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Biological Testing of Sediment for the Olympia Harbor Navigation Improvement Project, 1988

Geoduck, Amphipod, and Echinoderm Bioassays

J. A. Ward
J. Q. Word
L. D. Antrim

May 1989

Prepared for
the U.S. Army Corps of Engineers
Seattle District
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BIOLOGICAL TESTING OF SEDIMENT FOR THE OLYMPIA HARBOR NAVIGATION IMPROVEMENT PROJECT, 1988

GEODUCK, AMPHIPOD, AND ECHINODERM BIOASSAYS

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Sequim, Washington 98382

May 1989

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Pacific Northwest Laboratory
Richland, Washington 99352

SUMMARY

The Olympia Harbor Navigation Improvement Project requires the dredging of approximately 330,000 cubic yards (cy) of sediment from the harbor entrance channel and 205,185 cy from the turning basin. Puget Sound Dredged Disposal Analysis (PSDDA) partial characterization studies were used to plan a full sediment characterization in which chemical analyses and biological testing of sediments evaluated the suitability of the dredged material for unconfined, open-water disposal. The U.S. Army Corps of Engineers (COE), Seattle District, contracted with NOAA/NMFS, Environmental Conservation Division, to perform the chemical analysis and Microtox bioassay tests, and with the Battelle/Marine Sciences Laboratory (MSL) in Sequim to perform flow-through solid-phase bioassays utilizing juvenile (8 to 10 mm) geoduck clams, *Panopea generosa*, and static solid phase bioassays using the phoxocephalid amphipod, *Rhepoxynius abronius*, developing embryos and gametes of the purple sea urchin, *Strongylocentrotus purpuratus*, and the larvae of the Pacific oyster *Crassostrea gigas*.

The results of the geoduck protocol development test showed that juvenile geoducks are capable of surviving in both coarse and fine-grained substrates for a period exceeding 10 days, with an optimum sediment depth in the test containers of 2 cm. The definitive test results indicated no significant difference between Olympia Harbor (test) and reference sediment treatments.

Amphipod tests indicated that none of the test treatments were statistically different from reference sediment treatments. In comparison to control sediment treatments, four of the test treatments had statistically significant responses which ranged from 22 to 31% below control treatment response.

The sea urchin larval development tests showed that none of the test treatments were statistically different from the reference sediment treatment. However, all of the test treatments had at least a 40% greater mortality (statistically significant) in comparison to control treatments. The sea urchin sperm cell test indicated no significant differences between test and reference treatments. The oyster larvae tests were not valid due to unacceptably high control mortality.

When the results of the above biological tests were evaluated under PSDDA guidelines, it was found that all the tested sediment treatments from Olympia Harbor are suitable for unconfined open-water disposal.

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Special thanks to Dr. John Strand for his assistance in biological testing and in the review of this document. Thank you all.

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1.0 INTRODUCTION

The Olympia Harbor Navigation Improvement Project requires dredging of approximately 330,000 cubic yards (cy) of sediment from the entrance channel and 205,185 cy from the turning basin. The Puget Sound Dredged Disposal Analysis (PSDDA) process requires a review of existing information to assess the appropriate breadth of characterization studies of the proposed dredged sediment. The review revealed limited sediment data and a variety of potential contaminant sources, including wood product manufacturers, oil companies, port terminals, marinas, and wastewater outfalls. Thus, both partial and full characterizations of chemical contaminants and sediment toxicity were conducted at the proposed dredging site. For the full characterization, the U.S. Army Corps of Engineers (COE), Seattle District, contracted with the National Oceanographic and Atmospheric Administration/National Marine Fisheries Service (NOAA/NMFS), Environmental Conservation Division, to perform the sample compositing and distribution, the chemical analyses, and the saline Microtox biological testing. Battelle/Marine Sciences Laboratory (MSL)[a] in Sequim, Washington, was contracted to perform the bivalve larvae, juvenile bivalve, amphipod, and echinoderm sperm cell and larval development biological testing. Testing with juvenile bivalves included refinement of methodology for a 10-day geoduck (*Panopea generosa*) biological test. Sampling locations and the sediment compositing scheme for this project are presented in Figure 1.1.

The MSL performed biological testing on samples of sediment collected by both COE (Olympia Harbor) and by MSL (Sequim Bay, West Beach, and Point Whitney). Test sediment was composited by NOAA/NMFS personnel, and aliquots for chemistry and biological testing were distributed to each laboratory. This report contains a description of the juvenile bivalve (geoduck) methodology developed for this program. Detailed descriptions are included of the biological testing protocols employed for the sea urchin development and

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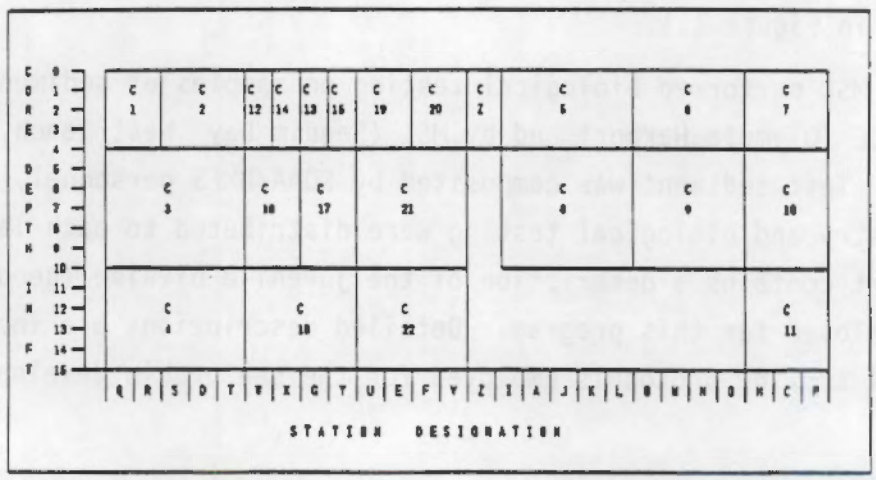


FIGURE 1.1. Sampling Stations and Compositing Scheme for the Olympia Harbor Navigation Improvement Project, November, 1988. (Composites associated with the 0-4 ft depth and composite C-17 were tested by MSL.)

sperm cell tests, the oyster embryo tests, and the amphipod tests. The results are compared to the biological testing guidelines for sediment disposal established by PSDDA.

2.0 MATERIALS AND METHODS

The solid phase of a dredged material was represented in these tests by a layer of sediment collected from the Olympia Harbor dredging site. This layer was intended to provide an approximation of exposure conditions that might be experienced by benthic organisms living in sediment near or within a disposal site. Test sediments were collected in Budd Inlet, Olympia, Washington, by the U.S. Army Corps of Engineers, composited by NOAA/NMFS personnel, and delivered to the MSL for biological testing. Sediment chemistry was conducted by the NOAA/NMFS, Environmental Conservation Division. One sediment treatment from Olympia Harbor arrived after biological testing had been initiated and necessitated a second round of testing. Control sediments for the amphipod tests were obtained by MSL personnel from West Beach, Whidbey Island, Washington. Geoduck control sediment was collected from Point Whitney at the Washington State Department of Fisheries Shellfish Laboratory near Brinnon, Washington. Reference sediment was obtained from Sequim Bay. The West Beach and Sequim Bay sediments were composited by NOAA/NMFS and returned to the MSL for biological testing. The Point Whitney sediment was used only during the geoduck methods testing and was not composited by NOAA/NMFS.

2.1 TEST OBJECTIVES AND EXPERIMENTAL DESIGN

The objective of these tests was to evaluate sediments proposed for dredging through biological testing. Three sensitive marine invertebrates were used during these tests: 8- to 10-mm juvenile geoducks, a phoxocephalid amphipod (*Repoxynius abronius*), and the gametes and developing embryos of the purple sea urchin (*Strongylocentrotus purpuratus*). Three tests were also conducted with the developing embryos of the Pacific oyster (*Crassostrea gigas*), but the tests were invalid due to poor survival in control treatments. Test failure was probably due to the time of the year, as the test was attempted outside of the annual cycle when oysters naturally spawn. Sea urchin tests were conducted as a substitute for the failed oyster test.

Selection of these animals allowed us to examine effects with respect to differences in feeding habits, life history, and life stage. The

experimental design is summarized in Table 2.1. Experiments involved a flow-through geoduck test, which examined the effects of sediment type and depth on survival (Protocol Development Test), and a flow-through geoduck test evaluating seven sediments from areas in Olympia Harbor proposed for dredging (Definitive Test). Also included were two static amphipod and two static sea urchin larval tests, which evaluated a total of 13 sediment treatments from locations in Olympia Harbor where dredging has been proposed. A static sea urchin sperm cell test evaluating 12 of the Olympia Harbor sediment treatments was also included.

The experimental design was devised to fulfill testing requirements of PSDDA (1988). Overall guidance for the studies was provided by the U.S. Environmental Protection Agency (EPA) Region 10, Puget Sound Estuary Program (PSEP 1986). Appropriate literature and/or protocols are also found in EPA (1976 and 1978), APHA (1985), and ASTM (1980a,b).

2.2 CONTROL AND REFERENCE SEDIMENT SAMPLE COLLECTION AND HANDLING

Control sediment for the amphipod biological testing was collected from West Beach, Whidbey Island, by MSL staff. The West Beach sediment was collected on November 14, 1988, at the same time as the collection of amphipods. An EPA-designed amphipod dredge was towed parallel to the shore in approximately 3 m of water, near the western edge of Whidbey Island (Figure 2.1). The sediment and amphipods were placed in clean coolers and immediately transported to the MSL.

Reference sediment samples from Sequim Bay were collected on November 14, 1988, using a ponar petite grab. The sediment was stored in a clean 5-gallon bucket for transport to the MSL. Station positioning in Sequim Bay was conducted using range fixes to reference landmarks, Loran C, and water-depth confirmation. The location of the sampling station is shown in Figure 2.2.

West Beach control material and Sequim Bay reference material was stored at the MSL until compositing by NOAA/NMFS personnel. All control, reference, and test sediment was then divided between NOAA/NMFS and MSL personnel and stored at the MSL or the NOAA/NMFS laboratory in clean 1-liter jars at 4°C

TABLE 2.1. Experimental Design for PSDDA Bioassay Tests

Test	Type(a)	Sediments Tested(b)	Reference	Controls(c)	Replicates Per Treatment	Organisms per Replicate	Test Duration
Geoduck Methods Development	FT	PW, SB, WB	N/A	N/A	2	9	10 d
Geoduck Definitive Test	FT	C1, C2, C12, C13, C14, C,16 CZ	SB	PW	4	10	10 d
Amphipod Test 1-A	S	N/A		SW, Pos1	5	20	4 d
Amphipod Test 1-B	S	C1, C2, C5, C6, C7, C12, C13, C14, C16, C19, C20, CZ	SB	WB	5	20	10 d
Amphipod Test 2-A	S	N/A		SW, Pos2	5	20	4 d
Amphipod Test 2-B	S	C17	SB	WB	5	20	10 d
Sea Urchin Embryo Test 1	S	C1, C2, C5, C6, C7, C12, C13, C14, C15, C19, C20, CZ	SB	SW, Pos2	5	~30/mL	72 h
Sea Urchin Embryo Test 2	S	C17	SB	SW, Pos2	5	~30/mL	72 h
Sea Urchin Urchin Sperm Cell	S	C1, C2, C5, C6, C7, C12, C13, C14, C15, C19, C20, CZ	SB	SW, Pos2	5	~2000	1 h
Pacific Oyster Embryo Tests 1-3(d)	S	C1, C2, C5, C6, C7, C12, C13, C14, C16, C19, C20, CZ	SB	SW, Pos2	5	~30/mL	48 h

(a) FT is flow-through and S is static test.

(b) PW is Point Whitney; SB is Sequim Bay; WB is West Beach; C are Budd Inlet sediment treatments; N/A is Not Applicable.

(c) SW is seawater-only control; Pos1 is NaPCP (positive) control, Pos2 is $CdCl_2$ (positive) control.

(d) These tests were not successful due to high mortalities in control and reference treatments.

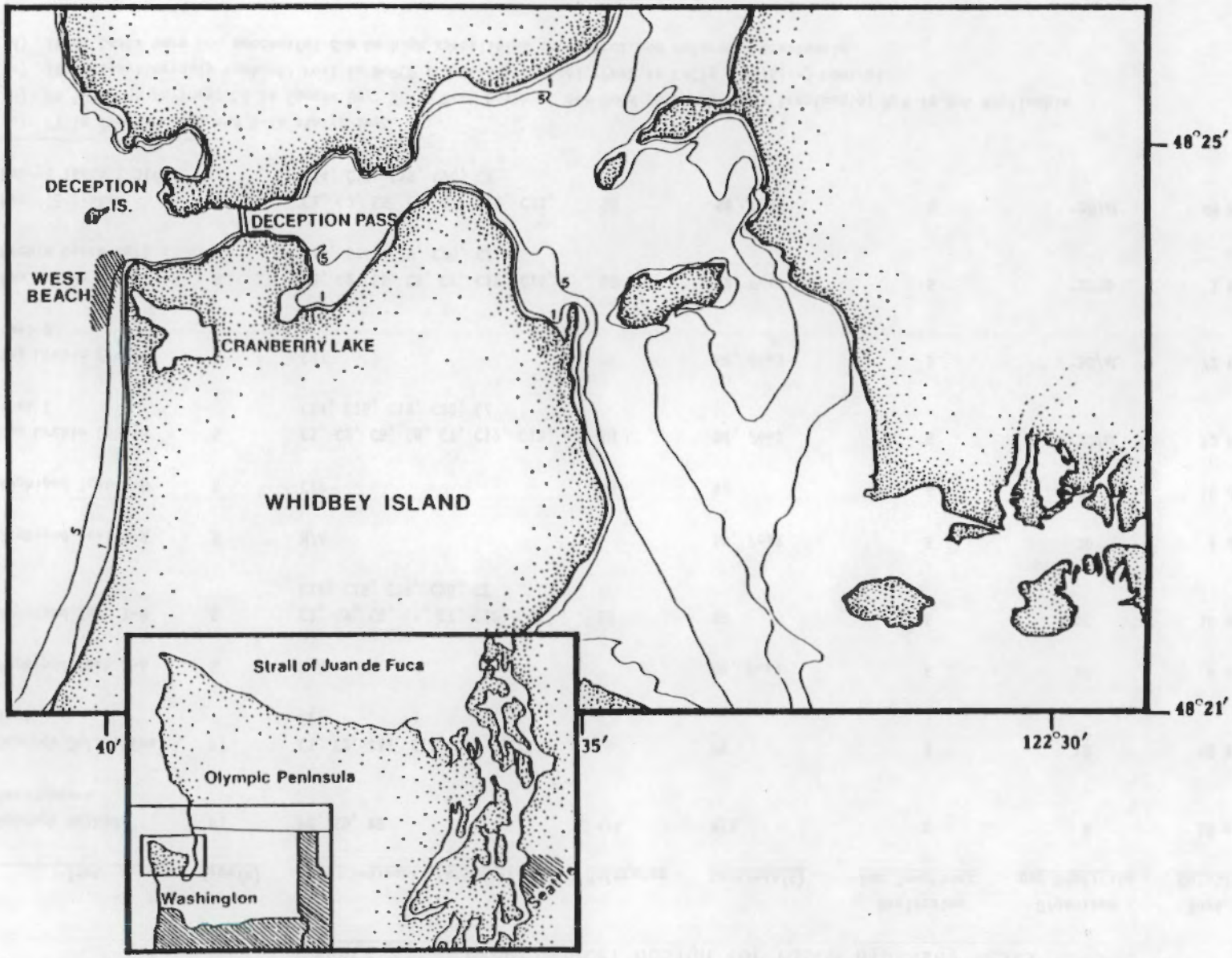


FIGURE 2.1. Location of West Beach, Whidbey Island, Amphipod Control Sampling Station

