

Final Report  
on  
TOXICITY TESTING OF SEDIMENT PORE WATER FROM HOOD CANAL  
AND SURROUNDING AREAS, PSAMP 2004 AND RETESTING OF  
POREWATER FROM THE SAN JUAN ISLANDS,  
STRAIT OF JUAN DE FUCA, AND ADMIRALTY INLET, WASHINGTON  
PSAMP 2003

submitted to

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

February 7, 2005

from

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**Final Report**

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**Toxicity Testing of Sediment Pore Water from Hood Canal and Surrounding Areas,  
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Strait of Juan de Fuca and Admiralty Inlet, Washington  
PSAMP 2003**

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Table 7. Summary of station means and statistical significance for the sea urchin (*Strongylocentrotus purpuratus*) fertilization assays conducted at two exposure periods and two temperatures for the PSAMP 2003 study tested in 2003 and 2005.

## FIGURES

Figure 1. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results for samples collected in the 2004 PSAMP program in Hood Canal and surrounding areas. Color differentiation of circles and diamonds indicates those stations that were significantly different from the reference (Dunnett's *t*-test,  $\alpha \leq 0.05$  and detectable significance criteria applied).

Figure 2. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results for samples collected in the 2003 PSAMP program in the San Juan Islands, Strait of Juan de Fuca and Admiralty Inlet, Washington and retested in 2005 with a 60 minute exposure period at 15°C. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's *t*-test,  $\alpha \leq 0.05$  and detectable significance criteria applied).

Figure 3. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results for samples collected in the 2003 PSAMP program in the San Juan Islands, Strait of Juan de Fuca and Admiralty Inlet, Washington. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's *t*-test,  $\alpha \leq 0.05$  and detectable significance criteria applied).

## ATTACHMENTS

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

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

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

## APPENDIX

Appendix 1. Chain of custody sheets from incoming samples arriving at the USGS Marine Ecotoxicology Research Station from June 8<sup>th</sup> -16<sup>th</sup>, 2004.

## INTRODUCTION

The Washington Department of Ecology annually determines the quality of recently deposited sediments in Puget Sound as a part of the Puget Sound Ambient 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. 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. Sediment samples were collected by personnel from the Washington Department of Ecology, in June of 2003 and 2004 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 until just prior to testing when water quality parameters were measured and adjusted, if necessary. A dilution series (100, 50 and 25%) test design was used to determine the toxicity of sediment porewater samples.

The specific objectives of this study were to:

- Extract sediment pore water from all 30 sediment samples as soon as possible after receipt of the samples using a pneumatic extraction device.
- Measure water quality parameters (salinity, dissolved oxygen, pH, sulfide, temperature, and ammonia) of thawed porewater samples prior to testing and adjust salinity, temperature and dissolved oxygen, if necessary.
- Conduct the fertilization toxicity test with pore water using the sea urchin (*S. purpuratus*) gametes.
- Perform side by side comparisons of two assay exposure periods and temperatures with 20 porewater samples collected in 2004.
- Retest the 41 samples collected in 2003 using the 60 minute exposure period at 15°C and compare the results to the 2003 results with an exposure period of 40 minutes at 12°C.
- 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 in 2004 from 30 stations in areas surrounding Hood Canal and Dabob Bay, Washington. 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 6 days from the time of field collection of sediment, and within 12 hours of arrival at the Corpus Christi laboratory.

### Toxicity Testing

#### Sediment Porewater Extraction Procedure

Pore water was extracted from the sediments using a pneumatic extraction device. This extractor is made of polyvinyl chloride (PVC) and uses a 5  $\mu\text{m}$  polyester filter. It is the same device used in previous sediment quality assessment surveys (Carr and Chapman, 1992; 1995; Carr et al., 1996a; 1996b; NBS, 1993; 1994; 1995a; 1995b; USFWS, 1992; USGS, 1997a; 1997b; 1998; 1999a; 1999b; 2000a; 2000b; 2001a; 2001b; 2001c; 2002a; 2002b; 2002c; 2002d; 2003a; 2003b). The apparatus and extraction procedures are detailed in SOP F10.9 (Attachment 1). After extraction, the porewater samples were centrifuged in polycarbonate bottles at 1200  $\times g$  for 20 minutes to remove any suspended particulate material; the supernatant was collected and frozen at  $-20^{\circ}\text{C}$ .

Two days before conducting a toxicity test, the samples were moved from the freezer to a refrigerator at  $4^{\circ}\text{C}$ . One day prior to testing, samples were thawed in a tepid ( $20^{\circ}\text{C}$ ) water bath. Temperature of the samples was maintained at room temperature  $20 \pm 3^{\circ}\text{C}$ . Sample salinity was measured and adjusted to  $30 \pm 1\text{‰}$ , if necessary, using purified deionized water or concentrated brine (see SOP F10.12, Attachment 2). Other water quality measurements (dissolved oxygen, pH, sulfide and ammonia concentrations) were made. Temperature and dissolved oxygen (DO) were measured with YSI<sup>®</sup> meters; salinity was measured with a Reichert<sup>®</sup> or American Optical<sup>®</sup> refractometer; and pH, sulfide (as  $\text{S}^{-2}$ ), and total ammonia (expressed as nitrogen; TAN) were measured with Orion<sup>®</sup> meters and their respective probes. Unionized ammonia (expressed as nitrogen) concentrations (UAN) were calculated for each sample using the respective salinity, temperature, pH, and TAN values. Any samples containing less than 80% DO saturation were gently aerated by stirring the sample on a magnetic stir plate. Following water quality measurements and adjustments, the samples were stored overnight at  $4^{\circ}\text{C}$  but returned to  $15 \pm 1^{\circ}\text{C}$ , or  $12 \pm 1^{\circ}\text{C}$  before the start of the toxicity tests.

#### Toxicity Testing with Sea Urchins

Toxicity of the sediment pore water collected in 2003 and 2004 and reference toxicants was determined using the fertilization test with the sea urchin *S. purpuratus* following the procedures outlined in SOP F10.6 (Attachment 3). SOP F10.6 was written for use specifically with the sea

urchin *Arbacia punctulata* and had to be modified slightly for use with *S. purpuratus*. Unlike *A. punctulata*, *S. purpuratus* cannot be induced to spawn using electrical stimulation. Therefore spawning was induced by injecting 0.5-3 ml of 0.5 M potassium chloride (in 0.5 increments) into the coelomic cavity. In addition, samples were tested at 15°C instead of 20°C as was the standard procedure for *Arbacia punctulata*. An additional test conducted with 20 of the 30 pore waters collected in 2004 utilized two additional changes made to the protocol last year which included exposure test time reduced from the stated SOP of 60 minutes (30 minutes sperm + 30 minutes sperm/egg) to 40 minutes (20 minutes sperm + 20 minutes sperm/egg) and test temperature further reduced from 15°C to 12°C. Test temperatures were maintained by incubating the pore waters, dilution waters and the test vials in an environmental chamber.

*Strongylocentrotus purpuratus* urchins were obtained from Marinus Scientific, Garden Grove, CA. Each of the porewater samples was tested in a dilution series design at 100, 50, and 25% of the water quality adjusted sample with 5 replicates per treatment. Dilutions were made with 0.45 µm filtered seawater. A reference porewater sample collected from Aransas Bay, Texas, which had been handled identically to the test samples, was included with each toxicity test as a negative control. This site is far removed from any known sources of contamination and has been used previously as a reference site (USGS, 2002a; 2003a; 2003b; 2003c; 2003d). In addition, a dilution water blank of filtered seawater was also included in each test and a reconstituted brine blank (brine with purified deionized water) was included. A dilution series test with sodium dodecyl sulfate (SDS) was also included in each assay as a positive control to evaluate overall test sensitivity.

#### 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 or modified arcsine square root transformed data with the aid of SAS (1989). The trimmed Spearman-Kärber method (Hamilton et al., 1977) with Abbott's correction (Morgan, 1992) was used to calculate EC<sub>50</sub> (50% effective concentration) values for reference toxicant tests. Prior to statistical analysis, the transformed data sets were screened for equal variance using SAS/LAB<sup>®</sup> Software (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:  $cv = t(df_{\text{Error}}, .05/(2 \times n))$ .

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 detect a significant effect while minimizing type I errors.. The DSC value for the sea urchin fertilization assay at  $\alpha = 0.05$  is 15.5%. At  $\alpha = 0.01$ , the DSC value is 19%. The DSC was developed using

the sea urchin *A. punctulata*, but was used to evaluate this data to aid in comparison to previous studies.

## 2004 RESULTS

### Porewater Quality Measurements

The sea urchin fertilization tests were performed with pore water from all 41 stations. Two separate tests using different temperatures (15 and 12°C) and exposure times (60 and 40 minutes) were performed on the same day with the same urchin gametes to minimize test variability. To satisfy the test salinity requirement of  $30 \pm 1.0$  ‰, twenty-seven samples required minor salinity adjustment with purified Milli-Q water. Salinities ranged from 30 to 32 ‰. Table 1 reports the values for all the water quality measurements conducted. Initial dissolved oxygen was > 80% in all the samples. Sulfide concentrations were detectable in low levels in samples 48, 92, 112, and 120. Total ammonia as nitrogen (TAN) ranged from 0.342 to 4.65 mg/L. The unionized fraction (the most toxic fraction) ranged from 2.6 to 65.8 µg/L. No samples exceeded the NOEC for unionized ammonia (0.17 mg/L) for *S. purpuratus* (Bailey et al. 1995).

### Sea Urchin Toxicity Testing

Raw data and means from the fertilization tests are given in Table 2 and 3. Three data points in the first test (60-minute exposure at 15°C) and two data points in the second (40-minute exposure at 12°C) were determined to be outliers (SAS 1992). Modifications were made to the transformations used to analyze the data from the second test (40 minute exposure at 12°C). Arcsine square root transformed data taken to the 1.5<sup>th</sup> power were used on the 100 and 50% dilution data to meet the assumptions of the analysis. The Log of the arcsine square root transformed data was used on the 25% data in order to meet the assumptions of that analysis. EC<sub>50</sub> values for the SDS positive controls were 3.69 and 3.54 mg/L (for tests one and two, respectively) which are similar to the mean for this species in our laboratory of 3.50 mg/L (95% CL 1.18-5.82). Unlike previous years in this laboratory, only a slight increase in sperm/egg ratio was required to achieve acceptable control survivals in the longer exposure test (60 minutes) than in the shorter test (40 minutes). Toxicity results from the two protocols can be compared in Table 4. Five samples were found to be toxic using both of the protocols (48, 56, 92, 96 and 112). In addition, sample 255 was found to be significantly different from the reference but did not meet the significance criterion for toxicity. Some slight differences were apparent in the level of toxicity of the dilutions of those samples when compared to each other, with the longer exposure time (60 minutes at 15°C) being slightly more toxic. This would be expected given that the sperm dilutions used to perform each test were similar and that the extra exposure time would be expected to kill more sperm in those toxic samples resulting in lower fertilization rates. Figure 1 gives a map of the stations and the results of the toxicity testing for both tests.

## 2003 RETEST RESULTS

## Porewater Quality Measurements

The sea urchin fertilization tests were performed with pore water from all 41 stations. To satisfy the test salinity requirement of  $30 \pm 1.0$  ‰, thirty samples required minor salinity adjustment with purified Milli-Q water. One sample required brine addition. Salinities ranged from 28 to 34 ‰. Table 5 reports the values for all the water quality measurements conducted. Initial dissolved oxygen was  $> 80\%$  in all the samples with the exception of samples #313, 441, 801, and 1289 which had to be aerated by stirring the day before the test and again the day of the test. Detectable concentrations of sulfide were observed in ten samples. Sulfide concentrations, dissolved oxygen, and pH of samples were remeasured on the day of the test in those samples that required aeration following the stirring process. Total ammonia as nitrogen (TAN) ranged from 0.445 to 6.34 mg/L. The unionized fraction (the most toxic fraction) ranged from 25.9 to 419.4  $\mu\text{g/L}$ . Seven samples (#313, 337, 433, 441, 681, 801, 1289) exceeded the NOEC for unionized ammonia (Bailey et al. 1995).

## Sea Urchin Toxicity Testing

Raw data and means from the fertilization tests are given in Table 6. No data points were determined to be outliers (SAS 1992). Unlike the previous tests in 2003 a high sperm/egg ratio was required in this test to achieve acceptable control fertilization in the pretest, indicating a high level of variability in the viability of the sperm for the longer test (60 min.) at the higher temperatures (15 °C). As a result the  $\text{EC}_{50}$  value for the SDS positive control was considerably higher for this test at 5.70 mg/L (5.35-6.08) which is near the upper limit of our confidence limits in our laboratory of 3.50 mg/L (95% CL 1.88-5.80) indicating a reduction in sensitivity. The  $\text{EC}_{50}$  values for the SDS positive controls were 2.49 and 2.76 mg/L for the 2003 testing period. The reduction in exposure time and temperature used in last years testing of these samples played an important role by allowing for the reduction of the overall quantity of sperm necessary to achieve satisfactory control fertilization rates while maintaining positive control (SDS)  $\text{EC}_{50}$ 's closer to the laboratory control mean.

Only seven of the 41 samples were found to be toxic in the retesting in 2005 compared to twenty of the 41 samples tested (48.8%) under the 40 minute 12° C protocol tested in 2003 (Table 7). In addition, none of the samples were found to be toxic beyond the 100% concentration this year compared to eight and four samples that were still toxic at the 50% dilution at the 25% concentration, respectively for the 2003 testing. The toxic sites from retesting with the 60 minute, 15°C protocol were # 363 and # 521 located in Discovery Bay, # 305 in Mackaye Harbor, # 369 located in Lopez Sound / Hunter Bay and # 1313, #801 and #1289 located in Sequim Bay (Figure 2). These sites were some of the most toxic in the testing that was done in 2003 (Figure 3.)

## DISCUSSION

A primary objective of this study was to compare the old (60 min. exposure at 15°C) and new (40 min. exposure at 12°C) test protocols. The best data set for making this comparison is from the 2004 collection where 20 samples were compared side-by-side and tested on the same day using gametes from the same sea urchins. Of the 20 samples tested, five were toxic using both protocols with the old (60 min./15°C) protocol being slightly more sensitive (i.e., toxicity

observed in a more dilute sample) than the new protocol in four of the five toxic samples. As mentioned in the results section, the sperm/egg ratio for the two different protocols for these tests was very similar and considerably lower than has been used in previous years to achieve an acceptable control fertilization in the pretest. Because the sperm/egg ratios were similar, it is not surprising that the old protocol with the longer exposure period resulted in more toxicity than the new protocol.

