooler	6/12/09	
D.C. walk-in cooler	6/15/09	HQ mailroom (FedEx)	6/15/09	
		[illegible]	6/16/09	

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/16/2009	458	OK	Urchin Fertilization Test	OK			OK
6/16/2009	370	OK	Urchin Fertilization Test	OK			OK 5°C
6/16/2009	298	OK	Urchin Fertilization Test	OK			OK
6/16/2009	226	OK	Urchin Fertilization Test	OK			OK
6/16/2009	426	OK	Urchin Fertilization Test	OK			OK 5°C

Relinquished By	Date	Received By	Date	Comments
Sandra Weakland	6/16/09	OC walk in cooler	6/16/09	
OC walk in cooler	6/17/09	Sandra Weakland	6/17/09	
Sandra Weakland	6/17/09	Fed ex Shipper	6/17/09	
		[illegible]	6/18/09	processed 6-18-09

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/17/2009	330	#N/A	Urchin Fertilization Test	OK			#N/A
6/17/2009	498	#N/A	Urchin Fertilization Test	OK			#N/A
6/17/2009	316	OK	Urchin Fertilization Test	OK			OK
6/17/2009	446	OK	Urchin Fertilization Test	OK			OK
6/17/2009	210	OK	Urchin Fertilization Test	OK			OK
6/17/2009	218	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/17/09	O.C. walk-in cooler	6/17/09	
O.C. walk in cooler	6/22/09	Sandra Weakland	6/22/09	
Sandra Weakland	6/22/09	Fed Ex Shipper	6/22/09	
		[illegible]	6/23/09	processed 6-24-09

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/18/2009	186	OK	Urchin Fertilization Test	OK			OK
6/18/2009	038	OK	Urchin Fertilization Test	OK			OK
6/18/2009	126	OK	Urchin Fertilization Test	OK			OK
6/18/2009	070	OK	Urchin Fertilization Test	OK			OK processed 6-24-09
6/18/2009	006	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
J. Fairbridge	6/18/09	O.C. walk-in cooler	6/18/09	
OC walk-in cooler	6/22/09	Sandra Weakland	6/22/09	
Sandra Weakland	6/22/09	O.C. FedEx Shipper	6/22/09	
		[illegible]	6-23-09	processed 6-25-09

PSAMP Spatial 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/19/2009	058	OK	Urchin Fertilization Test	OK			OK
6/19/2009	062	OK	Urchin Fertilization Test	OK			OK
6/19/2009	094	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
Maggie Dietz	6/19/09	walk-in cooler - OC		
walk in cooler @ OC	6/22/09	Sandra Weakland	6/22/09	
Sandra Weakland	6/22/09	OC Fed Ex Shipper	6/22/09	
		[illegible]	6-23-09	processed 6-25-09

Urban Waters 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/4/2009	126	OK	Urchin Fertilization Test	OK			OK
6/4/2009	125	OK	Urchin Fertilization Test	OK			OK
6/4/2009	124	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/4/09	O.C. walk-in cooler	6/4/09	
O.C. walk-in cooler	6/8/09	HQ mailroom (FedEx)	6/8/09	Processed 6/10/09
		M. Bi	6/9/09	

Urban Waters 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/9/2009	142	OK	Urchin Fertilization Test	OK			OK
6/9/2009	143	OK	Urchin Fertilization Test	OK			OK
6/9/2009	144	OK	Urchin Fertilization Test	OK			OK
6/9/2009	145	OK	Urchin Fertilization Test	OK			OK
6/9/2009	146	OK	Urchin Fertilization Test	OK			OK
6/9/2009	147	OK	Urchin Fertilization Test	OK			OK
6/9/2009	148	OK	Urchin Fertilization Test	OK			OK
6/9/2009	149	OK	Urchin Fertilization Test	OK			OK
6/9/2009	150	OK	Urchin Fertilization Test	OK			OK
6/9/2009	151	OK	Urchin Fertilization Test	OK			OK
6/9/2009	152	OK	Urchin Fertilization Test	OK			OK
6/9/2009	153	OK	Urchin Fertilization Test	OK			OK
6/9/2009	154	OK	Urchin Fertilization Test	OK			OK
6/9/2009	155	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
Sandra Weakland	6/9/09	OC walk in cooler	6/9/09	
OC walk in cooler	6/10/09	Sandra Weakland	6/10/09	
Sandra Weakland	6/10/09	FedEx Shipper	6/10/09	
		[illegible]	6/11/09	processed 6-11-09

Urban Waters 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/10/2009	168	OK	Urchin Fertilization Test	OK			OK
6/10/2009	166	OK	Urchin Fertilization Test	OK			OK 5.5°C
6/10/2009	167	OK	Urchin Fertilization Test	OK			OK
6/10/2009	170	OK	Urchin Fertilization Test	OK			OK 4°C
6/10/2009	171	OK	Urchin Fertilization Test	OK			OK
6/10/2009	169	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/10/09	O.C. walk-in cooler	6/10/09	
O.C. walk-in cooler	6/15/09	HQ mailroom (FedEx)	6/15/09	
		[illegible]	6-16-09	

Urban Waters 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/12/2009	158	OK	Urchin Fertilization Test	OK			OK 5.6°C
6/12/2009	157	OK	Urchin Fertilization Test	OK			OK
6/12/2009	156	OK	Urchin Fertilization Test	OK			OK
6/12/2009	159	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
D. Partridge	6/12/09	O.C. walk-in cooler	6/12/09	
O.C. walk-in cooler	6/15/09	HQ mailroom (FedEx)	6/15/09	
		[illegible]	6-16-09	

Urban Waters 2009

Urchin Fertilization Test

Date	Station		Parameter				
6/15/2009	165	OK	Urchin Fertilization Test	OK			OK
6/15/2009	164	OK	Urchin Fertilization Test	OK			OK 6°C
6/15/2009	161	OK	Urchin Fertilization Test	OK			OK 5°C
6/15/2009	160	OK	Urchin Fertilization Test	OK			OK 6°C
6/15/2009	162	OK	Urchin Fertilization Test	OK			OK
6/15/2009	163	OK	Urchin Fertilization Test	OK			OK

Relinquished By	Date	Received By	Date	Comments
Sandra Weakland	6/15/09	QC walk in cooler	6/15/09	
QC walk in cooler	6/17/09	Sandra Weakland	6/17/09	
Sandra Weakland	6/17/09	FedEx Shipper	6/17/09	
		[illegible]	6/18/09	processed 6-18-09