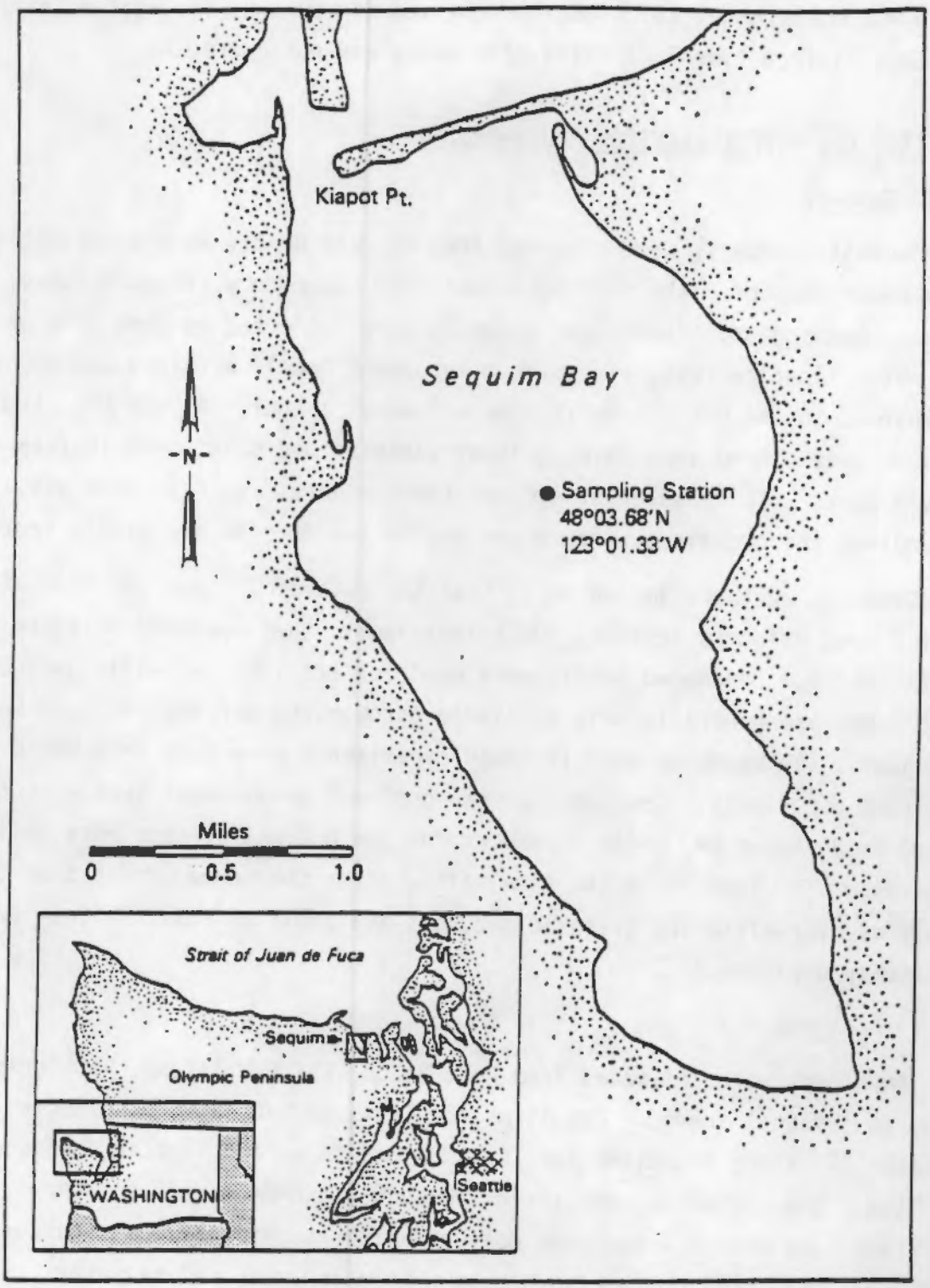


FIGURE 2.2. Location of Sequim Bay Reference Sediment Sampling Station

until used. Additional aliquots of NOAA/NMFS stored sediment were delivered to the MSL on December 16, 1988, for the second round of biological testing. The PSODA storage time limitation of 6 weeks was not exceeded.

2.3 TEST ORGANISM COLLECTION AND HANDLING

2.3.1 Geoduck

Juvenile geoducks were obtained from Mr. Hal Beatty at the Washington State Department of Fisheries (WDF) Shellfish Laboratory (Point Whitney) near Brinnon, Washington. Individual geoducks were collected by hand from an open-water floating raft, placed on moist paper towels within a cooler, and transported to the MSL. Transit time was about 1 hour. At the MSL, the bivalves were placed into Point Whitney sediment and maintained in flow-through tanks with seawater at ambient temperatures. Extreme care was taken in handling the organisms because the shells are fragile and easily broken.

Geoducks were acclimated to 15°C at the rate of 2°C/day and held at least 2 days prior to testing. Only individuals that appeared relatively healthy and had undamaged shells were used for testing. Juvenile geoducks (2 to 5 mm) are generally only available through the WDF program from April to August. The geoducks used in these experiments were part of a WDF over-wintering experiment. Geoducks for the protocol development test were collected on November 14, 1988. Geoducks for the definitive test were collected on November 22, 1988, from the same raft. After the second collection the supply of over-wintering juvenile geoducks available at Point Whitney was completely depleted.

2.3.2 Amphipod

Amphipods were collected from West Beach, Whidbey Island, Washington, using an infaunal dredge. Organisms for Tests 1A and 1B were collected on November 14, 1988; organisms for Tests 2A and 2B were collected on December 17, 1988. The sediments were sieved through a 1.0-mm screen to remove predators, and within 4 hours of collection the amphipods were transported to MSL in large tubs containing sieved native sediment and seawater. At the MSL, amphipods and sediment were transferred to holding tanks integrated into the MSL's flow-through seawater system. The seawater was gradually

increased from the West Beach ambient temperature (9°C) to 15°C over a period of 24 hours. Water quality was checked daily in holding tanks. Animals were not fed before or during the testing periods.

2.3.3 Purple Sea Urchin

Sea urchins were obtained from Marinus, Inc., of Long Beach, California, and held in the laboratory in flowing seawater. Sea urchins for Test 1 were shipped to MSL on December 9, 1988; sea urchins for Test 2 arrived at MSL on December 19, 1988. Organisms were not fed prior to testing. Sea urchins for Test 2 had been shipped on December 16, 1989, but because fog delayed delivery, they arrived in poor condition. Some sea urchins had spawned and had to be discarded. Others spawned immediately upon introduction into flowing seawater. Sea urchins suitable for testing were separated and used immediately for Test 2 without introduction into flowing seawater.

2.3.4 Pacific Oyster

Oysters were obtained from two sources: a private company (Brezina and Associates) in California provided animals from Vancouver Island, Canada, and Monterey, California, which were used for the first and second tests; a commercial oyster grower (Coast Oyster Company) located in Quilcene, Washington, provided oysters for the third test. In each case, the oysters had been conditioned for a period of four to six weeks in 20°C seawater with a salinity of 26°/₀₀ and fed a mixture of algae to provide nutrition and to hasten sexual maturity. The oysters were packed in wet paper and in a cooler, transported to MSL, and placed in flow-through holding tanks at 26°/₀₀ salinity and 20°C. Oysters for the first test arrived at MSL on November 17, 1988; oysters for the second test arrived on November 28, 1988; oysters for the third test arrived on December 6, 1988. The gonadal development in individuals from all three shipments indicated that the organisms were in poor spawning condition. In some cases, oysters appeared over- or under-conditioned, with females containing few fertile "pear-shaped" eggs and males displaying poor gonadal development. For all three tests, organisms with the best apparent gonadal development were used.

2.4 LABWARE PREPARATION

2.4.1 Glass, Plastic, Nytex, PVC, and Pyrex®

Labware was washed either in a Forma laboratory dishwasher with Forma Soap Solution 2 or by hand in warm, soapy (Alconox) water. Washing was followed by five rinses with deionized water and open air drying. After being washed, the labware was soaked in a solution of 5% nitric acid (HNO_3, Baker Instra-analyzed® grade) for at least 4 hours. The labware was then rinsed five times with deionized water and air dried.

2.4.2 Stainless Steel and Titanium

Stainless steel and titanium implements were washed with warm, soapy water, rinsed five times with deionized water, allowed to air dry, rinsed five times with methylene chloride, and allowed to dry under a laboratory hood.

2.5 LABORATORY INSTRUMENTATION

Temperature control was established by using the MSL's seawater boiler coupled to a YSI Temperature Controller. This controller moderated the mixing of the warmed (28°C) seawater with the colder (9°C) incoming seawater. Temperatures during biological tests were recorded with Fisher Model 15-077-8 Digital Thermometers calibrated against an ErTco L-68397 laboratory standard thermometer at 20°C. The pH was measured with Orion Research Model SA-250 pH meters, calibrated against RICCA Chemical Co. pH 7 buffer solution, and High Purity Chemical/Your Chemical Source buffer solution of pH 10. Salinity was measured with Reichert Model 10419 refractometers calibrated against an International Association of Physical Oceanographers (IAPO) Standard Seawater Sample P46 (which has a salinity of 35.000°/₀₀ and chlorinity of 19.377°/₀₀). Dissolved oxygen was measured with YSI Model 57 dissolved oxygen probes. Dissolved oxygen and pH meters were calibrated before each use; refractometers and thermometers were calibrated on a monthly basis.

® Pyrex is a registered trademark of Dow Corning Corporation, Midland, Michigan. Baker Instra-analyzed is a registered trademark of J. T. Baker Chemical Company, Phillipsburg, New Jersey.

2.6 BIOLOGICAL TEST PROCEDURES

The laboratory at MSL contained all the facilities required for the flow-through and static biological tests. Three water baths contained the test equipment for the following biological tests:

- flow-through geoduck test
- static amphipod test
- static sea urchin larvae and sperm cell test
- static oyster larvae test.

The facilities provided air, temperature control, lighting, and flow-through water supply as needed, as well as warning signals for potential equipment malfunctions. Static biological tests were conducted in a water bath with temperature-controlled flowing water (described above) to maintain constant temperature in the test chambers. Diagrams of the flow-through containers and static testing layouts are presented in Figures 2.3 and 2.4. The static exposure jar was similar to the flow-through design depicted in Figure 2.3 except it lacked discharge and overflow ports.

2.6.1 Geoduck Test Protocols

Protocol Development Test

The methods development test was designed to determine the optimum sediment depth and grain size that would sustain the geoducks during a 10-day test under flow-through conditions. The test was conducted in 1-L jars filled with either 1.5 cm, 2.0 cm, or 5 cm of clean material. Clean material used included coarse sediment from Point Whitney (PW), where geoducks are cultured, and fine sediment from Sequim Bay (SB). Two replicate jars of each sediment type and depth were tested and placed into randomly selected positions on a water table. After sediment was distributed into each test container, flow-through seawater was initiated at a rate of 40 mL/min (± 5.0 mL/min). Containers were allowed to equilibrate for 2 hours. After equilibration, nine juvenile geoducks were added to each container by gently dropping them through the water column onto the sediment surface. The test extended for a period of 10 days. The animals were allowed to burrow

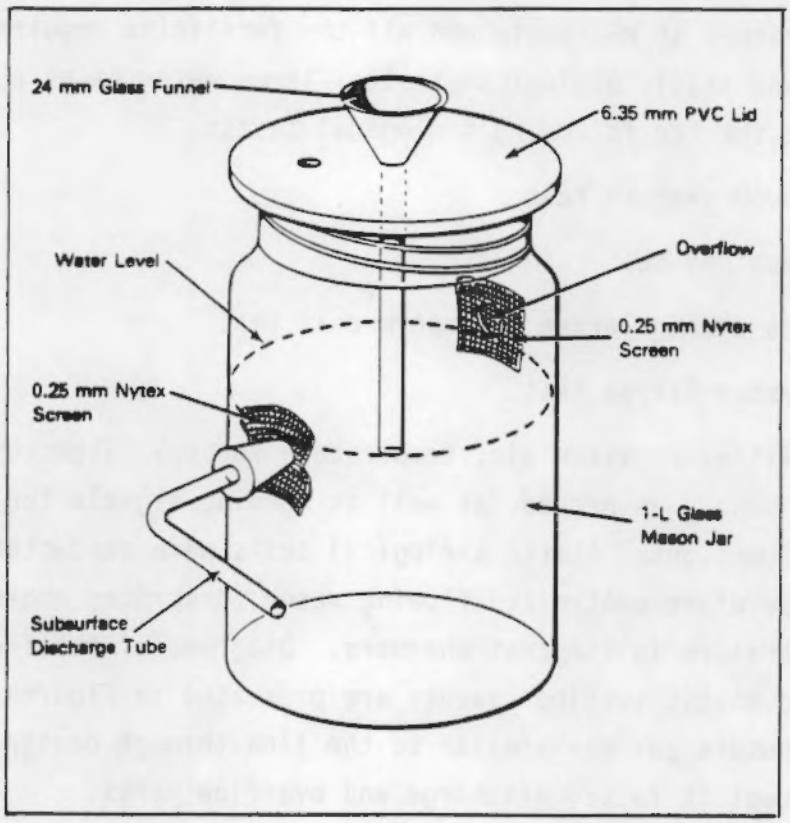


FIGURE 2.3. Flow-Through Exposure Jar

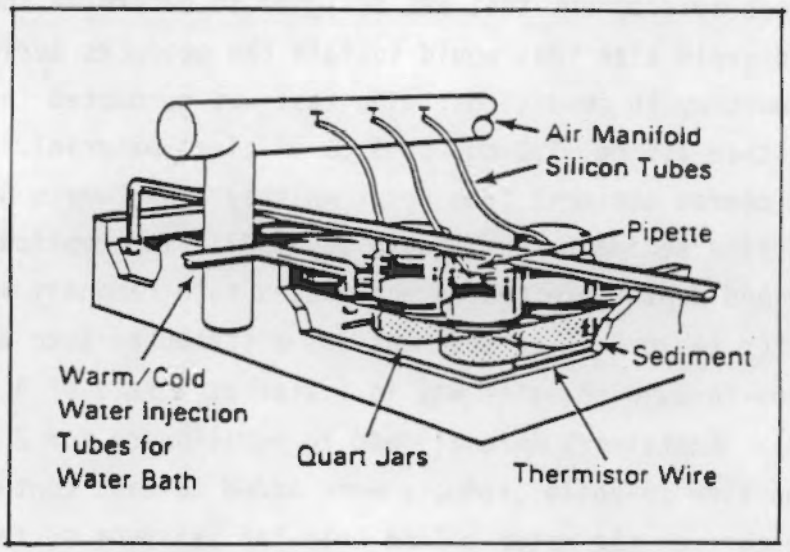


FIGURE 2.4. Jar Arrangement on Water Table

naturally and were observed periodically throughout the test. No food was provided to the bivalves at any time during holding or testing. Nevertheless, the organisms had access to food from the seawater and sediments in the test containers. Obviously dead animals were removed and preserved. Test conditions included water temperature of 15° ± 1°C, salinity of Sequim Bay ambient seawater (31°/₀₀ ± 1.0°/₀₀), pH of Sequim Bay ambient seawater (8.00 ± 0.5) and D.O. of ≥6.0 mg/L).

At the end of the test, the bivalves were gently sieved from the sediment, transferred to clean finger bowls, and mortality was determined. A pressure test was applied to the geoducks to evaluate mortality. An individual was considered alive if it closed its shell after gentle probing. If a shell continued to gape after pressure from a probe, the individual was considered dead.

Definitive Test

The definitive test evaluated the proposed dredged material from Olympia Harbor and was conducted in a fashion similar to the methods development test, with a few modifications. For fine-grained sediment treatments, the jars were filled to a depth of 2 cm with sediment and allowed to equilibrate for 2 hours under flow-through conditions. After equilibration, the geoducks were introduced into the jars through the water column onto the sediment surface. The animals usually burrowed rapidly. For coarse sediment treatments, one-half of the sediment (1-cm depth) was added to each jar and allowed to equilibrate for 2 hours; then, the geoducks were transferred to the sediment as described above. Immediately after their transfer, the bivalves were buried by gently adding the remainder of the sediment on top of the bivalves. This process was necessary as it was discovered during the protocol development test that the juvenile geoducks had difficulty establishing themselves in coarse substrates when introduced onto the sediment surface. Because this action was used only for coarse sediment which settled quickly, little suspended material was lost from the system.

Replication was increased to four jars per sediment treatment, and 10 geoducks were added to each jar. Water quality and animal behavior were monitored daily in each jar. Daily observations included checks on animal behavior (e.g., gaping or burrowing) and measurements of salinity,

temperature, dissolved oxygen, and pH. No food was supplied. Dead geoducks were removed daily during the exposure.

At the end of the 10-day exposure period, the contents of each jar were carefully passed through 1.0-mm Nytex sieves, and animals were counted and mortality determined as in the methods development test.

2.6.2 Amphipod Test Protocols

The amphipod static biological test was conducted in 1-L glass jars following protocols described in Swartz et al. (1985) and Puget Sound Protocols (PSEP 1986). During Test 1, a total of 14 sediment treatments were tested: 12 from Olympia Harbor, a reference sediment from Sequim Bay, and a control sediment from West Beach, Washington (Table 2.1). Three days before the test involving the sediment treatments, a positive (toxic) control containing the reference toxicant NaPCP (sodium pentachlorophenol) in a range of dilutions was also run as a separate 4-day test of amphipod response. During Test 1, 75 test containers were randomly arranged on a water table containing a 15°C water bath. Each container was layered with 3 cm (150 mL) of treatment, control, or reference sediment. Five replicate containers per sediment treatment were tested. After sediment addition, the jars were slowly filled to the 800-mL mark with filtered (0.45-μm) Sequim Bay seawater. At test initiation, the amphipods were carefully sieved from holding sediments, counted, and allocated randomly to exposure jars. A total of 20 amphipods were placed in each jar. Daily observations included the number of amphipods present on the sediment and water surface, the number of dead individuals observed, and the pH, D.O., temperature, and salinity of the water in each container. During daily observations, amphipods trapped at the water surface were gently pushed into the water column using clean glass pipettes. Dead animals were counted but not removed during testing. No food was supplied to the amphipods during holding or test periods beyond that available from sediments and seawater.