The retesting of the 41 samples from 2003 with the old protocol was conducted on a different day with a different batch of sea urchins. We conducted numerous pretests with combinations of gametes from nearly two dozen urchins in an attempt to locate the most viable gametes for this test. Unfortunately, based on the results from several pretests, the sperm/egg ratio necessary to achieve acceptable fertilization was considerably higher (4.5 to 5 times) than was used in the comparison test with the 2004 samples. When the actual test was run, this higher sperm/egg ratio resulted in an SDS EC<sub>50</sub> which was more than twice the EC<sub>50</sub> value in the original test with new protocol (40min./12°C). On the basis of this reference toxicant comparison alone, we would predict that the retesting with the old protocol would be less sensitive than the original test with the new protocol. Only seven of the 21 samples identified as toxic using the new protocol were found to be toxic using the old protocol during the retest. Six of the seven toxic samples using the old protocol in the retest were toxic at a 50 or 25% dilution in the original test with the new protocol but were only toxic in the 100% sample in the retest. Of the 14 samples found to be toxic using the new protocol but not with the old protocol in the retest, 11 were only toxic in the 100% samples in the original test. It would appear that the difference between the results from the two protocols can be accounted for by the loss of sensitivity with the old protocol resulting from the higher sperm/egg ratio as indicated by the higher EC<sub>50</sub> value for the reference toxicant test. In other words, if the reference toxicant tests results had been comparable for the two protocols, the results from the porewater tests would also have likely compared well.

The problem we experienced with the retesting of the 41 samples with the old protocol demonstrates the reason why we recommended changing to the new protocol. Because the viability of sperm from *S. purpuratus* is highly variable, it is difficult to determine the best sperm/egg ratio even when numerous pretests are conducted. The reduced exposure time and the lower temperature used in the new protocol minimizes these problems and allows the optimum sperm/egg ratio to be determined with a greater degree of precision. On the rare occasion when we are able to achieve an acceptable sperm/egg ratio using the old protocol at a level comparable to levels normally used with the new protocol, it appears that the methods compared well with the old protocol being slightly more sensitive because of the longer exposure duration. We recommend, therefore, based on the information obtained from these two comparison studies, that when the reference toxicant EC<sub>50</sub>s for a particular test fall within the normal range of  $\pm$  one standard deviation of the mean ( $3.50 \pm 1.16$ ) that data from either protocol can be compared directly without any adjustment

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- U.S. Geological Survey (USGS). 2003b. Toxicity testing of sediments from intertidal wetlands for the Washington Coastal EMAP 2002. Report Submitted by the USGS to the Washington State Department of Ecology, Olympia Washington. 8pp. + 7 tables, 2 figures, 3 attachments and 1 appendix.
- U.S. Geological Survey (USGS). 2003c. Toxicity testing of sediments near publicly owned treatment works and industrial discharges in the Mid-Atlantic region. Report Submitted by the USGS to the National Oceanic and Atmospheric Administration (NOAA), Center for Coastal Monitoring and Assessment, Silver Springs, MD. 8pp. + 7 tables, 2 figures, 5 attachments and 1 appendix.
- U.S. Geological Survey (USGS). 2003d. Toxicity testing of sediments from the Hawaii Coastal EMAP Study. Report Submitted by the USGS to the US Geological Survey's Biomonitoring of Environmental Status and Trends Program, Fort Collins, Colorado, 6 pp, + 5 tables, 4 figures and 4 attachments.

**TABLES 1-7**

**Table 1. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples from Hood Canal and surrounding areas collected in the 2004 PSAMP study.**

Station	Salinity <sup>1</sup> ‰	DO <sup>2</sup> (mg/L)	% DO <sup>3</sup>	pH	TAN <sup>4</sup> (mg/L)	UAN <sup>5</sup> ( $\mu$ g/L)	Sulfide <sup>6</sup> (mg/L)	% OUS <sup>7</sup>
TXREF <sup>8</sup>	25	8.09	93.2	7.818	0.437	9.1	<0.01	94.0
8	31.5	7.86	90.4	7.638	1.12	15.5	<0.01	95.0
24	32	7.99	91.3	7.627	0.947	12.8	<0.01	93.8
32	32	7.97	90.7	7.651	0.997	14.2	<0.01	93.8
48	32	8.29	94	7.848	1.58	35.2	0.01	93.8
56	31.5	8.17	92.8	7.764	0.833	15.4	<0.01	95.0
60	32	8.21	93.2	7.707	0.64	10.4	<0.01	93.8
64	31.5	8.07	91.7	7.641	0.634	8.9	<0.01	95.0
75	32	8.14	92.2	7.794	2.06	40.7	<0.01	93.8
80	31.5	8.20	92.7	7.719	0.558	9.3	<0.01	95.0
88	32	8.04	90.9	7.693	2.03	31.9	<0.01	93.8
92	32	8.29	93.6	7.849	1.38	30.8	0.01	93.8
96	32	8.46	95.4	7.778	0.866	16.5	<0.01	93.8
112	32	8.07	91.1	7.961	2.07	59.5	0.01	93.8
118	31	8.22	92.8	7.731	0.781	13.4	<0.01	100.0
120	32	8.21	92.6	7.703	0.361	5.8	0.16	93.8
124	31.5	8.39	95	7.616	2.73	36.0	<0.01	95.0
128	31.5	8.22	92.8	7.618	0.49	6.5	<0.01	95.0
144	31.5	8.22	92.9	7.745	1.36	24.0	<0.01	95.0
152	32	8.26	93.6	7.765	1.06	19.6	<0.01	93.8
184	32	8.01	90.9	7.598	0.653	8.3	<0.01	93.8
188	32	8.19	92.8	7.78	3.48	66.6	<0.01	93.8
203	32	8.30	94.1	7.689	2.19	34.1	<0.01	93.8
216	32	8.13	92.2	7.763	1.63	30.0	<0.01	93.8
224	32	8.16	92.4	7.611	0.601	7.8	<0.01	93.8
248	32	8.35	94.4	7.656	0.784	11.3	<0.01	93.8
252	30.5	8.24	93.3	7.647	4.65	65.8	<0.01	100.0
288	30	8.07	91.5	7.656	3.62	52.3	<0.01	100.0
296	32	8.59	97.4	7.374	0.342	2.6	<0.01	93.8
323	32	8.30	94.2	7.841	0.935	20.5	<0.01	93.8
336	32	7.81	88.7	7.795	0.833	16.5	<0.01	93.8

**Table 1. Continued.**

Station	Salinity <sup>1</sup> ‰	DO <sup>2</sup> (mg/L)	% DO <sup>3</sup>	pH	TAN <sup>4</sup> (mg/L)	UAN <sup>5</sup> ( $\mu$ g/L)	Sulfide <sup>6</sup> (mg/L)	% OUS <sup>7</sup>
MFS <sup>9</sup>	38	8.00	91.2	8.178	< 0.1	< 4.6	<0.01	78.9
Recon <sup>10</sup>	108	7.90	90.6	8.045	< 0.1	< 3.5	<0.01	26.4

<sup>1</sup> Salinity of sample prior to adjustment. Sample adjusted to  $30 \pm 1‰$

<sup>2</sup> Dissolved oxygen

<sup>3</sup> Percent saturation of dissolved oxygen

<sup>4</sup> Total ammonia as nitrogen

<sup>5</sup> Unionized ammonia

<sup>6</sup> Measured as S<sup>-2</sup>

<sup>7</sup> Percent of original sample after salinity adjustment

<sup>8</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>9</sup> Millipore filtered seawater diluent

<sup>10</sup> Reconstituted brine (concentrated brine diluted to 30 ‰ with reagent grade MilliQ water)

**Table 2. Sea urchin fertilization test raw data and means for sediment porewater samples collected in the 2004 PSAMP study and tested at 15°C for 60 minutes. Asterisks denote statistically differences (Dunnett's t-test) and detectable significance criteria between test and reference stations (\*alpha < 0.05, \*\*alpha ≤ 0.01). Plus signs denote only statistical differences (+alpha < 0.05, ++alpha ≤ 0.01).**

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
TX REF <sup>3</sup>	100	91	90	94	89	95	94.2	3.22		100.0
		98	97	95	95	98				
	50	96	96	93	96	94	95.0	1.41		100.0
		94	97	95	96	93				
	25	93	93	94	93	98	95.5	2.37		100.0
		97	96	94	99	98				
8	100	94	97	98	99	90	95.6	3.65		101.5
	50	100	97	98	95	96	97.2	1.92		102.3
	25	96	96	98	93	100	96.6	2.61		101.2
24	100	96	99	98	94	95	96.4	2.07		102.3
	50	94	96	95	98	95	95.6	1.52		100.6
	25	95	98	98	99	98	97.6	1.52		102.2
32	100	99	100	99	99	100	99.4	0.55		105.5
	50	94	97	96	91	97	95.0	2.55		100.0
	25	95	98	97	96	94	96.0	1.58		100.5
48	100	0	8	3	1	0	2.4	3.36	**	2.5
	50	19	12	10	26	28	19.0	8.06	**	20.0
	25	70	57	57	62	62	61.6	5.32	**	64.5
56	100	35	43	42	55	62	47.4	10.88	**	50.3
	50	83	86	89	80	82	84.0	3.54	++	88.4
	25	93	96	97	99	97	96.4	2.19		100.9
60	100	94	89	96	98	100	95.4	4.22		101.3
	50	95	95	91	96	94	94.2	1.92		99.2
	25	96	96	96	96	97	96.2	0.45		100.7
64	100	94	97	99	98	95	96.6	2.07		102.5
	50	95	94	98	94	94	95.0	1.73		100.0
	25	92	98	97	88	99	94.8	4.66		99.3

**Table 2. Continued.**

Station	% WQAS <sup>2</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
75	100	98	97	97	95	96	96.6	1.14		102.5
	50	93	88	95	93	90	91.8	2.77		96.6
	25	91	91	95	93	92	92.4	1.67		96.8
80	100	99	97	96	93	93	95.6	2.61		101.5
	50	96	93	96	96	95	95.2	1.30		100.2
	25	97	98	99	93	96	96.6	2.30		101.2
88	100	98	99	97	96	99	97.8	1.30		103.8
	50	94	92	96	88	92	92.4	2.97		97.3
	25	95	97	92	93	94	94.2	1.92		98.6
92	100	4	2	4	5	7	4.4	1.82	**	4.7
	50	44	27	25	50	38	36.8	10.76	**	38.7
	25	86	85	91	87	87	87.2	2.28	++	91.3
96	100	56	48	67	69	68	61.6	9.24	**	65.4
	50	68	64	75	63	67	67.4	4.72	**	70.9
	25	92	89	96	94	95	93.2	2.77		97.6
112	100	0	0	0	0	0	0.0	0.00	**	0.0
	50	12	3	8	9	2	6.8	4.21	**	7.2
	25	69	34	40	34	47	44.8	14.55	**	46.9
118	100	98	98	100	100	99	99.0	1.00		105.1
	50	98	98	100	98	100	98.8	1.10		104.0
	25	94	99	97	96	98	96.8	1.92		101.4
120	100	96	97	92	92	94	94.2	2.28		100.0
	50	96	95	88	95	97	94.2	3.56		99.2
	25	98	96	96	99	97	97.2	1.30		101.8
124	100	95	97	92	94	94	94.4	1.82		100.2
	50	92	95	94	96	96	94.6	1.67		99.6
	25	90	92	94	97	93	93.2	2.59		97.6
128	100	99	97	100	100	99	99.0	1.22		105.1
	50	94	100	99	99	98	98.0	2.35		103.2
	25	95	99	99	100	99	98.4	1.95		103.0
144	100	89	90	96	98	93	93.2	3.83		98.9
	50	93	97	96	95	91	94.4	2.41		99.4
	25	97	95	94	90	99	95.0	3.39		99.5

Table 2. Continued.

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
152	100	99	92	97	98	98	96.8	2.77		102.8
	50	95	94	91	98	90	93.6	3.21		98.5
	25	93	95	94	93	94	93.8	0.84		98.2
184	100	98	94	97	98	95	96.4	1.82		102.3
	50	94	94	90	94	97	93.8	2.49		98.7
	25	93	97	90	97	97	94.8	3.19		99.3
188	100	99	93	92	96	94	94.8	1.71		100.6
	50	88	93	91	94	93	91.8	2.39		96.6
	25	89	94	92	93	90	91.6	2.07		95.9
203	100	96	95	98	95	98	96.4	1.52		102.3
	50	94	94	91	96	96	94.2	2.05		99.2
	25	92	94	90	95	93	92.8	1.92		97.2
216	100	94	78	90	88	94	88.8	6.57		94.3
	50	92	99	95	93	94	94.6	2.70		99.6
	25	96	95	99	92	94	95.2	2.59		99.7
224	100	99	98	99	100	100	99.2	0.84		105.3
	50	97	96	95	94	99	96.2	1.92		101.3
	25	98	95	98	100	98	97.8	1.79		102.4
248	100	97	100	98	98	99	98.4	1.14		104.5
	50	100	97	99	98	99	98.6	1.14		103.8
	25	96	96	97	99	96	96.8	1.30		101.4
252	100	77	78	82	84	81	80.4	2.88	++	85.4
	50	96	91	94	86	90	91.4	3.85		96.2
	25	88	95	96	96	87	92.4	4.51		96.8
288	100	89	96	98	98	97	95.6	3.78		101.5
	50	97	92	94	91	99	94.6	3.36		99.6
	25	97	95	91	97	95	95.0	2.45		99.5
296	100	100	96	96	95	98	97.0	2.00		103.0
	50	96	97	100	98	97	97.6	1.52		102.7
	25	95	97	97	98	98	97.0	1.22		101.6
323	100	99	99	100	100	99	99.4	0.55		105.5
	50	96	99	99	98	100	98.4	1.52		103.6
	25	98	98	94	97	98	97.0	1.73		101.6

**Table 2. Continued.**

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
336	100	99	100	98	99	98	98.8	0.84		104.9
	50	98	94	97	100	95	96.8	2.39		101.9
	25	98	96	99	97	96	97.2	1.30		101.8
MFS <sup>4</sup>	100	79	89	70	57	91	87.2	14.21		92.6
		99	97	93	98	99				
RECON <sup>5</sup>	100	92	93	93	89	97	92.8	2.86		98.5
SDS <sup>6</sup>	10	0	0	0	0	0	0.0	0.00	**	0.0
	5	2	2	6	9	10	5.8	3.77	**	6.2
	2.5	98	97	57	99	99	98.3	0.96		104.3
	1.25	97	100	94	99	97	97.4	2.30		103.4

<sup>1</sup> Percent of water quality adjusted porewater sampled

<sup>2</sup> Significant difference from reference denoted as asterisks or plus signs