At the end of the 10-day exposure, the animals were gently sieved from the sediment through a 0.5-mm Nytex screen and transferred to small finger bowls for mortality determination. For this test, death was defined as a lack of pleopod movement after stimulation with a glass probe.

The positive (toxic) control test consisted of six concentrations of NaPCP and one seawater-only exposure. Three replicate jars were set up for each concentration, resulting in a total of 21 test containers. After each concentration range was prepared, the jars were placed randomly on a water table and 10 amphipods were added to each container. Daily observations included the number of dead individuals observed, and the pH, D.O., temperature, and salinity of the test water. Experimental conditions during this test were identical to those in the sediment test. At the end of 96 hours, the test was terminated by sieving and counting the amphipods, as described above.

During Test 2, the number of treatments totaled five, including one sediment from Olympia Harbor, a reference sediment (Sequim Bay), a control sediment (West Beach), a positive control of $CdCl_2$ (2.00 mg/L), and a seawater-only control (Table 2.1). The sediment treatment exposures were tested for 10 days; the toxic and seawater-only controls were tested for 4 days. Testing protocols and conditions were identical to those described above. Replication, randomization, test initiation, and test termination followed the protocols previously discussed.

2.6.3 Sea Urchin Test Protocols

Three tests involving the gametes or developing larvae of sea urchins were run during this project. The first test involved an evaluation of sediment treatments from Olympia Harbor using the development of the sea urchin following the procedures outlined in Dinnel and Stober (1985). At the same time, a sperm cell test was conducted, using water overlying the sediment treatment in the embryo test containers. The sperm cell test followed the procedures in Dinnel, Link, and Stober (1987). Later in the project, an additional sea urchin larval test was conducted, evaluating one sediment treatment from Olympia Harbor, as well as reference sediment, control sediment, positive controls, and a seawater control (Table 2.1).

Sea Urchin Larval Test Protocols

Larval Test 1. During Test 1, a total of 17 treatments were examined, including 12 sediment treatments from Olympia Harbor, one reference sediment (Sequim Bay), one amphipod control sediment (West Beach), two positive

controls of $CdCl_2$ (1.3 mg/L and 0.27 mg/L), and a seawater-only control. Each sediment treatment consisted of five replicate containers. Fifteen grams (wet) of test sediment were added to each jar, and the jars were filled to a total volume of 750 mL with filtered (0.45-μm) seawater from the Strait of Juan de Fuca. Sediments were suspended by vigorous stirring, after which the jars were placed in randomly designated positions on the 12°C water table and allowed to equilibrate for 4 hours. During the equilibration period the sea urchins were spawned as described below.

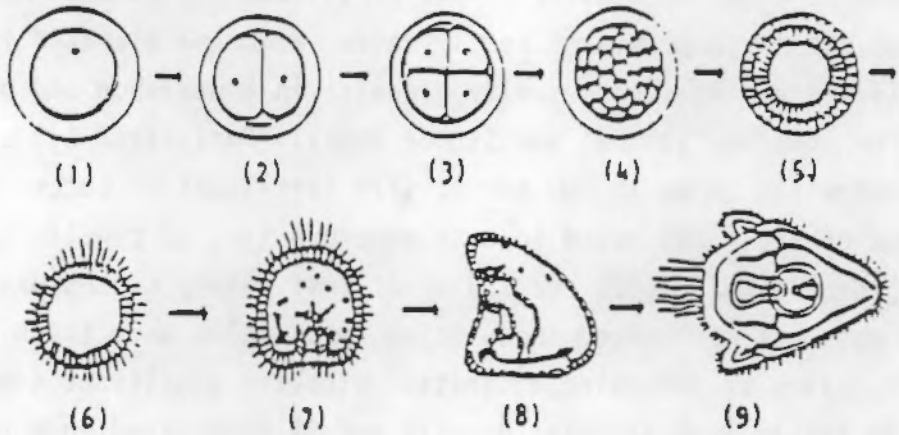
Adult sea urchins were placed over 150-mL beakers, oral side up, and injected with 1 mL of 0.5-M KCl through their peristomal membrane. Spawning occurred within 15 min and lasted approximately 45 min. Eggs from each female were evaluated for normal, round granular shape, and sperm from each male were examined for motility. Normal eggs from two females were combined in a 1500-mL beaker, washed three times with filtered seawater, and diluted to a total volume of 1000 mL with filtered seawater. Motile sperm from a single male was transferred to a 250-mL beaker and diluted to a total volume of 200 mL with filtered seawater. Egg density in the stock was determined by removing a 1-mL aliquot of the egg stock and diluting this to a final volume of 100 mL in a volumetric flask with a mixture of 5% formalin. One-mL aliquots of this diluted stock were counted under a compound microscope using a Sedgewick-Rafter counting cell. Sperm density in the stock was determined by removing a 1-mL aliquot of the sperm stock, then preserving it in a mixture of 10 mL of glacial acetic acid and 89 mL of seawater, giving a final dilution of 100:1. Aliquots of the dilute sperm cell suspension were counted at 400x under a compound microscope, using a hemocytometer to estimate sperm density in the original sperm stock.

After gamete stock densities were determined, an appropriate volume of the sperm suspension was added to the egg stock to provide a sperm:egg ratio of 200:1. This is considered an optimum ratio to achieve an acceptable percentage of fertilization, yet minimize the potential for polyspermy (Dinnel and Stober 1985). The egg and sperm were incubated for 20 min at 12°C, with gentle mixing using a perforated plunger. A 1-mL aliquot was removed from the stock and diluted to a total volume of 100 mL with 10% formalin in seawater. One-mL aliquots of this solution were examined using

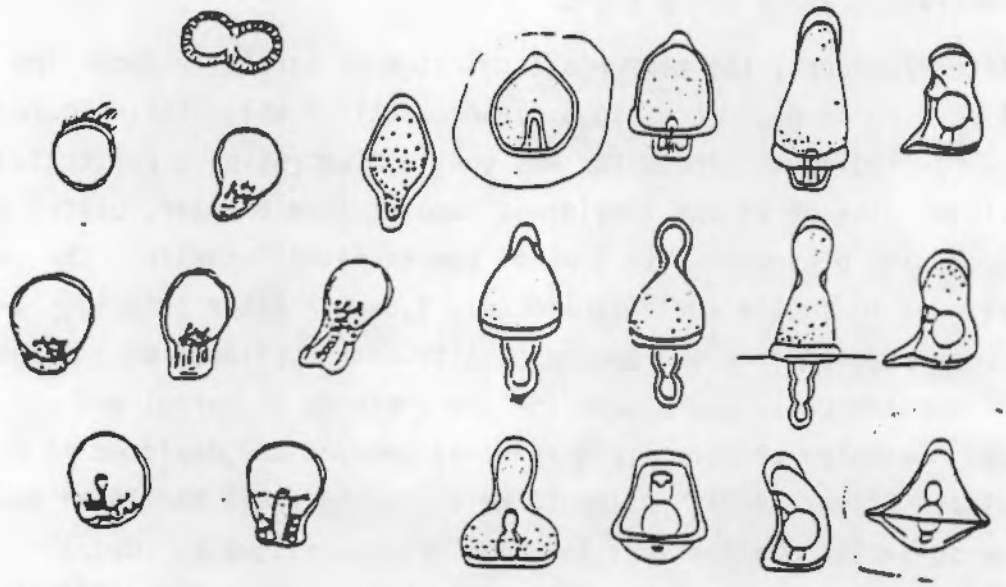
a Sedgewick-Rafter counting cell to determine percent fertilization and to estimate the embryo stocking density in the fertilized egg stock. Fertilized sea urchin eggs display a prominent fertilization membrane elevated from the egg, while unfertilized eggs are usually granular in appearance and display no fertilization membrane (Dinnel and Stober 1985). Fertilized egg stock density was determined to be 16,000 per mL (77% fertilization success). A total of 1.5 mL of stock was added to each exposure jar, to provide a fertilized egg density of 24,000 per 750 mL of test water, or approximately 32 fertilized eggs per mL. After inoculation, subsamples were taken from seawater control jars to determine an initial stocking density at time 0. For Test 1, the actual mean stocking density was 29 fertilized eggs per mL ($n = 5$, $sd = 4.8$).

Water quality checks performed daily on each jar included measurements of D.O., pH, salinity, and temperature. Acceptable ranges for these parameters included D.O. $\geq$6.0 mg/L, pH of 8.0 $\pm$ 0.5, salinity of 31.0°/$_{oo}$ $\pm$ 1.0, and temperature of 12.0°C $\pm$ 1.0°C.

After 72 hours, the test was terminated by carefully decanting the liquid portion of each jar into a clean container while taking care not to disturb the sediment. The water was gently mixed using a perforated plunger and a 10-mL aliquot of the liquid was removed from the jar, placed in a 20-mL test tube, and preserved with 1 mL of concentrated formalin. The samples were allowed to settle for approximately 3 days. After settling, 1-mL aliquots were removed from the bottom of a test tube, placed on a Sedgewick-Rafter counting cell, and scored for the presence of normal and abnormal embryos. Examples of normal and abnormal embryos are depicted in Figure 2.5 (Dinnel and Stober 1985). Aliquots were removed until the total number of embryos collected changed less than 10% between aliquots. Usually, the majority of embryos were captured and counted in three 1-mL aliquots. No fewer than three 1-mL aliquots were counted. The initial stocking density of containers was calculated in the same manner.



(a) Normal Development



(b) Abnormal Development

FIGURE 2.5. Examples of Normal Development (a) and Abnormal Development (b) in Sea Urchin Embryos (Dinnel and Stober 1985). (1) Fertilized Egg, (2) and (3) Early Cleavage, (4) Morula, (5) and (6) Blastula, (7) Gastrula, (8) Prism, and (9) Pluteus

Larval Test 2. During Test 2, one sediment treatment from Olympia Harbor, one reference sediment treatment, an amphipod control sediment treatment, a positive control (1.33 mg/L $CdCl_2$), and a seawater-only control were tested using procedures identical to those described above. Fertilization rate estimated in Test 2 was 88%. The estimated stocking density was 33 fertilized eggs per mL. The actual mean stocking density at Time 0 was determined to be 27 fertilized eggs per mL of test water (n = 5, sd = 2.7).

Sea Urchin Sperm Cell Test Protocols

The sea urchin sperm cell test was run concurrently with the first larval test following protocols described in Dinnel, Link, and Stober (1987). We used 10 mL of test water from each of the exposure jars from the sea urchin larval test (before developing embryos were added) as test media and used the same sperm and egg stocks from sea urchin larval Test 1. A portion of the egg stock was volumetrically diluted to a density of 2000 eggs/mL, and a portion of the sperm suspension was diluted to a density of 200,000 sperm/0.1 mL.

The test containers were 15-mL scintillation vials. A total of 0.2 mL of sperm stock (400,000 sperm) was added to each vial and exposed to the treatments at 12°C for 60 min. After sperm exposure, 1 mL of egg stock containing approximately 2000 eggs was added to each vial. Fertilization was allowed to occur for 20 minutes, after which the contents of each vial was preserved with 1 mL of concentrated formaldehyde.

Percentage of fertilization was assessed by scoring a subsample of approximately 100 eggs from each scintillation vial for the presence or absence of a fertilization membrane. Eggs with a partial fertilization membrane or exhibiting damage were counted as unfertilized.

2.6.4 Oyster Larval Test Protocols

The oyster larval bioassays were conducted prior to the sea urchin tests, following the procedures detailed in EPA's Puget Sound Protocols (PSEP 1986). These tests were performed three times and resulted in inadequate survival in control sediment treatments for each test. Procedures for Test 1 were similar to those employed for the sea urchin tests. During these tests, 15 g of (wet) sediment was added to each 1-L jar, the jars were filled to the

750-mL mark with filtered seawater, and the sediment was suspended through vigorous stirring. To minimize interference by natural toxins in the water, seawater used for these tests was collected from the Strait of Juan de Fuca, distant from the nearshore environment. As a further precaution, the seawater was filtered (0.45 μm) before use. The jars were then placed in randomly designated positions on a water table and the sediment was allowed to settle for 1 hour. While the sediment was settling, the oysters were spawned as described below.

Oyster larvae used for the biological testing were spawned by a technique commonly called "strip spawning." Using this technique, the oysters were opened by cutting the adductor muscles, and the gonad was sliced open with a clean scalpel. The contents of an individual's gonad were washed into a Pyrex baking dish using a squeeze bottle containing seawater. These gametes were examined under a compound microscope to determine sex and state of development. Normal-appearing, pear-shaped eggs, and viable sperm were saved and mixed in a 1500-mL Pyrex beaker containing 1200 mL of seawater. They were incubated for 1 hour at 20°C, aerated, and frequently mixed with a perforated plunger. After 1 hour, fertilization success and egg density in the stock was determined by volumetric dilution and microscopic examination via a Sedgewick-Rafter counting cell.

In Test 1, each test jar was inoculated with approximately 30,500 fertilized eggs, to provide a fertilized egg density of approximately 40/mL. Fertilization success was approximately 60%. The actual egg density at time 0 was 38 eggs/mL ($n = 5$, $sd = 3.8$). This egg density was determined by homogenizing a seawater control jar using a perforated plunger, removing a 1-mL aliquot, and diluting to a total volume of 100 mL with 5% formalin in seawater. Replicate counts were performed on 1-mL aliquots of this mixture to determine the fertilized egg density.

After 48 hours of exposure at 20°C, a 30-mL subsample was obtained from each test jar after thorough homogenization using a perforated plunger. The subsample was placed in a 50-mL centrifuge tube, and the contents were preserved with 10 mL of 10% formalin in seawater. After preservation, each sample was centrifuged for 10 min at 1750 rpm (740 x g) to consolidate the

larvae at the bottom of the centrifuge tube. Two mL of solution were pipetted from the bottom of each tube, transferred to a Sedgewick-Rafter counting cell in 1-mL aliquots, and examined at 100x magnification.

Samples were scored for the appearance of normal D-shaped larvae, abnormally developed larvae, blastula stage larvae, and total number of larvae (see Figure 2.6). The following descriptions explain the four categories used for quantification and data analysis

- Normal D-shaped larvae - Characterized by the presence of a straight or arched hinge, the larvae are relatively large and well developed.
- Normal, but slightly delayed D-shaped larvae - Although they have a straight hinge, they do not have a well-developed, smooth shell.
- Abnormally developed larvae - Round, oblong, or irregularly shaped with no evidence of straight hinge formation, or small, crenelated masses that are approximately 1/3 the size of a normal D-shaped larvae.
- Blastula stage - Spheres, approximately 1/2 to 2/3 the size of a normal D-shaped larvae.

After failure of the first test, efforts were made to improve chances for success. Conditioned oysters from Canada were used for the first test at a salinity of 28°/₀₀. For the second test, we used conditioned oysters from California, where bivalves might have been nearer natural spawning periods. Filtered seawater collected from the Strait of Juan de Fuca was used and adjusted to a salinity of 25°/₀₀, at which spawning success might be improved (Woelke 1972). For the third test, conditioned oysters from Washington State were tried along with seawater conditions as in the second test. In all three tests, inadequate survival in control treatments invalidated the test results. The results were probably due to the season of the year, as oysters typically do not spawn in November and December, even in a conditioned state.

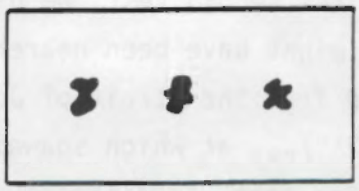
2.7 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

Quality assurance/quality control (QA/QC) procedures followed for these studies were consistent with PSDDA requirements (PSDDA 1988) and EPA

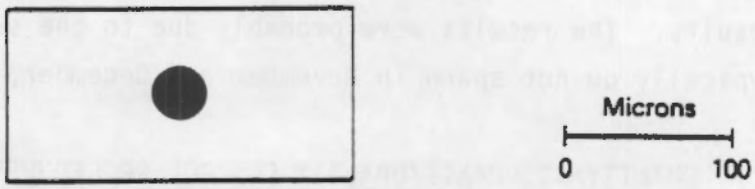
(a) Normal D Larvae

(b) Normal, But Slightly Delayed D Larvae

(c) Abnormal, Slightly Delayed Larvae



(d) Abnormal, Little or No Development



(e) Blastula Stage

FIGURE 2.6. Examples of Normal (a-b), Abnormal (c-d), and Blastula Stage (e) D-Shaped Larvae of the Pacific Oyster (Word et al. 1988)

protocols (PSEP 1986). The procedures followed were presented by Pacific Northwest Laboratory's (PNL) Quality Engineering Division as QA Plan, Number EES-20, Revision 1. A member of PNL's quality engineering staff was present throughout these studies to ensure that accepted procedures were followed. The PNL Laboratory Record Books (LRBs) were used for all phases of this project. These LRBs were assigned to each portion of the study and served as records of day-to-day activities during the research. All entries in the LRBs were signed, dated, and reviewed by both the project manager, Jack Q. Word, and the quality assurance engineer, Rob Cuello. The following discussion summarizes QA/QC procedures followed for this study.

2.7.1 Sediment Sampling, Storage, and Tracking

All sediments collected for these studies were stored in glass containers at 4°C and never frozen. Tracking forms were developed and unique labels attached to each sample.

2.7.2 Biological Testing: Care and Handling of Test Organisms

Test organisms were handled carefully during collection and transfer to test containers. Organisms shipped to MSL were gradually equilibrated to ambient conditions and kept in their native sediment whenever possible. No food was provided to organisms other than what was available in sediments or flowing seawater in the holding tanks and test containers. Organisms were transferred to test containers by pipetting, netting, or quantitative volumetric transfer. A short summary of collection and handling of each test species is included in Section 2.3.

2.7.3 Species Selection and Identification

Selection of species was consistent with the PSDDA requirements (PSDDA 1988) and included the use of juvenile forms, burrowing invertebrates, and a larval (planktonic) form. Representatives of all test organisms were taxonomically identified by qualified experts at MSL before use in bioassays.

2.7.4 Water Quality Checks

During all bioassay tests, water quality checks were performed to ensure that acceptable experimental conditions were maintained. These conditions included a stable temperature (±1.0°C), a minimum dissolved oxygen limit of

4.0 mg/L, a variation in pH between individual containers of no more than 0.5 units, a variation in salinity of no more than 1.0°/₀₀, and 14 hours of light per day. These limits and values are consistent with those outlined in the PSDDA and PSEP protocols (PSDDA 1988, PSEP 1986). Actual water quality data for each biological test are presented in Appendixes A through C. Water quality instruments were calibrated according to the manufacturer's specifications or accepted PNL protocols (see Section 2.5).

2.8 STATISTICAL DESIGN, DATA ANALYSIS, AND INTERPRETATION

2.8.1 Statistical Analysis

The purpose of statistical analyses was to determine the statistical significance of the response for each sediment treatment from the Puget Sound test samples compared with control and reference area sediments from West Beach, Point Whitney, and Sequim Bay. The biological test results were compared by analysis of variance (ANOVA) tests on the arcsine square root of the proportion surviving to the end of the test, or in the case of the sea urchin sperm cell test, the arcsine square root of the proportion of eggs fertilized. The transformation of arcsine square root was used to stabilize the within-class variances to meet the assumptions of the ANOVA. If ANOVA indicated a significant difference between samples ($\alpha = 0.05$), a multiple comparisons analysis was made using Tukey's Honestly Significant Difference (HSD) test for all possible comparisons (Steel and Torrie 1980). Tukey's HSD is a conservative multiple comparisons test which uses an experiment-wide error rate. Tukey's HSD provides more information about how each sediment treatment compares with every other one, as opposed to the more limited comparisons to a single control in Dunnet's test. The analysis results in a grouping of treatments (given alphabetic codes in the tables) that are not significantly different from each other. An individual treatment can be included in multiple groups and overlap can occur between groups.

Sediments from the locations where the animals were collected were used as control treatments. Thus, control station designation was specific to each biological test. For the juvenile bivalve (geoduck) test, Point Whitney was the control station. West Beach was the control station for the amphipod test. Because the sea urchins inhabit hard substrates, no appropriate

sediment control treatment was available for these tests. A seawater-only treatment served as a control for the sea urchin tests. Sequim Bay was a reference station for all biological tests. Most tests also included West Beach sediment (the amphipod control station) as a "uncontaminated" sediment which was incorporated into statistical analyses for comparison only, and not as a formal control or reference station.

2.8.2 Randomization

All biological testing was conducted using completely random designs. Organisms were randomly allocated to treatments, and treatments were randomly allocated to positions on the water tables. Separate random-number tables were generated for each of the biological tests for this purpose, using the discrete uniform random-number generator available in Lotus 1-2-3.®

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3.0 RESULTS

3.1 GEODUCK TESTS

Two tests involving geoducks were conducted: a protocol development test and an Olympia Harbor definitive test. The protocol development test was performed to determine the optimum sediment depth and grain size that would sustain juvenile geoducks for 10 days under flow-through conditions. Two sediment treatments at three sediment depths were tested. Sediment from the Washington State Department of Fisheries Shellfish Laboratory (Point Whitney) was used to hold juvenile geoducks prior to testing and served as the coarse-grained treatment during testing. Sediment from Sequim Bay served as the fine-grained treatment. The results of this test were used to develop the experimental design for the definitive test, which evaluated sediment treatments from Olympia Harbor, West Beach, and Sequim Bay. Data on mortality and water quality monitoring are presented in Appendix A.

3.1.1 Protocol Development Test

The results of the protocol development test are presented in Table 3.1. Survival of juvenile geoducks was slightly higher in Sequim Bay sediment than in Point Whitney sediment. Geoduck survival was also higher in the 1.5- and 2.0-cm sediment depths than in the 5.0-cm depth in each sediment type. It was noted during the experiment that all juvenile geoducks were able to burrow into the finer Sequim Bay material, while only about 40% of the bivalves were able to burrow into the coarser Point Whitney sediment. This may explain the slightly higher survival in the Sequim Bay sediment. Given these results, a sediment depth of 2.0 cm was selected for the definitive test. Further, with coarse sediments we decided to bury the bivalves by placing 1 cm of coarse sediment in the exposure jar, adding the geoducks, and then adding the remaining 1 cm to cover the animals. The animals were able to assume a normal vertical position when buried.

TABLE 3.1. Survival of Geoducks During the 10-Day Flow-Through Protocol Development Test

Sediment Treatment	Sediment Depth (cm)	Rep.	Alive	Dead	Total	Proportion Surviving
Point Whitney (PW)	1.5	1	8	1	9	0.89
Point Whitney (PW)	1.5	2	5	4	9	0.56
Point Whitney (PW)	2.0	1	7	2	9	0.78
Point Whitney (PW)	2.0	2	8	1	9	0.89
Point Whitney (PW)	5.0	1	7	2	9	0.78
Point Whitney (PW)	5.0	2	5	4	9	0.56
Total (all depths)	---	-	40	14	54	0.74
Total (1.5 cm depth)	---	-	13	5	18	0.72
Total (2.0 cm depth)	---	-	15	3	18	0.83
Total (5.0 cm depth)	---	-	12	5	18	0.67
Sequim Bay (SB)	1.5	1	8	1	9	0.89
Sequim Bay (SB)	1.5	2	8	1	9	0.89
Sequim Bay (SB)	2.0	1	7	2	9	0.78
Sequim Bay (SB)	2.0	2	8	1	9	0.89
Sequim Bay (SB)	5.0	1	5	4	9	0.56
Sequim Bay (SB)	5.0	2	8	1	9	0.89
Total (all depths)	---	-	44	10	54	0.81
Total (1.5 cm depth)	---	-	16	2	18	0.89
Total (2.0 cm depth)	---	-	15	3	18	0.83
Total (5.0 cm depth)	---	-	13	5	18	0.72

3.1.2 Definitive Test

The results of the definitive test are summarized in Table 3.2 and show that the mean proportion of juvenile geoducks surviving the 10-day exposure ranged from a low of 0.58 in the West Beach (amphipod control) sediment to a high of 0.85 in Olympia Harbor sediment treatment C-12. The mean proportion survival in Sequim Bay reference sediment decreased to 0.70 from the 0.81 observed in the same sediment during the protocol development test. Some of this difference in response may be due to holding periods for the reference sediments. Because the reference sediment for both tests was collected at one sampling time, the sediment holding time was 12 days longer for the second test than for the first test. A slight odor of hydrogen sulfide was detectable in the sediment used for the second test, and this compound may be responsible for decrease in survival in reference sediments.

Although control sediment treatments from Point Whitney were not included in the definitive test, the 2.0-cm-treatment results from the protocol development test were included as a control in statistical analyses. ANOVA of the transformed data showed no significant differences between the Olympia Harbor, control, reference, and West Beach sediment treatments (Tables 3.2 and 3.3, and Figure 3.1). Mean proportion survival in Sequim Bay reference treatments (2.0-cm depth) from the protocol development test was 0.81 and would not be statistically different from other treatments if it were included in this analysis. The relatively low survival in the Sequim Bay reference sediment treatment and Point Whitney control sediment biases statistical comparisons with Olympia Harbor sediment treatments and makes the definitive test suspect as a sensitive measure of the toxicity of the Olympia Harbor sediment treatments.

To supply organisms for these tests, we used all the remaining juvenile geoducks available at the State Shellfish Laboratory at Point Whitney. The bivalves were growing on an unmaintained, in-situ raft. Because this was the sole source and a limited supply of juvenile geoducks, we were not able to procure large quantities of these organisms. We feel that the sensitivity and success of this biological test will be improved with the use of an abundant stock of juvenile geoducks which have been reared under more controlled conditions.

TABLE 3.2. Summary and Statistical Grouping Information for the 10-Day Flow-Through Geoduck Bioassay (All sediment treatments were in same statistical group and thus were not significantly different.)

Sediment Treatment	Mean Proportion Surviving	SD
WB (Amphipod Control)	0.58	0.10
C-15	0.59	0.22
C-2	0.60	0.32
C-1	0.65	0.10
Sequim Bay Reference	0.70	0.23
C-Z	0.73	0.10
C-14	0.73	0.13
C-13	0.73	0.17
Point Whitney Control	0.83	0.08
C-12	0.85	0.13

TABLE 3.3. Balanced One-Way ANOVA Using the Arcsine Square Root Transformed Proportion of Geoducks Surviving a 10-Day Flow-Through Exposure

Source of Variation	Sum of Squares	d.f.	Mean Square	F-Ratio	Significance Level
Between Groups	0.4055	8	0.0507	1.1270	0.3771
Within Groups	1.2141	27	0.0450		
Total (Corrected)	1.6196				

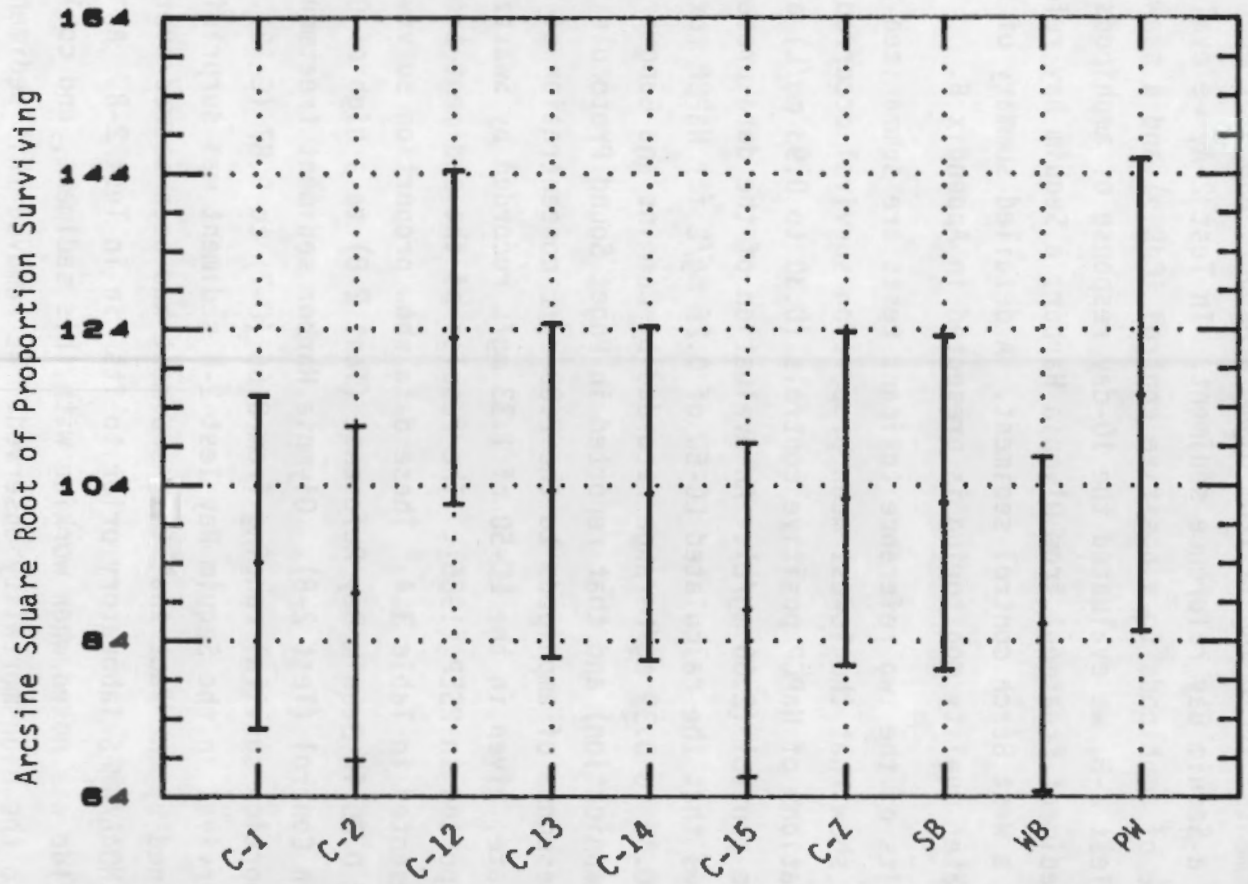

FIGURE 3.1. 95% Confidence Intervals for Arcsine Square Root Transformed Survival of Geoducks After 10-Day Flow-Through Exposure

3.2 AMPHIPOD TESTS

Four tests were conducted with amphipods. In Test 1-A, we evaluated the response of amphipods after a 4-day exposure to the reference toxicant NaPCP under six toxicant concentrations and seawater only. In Test 1-B, we evaluated the response of amphipods after a 10-day exposure to 14 solid-phase sediment treatments, including 12 from Olympia Harbor, one West Beach control sediment, and a Sequim Bay reference sediment. In Test 2-A, we evaluated the 4-day response of amphipods to a positive control ($CdCl_2$) and a seawater-only control. In Test 2-B, we evaluated the 10-day response of amphipods in one solid phase sediment treatment from Olympia Harbor, a Sequim Bay reference sediment, and a West Beach control sediment. A detailed summary of test results and water quality monitoring is presented in Appendix B.

The results of the two reference toxicant tests are summarized in Table 3.4 and show that the lowest mean proportion survival occurred in the high concentrations of NaPCP positive controls (0.30 to 0.93 mg/L) and in the $CdCl_2$ positive control (2.00 mg/L). An evaluation of the data presented in Table 3.4 shows that the calculated LC-50 of 0.26 mg/L for NaPCP toxicity is close to the 0.30 to 0.39 mg/L range recorded by Cummins and Gangmark (personal communication) and that reported in Puget Sound Protocols (PSEP 1986). The response of amphipods to the 2.00 mg/L concentration of $CdCl_2$ is also appropriate, given in the LC-50 of 1.33 mg/L recorded by Swartz et al. (1985) and reported in PSEP (1986). The results of the sediment treatment tests are presented in Table 3.4. These data show proportion survival ranged from a low of 0.00 in Sequim Bay Reference (Test 2-B) to a high of 0.98 in the West Beach Control (Test 2-B). Olympia Harbor sediment treatments displayed a proportion survival ranging from 0.66 (C-Z) to 0.87 (C-12). The low proportion survival in the Sequim Bay Test 2-B sediment was surprising, but may be explained by the fact that this sediment had been stored over two weeks at the NOAA/NMFS laboratory prior to its use in Test 2-B. An odor of hydrogen sulfide was noted when working with this sediment, and could have contributed to the high mortality observed. We removed this sediment treatment from the analysis before running the ANOVA on these data (discussed below).

TABLE 3.4. Summary Information for the Amphipod Reference Toxicant Tests

Sediment Treatment	Test	Mean Proportion Surviving	SD
NaPCP (0.64 mg/L)	1-A	0.00	0.00
NaPCP (0.93 mg/L)	1-A	0.00	0.00
NaPCP (0.442 mg/L)	1-A	0.00	0.00
NaPCP (0.30 mg/L)	1-A	0.20	0.00
NaPCP (0.145 mg/L)	1-A	0.90	0.10
NaPCP (0.21 mg/L)	1-A	1.00	0.00
Seawater Only	1-A	1.00	0.00
$CdCl_2$ (2.00 mg/L)	2-A	0.01	0.01
Seawater Only	2-A	0.91	0.04

Calculated LC-50 for NaPCP: 0.26 mg/L

The results of the tests were analyzed by ANOVA on the transformed data. Statistically significant differences were found between the sediment treatments (Table 3.5). Since there were significant differences, we applied Tukey's HSD Test and developed statistical groupings for sediment treatments.

The statistical groupings listed in Table 3.6, analyzed in Table 3.7, and shown in Figure 3.2 reveal that none of the Olympia Harbor sediment treatments were significantly different from the Sequim Bay reference treatment. In comparison to the West Beach control treatments, a total of four Olympia Harbor sediment treatments showed a significantly reduced survival (C-Z, C-20, C-5, and C-17).