<sup>3</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>4</sup> Millipore filtered seawater diluent

<sup>5</sup> Reconstituted brine (concentrated brine diluted to 30 ‰ with reagent grade MilliQ water)

<sup>6</sup> Sodium Dodecyl Sulfate positive reference control

 = Value is an outlier and was omitted from statistical analysis

**Table 3. Sea urchin fertilization test raw data and means for sediment porewater samples collected in the 2004 PSAMP study and tested at 12°C for 40 minutes. Asterisks denote statistically differences (Dunnett's t-test) and detectable significance criteria between test and reference stations (\*alpha < 0.05, \*\*alpha ≤ 0.01). Plus signs denote only statistical differences (+alpha < 0.05, ++alpha ≤ 0.01).**

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
TX REF <sup>3</sup>	100	95	95	92	95	97	95.4	1.84		101.3
		99	96	96	94	95				
	50	96	95	93	95	98	96.2	1.81		101.3
		95	96	98	99	97				
	25	100	98	99	96	98	97.6	1.78		102.2
		98	99	94	98	96				
24	100	99	96	97	97	98	97.4	1.14		103.4
	50	96	98	96	94	98	96.4	1.67		101.5
	25	97	94	99	96	99	97.0	2.12		101.6
32	100	100	97	99	98	99	98.6	1.14		104.7
	50	96	99	98	97	96	97.2	1.30		102.3
	25	97	96	97	96	99	97.0	1.22		101.6
48	100	41	12	40	15	15	24.6	14.57	**	26.1
	50	73	60	68	70	61	66.4	5.68	**	69.9
	25	86	86	82	89	79	84.4	3.91	++	88.4
56	100	63	72	84	80	81	76.0	8.51	*	80.7
	50	98	94	94	92	92	94.0	2.45		98.9
	25	99	96	96	99	98	97.6	1.52		102.2
60	100	100	99	99	98	97	98.6	1.14		104.7
	50	96	94	100	100	99	97.8	2.68		102.9
	25	95	97	97	100	100	97.8	2.17		102.4
64	100	100	99	99	97	99	98.8	1.10		104.9
	50	97	98	100	99	96	98.0	1.58		103.2
	25	99	97	99	99	100	98.8	1.10		103.5
80	100	98	97	98	99	97	97.8	0.84		103.8
	50	97	97	99	96	97	97.2	1.10		102.3
	25	96	97	96	93	99	96.2	2.17		100.7

**Table 3. Continued.**

Station	% WQAS <sup>2</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
88	100	97	92	99	99	96	96.6	2.88		102.5
	50	96	98	95	93	96	95.6	1.82		100.6
	25	95	98	93	94	93	94.6	2.07		99.1
92	100	34	16	34	24	60	27.0	8.72	**	28.7
	50	73	89	84	87	84	83.4	6.19	++	87.8
	25	96	96	95	92	94	94.6	1.67		99.1
96	100	76	73	85	84	84	80.4	5.50	*	85.4
	50	88	80	91	89	89	87.4	4.28	++	92.0
	25	98	97	99	97	94	97.0	1.87		101.6
112	100	4	2	5	11	6	5.6	3.36	**	5.9
	50	34	27	56	41	53	42.2	12.32	**	44.4
	25	84	89	88	89	80	86.0	3.94	++	90.1
118	100	97	97	100	99	99	98.4	1.34		104.5
	50	97	98	99	99	98	98.2	0.84		103.4
	25	95	96	95	97	99	96.4	1.67		100.9
120	100	94	97	97	98	97	96.6	1.52		102.5
	50	95	97	98	96	97	96.6	1.14		101.7
	25	100	99	100	99	99	99.4	0.55		104.1
128	100	100	99	98	98	97	98.4	1.14		104.5
	50	98	97	96	99	98	97.6	1.14		102.7
	25	98	99	98	98	100	98.6	0.89		103.2
144	100	98	97	96	96	99	97.2	1.30		103.2
	50	95	95	95	96	97	95.6	0.89		100.6
	25	97	100	98	97	98	98.0	1.22		102.6
184	100	98	98	97	97	98	97.6	0.55		103.6
	50	99	98	97	100	96	98.0	1.58		103.2
	25	98	98	100	99	97	98.4	1.14		103.0
188	100	98	98	98	99	98	98.2	0.50		104.2
	50	94	96	98	100	100	97.6	2.61		102.7
	25	98	93	97	97	76	96.3	2.22		100.8
224	100	98	100	100	99	100	99.4	0.89		105.5
	50	100	99	100	99	99	99.4	0.55		104.6
	25	96	99	100	97	100	98.4	1.82		103.0

**Table 3. Continued.**

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
323	100	100	99	98	99	98	98.8	0.84		104.9
	50	98	98	97	95	97	97.0	1.22		102.1
	25	98	97	98	100	94	97.4	2.19		102.0
336	100	99	100	100	99	100	99.6	0.55		105.7
	50	100	100	99	99	97	99.0	1.22		104.2
	25	97	98	97	97	99	97.6	0.89		102.2
MFS <sup>4</sup>	100	97	96	95	97	99	96.9	1.85		102.9
		99	95	96	100	95				
RECON <sup>5</sup>	100	97	96	97	97	93	96.0	1.73		101.9
SDS <sup>6</sup>	10	0	0	0	0	0	0.0	0.00	**	0.0
	5	0	0	2	1	0	0.6	0.89	**	0.6
	2.5	98	98	100	97	94	97.4	1.89		103.4
	1.25	100	98	98	97	96	97.8	1.48		103.8

<sup>1</sup> Percent of water quality adjusted porewater sampled

<sup>2</sup> Significant difference from reference denoted as asterisks or plus signs

<sup>3</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>4</sup> Millipore filtered seawater diluent

<sup>5</sup> Reconstituted brine (concentrated brine diluted to 30 ‰ with reagent grade MilliQ water)

<sup>6</sup> Sodium Dodecyl Sulfate positive reference control

☐ = Value is an outlier and was omitted from statistical analysis

Table 4. Summary of station means and statistical significance for the sea urchin fertilization assays (*Strongylocentrotus purpuratus*) conducted at two exposure periods and two temperatures for the PSAMP 2004 study.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C.					40-Minute Exposure period at 12° C.							
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	
8	100	95.6	3.65	101.5	94.2	3.22								
8	50	97.2	1.92	102.3	95.0	1.41								
8	25	96.6	2.61	101.2	95.5	2.37								
24	100	96.4	2.07	102.3	94.2	3.22		97.4	1.14	103.4	95.4	1.84		
24	50	95.6	1.52	100.6	95.0	1.41		96.4	1.67	101.5	96.2	1.81		
24	25	97.6	1.52	102.2	95.5	2.37		97.0	2.12	101.6	97.6	1.78		
32	100	99.4	0.55	105.5	94.2	3.22		98.6	1.14	104.7	95.4	1.84		
32	50	95.0	2.55	100.0	95.0	1.41		97.2	1.30	102.3	96.2	1.81		
32	25	96.0	1.58	100.5	95.5	2.37		97.0	1.22	101.6	97.6	1.78		
48	100	2.4	3.36	2.5	94.2	3.22	***	24.6	14.57	26.1	95.4	1.84	**	
48	50	19.0	8.06	20.0	95.0	1.41	**	66.4	5.68	69.9	96.2	1.81	**	
48	25	61.6	5.32	64.5	95.5	2.37	**	84.4	3.91	88.4	97.6	1.78	++	
56	100	47.4	10.88	50.3	94.2	3.22	***	76.0	8.51	80.7	95.4	1.84	*	
56	50	84.0	3.54	88.4	95.0	1.41	++	94.0	2.45	98.9	96.2	1.81		
56	25	96.4	2.19	100.9	95.5	2.37		97.6	1.52	102.2	97.6	1.78		
60	100	95.4	4.22	101.3	94.2	3.22		98.6	1.14	104.7	95.4	1.84		
60	50	94.2	1.92	99.2	95.0	1.41		97.8	2.68	102.9	96.2	1.81		
60	25	96.2	0.45	100.7	95.5	2.37		97.8	2.17	102.4	97.6	1.78		
64	100	96.6	2.07	102.5	94.2	3.22		98.8	1.10	104.9	95.4	1.84		
64	50	95.0	1.73	100.0	95.0	1.41		98.0	1.58	103.2	96.2	1.81		
64	25	94.8	4.66	99.3	95.5	2.37		98.8	1.10	103.5	97.6	1.78		
75	100	96.6	1.14	102.5	94.2	3.22								
75	50	91.8	2.77	96.6	95.0	1.41								
75	25	92.4	1.67	96.8	95.5	2.37								

Not Tested

Table 4 Continued.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C.						40-Minute Exposure period at 12° C.					
		Mean	SD	% of REF <sup>2</sup>		SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>		SD	Sig. <sup>3</sup>
				REF <sup>2</sup> Mean	Mean					REF <sup>2</sup> Mean	Mean		
80	100	95.6	2.61	101.5	94.2	3.22		97.8	0.84	103.8	95.4	1.84	
80	50	95.2	1.30	100.2	95.0	1.41		97.2	1.10	102.3	96.2	1.81	
80	25	96.6	2.30	101.2	95.5	2.37		96.2	2.17	100.7	97.6	1.78	
88	100	97.8	1.30	103.8	94.2	3.22		96.6	2.88	102.5	95.4	1.84	
88	50	92.4	2.97	97.3	95.0	1.41		95.6	1.82	100.6	96.2	1.81	
88	25	94.2	1.92	98.6	95.5	2.37		94.6	2.07	99.1	97.6	1.78	
92	100	4.4	1.82	4.7	94.2	3.22	**	27.0	8.72	28.7	95.4	1.84	**
92	50	36.8	10.76	38.7	95.0	1.41	**	83.4	6.19	87.8	96.2	1.81	++
92	25	87.2	2.28	91.3	95.5	2.37	++	94.6	1.67	99.1	97.6	1.78	
96	100	61.6	9.24	65.4	94.2	3.22	**	80.4	5.50	85.4	95.4	1.84	*
96	50	67.4	4.72	70.9	95.0	1.41	**	87.4	4.28	92.0	96.2	1.81	++
96	25	93.2	2.77	97.6	95.5	2.37		97.0	1.87	101.6	97.6	1.78	
112	100	0.0	0.00	0.0	94.2	3.22	**	5.6	3.36	5.9	95.4	1.84	**
112	50	6.8	4.21	7.2	95.0	1.41	**	42.2	12.32	44.4	96.2	1.81	**
112	25	44.8	14.55	46.9	95.5	2.37	**	86.0	3.94	90.1	97.6	1.78	++
118	100	99.0	1.00	105.1	94.2	3.22		98.4	1.34	104.5	95.4	1.84	
118	50	98.8	1.10	104.0	95.0	1.41		98.2	0.84	103.4	96.2	1.81	
118	25	96.8	1.92	101.4	95.5	2.37		96.4	1.67	100.9	97.6	1.78	
120	100	94.2	2.28	100.0	94.2	3.22		96.6	1.52	102.5	95.4	1.84	
120	50	94.2	3.56	99.2	95.0	1.41		96.6	1.14	101.7	96.2	1.81	
120	25	97.2	1.30	101.8	95.5	2.37		99.4	0.55	104.1	97.6	1.78	
124	100	94.4	1.82	100.2	94.2	3.22		Not Tested					
124	50	94.6	1.67	99.6	95.0	1.41							
124	25	93.2	2.59	97.6	95.5	2.37							
128	100	99.0	1.22	105.1	94.2	3.22							
128	50	98.0	2.35	103.2	95.0	1.41		97.6	1.14	102.7	96.2	1.81	
128	25	98.4	1.95	103.0	95.5	2.37		98.6	0.89	103.2	97.6	1.78	



Table 4 Continued.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C.					40-Minute Exposure period at 12° C.						
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>
288	100	95.6	3.78	101.5	94.2	3.22							
288	50	94.6	3.36	99.6	95.0	1.41							
288	25	95.0	2.45	99.5	95.5	2.37							
296	100	97.0	2.00	103.0	94.2	3.22							
296	50	97.6	1.52	102.7	95.0	1.41							
296	25	97.0	1.22	101.6	95.5	2.37							
323	100	99.4	0.55	105.5	94.2	3.22		98.8	0.84	104.9	95.4	1.84	
323	50	98.4	1.52	103.6	95.0	1.41		97.0	1.22	102.1	96.2	1.81	
323	25	97.0	1.73	101.6	95.5	2.37		97.4	2.19	102.0	97.6	1.78	
336	100	98.8	0.84	104.9	94.2	3.22		99.6	0.55	105.7	95.4	1.84	
336	50	96.8	2.39	101.9	95.0	1.41		99.0	1.22	104.2	96.2	1.81	
336	25	97.2	1.30	101.8	95.5	2.37		97.6	0.89	102.2	97.6	1.78	

<sup>1</sup> Percent of water quality adjusted porewater sampled

<sup>2</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>3</sup> Asterisks denote statistical differences (Dunnett's t-test) and detectable significance criteria between test and reference stations (\*-alpha < 0.05, \*\*-alpha < 0.01). Plus signs denote only statistical differences (+alpha < 0.05, ++alpha < 0.01).