TABLE 3.5. Balanced One-Way ANOVA Using the Arcsine Square Root Transformed Proportion of Amphipods Surviving the 4-Day and 10-Day Static Exposures

Source of Variation	Sum of Squares	d.f.	Mean Square	F-Ratio	Significance Level
Between Groups	25.901	25	1.036	49.421	<0.05
Within Groups	1.866	89	0.021		
Total (Corrected)	27.767	114			

TABLE 3.6. Summary and Statistical Grouping Information for the 10-Day Amphipod Biological Tests (Positive Control, Seawater-Only, and Sequim Bay Test 2-B Reference Removed)

Sediment Treatment	Test	Mean Proportion Surviving	SD	Statistical Group(a)
Sequim Bay Reference	2-B	0.00	0.00	(b)
C-Z	1-B	0.66	0.11	A
C-20	1-B	0.70	0.24	A
C-5	1-B	0.72	0.12	A
C-17	2-B	0.75	0.14	A
C-2	1-B	0.79	0.12	AB
C-19	1-B	0.80	0.07	AB
C-1	1-B	0.80	0.08	AB
C-6	1-B	0.81	0.11	AB
C-7	1-B	0.83	0.06	AB
C-14	1-B	0.83	0.06	AB
C-13	1-B	0.80	0.21	AB
C-15	1-B	0.87	0.08	AB
C-12	1-B	0.87	0.09	AB
Sequim Bay Reference	1-B	0.92	0.04	AB
West Beach Control	1-B	0.97	0.03	B
West Beach Control	2-B	0.98	0.03	B

(a) Sediment treatments with the same statistical group are not statistically different from each other.
(b) Not included in analysis.

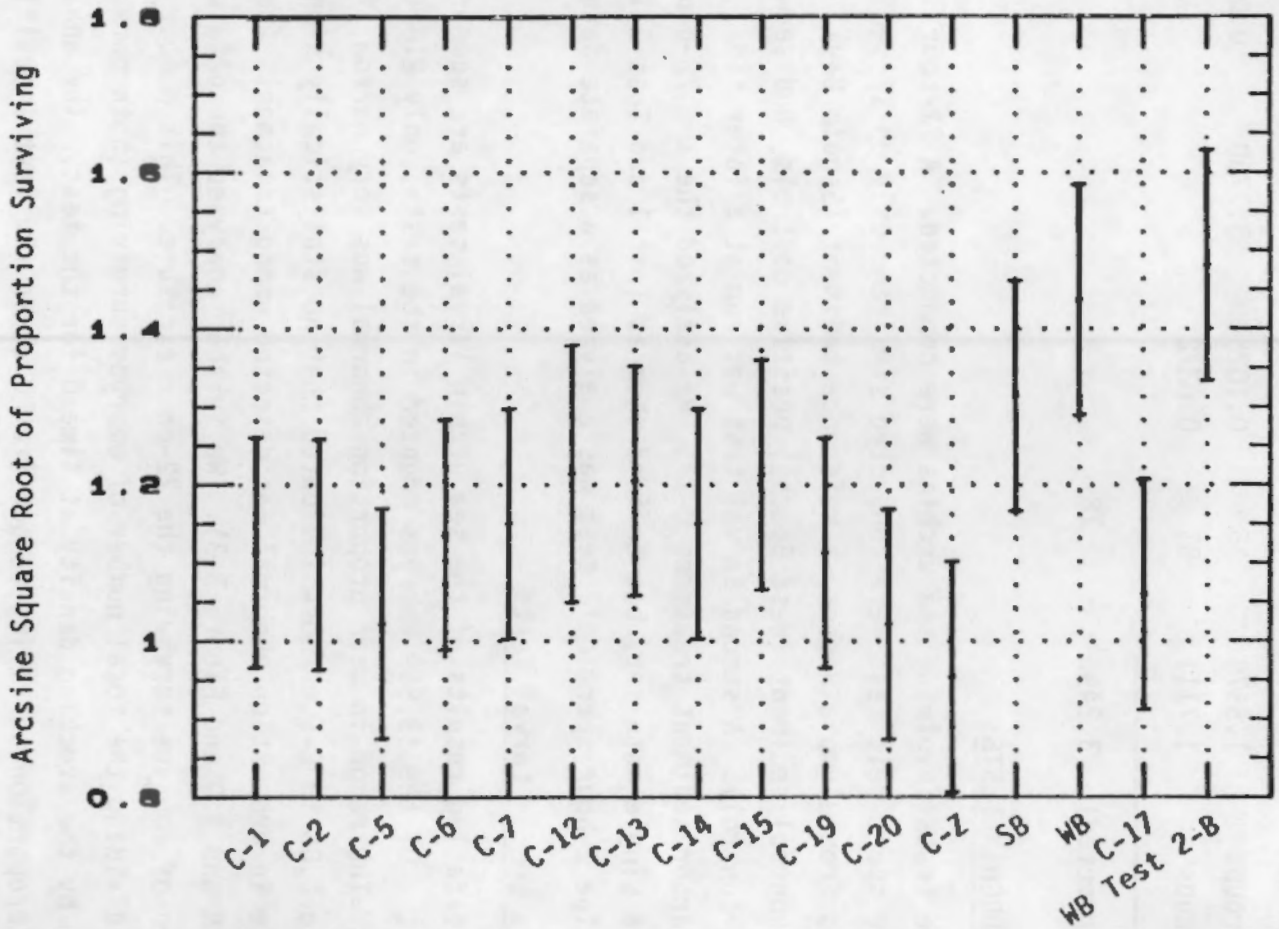

FIGURE 3.2. 95% Confidence Intervals for the Arcsine Square Root Transformed Survival of Amphipods After 10-Day Static Exposures (Positive Control, Seawater only, and Sequim Bay Test 2-B Reference Removed)

TABLE 3.7. Balanced One-Way ANOVA Using the Arcsine Square Root Transformed Proportion of Amphipods Surviving the 10-Day Static Exposures

Source of Variation	Sum of Squares	d.f.	Mean Square	F Ratio	Significance Level
Between Groups	1.5378	15	0.1025	3.7750	0.0001
Within Groups	1.7111	63	0.0272		
Total (Corrected)	3.2490	78			

3.3 SEA URCHIN TESTS

Three tests involving sea urchins were conducted. A 72-hour larval test and 1-hour sperm cell test were conducted simultaneously on 12 sediment treatments from Olympia Harbor, a reference sediment (Sequim Bay), the amphipod control sediment (West Beach), positive controls, and seawater only (negative control). A second larval test was run at a later time, evaluating Olympia Harbor sediment treatment C-17. We analyzed the two 72-hour larval tests as a single data set, but defined them as Test 1 and Test 2 in summary tables. The 1-hour sperm cell test was analyzed as a separate data set.

3.3.1 Sea Urchin Larval Tests

The detailed results of the sea urchin larval tests are summarized in Appendix C. Of the 13,569 embryos counted in the tests, only 218 (1.6%) were abnormal. The range in mean proportion abnormal was very narrow, from 0.00 in C-14 to 0.05 in C-5. ANOVA indicated that no statistically significant difference in proportion abnormal was detected among treatments (see Tables 3.8 and 3.9 and Figure 3.3). We further analyzed the data using the proportion of embryos surviving the 72-hour exposure. This number is calculated by dividing the total number of embryos surviving (both normal and abnormal) by the stocking density at Time 0 for the test. The analysis shows that the proportion surviving ranged from 0.00 in the Test 1 positive control (1.33 mg/L $CdCl_2$) to more than 0.86 in seawater-only, West Beach sediment, and positive controls of 0.27 mg/L $CdCl_2$. One unusual result from Test 2 was the high proportion survival in the 1.33 mg/L $CdCl_2$ positive control. One possible explanation is that the toxicity was associated with reduced pH and

TABLE 3.8. Summary and Statistical Group Information for Abnormality in the Sea Urchin Larval Test (All sediment treatments were in the same statistical group and thus not significantly different from each other.)

Sediment Treatment	Test	Mean Proportion Abnormal	SD
C-14	1	0.00	0.08
C-2	1	0.01	0.01
WB (Amphipod Control)	1	0.00	0.01
C-1	1	0.01	0.02
C-15	1	0.01	0.08
Sequim Bay Reference	2	0.01	0.01
C-20	1	0.01	0.01
Sequim Bay Reference	1	0.01	0.09
C-6	1	0.01	0.00
C-19	1	0.03	0.06
C-7	1	0.01	0.02
C-Z	1	0.02	0.03
C-13	1	0.02	0.02
Seawater only	1	0.03	0.02
C-12	1	0.03	0.04
C-5	1	0.05	0.07

TABLE 3.9. Balanced One-Way ANOVA Using the Arcsine Square Root Transformed Proportion of Abnormal Sea Urchin Embryos After 72-Hour Exposure

Source of Variation	Sum of Squares	d.f.	Mean Square	F-Ratio	Significance Level
Between Groups	0.1871	15	0.0125	1.4330	0.1598
Within Groups	0.5572	64	0.0087		
Total (Corrected)	0.7443	79			

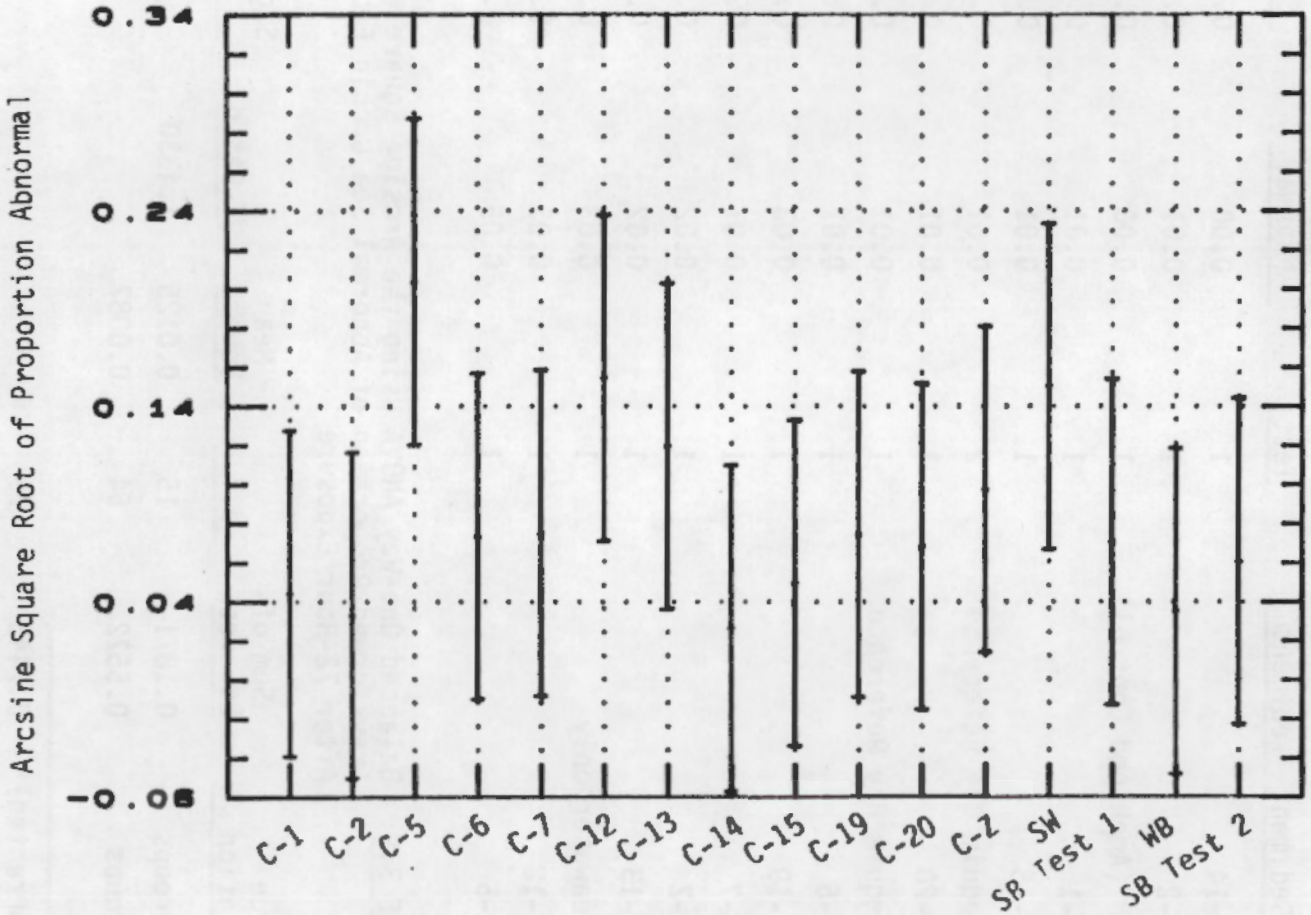

FIGURE 3.3. 95% Confidence Intervals for the Arcsine Square Root Transformed Abnormality of Sea Urchin Embryos After 72-Hour Exposure

not the cadmium concentration. The cadmium solution used for positive controls had a low pH. For Test 1, the sperm were added soon after dosing with cadmium. During Test 2, the containers equilibrated slightly longer after spiking, which may have allowed for better buffering of the solution by the seawater. It is unlikely that containers from Test 2 were not dosed because a reduced pH from the spike addition is clear from the water quality measurements (Appendix C).

Statistical comparison by ANOVA and Tukey's HSD (see Tables 3.10 and 3.11 and Figure 3.4) shows that there are statistically significant differences among the treatments. Tukey's groupings of these data indicate that all of the Olympia Harbor and Sequim Bay sediment treatments had significantly reduced survival as compared to seawater-only controls. None of the Olympia Harbor sediment treatments were significantly different from Sequim Bay reference sediment treatments. In comparison to the West Beach amphipod control (Test 1, 70% survival), reduced survival was noted in seven Olympia Harbor sediment treatments (C-7, C-17, C-13, C-20, C-12, C-15, and C-19). All Olympia Harbor and Sequim Bay sediment treatments had significantly reduced survival as compared to the West Beach amphipod control from Test 2 (86% survival).

3.3.2 Sea Urchin Sperm Cell Test

The results of the sea urchin sperm cell test are summarized in Table 3.12 and Appendix C. The mean proportion of fertilized eggs ranged from a low of 0.85 to a high of 0.97, with all Olympia Harbor sediment treatments displaying a mean proportion of fertilized eggs of 0.93 or greater. ANOVA results indicate that there are significant differences among treatments (Table 3.13 and Figure 3.5), but Tukey's statistical groupings show that only the high concentration of positive control was in a different statistical group from the other treatments. Thus, there are no significant differences among the Olympia Harbor sediment treatments, seawater-only controls, and Sequim Bay reference sediment treatments. All Olympia Harbor sediments tested show no effect on the success of sea urchin fertilization.

TABLE 3.10. Summary and Statistical Grouping Information for the 72-Hour Sea Urchin Larval Test

Sediment Treatment	Test	Mean Proportion Surviving	SD	Statistical Group(a)
$CdCl_2$ (1.33 mg/L)	1	0.00	0.00	A
Sequim Bay Reference	2	0.19	0.54	B
C-19	1	0.19	0.03	B
C-15	1	0.23	0.05	B
C-12	1	0.24	0.10	B
C-20	1	0.29	0.10	B
C-13	1	0.31	0.16	B
Sequim Bay Reference	1	0.31	0.16	B
C-17	2	0.31	0.08	B
C-7	1	0.34	0.20	B
C-14	1	0.35	0.10	BC
C-2	1	0.35	0.05	BC
C-5	1	0.37	0.16	BC
C-1	1	0.39	0.25	BC
C-Z	1	0.39	0.09	BC
C-6	1	0.46	0.11	BC
WB (Amphipod Control)	1	0.70	0.10	CD
Seawater only	2	0.86	0.08	DE
$CdCl_2$ (0.27 mg/L)	1	0.87	0.09	DE
WB (Amphipod Control)	2	0.86	0.14	DE
Seawater only	1	0.90	0.08	DE
$CdCl_2$ (1.33 mg/L)	2	0.96	0.09	E

(a) Sediment treatments within the same statistical group are not statistically different from each other.

TABLE 3.11. Balanced One-Way ANOVA Using the Arcsine Square Root Transformed Proportion of Sea Urchin Embryos Surviving a 72-Hour Static Exposure

Source of Variation	Sum of Squares	d.f.	Mean Square	F-Ratio	Significance Level
Between Groups	12.3851	21	0.5898	22.2310	<0.05
Within Groups	2.0102	86	0.0234		
Total (Corrected)	14.3953	107			

3.4 OYSTER TESTS

Three larval development tests involving oysters were conducted during this project. All three tests failed to produce adequate survival in reference sediment treatments and were therefore invalidated. The Pacific oyster is typically a late spring to early summer spawner under natural conditions. The spawning season can be extended under laboratory conditions, however, by artificial conditioning of the oysters to warmer temperatures. The oysters used for these tests were the first batch of artificially conditioned individuals available in the year. During the strip spawning procedures, gonadal development was poor. The females used for the tests displayed smaller gonads containing predominantly rounded eggs. Ripe females usually have large, milky gonadal material and produce pear-shaped eggs. Males did produce motile sperm but gonadal development did not appear good. We were able to find a few females with a majority of pear-shaped eggs and these eggs were used for the tests. We discovered that a large number of the pear-shaped eggs were still present after mixing of eggs and sperm. This is an indication that fertilization success was low. Oyster eggs usually round up after fertilization. During the test periods, fertilized eggs began development but most died between 24 hours and 48 hours.