**Table 5. Water quality parameters after salinity adjustment and original salinity of sediment porewater samples from the San Juan Islands, Strait of Juan de Fuca and Admiralty Inlet, Washington, collected in the 2003 PSAMP study and retested in 2005.**

Station	Salinity <sup>1</sup> ‰	DO <sup>2</sup> (mg/L)	% DO <sup>3</sup>	pH	TAN <sup>4</sup> (mg/L)	UAN <sup>5</sup> ( $\mu$ g/L)	Sulfide <sup>6</sup> (mg/L)	% OUS <sup>7</sup>
Tx Ref <sup>8</sup>	26	8.18	96.1	7.867	0.693	16.1	<0.01	95.2
297	30.5	8.12	95.4	7.651	3.06	43.7	<0.01	100.0
305	31	8.06	94.5	7.842	4.43	97.4	0.01	100.0
313	32	7.62	87.6	8.228	7.53	390.5	0.10	93.8
337	30	8.04	94.3	8.33	3.89	251.7	<0.01	100.0
345	28	8.04	94.3	8.274	1.92	110.1	<0.01	97.4
361	32.5	7.96	93.0	8.106	1.03	40.9	<0.01	92.6
363	32	7.73	91.2	7.875	2.78	65.9	<0.01	93.8
369	30.5	8.03	94.3	8.281	0.445	25.9	0.01	100.0
377	31	8.04	94.5	7.394	2.86	22.7	<0.01	100.0
409	31	8.45	98.8	8.442	3.64	299.1	<0.01	100.0
417	34	8.05	94.1	8.309	1.62	100.2	<0.01	88.2
421	30.5	8.08	94.5	7.758	4.21	76.6	<0.01	100.0
425	30	8.33	97.2	8.042	1.65	56.8	<0.01	100.0
433	31	8.14	95.2	8.197	3.72	180.3	<0.01	100.0
441	32	7.84	89.0	8.409	4.61	353.2	0.04	93.8
449	33.5	7.84	91.7	7.68	1.76	26.8	<0.01	89.6
459	32	8.1	94.8	7.581	2.39	29.1	<0.01	93.8
465	30.5	7.92	92.7	7.63	3.61	49.2	<0.01	100.0
491	32	7.91	92.9	7.923	1.09	28.8	<0.01	93.8
521	33	8.03	94.3	8.237	2.08	110.0	<0.01	90.9
523	32	7.99	93.5	7.554	2.76	31.6	<0.01	93.8
527	30	7.67	89.9	7.573	6.34	75.8	0.012	100.0
545	33	7.70	90.5	7.727	3.65	61.9	0.01	90.9
577	34	7.93	92.2	7.636	3.39	46.8	<0.01	88.2
587	32	8.3	96.6	8.076	1.54	57.2	<0.01	93.8
609	34	7.98	93.3	7.715	1.82	30.0	<0.01	88.2
649	33	8.04	94.2	7.535	0.95	10.4	<0.01	90.9
651	32	7.94	92.9	7.689	4.22	65.7	<0.01	93.8
673	34	7.86	92.0	7.681	2.83	43.3	<0.01	88.2

**Table 5. Continued.**

Station	Salinity <sup>1</sup> ‰	DO <sup>2</sup> (mg/L)	% DO <sup>3</sup>	pH	TAN <sup>4</sup> (mg/L)	UAN <sup>5</sup> ( $\mu$ g/L)	Sulfide <sup>6</sup> (mg/L)	% OUS <sup>7</sup>
681	32	7.91	92.8	8.093	5.1	196.6	0.010	93.8
705	34	7.88	92.3	7.887	1.14	27.7	<0.01	93.8
715	32	7.86	92.4	8.261	1.1	61.3	<0.01	93.8
747	32	7.82	91.7	7.651	1.55	22.1	<0.01	93.8
777	33	7.72	90.4	8.266	1.35	76.1	<0.01	90.9
801	32	7.66	86.6	8.377	5.86	419.4	0.60	93.8
1033	33	7.81	91.5	7.911	1.04	26.7	<0.01	90.9
1161	32.5	8.08	94.6	8.294	1.49	89.2	<0.01	92.6
1193	33	8	93.5	7.65	1.16	16.6	<0.01	90.9
1289	32	7.67	85.5	8.257	6.04	333.7	0.85	93.8
1313	32	7.84	91.8	8.21	1.6	80.1	0.18	93.8
1387	32	7.92	92.9	8.386	1.55	113.1	<0.01	93.8
MFS <sup>9</sup>	38	7.3	85.6	8.141	<0.1	< 4.3	<0.01	78.9
Recon <sup>10</sup>	108	7.4	86.8	7.848	<0.1	<2.2	<0.01	26.4

<sup>1</sup> Salinity of sample prior to adjustment. Sample adjusted to  $30 \pm 1‰$

<sup>2</sup> Dissolved oxygen

<sup>3</sup> Percent saturation of dissolved oxygen

<sup>4</sup> Total ammonia as nitrogen

<sup>5</sup> Unionized ammonia

<sup>6</sup> Measured as  $S^{-2}$

<sup>7</sup> Percent of original sample after salinity adjustment

<sup>8</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>9</sup> Millipore filtered seawater diluent

<sup>10</sup> Reconstituted brine (concentrated brine diluted to 30 ‰ with reagent grade MilliQ water)

**Table 6. Sea urchin fertilization test raw data and means for sediment porewater samples from the San Juan Islands, Strait of Juan de Fuca and Admiralty Inlet collected in the 2003 PSAMP study and retested in 2005. Asterisks denote statistically significant differences (Dunnett's *t*-test) and detectable significance criteria between test and reference stations (\* $\alpha \leq 0.05$ , \*\* $\alpha \leq 0.01$ ). Plus signs denote only statistical differences (+ $\alpha \leq 0.05$ , ++ $\alpha \leq 0.01$ ).**

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
TX REF <sup>3</sup>	100	98	99	99	100	99	99.2	0.63		100.0
		99	99	99	100	100				
	50	98	99	100	98	100	99.2	0.92		100.0
		100	98	99	100	100				
	25	97	99	99	100	100	98.9	1.10		100.0
		97	100	99	99	99				
297	100	97	100	96	100	99	98.4	1.82		99.2
	50	99	100	98	99	99	99.0	0.71		99.8
	25	99	97	99	99	100	98.8	1.10		99.9
305	100	80	80	76	87	86	81.8	4.60	*	82.5
	50	100	98	97	100	100	99.0	1.41		99.8
	25	99	100	100	99	99	99.4	0.55		100.5
313	100	90	86	90	87	89	88.4	1.82	++	89.1
	50	99	100	97	100	99	99.0	1.22		99.8
	25	98	99	100	100	100	99.4	0.89		100.5
337	100	99	99	100	99	99	99.2	0.45		100.0
	50	98	99	99	100	98	98.8	0.84		99.6
	25	99	100	99	100	99	99.4	0.55		100.5
345	100	100	99	96	98	99	98.4	1.52		99.2
	50	97	98	100	99	97	98.2	1.30		99.0
	25	98	99	100	100	99	99.2	0.84		100.3
361	100	99	99	99	99	98	98.8	0.45		99.6
	50	100	99	100	100	100	99.8	0.45		100.6
	25	100	99	98	99	98	98.8	0.84		99.9
363	100	67	61	54	46	45	54.6	9.50	**	55.0
	50	96	98	99	99	98	98.0	1.22		98.8
	25	96	100	98	96	99	97.8	1.79		98.9

Table 6. Continued.

Station	% WQAS <sup>2</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
369	100	51	42	48	43	27	42.2	9.26	**	42.5
	50	98	94	93	91	96	94.4	2.70	++	95.2
	25	98	97	96	95	92	95.6	2.30	++	96.7
377	100	96	99	97	100	99	98.2	1.64		99.0
	50	98	99	100	100	98	99.0	1.00		99.8
	25	100	99	97	99	98	98.6	1.14		99.7
409	100	91	83	87	91	84	87.2	3.77	++	87.9
	50	96	95	92	94	96	94.6	1.67	++	95.4
	25	96	97	100	96	95	96.8	1.92		97.9
417	100	98	100	100	98	94	98.0	2.45		98.8
	50	100	100	99	99	99	99.4	0.55		100.2
	25	99	99	99	97	97	98.2	1.10		99.3
421	100	100	100	100	99	99	99.6	0.55		100.4
	50	99	100	100	99	99	99.4	0.55		100.2
	25	100	99	99	100	100	99.6	0.55		100.7
425	100	100	99	100	99	99	99.4	0.55		100.2
	50	99	100	99	100	99	99.4	0.55		100.2
	25	99	99	99	100	100	99.4	0.55		100.5
433	100	99	99	100	100	100	99.6	0.55		100.4
	50	100	99	100	100	100	99.8	0.45		100.6
	25	98	99	98	97	100	98.4	1.14		99.5
441	100	92	92	91	97	92	92.8	2.39	++	93.5
	50	98	98	97	100	99	98.4	1.14		99.2
	25	97	99	99	99	100	98.8	1.10		99.9
449	100	99	99	99	98	100	99.0	0.71		99.8
	50	98	100	100	98	100	99.2	1.10		100.0
	25	100	100	100	100	100	100.0	0.00		101.1
459	100	100	98	99	99	100	99.2	0.84		100.0
	50	99	100	98	98	100	99.0	1.00		99.8
	25	99	98	100	97	98	98.4	1.14		99.5
465	100	97	99	99	98	99	98.4	0.89		99.2
	50	100	99	97	99	97	98.4	1.34		99.2
	25	98	100	98	98	99	98.6	0.89		99.7

Table 6. Continued.

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
491	100	100	100	100	100	99	99.8	0.45		100.6
	50	99	99	100	100	100	99.6	0.55		100.4
	25	100	100	98	97	100	99.0	1.41		100.1
521	100	75	6	10	10	6	8.0	2.31	**	8.1
	50	97	100	100	100	100	99.4	1.34		100.2
	25	99	99	99	98	99	98.8	0.45		99.9
523	100	98	100	100	97	98	98.6	1.34		99.4
	50	99	98	99	100	98	98.8	0.84		99.6
	25	99	100	99	99	100	99.4	0.55		100.5
527	100	94	89	90	76	82	86.2	7.16	++	86.9
	50	99	98	98	100	98	98.6	0.89		99.4
	25	99	98	100	99	99	99.0	0.71		100.1
545	100	100	100	97	100	100	99.4	1.34		100.2
	50	100	100	99	98	98	99.0	1.00		99.8
	25	100	100	99	100	99	99.6	0.55		100.7
577	100	99	100	99	100	100	99.6	0.55		100.4
	50	100	100	99	100	98	99.4	0.89		100.2
	25	99	100	100	100	100	99.8	0.45		100.9
587	100	100	98	99	99	100	99.2	0.84		100.0
	50	100	100	98	99	99	99.2	0.84		100.0
	25	99	98	99	99	99	98.8	0.45		99.9
609	100	99	100	100	98	100	99.4	0.89		100.2
	50	100	98	100	100	100	99.6	0.89		100.4
	25	100	99	99	100	99	99.4	0.55		100.5
649	100	100	98	100	99	100	99.4	0.89		100.2
	50	100	99	100	100	100	99.8	0.45		100.6
	25	100	100	100	97	99	99.2	1.30		100.3
651	100	99	99	100	100	98	99.2	0.84		100.0
	50	98	98	100	99	99	98.8	0.84		99.6
	25	100	100	100	99	100	99.8	0.45		100.9
673	100	99	99	100	99	100	99.4	0.55		100.2
	50	100	98	98	99	100	99.0	1.00		99.8
	25	98	100	99	99	100	99.2	0.84		100.3

Table 6. Continued.

Station	% WQAS <sup>2</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
681	100	81	87	88	86	81	84.6	3.36	++	85.3
	50	100	97	96	100	100	98.6	1.95		99.4
	25	100	98	100	99	99	99.2	0.84		100.3
705	100	100	97	99	99	97	98.4	1.34		99.2
	50	99	100	99	99	100	99.4	0.55		100.2
	25	100	100	99	99	100	99.6	0.55		100.7
715	100	99	98	99	97	100	98.6	1.14		99.4
	50	100	100	99	100	99	99.6	0.55		100.4
	25	100	99	100	98	100	99.4	0.89		100.5
747	100	100	100	98	99	99	99.2	0.84		100.0
	50	100	100	99	100	99	99.6	0.55		100.4
	25	99	100	100	100	99	99.6	0.55		100.7
777	100	99	100	100	98	100	99.4	0.89		100.2
	50	100	100	99	100	100	99.8	0.45		100.6
	25	98	100	99	100	100	99.4	0.89		100.5
801	100	66	71	61	62	65	65.0	3.94	**	65.5
	50	99	99	100	99	98	99.0	0.71		99.8
	25	99	99	100	100	99	99.4	0.55		100.5
1033	100	96	100	100	99	99	98.8	1.64		99.6
	50	99	100	98	100	99	99.2	0.84		100.0
	25	100	99	100	99	99	99.4	0.55		100.5
1161	100	97	99	100	100	100	99.2	1.30		100.0
	50	100	99	100	100	100	99.8	0.45		100.6
	25	98	99	100	100	98	99.0	1.00		100.1
1193	100	99	100	100	100	100	99.8	0.45		100.6
	50	99	99	100	100	99	99.4	0.55		100.2
	25	100	99	100	100	100	99.8	0.45		100.9
1289	100	88	82	83	80	75	81.6	4.72	*	82.3
	50	99	99	99	99	100	99.2	0.45		100.0
	25	100	100	100	100	99	99.8	0.45		100.9
1313	100	12	17	22	29	24	20.8	6.53	**	21.0
	50	99	99	99	100	99	99.2	0.45		100.0
	25	99	100	99	99	99	99.2	0.45		100.3

**Table 6. Continued.**

Station	% WQAS <sup>1</sup>	% Fertilized					Mean	SD	Sig. <sup>2</sup>	% of TX Ref <sup>3</sup>
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				
1387	100	100	100	99	100	100	99.8	0.45		100.6
	50	100	100	100	99	98	99.4	0.89		100.2
	25	100	100	98	100	98	99.2	1.10		100.3
MFS <sup>4</sup>	100	98	100	96	98	98	98.5	1.18		99.3
		98	99	100	99	99				
RECON <sup>5</sup>	100	99	99	96	100	99	98.6	1.52		99.7
SDS <sup>6</sup>	10	0	2	0	0	0	0.4	0.89	**	0.4
	5	66	68	73	66	69	68.4	2.88	**	69.0
	2.5	97	99	100	98	99	98.6	1.14		99.7
	1.25	99	100	na	100	100	99.8	0.50		100.9

<sup>1</sup> Percent of water quality adjusted porewater sampled

<sup>2</sup> Significant difference from reference denoted as asterisks or plus signs

<sup>3</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>4</sup> Millipore filtered seawater diluent

<sup>5</sup> Reconstituted brine (concentrated brine diluted to 30 ‰ with reagent grade MilliQ water)

<sup>6</sup> Sodium Dodecyl Sulfate positive reference control

na = data not available due to technical error

**Table 7. Summary of station means and statistical significance for the sea urchin fertilization assays (*Strongylocentrotus purpuratus*) conducted at two exposure periods and two temperatures for the PSAMP 2003 study tested in 2003 and 2005.**

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C tested in 2005					40-Minute Exposure period at 12° C tested in 2003						
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>
297	100	98.4	1.82	99.2	99.2	0.63		94.2	1.92	100.6	93.6	4.04	
297	50	99.0	0.71	99.8	99.2	0.92		96.6	1.34	110.4	87.5	1.73	
297	25	98.8	1.10	99.9	98.9	1.10		91.6	1.67	103.2	88.8	5.17	
305	100	81.8	4.60	82.5	99.2	0.63	*	71.8	11.78	76.7	93.6	4.04	**
305	50	99.0	1.41	99.8	99.2	0.92		87.4	5.32	99.9	87.5	1.73	
305	25	99.4	0.55	100.5	98.9	1.10		84.8	2.05	95.5	88.8	5.17	
313	100	88.4	1.82	89.1	99.2	0.63	++	50.8	8.98	54.3	93.6	4.04	**
313	50	99.0	1.22	99.8	99.2	0.92		77.4	5.90	88.5	87.5	1.73	+
313	25	99.4	0.89	100.5	98.9	1.10		81.0	5.57	91.2	88.8	5.17	
337	100	99.2	0.45	100.0	99.2	0.63		52.8	10.11	56.4	93.6	4.04	**
337	50	98.8	0.84	99.6	99.2	0.92		82.8	2.17	94.6	87.5	1.73	
337	25	99.4	0.55	100.5	98.9	1.10		85.8	4.32	96.6	88.8	5.17	
345	100	98.4	1.52	99.2	99.2	0.63		56.6	7.99	60.5	93.6	4.04	**
345	50	98.2	1.30	99.0	99.2	0.92		75.0	3.39	85.7	87.5	1.73	++
345	25	99.2	0.84	100.3	98.9	1.10		78.6	3.05	88.5	88.8	5.17	++
361	100	98.8	0.45	99.6	99.2	0.63		57.8	5.72	61.8	93.6	4.04	**
361	50	99.8	0.45	100.6	99.2	0.92		75.8	4.97	86.6	87.5	1.73	++
361	25	98.8	0.84	99.9	98.9	1.10		78.0	0.82	87.8	88.8	5.17	++
363	100	54.6	9.50	55.0	99.2	0.63	**	2.4	1.67	2.6	93.6	4.04	**
363	50	98.0	1.22	98.8	99.2	0.92		17.2	9.31	19.7	87.5	1.73	**
363	25	97.8	1.79	98.9	98.9	1.10		52.8	5.59	59.5	88.8	5.17	**
369	100	42.2	9.26	42.5	99.2	0.63	**	1.8	0.84	1.9	93.6	4.04	**
369	50	94.4	2.70	95.2	99.2	0.92	++	24.8	10.57	28.3	87.5	1.73	**
369	25	95.6	2.30	96.7	98.9	1.10	++	48.4	7.83	54.5	88.8	5.17	**

Table 7 Continued.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C tested in 2005						4-Minute Exposure period at 12° C tested in 2003					
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>
377	100	98.2	1.64	99.0	99.2	0.63		81.6	4.39	87.2	93.6	4.04	++
377	50	99.0	1.00	99.8	99.2	0.92		81.8	5.72	93.5	87.5	1.73	
377	25	98.6	1.14	99.7	98.9	1.10		82.6	2.70	93.0	88.8	5.17	
409	100	87.2	3.77	87.9	99.2	0.63	++	73.2	4.76	78.2	93.6	4.04	**
409	50	94.6	1.67	95.4	99.2	0.92	++	80.6	5.32	92.1	87.5	1.73	
409	25	96.8	1.92	97.9	98.9	1.10		84.5	4.65	95.2	88.8	5.17	
417	100	98.0	2.45	98.8	99.2	0.63		94.4	3.13	100.9	93.6	4.04	
417	50	99.4	0.55	100.2	99.2	0.92		96.2	0.84	109.9	87.5	1.73	
417	25	98.2	1.10	99.3	98.9	1.10		93.0	3.00	104.7	88.8	5.17	
421	100	99.6	0.55	100.4	99.2	0.63		83.4	4.88	89.1	93.6	4.04	++
421	50	99.4	0.55	100.2	99.2	0.92		90.0	2.74	102.9	87.5	1.73	
421	25	99.6	0.55	100.7	98.9	1.10		91.2	2.86	102.7	88.8	5.17	
425	100	99.4	0.55	100.2	99.2	0.63		92.4	1.95	98.7	93.6	4.04	
425	50	99.4	0.55	100.2	99.2	0.92		92.0	2.35	105.1	87.5	1.73	
425	25	99.4	0.55	100.5	98.9	1.10		93.0	3.81	104.7	88.8	5.17	
433	100	99.6	0.55	100.4	99.2	0.63		62.4	11.93	66.7	93.6	4.04	**
433	50	99.8	0.45	100.6	99.2	0.92		88.2	4.55	100.8	87.5	1.73	
433	25	98.4	1.14	99.5	98.9	1.10		86.6	7.54	97.5	88.8	5.17	
441	100	92.8	2.39	93.5	99.2	0.63	++	86.0	3.00	91.9	93.6	4.04	+
441	50	98.4	1.14	99.2	99.2	0.92		86.4	3.91	98.7	87.5	1.73	
441	25	98.8	1.10	99.9	98.9	1.10		81.8	5.07	92.1	88.8	5.17	
449	100	99.0	0.71	99.8	99.2	0.63		87.6	4.10	93.6	93.6	4.04	
449	50	99.2	1.10	100.0	99.2	0.92		87.8	5.89	100.3	87.5	1.73	
449	25	100.0	0.00	101.1	98.9	1.10		87.6	4.34	98.6	88.8	5.17	
459	100	99.2	0.84	100.0	99.2	0.63		88.2	5.02	94.2	93.6	4.04	
459	50	99.0	1.00	99.8	99.2	0.92		88.8	2.39	101.5	87.5	1.73	
459	25	98.4	1.14	99.5	98.9	1.10		90.6	3.05	102.0	88.8	5.17	

Table 7 Continued.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C tested in 2005					40-Minute Exposure period at 12° C tested in 2003						
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>
465	100	98.4	0.89	99.2	99.2	0.63		59.0	4.95	63.0	93.6	4.04	**
465	50	98.4	1.34	99.2	99.2	0.92		75.8	4.32	86.6	87.5	1.73	++
465	25	98.6	0.89	99.7	98.9	1.10		82.0	2.00	92.3	88.8	5.17	
491	100	99.8	0.45	100.6	99.2	0.63		91.6	2.97	97.9	93.6	4.04	
491	50	99.6	0.55	100.4	99.2	0.92		93.0	2.45	106.3	87.5	1.73	
491	25	99.0	1.41	100.1	98.9	1.10		85.4	4.98	96.2	88.8	5.17	
521	100	8.0	2.31	8.1	99.2	0.63	**	0.0	0.00	0.0	93.6	4.04	**
521	50	99.4	1.34	100.2	99.2	0.92		5.0	3.39	5.7	87.5	1.73	**
521	25	98.8	0.45	99.9	98.9	1.10		31.8	4.21	35.8	88.8	5.17	**
523	100	98.6	1.34	99.4	99.2	0.63		92.8	1.64	99.1	93.6	4.04	
523	50	98.8	0.84	99.6	99.2	0.92		93.6	3.78	107.0	87.5	1.73	
523	25	99.4	0.55	100.5	98.9	1.10		93.6	1.14	105.4	88.8	5.17	
527	100	86.2	7.16	86.9	99.2	0.63	++	54.4	7.57	57.4	94.8	1.48	**
527	50	98.6	0.89	99.4	99.2	0.92		86.6	2.97	99.1	87.4	4.83	
527	25	99.0	0.71	100.1	98.9	1.10		90.8	2.86	96.2	94.4	2.70	
545	100	99.4	1.34	100.2	99.2	0.63		94.4	2.07	99.6	94.8	1.48	
545	50	99.0	1.00	99.8	99.2	0.92		95.6	2.97	109.4	87.4	4.83	
545	25	99.6	0.55	100.7	98.9	1.10		93.8	2.17	99.4	94.4	2.70	
577	100	99.6	0.55	100.4	99.2	0.63		96.8	1.10	102.1	94.8	1.48	
577	50	99.4	0.89	100.2	99.2	0.92		95.6	2.07	109.4	87.4	4.83	
577	25	99.8	0.45	100.9	98.9	1.10		93.0	2.74	98.5	94.4	2.70	
587	100	99.2	0.84	100.0	99.2	0.63		97.0	2.12	102.3	94.8	1.48	
587	50	99.2	0.84	100.0	99.2	0.92		96.8	1.30	110.8	87.4	4.83	
587	25	98.8	0.45	99.9	98.9	1.10		94.8	2.59	100.4	94.4	2.70	
609	100	99.4	0.89	100.2	99.2	0.63		94.4	3.36	99.6	94.8	1.48	
609	50	99.6	0.89	100.4	99.2	0.92		92.6	1.52	105.9	87.4	4.83	
609	25	99.4	0.55	100.5	98.9	1.10		93.2	2.28	98.7	94.4	2.70	

Table 7 Continued.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C tested in 2005					40-Minute Exposure period at 12° C tested in 2003						
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	SD	Sig. <sup>3</sup>
649	100	99.4	0.89	100.2	99.2	0.63		62.4	8.20	65.8	94.8	1.48	**
649	50	99.8	0.45	100.6	99.2	0.92		84.2	3.56	96.3	87.4	4.83	
649	25	99.2	1.30	100.3	98.9	1.10		90.2	2.59	95.6	94.4	2.70	
651	100	99.2	0.84	100.0	99.2	0.63		93.3	2.87	98.4	94.8	1.48	
651	50	98.8	0.84	99.6	99.2	0.92		93.0	2.92	106.4	87.4	4.83	
651	25	99.8	0.45	100.9	98.9	1.10		91.0	2.45	96.4	94.4	2.70	
673	100	99.4	0.55	100.2	99.2	0.63		87.4	7.37	92.2	94.8	1.48	+
673	50	99.0	1.00	99.8	99.2	0.92		88.0	4.36	100.7	87.4	4.83	
673	25	99.2	0.84	100.3	98.9	1.10		83.4	8.91	88.3	94.4	2.70	++
681	100	84.6	3.36	85.3	99.2	0.63	++	38.4	4.22	40.5	94.8	1.48	**
681	50	98.6	1.95	99.4	99.2	0.92		71.8	6.91	82.2	87.4	4.83	*
681	25	99.2	0.84	100.3	98.9	1.10		82	4.36	86.9	94.4	2.70	++
705	100	98.4	1.34	99.2	99.2	0.63		92.8	3.42	97.9	94.8	1.48	
705	50	99.4	0.55	100.2	99.2	0.92		90.8	2.49	103.9	87.4	4.83	
705	25	99.6	0.55	100.7	98.9	1.10		86.2	4.44	91.3	94.4	2.70	+
715	100	98.6	1.14	99.4	99.2	0.63		97.2	3.11	102.5	94.8	1.48	
715	50	99.6	0.55	100.4	99.2	0.92		97.4	0.89	111.4	87.4	4.83	
715	25	99.4	0.89	100.5	98.9	1.10		94.4	1.14	100.0	94.4	2.70	
747	100	99.2	0.84	100.0	99.2	0.63		92.6	3.78	97.7	94.8	1.48	
747	50	99.6	0.55	100.4	99.2	0.92		91.3	1.71	104.4	87.4	4.83	
747	25	99.6	0.55	100.7	98.9	1.10		90.4	6.50	95.8	94.4	2.70	
777	100	99.4	0.89	100.2	99.2	0.63		86.2	2.59	90.9	94.8	1.48	++
777	50	99.8	0.45	100.6	99.2	0.92		90.6	3.65	103.7	87.4	4.83	
777	25	99.4	0.89	100.5	98.9	1.10		92.6	4.83	98.1	94.4	2.70	
801	100	65.0	3.94	65.5	99.2	0.63	**	16.4	7.77	17.3	94.8	1.48	**
801	50	99.0	0.71	99.8	99.2	0.92		54.6	18.54	62.5	87.4	4.83	**
801	25	99.4	0.55	100.5	98.9	1.10		90.3	2.22	95.6	94.4	2.70	

Table 7 Continued.

Station	WQAS <sup>1</sup>	60-Minute Exposure period at 15° C tested in 2005					40-Minute Exposure period at 12° C tested in 2003				
		Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	Sig. <sup>3</sup>	Mean	SD	% of REF <sup>2</sup>	REF <sup>2</sup> Mean	Sig. <sup>3</sup>
1033	100	98.8	1.64	99.6	99.2		68.8	5.76	72.6	94.8	**
1033	50	99.2	0.84	100.0	99.2		83.0	3.81	95.0	87.4	
1033	25	99.4	0.55	100.5	98.9		86.4	7.44	91.5	94.4	+
1161	100	99.2	1.30	100.0	99.2		34.4	6.69	36.3	94.8	**
1161	50	99.8	0.45	100.6	99.2		71.2	6.87	81.5	87.4	*
1161	25	99.0	1.00	100.1	98.9		83.0	3.46	87.9	94.4	++
1193	100	99.8	0.45	100.6	99.2		52.4	11.26	55.3	94.8	**
1193	50	99.4	0.55	100.2	99.2		80	7.58	91.5	87.4	
1193	25	99.8	0.45	100.9	98.9		85.6	7.89	90.7	94.4	+
1289	100	81.6	4.72	82.3	99.2	*	2.2	2.17	2.3	94.8	**
1289	50	99.2	0.45	100.0	99.2		32.5	11.68	37.2	87.4	**
1289	25	99.8	0.45	100.9	98.9		80.2	4.82	85.0	94.4	++
1313	100	20.8	6.53	21.0	99.2	**	0.8	1.30	0.8	94.8	**
1313	50	99.2	0.45	100.0	99.2		13.8	4.44	15.8	87.4	**
1313	25	99.2	0.45	100.3	98.9		41.0	11.11	43.4	94.4	**
1387	100	99.8	0.45	100.6	99.2		83.2	4.97	87.8	94.8	++
1387	50	99.4	0.89	100.2	99.2		78.0	3.54	89.2	87.4	+
1387	25	99.2	1.10	100.3	98.9		84.0	3.39	89.0	94.4	++

<sup>1</sup> Percent of water quality adjusted porewater sample

<sup>2</sup> Reference pore water extracted from sediment collected in Aransas Bay, Texas

<sup>3</sup> Asterisks denote statistical differences (Dunnnett's t-test) and detectable significance criteria between test and reference stations (\*alpha < 0.05, \*\*alpha < 0.01). Plus signs denote only statistical differences (+alpha < 0.05, ++alpha < 0.01).