The results exceed PSDDA guidelines for control mortality (see Discussion section), which invalidates the tests. Thus, the data cannot be used to evaluate Olympia Harbor sediments.

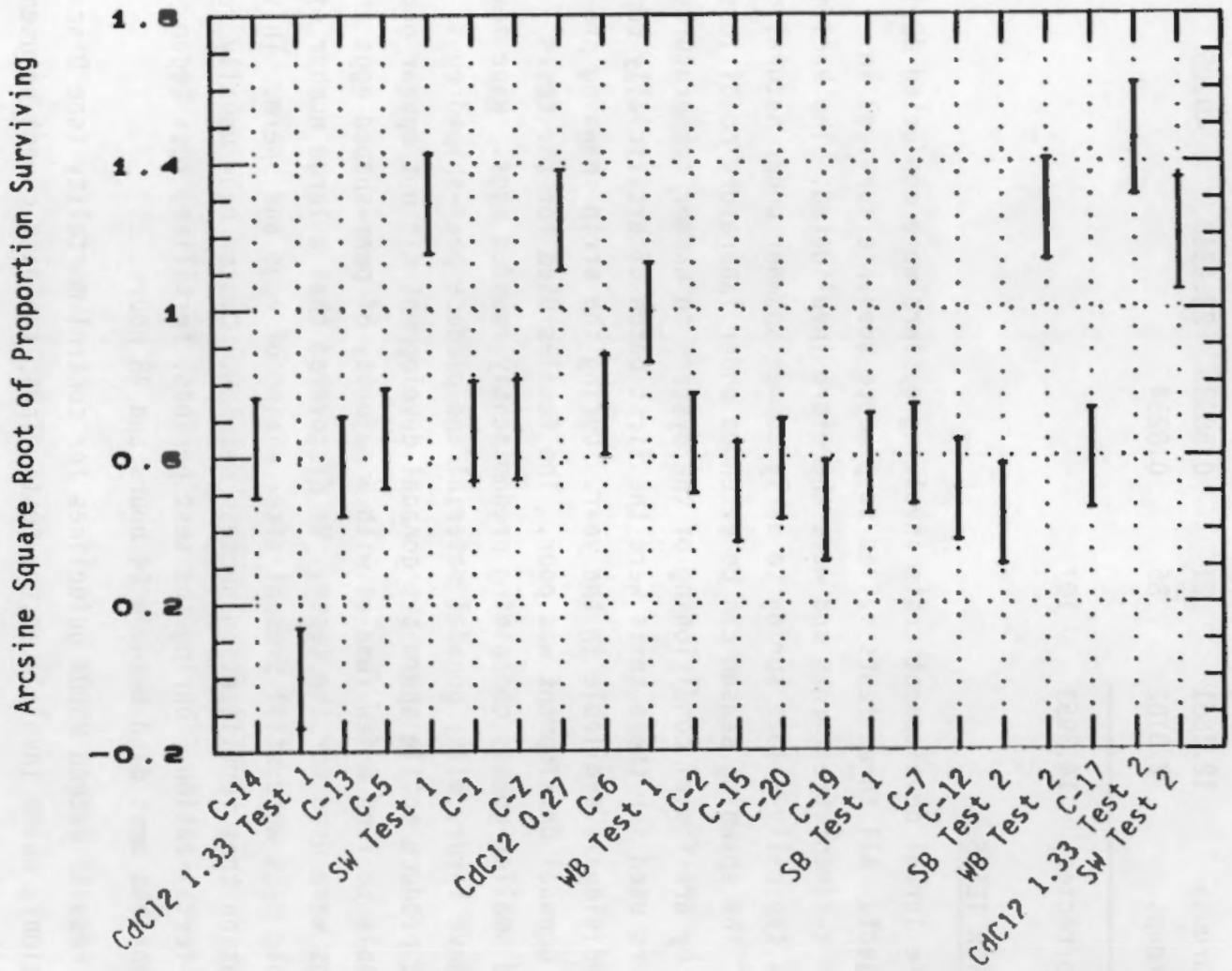

FIGURE 3.4. 95% Confidence Intervals for Arcsine Square Root Transformed Survival of Sea Urchin Embryos After 72-Hour Static Exposure

TABLE 3.12. Summary and Statistical Grouping Information for the Sea Urchin Sperm Cell Test

Sediment Treatment	Mean Proportion Fertilized	SD	Statistical Group(a)
$CdCl_2$ (1.33 mg/L)	0.85	0.09	A
C-5	0.93	0.04	AB
Sequim Bay Reference	0.94	0.02	AB
C-7	0.94	0.02	AB
C-12	0.94	0.02	AB
C-19	0.94	0.04	AB
C-Z	0.94	0.04	AB
C-15	0.95	0.05	B
C-13	0.96	0.02	B
C-2	0.96	0.02	B
C-1	0.96	0.03	B
C-20	0.96	0.04	B
C-14	0.96	0.03	B
$CdCl_2$ (0.27 mg/L)	0.97	0.01	B
WB (Amphipod Control)	0.97	0.01	B
Seawater only	0.97	0.02	B
C-6	0.97	0.02	B

(a) Sediment treatments with the same statistical group are not statistically different from each other.

TABLE 3.13. Balanced One-Way ANOVA Using the Arcsine Square Root Transformed Proportion of Sea Urchin Eggs Fertilized After Sperm Exposure to Treatments

Source of Variation	Sum of Squares	d.f.	Mean Square	F Ratio	Significance Level
Between Groups	0.2317	16	0.0145	2.5700	0.0036
Within Groups	0.3831	68	0.0056		
Total (Corrected)	0.6148	84			

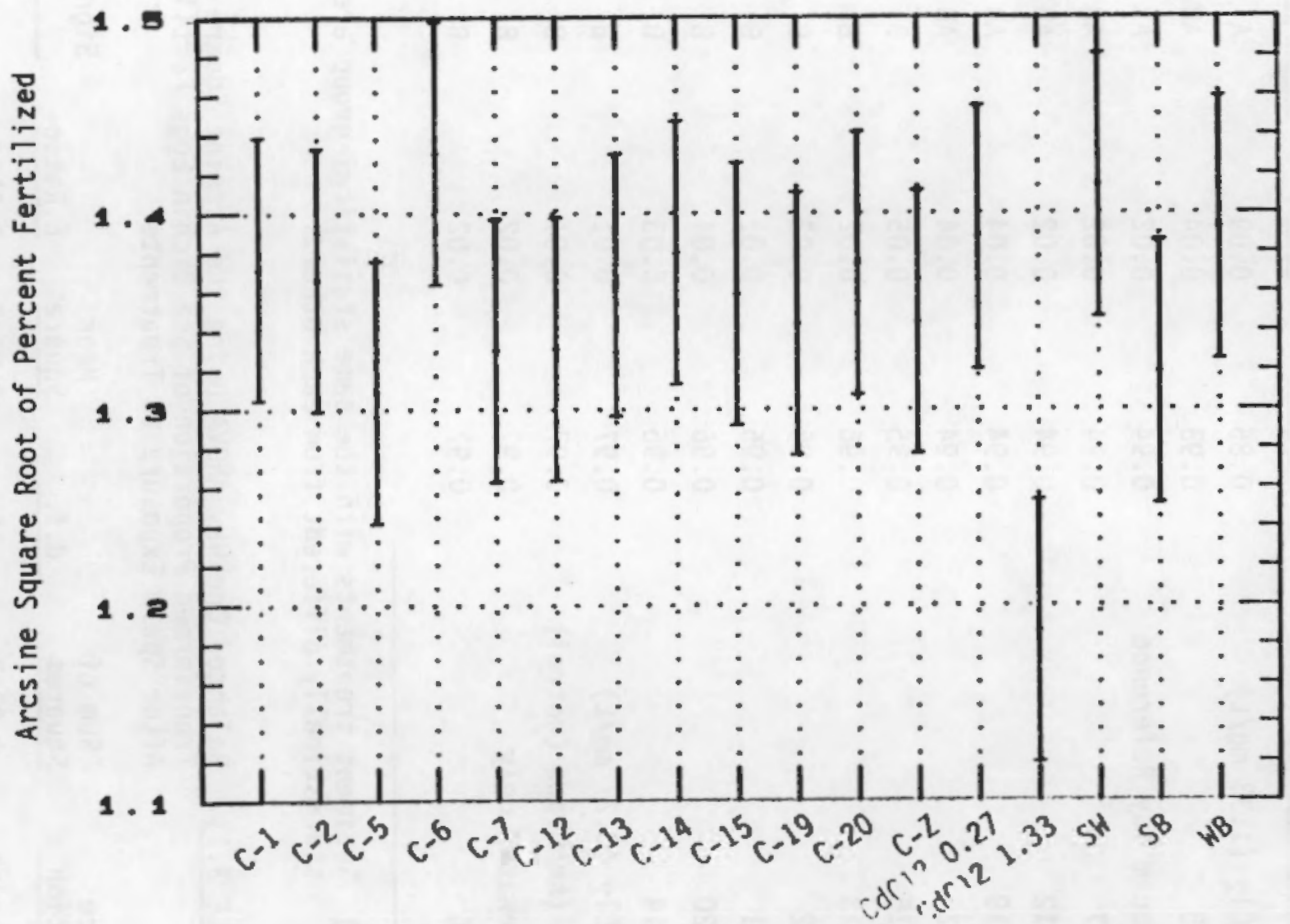

FIGURE 3.5. 95% Confidence Intervals for Arcsine Square Root Transformed Percent Fertilization After Sea Urchin Sperm Exposure to Treatments

4.0 DISCUSSION

4.1 OVERVIEW

The experimental design for this program satisfied PSDDA characterization criteria (PSDDA 1988) for the evaluation of sediments proposed for dredging. All tests except the bivalve larvae tests satisfied internal QA/QC requirements for test conduct, water quality parameters, and survival in reference and control sediments. Inadequate survival in the controls invalidated the bivalve larvae tests. The juvenile geoduck test still needs refinement. QA/QC criteria have not been established for background mortality in reference and control sediments or for the geoduck's response to reference toxicants. Relatively poor survival in reference sediment treatments may be attributable to hydrogen sulfide buildup in sediments over the storage period, and this makes interpretations of this test suspect. Summary observations of each test follow. Sediment quality evaluations are presented in Section 4.2.

4.1.1 Geoduck Tests

The results of the geoduck tests indicate that more methods development is in order. What is required is clearly outside the scope of Battelle's contract. Additional work should include investigations on the ability of juvenile geoducks to establish themselves in test sediments, better techniques of removing the juveniles from their native holding sediment, the appropriate duration for an acute flow-through test, and the effect of fine-grain sediment upon the health of the animals. Additional experimental design modifications might include using a container large enough to accommodate 20 organisms and the use of younger individuals (<5 mm). It is also possible that unless the organisms are securely positioned in the sediment, they will become subject to adductor muscle fatigue and gape, and thus are more likely to perish during a 10-day test. This phenomenon has been observed in another bivalve, the razor clam, *Siliqua patula* (Dr. R. E. Elston, personal communication).

4.1.2 Amphipod Tests

The tests involving amphipods were successful and included survival of 90% or greater in 4-day tests (seawater only) and 10-day tests (reference and control sediments), and a range of response in test sediments. The response of the amphipods to the reference toxicant NaPCP produced a calculated 96-hour LC-50 of 0.27 mg/L. This is very close to reported ranges of 0.30 to 0.39 mg/L reported in Puget Sound Protocols (PSEP 1986). The 2.0 mg/L concentration of the reference toxicant produced a 0.01 mean proportion survival in the second test, a response consistent with the reported LC-50 of 1.61 mg/L for this toxicant. One unusual occurrence was the high mortality in the Sequim Bay reference sediment during Test 2. This contrasts sharply in comparison to the high survival recorded in Sequim Bay reference during Test 1. All parameters concerning this sediment (compositing, storage container, test conduct) were the same except the holding time. The sediment used for the second test was stored approximately 2 weeks longer than for the first test. When this material was removed from the 4°C coldroom and used in the second biological test, an odor of hydrogen sulfide was noticed. This is typical when sediments high in organic carbon are stored for any length of time. Whether the extended storage period influenced the biological testing is not known.

4.1.3 Sea Urchin Tests

The tests involving sea urchins were initiated after three invalid tests with the Pacific oyster. The 72-hour development test served as a substitute for the larval oyster test. One unexpected result in the sea urchin larval tests was the difference between the two tests in survival at the high concentration of cadmium. It is possible that temporary fluctuations in pH shortly after cadmium spiking were responsible for these differences in survival.

Subsequent to the June, 1988, PSDDA Report, an interpretative clarification was released for the bivalve larvae test. Under this clarification, both mortality and abnormality are computed in accordance with ASTM and PSEP protocols, and are then applied to the PSDDA guidelines. Control results are unacceptable if mortality is >39% or if abnormality is >10%. Reference sediment results are unacceptable if mortality is >20% (absolute) over control

mortality, or if abnormality is >20% (absolute) over control abnormality. If we apply these criteria to the sea urchin larval test, we find that the seawater-only control and the coarse-grained West Beach reference sediment met the criteria and thus validated the tests. The fine-grained Sequim Bay reference sediment was not acceptable since the mortality in that treatment was greater than 20% over control, probably caused by a grain-size effect (discussed below).

The low proportion of survival in the Sequim Bay reference sediment for Tests 1 and 2 suggests that there may be a grain-size-related impact associated with the sea urchin larval test because of variable water column suspension times following agitation. The mean proportions surviving in Sequim Bay reference sediment were 0.31 in Test 1 and 0.19 in Test 2. Mean proportion survival in Olympia Harbor sediment treatments ranged from 0.19 to 0.46. Most of the sediment treatments from Olympia Harbor appeared similar in grain size to the Sequim Bay reference, in that they were composed primarily of silt and clay with some shell material. These sediment treatments remained in suspension for hours or days after agitation. Survival in West Beach sediment treatments was 0.70 and 0.86 in Tests 1 and 2, respectively. The West Beach amphipod control sediment was the most coarse of the sediments used in this test and was composed primarily of fine to medium sand, which settled out within minutes after suspension.

Given these observations, the toxicity of Olympia Harbor sediment treatments relative to reference sediment and seawater-only controls must be interpreted with caution. It is possible that chemical toxicity may be masked by a grain-size effect. Several authors have noted that with echinoderm biological testing it is necessary to exclude the effects of grain size and to evaluate toxicity solely related to chemical content of sediment samples (Ross, Dinnel, and Stober 1984; Dinnel and Stober 1985). An improvement of the technique might be to expose the embryos to elutriate produced by centrifugation after sediment suspension, as described in Word et al. (1988).

The sea urchin sperm cell test was not sensitive enough to produce a statistical difference between the treatment, control, reference, and amphipod control sediments.

4.2 SEDIMENT QUALITY EVALUATION

Disposal guidelines have been established by PSDDA to evaluate sediments proposed for unconfined open-water disposal. These guidelines are summarized in Figure II.2-4 of "Evaluation Procedures Technical Appendix Phase 1 (Central Puget Sound)," PSDDA, June, 1988. Applicable guidelines are summarized below:

Guideline 1

Is toxicity in amphipod, juvenile bivalve (geoduck), or sea urchin larval test >30% over, and statistically different from reference?

If Yes, material is unsuitable for unconfined open-water disposal.
If No, see Guideline 2.

Guideline 2

Are there any two amphipod, juvenile bivalve (geoduck), sea urchin larval, and Microtox tests that are both "statistically significant" from reference? This requires both a statistical difference from reference and total mortality response that is greater than 20 percent over control.

If Yes, material is unsuitable for unconfined open-water disposal.
If No, see Guideline 3.

A summary of the results of all biological tests performed for this study by the Battelle MSL is presented in Table 4.1. This table shows that none of the sediment treatments were significantly different from the reference treatment for the geoduck, amphipods, sea urchin development, and sea urchin sperm cell tests. A total of four treatments (C-5, C-17, C-20, and C-Z) were significantly different from the control sediment in the amphipod test, and all sediment treatments were significantly different than control in the sea urchin development test.

For the purpose of determining whether the Olympia Harbor sediment treatments are acceptable for unconfined open-water disposal, comparisons are made between sediment treatments and reference sediment only. None of the tests showed a toxic effect which had >30% mortality and which was also statistically different from the reference. Therefore, under Guideline 1, the

TABLE 4.1. Summary of Biological Tests Conducted on Olympia Harbor Sediments. Values are Response in Comparison to Specific Reference and Control Sites with Statistical Significance (NS = no significant difference, SD = significantly different)

Sediment	Geoduck (a)				Amphipod (b)				Sea Urchin Development (c)								Sea Urchin Sperm Cell (d)			
									Mortality				Abnormality							
Treatment	Reference		Control		Reference		Control		Reference		Control		Reference		Control		Reference		Control	
C-1	5	NS	18	NS	12	NS	18	NS	-8	NS	51	SD	0	NS	2	NS	-2	NS	1	NS
C-2	10	NS	23	NS	13	NS	19	NS	-4	NS	55	SD	0	NS	2	NS	-2	NS	1	NS
C-5	Not Tested		Not Tested		20	NS	26	SD	-6	NS	53	SD	-4	NS	-2	NS	1	NS	4	NS
C-6	Not Tested		Not Tested		11	NS	17	NS	-15	NS	44	SD	0	NS	2	NS	-3	NS	0	NS
C-7	Not Tested		Not Tested		9	NS	15	NS	-3	NS	58	SD	0	NS	2	NS	0	NS	3	NS
C-12	-15	NS	-2	NS	5	NS	11	NS	7	NS	68	SD	-2	NS	0	NS	0	NS	3	NS
C-13	-3	NS	10	NS	12	NS	18	NS	0	NS	59	SD	-1	NS	1	NS	-2	NS	1	NS
C-14	-3	NS	10	NS	9	NS	15	NS	-4	NS	55	SD	1	NS	3	NS	-2	NS	1	NS
C-15	11	NS	24	NS	5	NS	11	NS	8	NS	67	SD	0	NS	2	NS	-1	NS	2	NS
C-17	Not Tested		Not Tested		17	NS	23	SD	0	NS	59	SD	Not Tested		Not Tested		Not Tested		Not Tested	
C-19	Not Tested		Not Tested		12	NS	18	NS	12	NS	71	SD	-2	NS	0	NS	0	NS	3	NS
C-20	Not Tested		Not Tested		12	NS	28	SD	2	NS	61	SD	0	NS	-2	NS	-2	NS	1	NS
C-Z	-3	NS	10	NS	26	NS	32	SD	-8	NS	51	SD	-1	NS	1	NS	0	NS	3	NS

(a) 10-day test; mortality and statistical significance compared to Sequim Bay reference and Point Whitney control.
(b) 10-day test; mortality and statistical significance compared to Sequim Bay reference and West Beach Test 2-B control.
(c) 72-hour test; mortality and statistical significance compared to Sequim Bay reference and seawater-only control.
(d) 1-hour test; mortality and statistical significance compared to Sequim Bay reference and seawater-only control.

Olympia Harbor sediments are suitable for unconfined open-water disposal. Two sediment treatments in the amphipod tests produced mortalities which were >20% over the reference (C-5 and C-Z), but neither were significantly different from the reference. Therefore, under Guideline 2, no sediment treatments displayed both statistical difference from the reference and a mortality >20% over reference in two types of toxicity tests. Based upon these sediment toxicity tests, all of the Olympia Harbor sediments are suitable for unconfined open-water disposal using Guidelines 1 and 2.

5.0 REFERENCES

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APPENDIX A

GEODUCK TEST DATA

TABLE A.1. Geoduck (*P. generosa*) Protocol Development Test

Sediment Depth	Sediment Treatment	Rep	Alive	Dead	Total
1.5	Point Whitney Control	1	8	1	9
1.5	Point Whitney Control	2	5	4	9
2.0	Point Whitney Control	1	7	2	9
2.0	Point Whitney Control	2	8	1	9
5.0	Point Whitney Control	1	7	2	9
5.0	Point Whitney Control	2	5	4	9
1.5	Sequim Bay Reference	1	8	1	9
1.5	Sequim Bay Reference	2	8	1	9
2.0	Sequim Bay Reference	1	7	2	9
2.0	Sequim Bay Reference	2	8	1	9
5.0	Sequim Bay Reference	1	5	4	9
5.0	Sequim Bay Reference	2	8	1	9

TABLE A.2. Geoduck (*P. generosa*) Definitive Test

Sediment Treatment	Rep	Alive	Dead	Total
C-1	1	6	4	10
C-1	2	8	2	10
C-1	3	6	4	10
C-1	4	6	4	10
C-2	1	2	8	10
C-2	2	5	5	10
C-2	3	9	1	10
C-2	4	8	2	10
C-12	1	9	1	10
C-12	2	7	3	10
C-12	3	10	0	10
C-12	4	8	2	10
C-13	1	7	3	10
C-13	2	5	5	10
C-13	3	8	2	10
C-13	4	9	1	10
C-14	1	9	1	10
C-14	2	7	3	10
C-14	3	7	3	10
C-14	4	6	4	10
C-15	1	8	2	10
C-15	2	5	4	9
C-15	3	3	7	10
C-15	4	7	3	10
C-Z	1	8	2	10
C-Z	2	8	2	10
C-Z	3	6	4	10
C-Z	4	7	3	10
Sequim Bay Reference	1	5	5	10
Sequim Bay Reference	2	9	1	10
Sequim Bay Reference	3	9	1	10
Sequim Bay Reference	4	5	5	10
For comparison only:				
WB Amphipod Control	1	5	5	10
WB Amphipod Control	2	7	3	10
WB Amphipod Control	3	6	4	10
WB Amphipod Control	4	5	5	10

TABLE A.3. Geoduck (*P. generosa*) Definitive Test - Water Quality Monitoring

		Temperature (°C)		Dissolved Oxygen (mg/L)		pH		Salinity (°/oo)		Flow Rate (mL/min)	
Sediment Treatment	Rep	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
C-1	1	15.0	16.0	7.5	8.1	7.72	8.07	31.0	32.0	36	44
C-1	2	14.8	15.7	7.6	8.1	7.71	8.07	31.0	32.0	35	44
C-1	3	14.8	15.7	7.3	8.1	7.70	8.06	31.0	32.0	36	44
C-1	4	14.7	15.6	7.5	7.9	7.72	8.07	31.0	32.0	35	45
C-2	1	14.8	15.7	7.5	8.2	7.70	8.07	31.0	32.0	35	44
C-2	2	14.8	15.7	7.5	7.9	7.72	8.06	31.0	32.0	36	45
C-2	3	14.8	15.7	7.5	8.1	7.70	8.07	31.0	32.0	36	44
C-2	4	14.7	15.6	7.5	7.9	7.72	8.07	31.0	32.0	36	45
C-12	1	14.9	15.8	7.4	8.1	7.71	8.05	31.0	32.0	36	44
C-12	2	14.8	15.8	7.4	8.0	7.70	8.05	31.0	32.0	36	44
C-12	3	14.8	15.7	7.5	8.1	7.71	8.07	31.0	32.0	36	45
C-12	4	14.9	15.6	7.5	8.0	7.71	8.06	31.0	32.0	36	44
C-13	1	14.6	15.8	7.5	8.1	7.70	8.09	31.0	32.0	36	45
C-13	2	14.7	15.6	7.5	8.0	7.70	8.06	31.0	32.0	35	44
C-13	3	14.8	15.7	7.4	8.0	7.71	8.07	31.0	32.0	36	44
C-13	4	14.8	15.6	7.2	8.0	7.71	8.06	31.0	32.0	35	45
C-14	1	14.8	15.8	7.4	8.0	7.70	8.06	31.0	32.0	36	45
C-14	2	14.9	15.8	7.4	8.1	7.70	8.07	31.0	32.0	35	45
C-14	3	14.8	15.7	7.5	8.0	7.71	8.07	31.0	32.0	36	44
C-14	4	14.8	15.7	7.4	8.2	7.71	8.05	31.0	32.0	37	42
C-15	1	15.1	16.1	7.4	7.9	7.72	8.07	31.0	32.0	35	42
C-15	2	14.8	15.7	7.4	8.0	7.71	8.06	31.0	32.0	36	44
C-15	3	14.9	15.9	7.4	8.1	7.72	8.07	31.0	32.0	35	45
C-15	4	14.9	16.0	7.4	8.1	7.72	8.07	31.0	32.0	35	44
C-Z	1	14.9	15.7	7.4	7.9	7.71	8.06	31.0	32.0	36	45
C-Z	2	14.8	15.8	7.5	8.1	7.72	8.07	31.0	32.0	36	45
C-Z	3	14.7	15.8	7.5	8.1	7.71	8.05	31.0	32.0	35	44
C-Z	4	15.0	16.0	7.5	8.1	7.71	8.07	31.0	32.0	36	44
Sequim Bay Reference	1	14.8	15.7	7.4	8.0	7.72	8.06	31.0	32.0	36	44
Sequim Bay Reference	2	14.8	15.8	7.4	8.0	7.72	8.05	31.0	32.0	36	44
Sequim Bay Reference	3	15.0	16.0	7.4	7.9	7.72	8.06	31.0	32.0	36	44
Sequim Bay Reference	4	14.7	15.6	7.4	7.9	7.72	8.05	31.0	32.0	37	44
West Beach Reference	1	14.9	16.0	7.4	8.1	7.72	8.05	31.0	32.0	36	44
West Beach Reference	2	14.9	15.8	7.4	8.1	7.71	8.07	31.0	32.0	36	45
West Beach Reference	3	14.9	15.8	7.4	8.0	7.72	8.07	31.0	32.0	36	45
West Beach Reference	4	14.9	15.8	7.5	8.0	7.71	8.06	31.0	32.0	36	44

APPENDIX B

AMPHIPOD TEST DATA

TABLE B.1. Amphipod (*R. abronius*) Test 1-A Reference Toxicant

PCP	Rep	Alive	Dead	Total
0.21 mg/L	1	10	0	10
0.21 mg/L	2	10	0	10
0.21 mg/L	3	10	0	10
0.30 mg/L	1	3	7	10
0.30 mg/L	2	2	8	10
0.30 mg/L	3	2	8	10
0.64 mg/L	1	0	15	15
0.64 mg/L	2	0	10	10
0.64 mg/L	3	0	10	10
0.93 mg/L	1	0	10	10
0.93 mg/L	2	0	10	10
0.93 mg/L	3	0	10	10
0.145 mg/L	1	10	0	10
0.145 mg/L	2	8	2	10
0.145 mg/L	3	9	1	10
0.442 mg/L	1	0	10	10
0.442 mg/L	2	0	10	10
0.442 mg/L	3	0	10	10
Seawater Only	1	10	0	10
Seawater Only	2	10	0	10
Seawater Only	3	10	0	10

TABLE B.2. Amphipod (*R. abronius*) Test 1-B

Sediment Treatment	Rep	Alive	Dead	Total
C-1	1	14	6	20
C-1	2	18	2	20
C-1	3	17	3	20
C-1	4	15	5	20
C-1	5	16	4	20
C-2	1	16	4	20
C-2	2	16	4	20
C-2	3	19	1	20
C-2	4	16	4	20
C-2	5	12	8	20
C-5	1	12	8	20
C-5	2	15	5	20
C-5	3	13	7	20
C-5	4	18	2	20
C-5	5	14	6	20
C-6	1	15	5	20
C-6	2	15	5	20
C-6	3	18	2	20
C-6	4	14	6	20
C-6	5	19	1	20
C-7	1	16	4	20
C-7	2	15	5	20
C-7	3	17	3	20
C-7	4	17	3	20
C-7	5	18	2	20
C-12	1	15	5	20
C-12	2	29	1	30
C-12	3	18	2	20
C-12	4	0	0	0
C-12	5	17	3	20
C-13	1	20	0	20
C-13	2	16	4	20
C-13	3	20	0	20
C-13	4	14	6	20
C-13	5	10	10	20

TABLE B.2. (contd)

Sediment Treatment	Rep	Alive	Dead	Total
C-14	1	17	3	20
C-14	2	16	4	20
C-14	3	18	2	20
C-14	4	17	3	20
C-14	5	15	5	20
C-15	1	18	2	20
C-15	2	17	3	20
C-15	3	15	5	20
C-15	4	19	1	20
C-15	5	18	2	20
C-19	1	15	5	20
C-19	2	18	2	20
C-19	3	15	5	20
C-19	4	15	5	20
C-19	5	17	3	20
C-20	1	14	6	20
C-20	2	7	13	20
C-20	3	12	8	20
C-20	4	18	2	20
C-20	5	19	1	20
C-Z	1	13	7	20
C-Z	2	11	9	20
C-Z	3	17	3	20
C-Z	4	13	7	20
C-Z	5	12	8	20
Sequim Bay Reference	1	20	0	20
Sequim Bay Reference	2	18	2	20
Sequim Bay Reference	3	18	2	20
Sequim Bay Reference	4	18	2	20
Sequim Bay Reference	5	18	2	20
West Beach Control	1	19	1	20
West Beach Control	2	19	1	20
West Beach Control	3	20	0	20
West Beach Control	4	20	0	20
West Beach Control	5	20	1	21

TABLE B.3. Amphipod (*R. abronius*) Test 2-A Reference Toxicant

Sediment Treatment	Rep	Alive	Dead	Total
$CDCl_2$	1	0	20	20
$CDCl_2$	2	1	39	40
$CDCl_2$	3	0	20	20
$CDCl_2$	4	0	20	20
$CDCl_2$	5	0	20	20
Seawater Only	1	19	1	20
Seawater Only	2	19	1	20
Seawater Only	3	18	2	20
Seawater Only	4	17	3	20
Seawater Only	5	18	2	20

TABLE B.4. Amphipod (*R. abronius*) Test 2-B

Sediment Treatment	Rep	Alive	Dead	Total
C-17	1	17	3	20
C-17	2	15	5	20
C-17	3	14	6	20
C-17	4	11	9	20
C-17	5	18	2	20
Sequim Bay Reference	1	0	20	20
Sequim Bay Reference	2	0	20	20
Sequim Bay Reference	3	0	20	20
Sequim Bay Reference	4	0	20	20
Sequim Bay Reference	5	0	20	20
WB Amphipod Control	1	20	0	20
WB Amphipod Control	2	19	1	20
WB Amphipod Control	3	20	0	20
WB Amphipod Control	4	19	1	20
WB Amphipod Control	5	20	0	20

TABLE B.5. Amphipod (*R. abronius*) Test 1-A Reference Toxicant - Water Quality Monitoring

NaPCP	Rep	Temperature (°C)		Dissolved Oxygen (mg/L)		pH		Salinity (°/₀₀)	
		Min	Max	Min	Max	Min	Max	Min	Max
0.21 mg/L	1	15.2	15.2	8.5	8.5	8.24	8.24	28.0	28.0
0.21 mg/L	2	15.7	16.2	8.0	9.7	7.99	8.26	28.0	28.0
0.21 mg/L	3	15.9	15.9	9.1	9.1	8.07	8.07	28.0	28.0
0.30 mg/L	1	15.2	15.2	7.6	7.6	8.17	8.17	29.0	29.0
0.30 mg/L	2	16.2	16.2	8.1	8.7	8.04	8.15	28.0	28.5
0.30 mg/L	3	15.9	15.9	8.7	8.7	8.04	8.04	28.0	28.0
0.64 mg/L	1	15.2	15.2	6.8	6.8	7.95	7.95	29.0	29.0
0.64 mg/L	2	15.8	16.2	7.9	8.4	8.01	8.02	28.0	29.0
0.64 mg/L	3	15.9	15.9	8.3	8.3	7.97	7.97	28.5	28.5
0.93 mg/L	1	15.2	15.2	6.5	6.5	7.97	7.97	29.0	29.0
0.93 mg/L	2	15.7	16.2	6.4	8.4	7.99	8.02	28.0	29.0
0.93 mg/L	3	15.9	15.9	8.1	8.1	7.99	7.99	30.0	30.0
0.145 mg/L	1	15.2	15.2	8.4	8.4	8.21	8.21	28.0	28.0
0.145 mg/L	2	15.7	16.2	8.4	9.6	8.01	8.22	28.5	29.0
0.145 mg/L	3	15.9	15.9	9.4	9.4	8.09	8.09	28.0	28.0
0.442 mg/L	1	15.2	15.2	7.5	7.5	8.12	8.12	28.0	28.0
0.442 mg/L	2	15.7	16.2	7.8	8.3	7.99	8.02	28.0	28.0
0.442 mg/L	3	15.9	15.9	8.3	8.3	7.97	7.97	28.0	28.0
NaPCP Reference	1	15.2	15.2	7.9	7.9	8.19	8.19	29.0	29.0
NaPCP Reference	2	15.8	16.2	8.3	9.7	7.97	8.21	28.5	29.0
NaPCP Reference	3	15.9	15.9	9.4	9.4	8.05	8.05	28.0	28.0