**FIGURES 1-3**

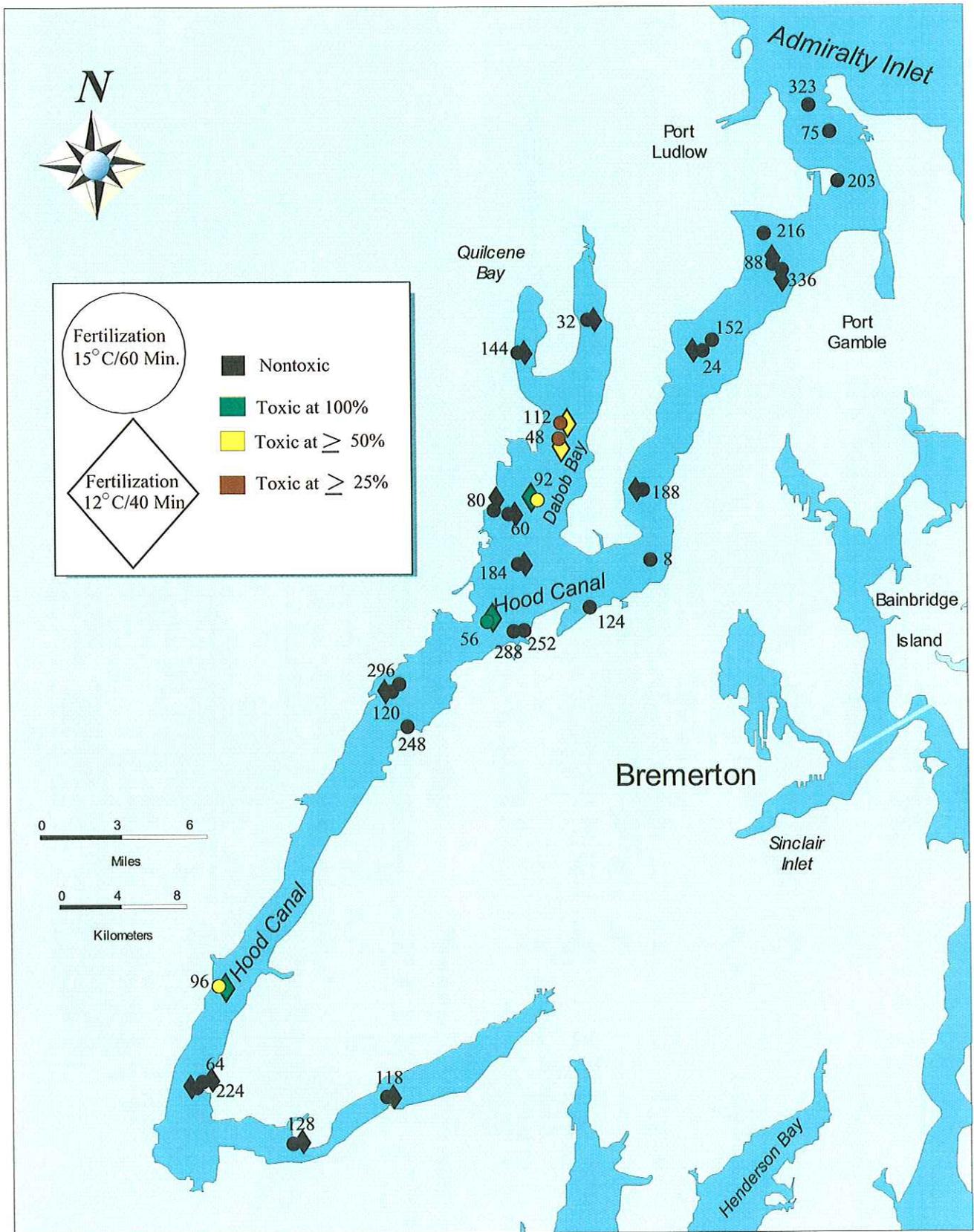


Figure 1. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results for samples collected in the 2004 PSAMP program in Hood Canal and surrounding areas. Color differentiation of circles and diamonds indicates those stations that were significantly different from the reference (Dunnett's  $t$ -test,  $\leq 0.05$  and detectable significance criteria applied).

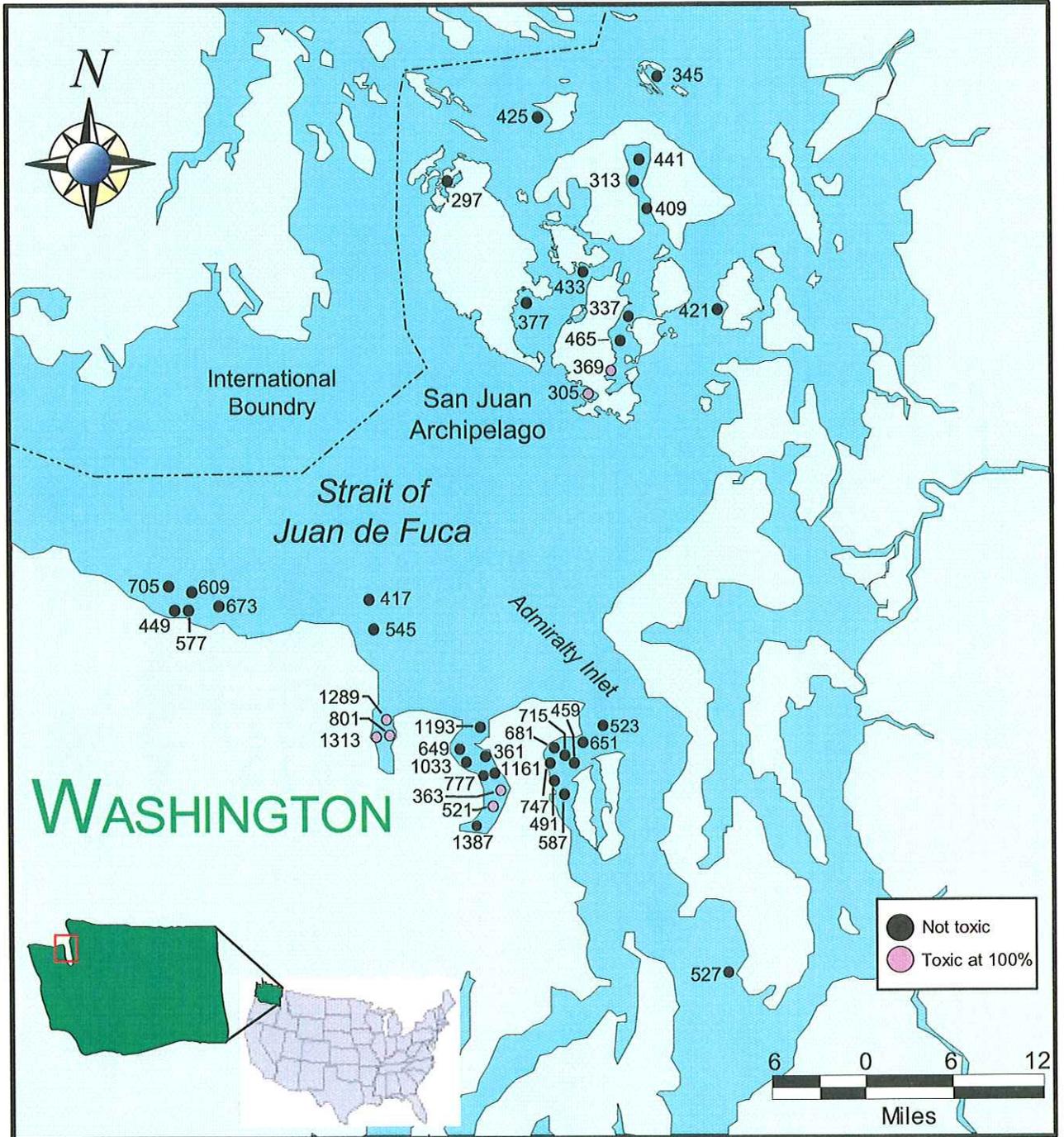


Figure 2. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results for samples collected in the 2003 PSAMP program in the San Juan Islands, Strait of Juan de Fuca and Admiralty Inlet, Washington and retested in 2005 with a 60 minute exposure period at 15°C. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's  $t$ -test,  $\leq 0.05$  and detectable significance criteria applied).

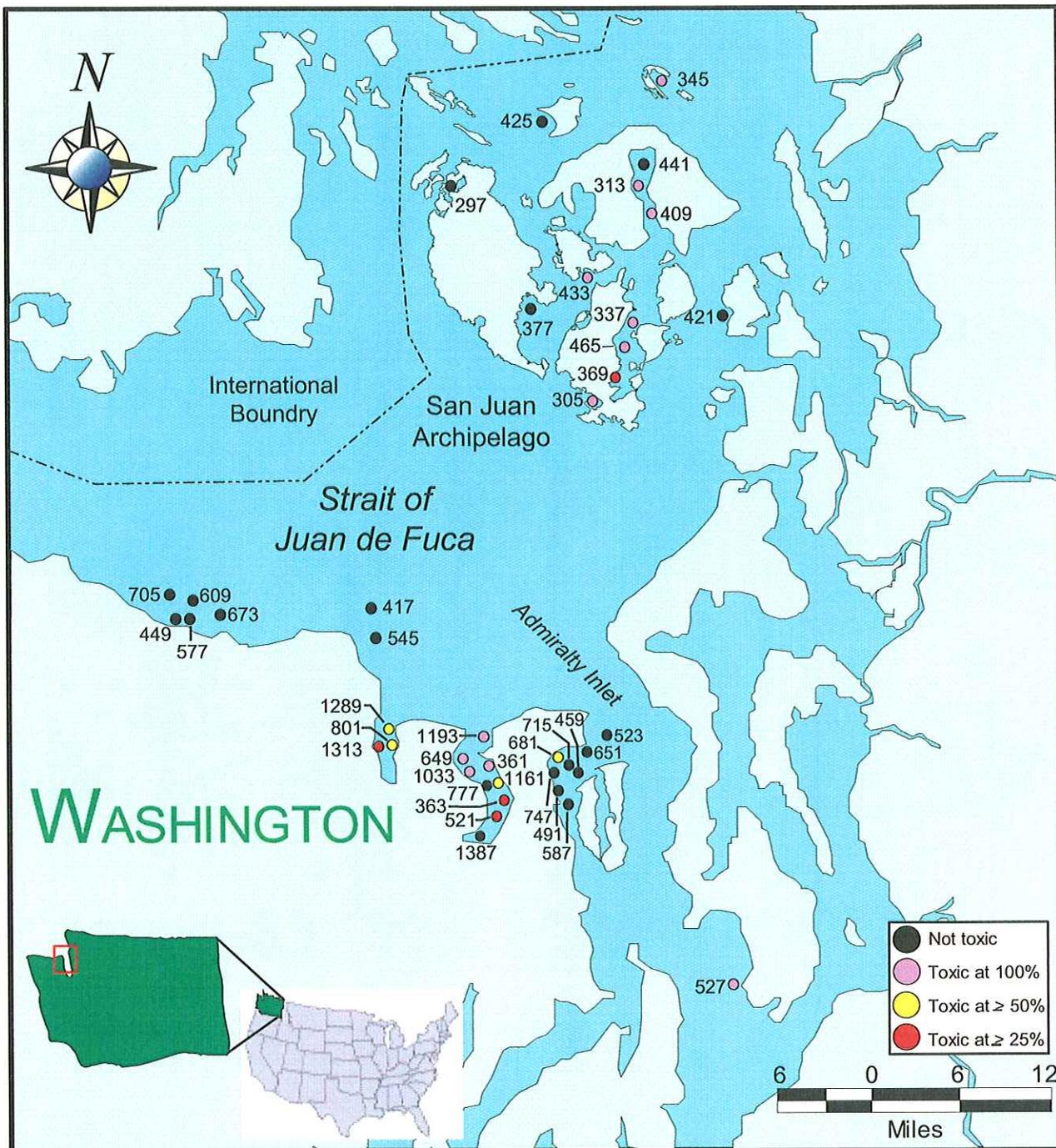


Figure 3. Sea urchin (*Strongylocentrotus purpuratus*) fertilization test results for samples collected in the 2003 PSAMP program in the San Juan Islands, Strait of Juan de Fuca and Admiralty Inlet, Washington. Color differentiation of circles indicates those stations that were significantly different from the reference (Dunnett's  $t$ -test,  $\leq 0.05$  and detectable significance criteria applied).

**ATTACHMENTS 1-3**

Date Prepared: May 5, 1990

Date Revised: June 10, 1994

## **EXTRACTION AND STORAGE OF POREWATER SAMPLES**

### **1.0 OBJECTIVE**

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

### **2.0 PREPARATION**

#### **2.1 Description of the Porewater Extraction System**

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

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

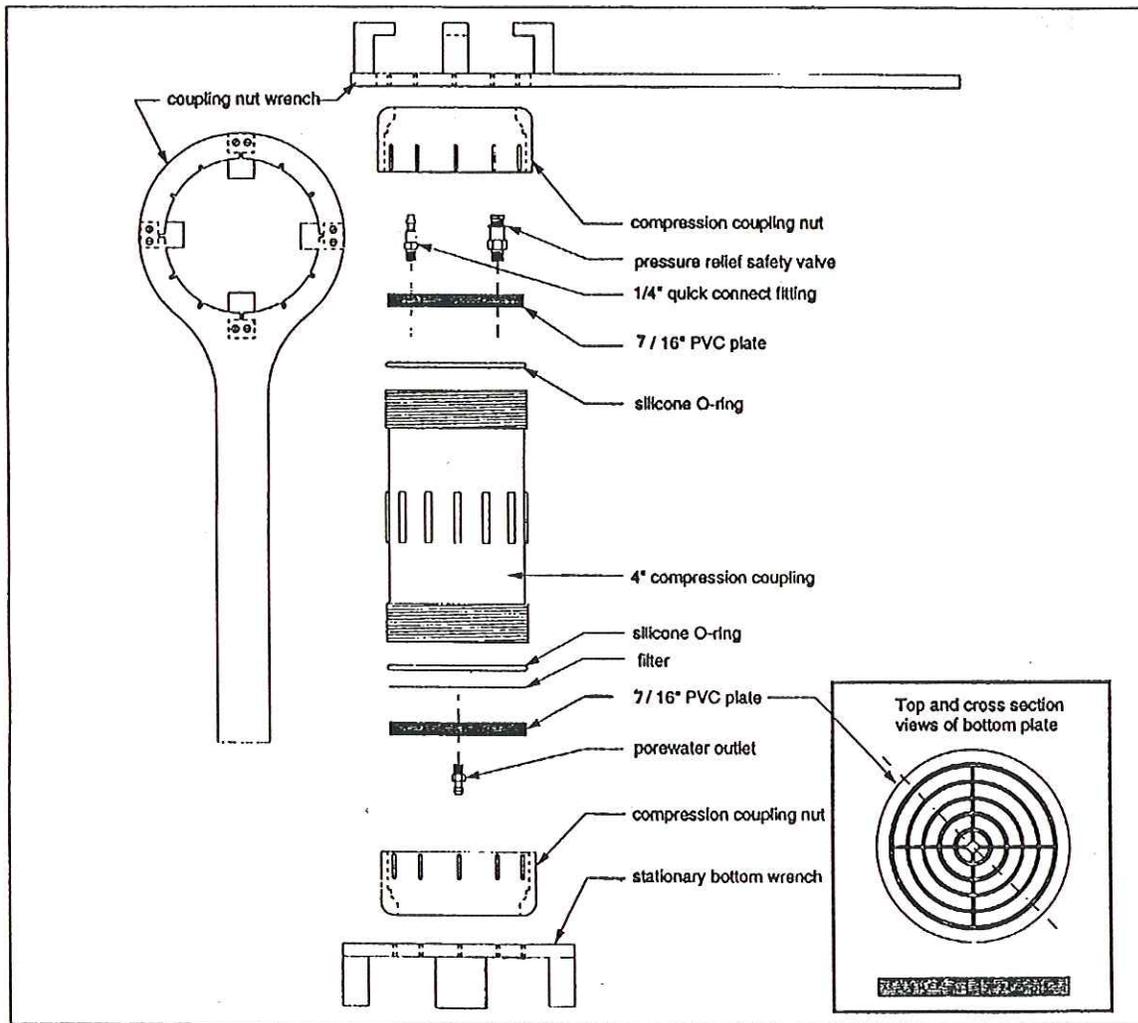


Figure 1. Sediment pore water squeeze extraction device.

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

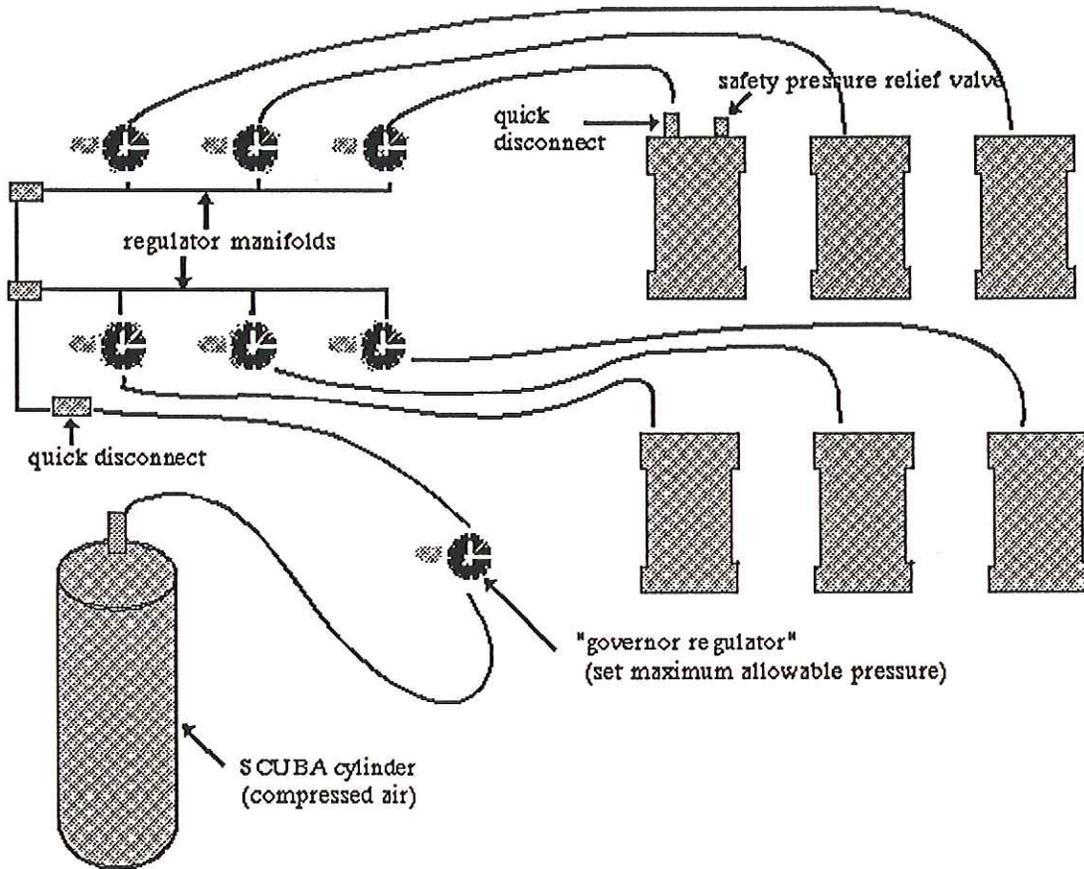


Figure 2. Schematic of sediment porewater pressure extraction system.

## 2.2 Equipment List

Supplies and equipment needed are listed in Attachment 1.

### 3.0 PROCEDURE

#### 3.1 Sediment Collection and Storage Considerations

Generally, surficial sediment samples are collected for porewater extraction. A homogenate of the upper ~2-10 cm sediment may be collected by multiple cores or grabs at a particular sampling station. (Further details of sediment sampling procedures are not within the scope of this SOP.) One liter of sediment will typically provide 100-200 mL pore water. However, a larger volume of 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), labelled, and stored on ice, in a cooler, or in a refrigerator until the samples are delivered and processed. Pore water should be extracted from the samples as soon as possible because the toxicity of sediments in storage may change over time. A sample tracking system should be maintained for each sediment sample collected and porewater sample extracted. All manipulations made on samples are recorded on the Sample History Data Form (Attachment 2).

#### 3.2 Load Extraction Cylinder

1. Assemble all parts of extraction cylinder except the top end compression coupling nut, top end plate and O-ring. Make sure filter is snugly in place beneath bottom O-ring (both over- and under-tightening will result in an improper seal). Place the extractor cylinder on the stand and position an appropriately labelled porewater sample container (usually an I-Chem® amber 250 mL or 125 mL glass jar cleaned to EPA standards, with Teflon® lid liner) underneath the outlet.
2. Ensure that the sediment sample is homogenized, by shaking, stirring with a clean Teflon® or plastic spatula or spoon, or by both.
3. Transfer sediment from the sample container/bag to the extractor by pouring and/or using a clean Teflon® or plastic spatula or spoon. If necessary, particularly when extracting pore water from sandy or shelly sediments, the spatula may be used to compress the sample in the cylinder to eliminate channelization. The amount of sediment to be transferred will depend on the texture of the sample. The cylinder may be filled nearly full with a sandy sediment. However, when extracting pore water from a clay sediment, a relatively impermeable layer of compressed clay will eventually form on the filter, so that extraction of a large volume of clay sediment at once would take an extremely long time. When extracting pore water from extremely fine grained sediments, the cylinder should be less than one-third filled. If additional pore water is needed, this process can be repeated by removing the sediment including

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

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

### 3.3 Porewater Extraction

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

1. Turn the SCUBA valve counter clockwise, pressurizing the first stage regulator and the intermediate-pressure hose (approximately 150 psi). An additional "governor" pressure regulator between the SCUBA tanks and the final second stage regulators which control pressure to the individual extractors should be set at maximum extractor pressure (~40 psi).
2. Ensure that all final pressure regulators are set to zero. Attach the hose from one of the pressure regulators on the pressure regulator manifold to the air inlet, using the quick disconnect fitting.
3. Slowly open the corresponding pressure regulator to a pressure of 5-10 psi. Check the first drops of porewater passing from the outlet for cloudiness. Occasionally, a small amount of sediment will pass through the porewater outlet, presumably around the filter. If this happens, wait until the pore water clears, discard the initial pore water collected, and continue.
4. Check the cylinder for leaks and if necessary tighten clamping nuts slightly.
5. As the flow of pore water decreases, pressure may be increased gradually to a maximum of 35-40 psi. When flow is less than or slows to less than 1-3 drops per minute, increase the pressure in 5-10 psi increments to maintain the flow. Allow the extraction to continue until sufficient pore water has been collected.
6. Disassemble the extractor, discard sediment, and rinse and wash appropriately all parts contacting sediment before placing a different sediment sample into the extractor.

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

### **3.4 Centrifugation of Porewater Samples**

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

1. After collection, keep the porewater samples refrigerated or chilled on ice until they are centrifuged.
2. Transfer the pore water from the glass sample jar to an appropriate centrifuge bottle (e.g., polycarbonate). Centrifuge at  $\geq 1200$  g for 20 minutes. Return the centrifuged sample to a rinsed and labelled glass jar, taking care not to disturb any material that may have settled on the bottom/sides of the centrifuge bottle.
3. If multiple jars of pore water were collected from a single sediment sample, they should be composited after centrifugation and redistributed to the glass jars before testing or storage.

### **3.5 Storage of Porewater Samples**

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

## **4.0 QUALITY CONTROL**

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

## 5.0 TRAINING

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

## 6.0 SAFETY

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

## 7.0 ATTACHMENTS

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

## 8.0 REFERENCES

- Carr, R.S. 1993. Sediment quality assessment survey of the Galveston Bay System. Galveston Bay National Estuary Program report, GBNEP-30, 101 pp.
- Carr, R.S., J.W. Williams, and C.T.B. Fragata. 1989. Development and evaluation of a novel marine sediment pore water toxicity test with the polychaete *Dinophilus gyrociliatus*. Environ. Toxicol. Chem. 8:533-543.
- Carr, R.S. and D.C. Chapman. 1992. Comparison of solid-phase and pore-water approaches for assessing the quality of marine and estuarine sediments. Chem. Ecol. 7:19-30.
- Carr, R.S. and Chapman. 1994. Improved device for extracting sediment pore water. National Biological Survey, Research Information Bulletin No. 38.

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

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

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

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

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Chief, Field Research Division

 6-28-94  
Joseph B. Hunn  
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



Date Prepared: March 14, 1991

Date Revised: May 17, 1994

## WATER QUALITY ADJUSTMENT OF SAMPLES

### 1.0 OBJECTIVE

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

### 2.0 PREPARATION

#### 2.1 Equipment and Labware

The supplies and equipment needed are listed in Attachment 1.

#### 2.2 Source of Dilution Water

For samples lower in salinity than target salinity, concentrated brine (~100‰) is added to increase salinity. Concentrated brine is prepared by heating (to 35-40°C) and gently aerating filtered natural seawater (1 µm) to concentrate the salts by evaporation. Prior to use, a 10% addition of reference pore water is added to the brine to replace lost trace elements. For samples higher in salinity than target salinity, Milli-Q, HPLC grade ultrapure water is added to decrease salinity.

### 3.0 PROCEDURES

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

#### 3.1 Preparation for Salinity Adjustment

1. Although fresh samples are routinely tested at the Corpus Christi field station, most of the samples tested are stored frozen in amber I-Chem® jars. If frozen, remove samples from freezer and allow them to thaw at room temperature or immerse them in a tepid water bath to thaw, ensuring that sample temperature does not exceed 25°C. The samples may be thawed the day of water quality adjustment (WQA) or may be transferred from the freezer to a refrigerator (4°C) the day before WQA and then completely thawed the following day. After thawing, allow the samples to come to room temperature. Generally, the samples should be maintained at the same temperature required for the toxicity test that will be conducted. The temperature requirement for most toxicity tests performed at this field station is 20±1°C, and room temperature should be maintained accordingly.
2. Turn bottled sample end over end a few times to mix thoroughly before measuring salinity. Using a salinity refractometer, measure salinity and record on Water Quality Adjustment Data Form (Attachment 2).
3. In order to make calculations for the salinity adjustment, the volume of the sample must be known. When porewater or other water samples are collected and transferred to amber jars for storage, they are commonly measured to an approximate volume (~110 mL, for example) prior to freezing. On the day of WQA, this volume should be recorded on the WQA data form for the respective samples. If the volume is unknown at this point, it should be measured using a graduated cylinder of appropriate size, and recorded on the data sheet.

#### 3.2 Salinity Adjustment

##### 3.21 Reducing the salinity of aqueous samples

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

- (i)  $(\text{target } \text{‰} \div \text{sample } \text{‰}) \times \text{sample vol. in mL} = A$
- (ii)  $\text{sample vol.} - A = B$
- (iii)  $\text{sample vol.} \div A = C$
- (iv)  $B \times C = \text{volume of HPLC water to add}$

### 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)  $((\text{target } \text{‰} - \text{sample } \text{‰}) \times \text{sample vol. in mL}) \div (\text{brine } \text{‰} - \text{target } \text{‰}) = \text{vol. of brine to add}$

### 3.3 Dissolved Oxygen Adjustment

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

### 3.4 Other Water Quality Determinations

1. Measure pH (SOP F10.21) and record on the Water Quality Adjustment Data Form.
2. Measure and record ammonia concentration (SOP F10.4).
3. Measure and record sulfide concentration if required.

## 4.0 DATA COLLECTION

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

## 5.0 QUALITY CONTROL

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

## 6.0 TRAINING

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

**7.0 SAFETY**

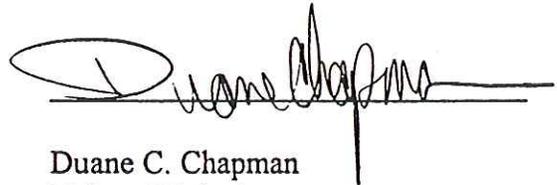
The NaOH solution used in the ammonia determination procedure is a highly caustic liquid. Care should be taken to avoid its contact with skin or clothing. Should such contact occur, quickly flush affected with water. A sink is present along the west wall of the dry lab, another is present along the east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

**8.0 ATTACHMENTS**

Attachment 1. Equipment List for Water Quality Adjustment

Attachment 2. Water Quality Adjustment Data Form

Prepared by:

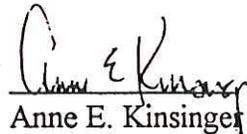


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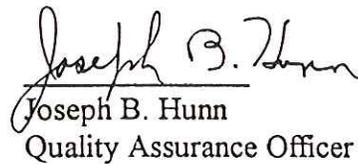
Approved by:



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Joseph B. Hunn  
Quality Assurance Officer

5-20-94

**ATTACHMENT 1**

**EQUIPMENT LIST FOR WATER QUALITY ADJUSTMENT**

Graduated cylinders

Pipetters

Latex gloves

Magnetic stirrer and stir bars

10 M NaOH

Concentrated brine (See section 2.2 for preparation)

HPLC ultrapure sterile water (J.T. Baker® #JT4218-2)

Salinity refractometer

Dissolved oxygen meter

pH electrode, buffer solutions, and meter

Ammonia electrode, standard solutions, and meter

Sulfide electrode, standard solutions, and meter

Data sheets

Hand calculator

**ATTACHMENT 2**

**WATER QUALITY ADJUSTMENT DATA FORM**

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STUDY PROTOCOL \_\_\_\_\_ INITIALS \_\_\_\_\_

SAMPLE DESIGNATION \_\_\_\_\_ DATE \_\_\_\_\_

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**A. Salinity Adjustment:**

Initial volume (mL) \_\_\_\_\_

Initial salinity (‰) \_\_\_\_\_

Vol. Baker® HPLC water added (mL) \_\_\_\_\_

Vol. \_\_\_\_‰ brine added (mL) \_\_\_\_\_

% of original sample \_\_\_\_\_

(initial vol./final vol. x 100)

**B. Character of Sample (after salinity adjustment):**

Final Volume (mL) \_\_\_\_\_

Final Salinity (‰) \_\_\_\_\_

pH \_\_\_\_\_

Dissolved oxygen (mg/L) \_\_\_\_\_

DO saturation (%) \_\_\_\_\_

Total ammonia (mg/L) \_\_\_\_\_

Sulfide (mg/L) \_\_\_\_\_

COMMENTS \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Corpus Christi SOP: F10.6

Page 1 of 16 pages

Date Prepared : April 10, 1990

Date Revised: March 10, 1995

## SEA URCHIN FERTILIZATION TOXICITY TEST

### 1.0 OBJECTIVE

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

### 2.0 TEST PREPARATION

#### 2.1 Test Animals

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

## 2.2 Dilution Water

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

Filtered (0.45 µm) seawater adjusted to 30 ‰ is used to wash eggs and is also used for sperm and egg dilutions. The acronym MFS (for Millipore® filtered seawater) is used for this filtered and salinity adjusted seawater.

## 2.3 Test System: Equipment

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

### 2.3.1 Equipment

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

### 2.3.2 Solutions

#### 10% Buffered Formalin:

1,620 mL sea water  
620 mL formaldehyde  
6.48 g  $\text{NaH}_2\text{P}_0_4$  or  $\text{KH}_2\text{PO}_4$  (mono)  
10.5 g  $\text{Na}_2\text{HPO}_4$  or  $\text{K}_2\text{HPO}_4$  (dibasic)

1 mL needed for each replicate. Fill the dispenser.

## 2.4 Collection and Preparation of Gametes

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

### 2.4.1 Selection of Urchins to be Used in Toxicity Test.

1. Take two or three females and place in shallow bowl, barely covering tests with seawater.
2. Stimulate release of eggs from gonopores of a female by touching test with electrodes from a 12V transformer.
3. Collect a few eggs from between spines using a 10 mL disposable syringe with a large gauge blunt-tipped needle attached. Discard the first small quantity of eggs expelled from each gonopore and continue collecting. Place a 2 to 5 drops of eggs onto a scintillation vial containing 10ml of filtered seawater. Rinse syringe and repeat for each female.
4. Select females which have round, well developed eggs, and which do not release clumps of eggs or undeveloped ovarian tissue.
5. Place 2-4 males in shallow bowl(s) with a small amount of seawater, leaving the upper  $\frac{1}{2}$  to  $\frac{1}{3}$  of the animals uncovered.
6. Stimulate release of sperm from gonopores by touching test with electrodes from 12V transformer (about 30 seconds each time). If sperm is watery, reject the animal and choose another. Sperm should be the consistency of condensed milk. Collect sperm using a pastuere pipette with a rubber bulb attached.

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

### 2.4.2 Obtain Eggs

1. Place selected female in large Carolina dish and add enough water to cover the urchin's test with approximately 1 cm of seawater. Stimulate release of eggs from female with 12V transformer.
2. Collect eggs as above using the 10 mL syringe. Remove needle before dispensing eggs into a disposable shell vial or other clean container capable of holding 25-50 mL. Collect enough eggs for pretest and test. If female stops giving eggs readily or starts giving chunky material, cease stimulation and collection of eggs from that female.
3. Add MFS to fill shell vials, gently mixing eggs. Allow eggs to settle to bottom of vial. Remove water with a pipette. Replace water, again gently mixing the eggs.
4. Repeat washing procedure.

### 2.4.3 Prepare Appropriate Egg Concentration

1. Put approximately 100 mL of 30 ‰ 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:

$$(\text{"egg count"} - 200/200) \times \text{Current Volume of Eggs} = \text{Volume seawater to add to stock (mLs)}$$

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

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

#### **2.4.4 Obtain Sperm**

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

#### **2.4.5 Prepare Appropriate Sperm Dilution**

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

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

### **3.0 TEST PROCEDURES**

1. Add 50  $\mu$ L appropriately diluted sperm to each vial. Record time of sperm addition. Sperm should be used within 30 minutes of wetting.
2. Incubate all test vials at  $20 \pm 2^\circ\text{C}$  for 30 minutes. At this point it is useful to set a timer for five to ten minutes prior to the end of the incubation period. This will notify the worker early enough to be ready to start the next step exactly on time.
3. While gently swirling the egg solution to maintain even mixing of eggs, use a 200  $\mu$ L pipetter to add 200  $\mu$ L diluted egg suspension to each vial. Pipette tips are cut back using

- a clean razor blade to prevent crushing the eggs during pipetting. Record time of egg addition.
4. Incubate for 30 minutes at  $20 \pm 2^\circ\text{C}$ . The timer may be used again at this point.
  5. Using the dispenser, add 1 mL of 10% buffered formalin to each sample.
  6. Vials may now be capped and stored overnight or for several days until evaluated. Fertilization membranes are easiest to see while eggs are fairly fresh, so evaluation within two to three days may decrease the time required for evaluation.
  7. If it is not possible to make the evaluations within several days or the membranes are difficult to discern, an optional technique may be employed. Make up a 200 ‰ NaCl solution (pickling salt) and add 2 to 4 drops of the solution to a 1 mL egg sample on a microscope slide. This solution causes the egg, but not the membrane, to shrink briefly thereby making the membrane easier to see. The effect only lasts for a short time (~5 min.) so the observations must be made immediately after the NaCl solution is added. If this optional technique is employed, it must be used on all samples in that test series.

#### 4.0 DATA COLLECTION AND TABULATION

1. Transfer approximately 1 mL eggs and water from bottom of test vials to counting slide. Observe eggs using compound microscope under 100X magnification. Dark field viewing is useful here in identifying fertilization membranes.
2. Count 100 eggs/sample using hand counter with multiple keys (such as a blood cell counter), using one key to indicate fertilized eggs and another to indicate unfertilized eggs. Fertilization is defined by the presence of fertilization membrane surrounding egg.
3. Calculate fertilization percentage for each replicate test:

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

## 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-Kärber method with Abbott's correction is recommended to calculate EC<sub>50</sub> values for dilution series tests (Hamilton et al. 1977)

## 6.0 QUALITY CONTROL

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

## 7.0 TRAINING

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

## 8.0 SAFETY

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

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

## 9.0 ATTACHMENTS

- Attachment 1. Equipment List for Fertilization Toxicity Test
- Attachment 2. Pretest to Insure Selection of Quality Gametes
- Attachment 3. Water Quality Adjustment Data Form
- Attachment 4. Sea Urchin Pretest Data Sheet
- Attachment 5. Sea Urchin Pretest Continuation Data Sheet
- Attachment 6. Sea Urchin Fertilization/Embryological Development Toxicity Test Gamete Data Sheet
- Attachment 7. Sea Urchin Fertilization Toxicity Test Fertilization Data Sheet

## 10.0 REFERENCES

- 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-Kärber 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*. 2<sup>nd</sup> edition. W.H. Freeman and Company, San Francisco, CA 859 pp.

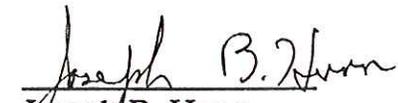
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Attachment 1

EQUIPMENT LIST FOR FERTILIZATION TOXICITY TEST

Large Carolina dishes (at least 2)  
20 mL KIMBLE scintillation vials (These should be type shipped with caps off, and without cap liners. If other brand or type is used, the vials should be tested for toxicity prior to use.)  
400 mL beaker or wide-mouthed thermos for holding vials of sperm  
250 mL beakers (4)  
Pasteur pipettes and latex bulbs  
plastic microcentrifuge tubes  
25 mL shell vials or equivalent  
Test tube rack (to hold shell vials)  
12V transformer with pencil type electrodes  
Styrofoam (or something to hold electrode tips)  
10 cc syringe with large diameter blunt ended needle (make by grinding sharp point off the needle with a grinding stone)  
Marking pens  
Ice  
10-100  $\mu$ L pipetter  
50-200  $\mu$ L 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  $\mu$ m) seawater, adjusted to 30 ‰  
Data sheets  
Baker reagent grade water  
Approximately 100 ‰ concentrated brine

## Attachment 2

## PRETEST TO INSURE SELECTION OF QUALITY GAMETES

1. Using the procedure in section 2.4.1, select 2 to 5 females and at least 2 male urchins to be used in the pretest.
2. Fill pretest vials with five mL of **reference** water. There should be at least two vials for each combination of male, female, and pretest sperm concentration (step 4 below). For example, in a pretest with two females, one male, and six pretest sperm concentrations, 24 vials (2 X 2 X 6) would be needed. Arrange and mark vials accordingly in a rack.
3. Perform steps 2.4.2 (egg collection) and 2.4.3 (egg dilution) for each female urchin. Make enough volume of the egg suspension to perform the pretest and the test.
4. Perform step 2.4.4 (sperm collection) for each male urchin or male combination. Prepare a dilution series of sperm concentrations which will bracket the 60-90% fertilization rate in the test. Sperm dilution will depend on the health and reproductive status of the male urchin, but in most cases the following "standard dilution" should be used:
  - 1: 250 (20  $\mu$ L dry sperm added to 5 mL MFS. This concentration is used only as stock solution to make up the rest of the dilution series and is not used full strength in the pretest.)
  - 1: 1250 (1 mL of 1:250 and 4 mL MFS)
  - 1: 2500 (1 mL of 1:250 and 9 mL MFS)
  - 1: 5000 (2 mL of 1:2500 and 2 mL MFS)
  - 1: 7500 (2 mL of 1:2500 and 4 mL MFS)
  - 1:10000 (3 mL of 1:7500 and 1 mL MFS)
  - 1:12500 (1 mL of 1:2500 and 4 mL MFS)

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

5. As in section 3.0 add 50  $\mu$ L of the diluted sperm to each pretest vial. Incubate for 30 minutes at approximately 20°C, and add 200  $\mu$ L of the egg suspension. Incubate for another 30 minutes, then fix with 1 mL of the buffered formalin solution.
6. As in section 4.0, obtain a fertilization rate for the vials. There is no need to count all vials, enough vials should be counted to determine a good male/female combination, and an appropriate sperm dilution factor. If more than one male/female combination is acceptable, this is a good opportunity to choose a female which exhibits easily visible fertilization membranes or in cases where there are many samples, to combine eggs from different females. The appearance of the fertilization membranes may vary among female urchins, and presence of easily visible membranes facilitates counting.

Attachment 3

**WATER QUALITY ADJUSTMENT DATA FORM**

STUDY PROTOCOL \_\_\_\_\_ INITIALS \_\_\_\_\_

SAMPLE DESIGNATION \_\_\_\_\_ DATE \_\_\_\_\_

A. Salinity Adjustment:

Initial volume (mL) \_\_\_\_\_

Initial salinity (‰) \_\_\_\_\_

Vol. Milli-Q water added (mL) \_\_\_\_\_

Vol. \_\_\_‰ brine added (mL) \_\_\_\_\_

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

B. Character of Sample (after salinity adjustment):

Volume (mL) \_\_\_\_\_

Salinity (‰) \_\_\_\_\_

pH \_\_\_\_\_

Dissolved oxygen (mg/L) \_\_\_\_\_

DO saturation (%) \_\_\_\_\_

Total ammonia (mg/L) \_\_\_\_\_

Sulfide (mg/L) \_\_\_\_\_

COMMENTS \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Attachment 4

**SEA URCHIN PRETEST DATA SHEET**

TEST ID \_\_\_\_\_ INITIALS \_\_\_\_\_

STUDY PROTOCOL \_\_\_\_\_ DATE \_\_\_\_\_

**EGGS**

Female number: \_\_\_\_\_

Collection time: \_\_\_\_\_

Count: \_\_\_\_\_

**SPERM**

Male number: \_\_\_\_\_

Collection time: \_\_\_\_\_

Dilution start time: \_\_\_\_\_

**TEST TIMES**

Sperm in: \_\_\_\_\_ Eggs in: \_\_\_\_\_ Formalin in: \_\_\_\_\_

**SPERM DILUTION** \_\_\_\_\_

**COMMENTS** \_\_\_\_\_

**% FERTILIZATION** Reference sample designation: \_\_\_\_\_

	Female #	Male #			
<u>Sperm Dilution</u>	<u>REP 1</u>	<u>REP 2</u>	<u>REP 3</u>	<u>REP 4</u>	
_____	_____	_____	_____	_____	
_____	_____	_____	_____	_____	
_____	_____	_____	_____	_____	
_____	_____	_____	_____	_____	

**% FERTILIZATION** Reference sample designation: \_\_\_\_\_

	Female #	Male #			
<u>Sperm dilution</u>	<u>REP 1</u>	<u>REP 2</u>	<u>REP 3</u>	<u>REP 4</u>	
_____	_____	_____	_____	_____	
_____	_____	_____	_____	_____	
_____	_____	_____	_____	_____	
_____	_____	_____	_____	_____	

Attachment 5

**SEA URCHIN PRETEST CONTINUATION DATA SHEET**

TEST ID \_\_\_\_\_ INITIALS \_\_\_\_\_

STUDY PROTOCOL \_\_\_\_\_ DATE \_\_\_\_\_

**% FERTILIZATION** Reference sample designation: \_\_\_\_\_

Female # \_\_\_\_\_ Male # \_\_\_\_\_

Sperm dilution	<u>REP 1</u>	<u>REP 2</u>	<u>REP 3</u>	<u>REP 4</u>
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____

**% FERTILIZATION** Reference sample designation: \_\_\_\_\_

Female # \_\_\_\_\_ Male # \_\_\_\_\_

Sperm dilution	<u>REP 1</u>	<u>REP 2</u>	<u>REP 3</u>	<u>REP 4</u>
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____

**% FERTILIZATION** Reference sample designation: \_\_\_\_\_

Female # \_\_\_\_\_ Male # \_\_\_\_\_

Sperm dilution	<u>REP 1</u>	<u>REP 2</u>	<u>REP 3</u>	<u>REP 4</u>
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____

**% FERTILIZATION** Reference sample designation: \_\_\_\_\_

Female # \_\_\_\_\_ Male # \_\_\_\_\_

Sperm dilution	<u>REP 1</u>	<u>REP 2</u>	<u>REP 3</u>	<u>REP 4</u>
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____
=====	_____	_____	_____	_____





## **APPENDIX**

Appendix 1. Chain of custody sheets from incoming samples arriving at the USGS Marine Ecotoxicology Research Station between June 8<sup>th</sup> and June 16<sup>th</sup>, 2004