TABLE B.6. Amphipod (*R. abronius*) Test 1-B - Water Quality Monitoring

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/oo) Min	Salinity (°/oo) Max
C-1	1	14.5	16.1	7.5	8.4	7.95	8.39	30.0	30.0
C-1	2	14.5	15.8	7.5	8.4	7.93	8.33	29.0	30.5
C-1	3	14.5	16.4	7.7	8.3	7.87	8.42	30.0	30.5
C-1	4	14.5	16.2	7.5	8.5	7.97	8.44	30.0	30.5
C-1	5	14.5	16.7	7.6	8.5	7.97	8.42	30.0	31.0
C-2	1	14.5	16.1	7.5	8.5	7.94	8.38	30.0	30.5
C-2	2	14.5	15.8	7.6	8.4	7.92	8.40	30.0	30.5
C-2	3	14.4	16.1	7.6	8.4	7.95	8.42	30.0	31.5
C-2	4	14.5	16.1	7.5	8.5	7.96	8.40	30.0	30.5
C-2	5	14.5	15.8	7.6	8.4	7.94	8.41	31.0	32.0
C-5	1	14.5	16.3	7.5	8.4	7.90	8.33	30.0	31.0
C-5	2	14.4	16.1	7.7	8.5	7.96	8.43	30.0	32.0
C-5	3	14.5	15.8	7.6	8.5	7.96	8.37	30.0	31.0
C-5	4	14.5	15.8	7.6	8.4	7.96	8.42	30.0	32.0
C-5	5	14.5	16.1	7.6	8.5	7.96	8.42	30.0	31.0
C-6	1	14.5	16.1	7.6	8.4	7.93	8.42	31.0	32.0
C-6	2	14.5	15.8	7.6	8.4	7.92	8.35	30.0	32.0
C-6	3	14.5	16.2	7.6	8.5	7.95	8.39	30.0	32.0
C-6	4	14.6	16.1	7.5	8.5	7.89	8.38	30.0	31.0
C-6	5	14.6	16.2	7.5	8.4	7.94	8.34	30.0	32.0
C-7	1	14.5	16.1	7.5	8.6	7.91	8.38	30.5	32.0
C-7	2	14.5	16.1	7.6	8.4	7.95	8.43	30.0	31.5
C-7	3	14.5	16.1	7.5	8.4	7.93	8.38	30.0	31.0
C-7	4	14.5	16.1	7.3	8.5	7.91	8.43	30.0	31.0
C-7	5	14.5	16.2	7.4	8.5	7.93	8.41	30.0	32.0
C-12	1	14.5	16.1	7.6	8.4	8.00	8.43	30.0	31.0
C-12	2	14.5	15.8	7.5	8.4	8.00	8.45	29.0	30.0
C-12	3	14.4	16.1	7.6	8.5	8.05	8.52	30.0	30.0
C-12	4	14.5	15.8	7.6	8.5	8.08	8.50	29.0	30.0
C-12	5	14.5	16.1	7.6	8.4	8.08	8.54	29.0	30.0
C-13	1	14.5	16.1	7.6	8.5	8.16	8.68	30.0	31.0
C-13	2	14.5	16.2	7.3	8.5	8.10	8.62	30.0	31.0
C-13	3	14.5	16.1	7.4	8.4	8.13	8.61	30.0	30.5
C-13	4	14.5	16.1	7.5	8.4	8.16	8.54	29.0	30.0
C-13	5	14.5	15.8	7.5	8.5	8.08	8.54	29.0	31.0

TABLE B.6. (contd)

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/₀₀) Min	Salinity (°/₀₀) Max
C-14	1	14.5	16.2	7.5	8.4	7.92	8.32	30.0	31.0
C-14	2	14.5	16.1	7.5	8.5	7.95	8.42	31.0	32.0
C-14	3	14.5	16.1	7.4	8.4	7.93	8.28	31.0	31.5
C-14	4	14.4	16.1	7.6	8.4	7.96	8.32	30.0	32.0
C-14	5	14.5	15.8	7.6	8.4	7.95	8.42	31.0	32.0
C-15	1	14.5	16.3	7.5	8.5	8.26	8.72	31.0	31.5
C-15	2	14.8	16.2	7.4	8.5	8.14	8.54	30.0	31.0
C-15	3	14.5	15.8	7.7	8.4	8.30	8.70	30.0	32.0
C-15	4	14.4	16.6	7.6	8.3	8.28	8.58	30.0	32.0
C-15	5	14.5	16.2	7.5	8.6	8.23	8.57	30.0	31.0
C-19	1	14.5	16.2	7.1	8.5	8.06	8.54	32.0	32.0
C-19	2	14.5	16.2	7.8	8.5	7.93	8.41	30.0	32.0
C-19	3	14.5	16.1	7.5	8.4	8.02	8.53	31.0	32.0
C-19	4	14.5	15.8	7.4	8.4	8.06	8.51	32.0	32.0
C-19	5	14.5	16.1	7.6	8.4	8.07	8.45	31.0	31.0
C-20	1	14.5	15.7	7.8	8.5	8.02	8.45	28.0	30.0
C-20	2	14.5	16.2	7.5	8.5	8.04	8.52	29.0	30.0
C-20	3	14.5	16.2	7.5	8.5	8.10	8.54	29.0	30.5
C-20	4	14.5	16.1	7.5	8.5	8.03	8.60	29.0	30.5
C-20	5	14.5	15.8	7.6	8.4	8.07	8.52	28.0	30.0
C-Z	1	14.5	15.8	7.4	8.4	7.98	8.42	31.0	32.0
C-Z	2	14.5	16.6	7.5	8.4	8.07	8.52	30.0	32.0
C-Z	3	14.4	16.1	7.6	8.5	8.05	8.57	30.5	32.0
C-Z	4	14.5	15.7	7.6	8.4	8.09	8.59	31.0	31.5
C-Z	5	14.5	15.8	7.5	8.4	8.08	8.51	30.0	32.0
Sequim Bay Reference	1	14.5	16.1	7.2	8.4	8.00	8.30	32.0	32.0
Sequim Bay Reference	2	14.5	16.5	7.4	8.4	8.07	8.44	32.0	32.0
Sequim Bay Reference	3	14.5	15.8	7.5	8.4	8.06	8.47	32.0	32.0
Sequim Bay Reference	4	14.4	16.2	7.5	8.3	8.06	8.41	32.0	32.0
Sequim Bay Reference	5	14.5	16.1	7.4	8.5	8.05	8.47	32.0	32.0
West Beach Control	1	14.5	16.1	7.5	8.5	7.98	8.38	31.0	32.0
West Beach Control	2	14.5	15.8	7.6	8.4	7.99	8.38	31.0	32.5
West Beach Control	3	14.5	15.8	7.6	8.5	7.98	8.39	31.0	32.0
West Beach Control	4	14.4	16.1	7.5	8.3	7.98	8.38	32.0	32.0
West Beach Control	5	14.5	16.2	7.5	8.5	7.98	8.33	31.5	32.0

TABLE B.7. Amphipod (*R. abronius*) Test 2-A Reference Toxicant Water Quality Monitoring

Sediment Treatment	Rep	Temperature (°C)		Dissolved Oxygen (mg/L)		pH		Salinity (‰)	
		Min	Max	Min	Max	Min	Max	Min	Max
$CdCl_2$	1	14.8	14.8	7.6	9.0	7.51	7.75	30.5	31.5
$CdCl_2$	2	14.8	14.8	7.4	8.7	7.51	7.72	31.0	31.5
$CdCl_2$	3	14.8	14.8	7.7	9.2	7.22	7.74	30.5	31.0
$CdCl_2$	4	14.8	14.8	7.2	8.7	7.17	7.74	31.5	32.0
$CdCl_2$	5	14.8	14.8	7.2	8.9	7.58	7.78	31.0	31.5
Seawater Only	1	14.8	14.8	7.3	8.9	8.21	8.32	30.5	31.0
Seawater Only	2	14.8	14.8	7.3	8.9	8.18	8.31	31.0	31.5
Seawater Only	3	14.8	14.8	7.6	9.1	8.21	8.32	31.0	31.5
Seawater Only	4	14.8	14.8	7.3	9.0	8.15	8.29	31.0	31.5
Seawater Only	5	14.8	14.8	7.2	8.9	8.21	8.32	31.5	31.5

TABLE B.8. Amphipod (*R. abronius*) Test 2-B - Water Quality Monitoring

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/oo) Min	Salinity (°/oo) Max
C-17	1	14.2	14.8	6.7	8.6	8.00	8.21	29.0	30.5
C-17	2	14.2	14.8	7.3	9.0	8.08	8.30	28.5	30.0
C-17	3	14.2	14.8	7.0	8.7	8.10	8.32	29.5	30.5
C-17	4	14.2	14.8	6.9	8.3	8.05	8.20	29.5	30.5
C-17	5	14.2	14.8	7.1	8.5	8.02	8.22	28.5	30.0
Sequim Bay Reference	1	14.2	14.8	6.7	8.0	8.09	8.25	31.0	32.0
Sequim Bay Reference	2	14.2	14.8	6.6	8.6	8.05	8.22	30.5	31.5
Sequim Bay Reference	3	14.2	14.8	6.6	8.2	8.10	8.20	31.5	32.5
Sequim Bay Reference	4	14.2	14.8	6.3	7.9	8.01	8.25	30.5	31.5
Sequim Bay Reference	5	14.2	14.8	5.0	7.2	7.97	8.19	31.0	32.0
West Beach Reference	1	14.2	14.8	7.1	8.8	8.06	8.27	31.0	32.0
West Beach Reference	2	14.2	14.8	7.1	8.8	8.04	8.24	31.0	31.5
West Beach Reference	3	14.2	14.8	7.0	8.6	8.09	8.27	31.0	32.0
West Beach Reference	4	14.2	14.8	7.0	8.7	8.10	8.30	31.0	32.0
West Beach Reference	5	14.2	14.8	7.2	8.9	8.06	8.29	31.0	32.0

APPENDIX C

SEA URCHIN TEST DATA

TABLE C.1. Sea Urchin (*S. purpuratus*) Larval Test 1

Sediment Treatment	Rep	Normal	Abnormal	Total	Prop Normal	Prop Abnormal	Prop. Surviving 290 per 10-mL Stocking Density
C-1	1	218	11	229	0.95	0.05	0.79
C-1	2	114	0	114	1.00	0.00	0.39
C-1	3	66	0	66	1.00	0.00	0.23
C-1	4	121	0	121	1.00	0.00	0.42
C-1	5	41	0	41	1.00	0.00	0.14
C-2	1	89	0	89	1.00	0.00	0.31
C-2	2	123	0	123	1.00	0.00	0.42
C-2	3	99	0	100	0.99	0.00	0.34
C-2	4	109	3	112	0.97	0.03	0.39
C-2	5	88	0	88	1.00	0.00	0.30
C-5	1	163	3	166	0.98	0.02	0.57
C-5	2	115	5	120	0.96	0.04	0.41
C-5	3	31	7	38	0.82	0.18	0.13
C-5	4	122	1	123	0.99	0.01	0.42
C-5	5	96	2	98	0.98	0.02	0.34
C-6	1	156	1	157	0.99	0.01	0.54
C-6	2	94	1	95	0.99	0.01	0.33
C-6	3	109	1	110	0.99	0.01	0.38
C-6	4	126	1	127	0.99	0.01	0.44
C-6	5	171	0	171	1.00	0.00	0.59
C-7	1	148	5	153	0.97	0.03	0.53
C-7	2	78	3	81	0.96	0.04	0.28
C-7	3	28	0	28	1.00	0.00	0.10
C-7	4	66	0	66	1.00	0.00	0.23
C-7	5	161	0	161	1.00	0.00	0.56
C-12	1	96	9	105	0.91	0.09	0.36
C-12	2	31	2	33	0.94	0.06	0.11
C-12	3	67	1	68	0.99	0.01	0.23
C-12	4	90	1	91	0.99	0.01	0.31
C-12	5	56	0	56	1.00	0.00	0.19
C-13	1	102	2	104	0.98	0.02	0.36
C-13	2	163	1	164	0.99	0.01	0.57
C-13	3	43	2	45	0.96	0.04	0.16
C-13	4	57	0	57	1.00	0.00	0.20
C-13	5	70	2	72	0.97	0.03	0.25

TABLE C.1. (contd)

Sediment Treatment	Rep	Normal	Abnormal	Total	Prop Normal	Prop Abnormal	Prop. Surviving 200 per 10-mL Stocking Density
C-14	1	73	0	73	1.00	0.00	0.26
C-14	2	114	2	116	0.98	0.02	0.40
C-14	3	134	0	134	1.00	0.00	0.46
C-14	4	111	0	111	1.00	0.00	0.38
C-14	5	69	0	69	1.00	0.00	0.24
C-15	1	63	0	63	1.00	0.00	0.22
C-15	2	67	1	68	0.99	0.01	0.23
C-15	3	63	1	64	0.98	0.02	0.22
C-15	4	51	0	51	1.00	0.00	0.18
C-15	5	93	0	93	1.00	0.00	0.32
C-19	1	46	7	53	0.87	0.13	0.18
C-19	2	43	0	43	1.00	0.00	0.15
C-19	3	59	0	59	1.00	0.00	0.20
C-19	4	66	0	66	1.00	0.00	0.23
C-19	5	57	0	57	1.00	0.00	0.20
C-20	1	58	0	58	1.00	0.00	0.20
C-20	2	46	1	47	0.98	0.02	0.16
C-20	3	107	0	107	1.00	0.00	0.37
C-20	4	112	1	113	0.99	0.01	0.39
C-20	5	99	1	100	0.99	0.01	0.34
C-Z	1	136	11	147	0.93	0.07	0.51
C-Z	2	131	0	131	1.00	0.00	0.45
C-Z	3	108	0	108	1.00	0.00	0.37
C-Z	4	93	1	94	0.99	0.01	0.32
C-Z	5	88	1	89	0.99	0.01	0.31
$CdCl_2$ (0.27 mg/L)	1	231	9	240	0.96	0.04	0.83
$CdCl_2$ (0.27 mg/L)	2	212	11	223	0.95	0.05	0.77
$CdCl_2$ (0.27 mg/L)	3	246	3	249	0.99	0.01	0.86
$CdCl_2$ (0.27 mg/L)	4	261	0	261	1.00	0.00	0.90
$CdCl_2$ (0.27 mg/L)	5	309	1	310	1.00	0.00	1.07
$CdCl_2$ (1.33 mg/L)	1	0	0	0	0.00	0.00	0.00
$CdCl_2$ (1.33 mg/L)	2	0	0	0	0.00	0.00	0.00
$CdCl_2$ (1.33 mg/L)	3	0	0	0	0.00	0.00	0.00
$CdCl_2$ (1.33 mg/L)	4	0	0	0	0.00	0.00	0.00
$CdCl_2$ (1.33 mg/L)	5	0	0	0	0.00	0.00	0.00

TABLE C.1. (contd)

Sediment Treatment	Rep	Normal	Abnormal	Total	Prop Normal	Prop Abnormal	Prop. Surviving 200 per 10-mL Stocking Density
Seawater-Only Control	1	268	18	286	0.94	0.06	0.99
Seawater-Only Control	2	239	4	243	0.98	0.02	0.84
Seawater-Only Control	3	280	8	288	0.97	0.03	0.99
Seawater-Only Control	4	239	2	241	0.99	0.01	0.83
Seawater-Only Control	5	250	3	253	0.99	0.01	0.87
Sequim Bay Reference	1	107	0	107	1.00	0.00	0.37
Sequim Bay Reference	2	109	2	111	0.98	0.02	0.38
Sequim Bay Reference	3	31	0	31	1.00	0.00	0.11
Sequim Bay Reference	4	54	1	55	0.98	0.02	0.19
Sequim Bay Reference	5	147	1	148	0.99	0.01	0.51
For comparison only:							
WB Amphipod Control	1	212	0	212	1.00	0.00	0.73
WB Amphipod Control	2	178	0	178	1.00	0.00	0.61
WB Amphipod Control	3	165	2	167	0.99	0.01	0.58
WB Amphipod Control	4	229	1	230	1.00	0.00	0.79
WB Amphipod Control	5	222	0	222	1.00	0.00	0.77

TABLE C.2. Sea Urchin (*S. purpuratus*) Larval Test 2

Sediment Treatment	Rep	Normal	Abnormal	Total	Prop Normal	Prop Abnormal	Prop. Surviving 270 per 10-mL Stocking Density
C-17	1	87	0	87	1.00	0.00	0.32
C-17	2	103	0	103	1.00	0.00	0.38
C-17	3	107	0	107	1.00	0.00	0.40
C-17	4	71	0	71	1.00	0.00	0.26
C-17	5	56	0	56	1.00	0.00	0.21
$CdCl_2$ (1.33 mg/L)	1	206	15	221	0.93	0.07	0.82
$CdCl_2$ (1.33 mg/L)	2	289	18	307	0.94	0.06	1.14
$CdCl_2$ (1.33 mg/L)	4	283	19	302	0.94	0.06	1.12
$CdCl_2$ (1.33 mg/L)	5	263	7	270	0.97	0.03	1.00
Seawater Only	2	226	0	226	1.00	0.00	0.84
Seawater Only	3	253	0	253	1.00	0.00	0.94
Seawater Only	4	247	0	247	1.00	0.00	0.91
Seawater Only	5	207	0	206	1.00	0.00	0.76
Sequim Bay Reference	1	32	0	32	1.00	0.00	0.12
Sequim Bay Reference	2	66	0	66	1.00	0.00	0.24
Sequim Bay Reference	3	36	1	37	0.97	0.03	0.14
Sequim Bay Reference	4	62	0	62	1.00	0.00	0.23
Sequim Bay Reference	5	52	1	53	0.98	0.02	0.20
For comparison only:							
WB Amphipod Control	1	194	0	194	1.00	0.00	0.72
WB Amphipod Control	2	232	0	232	1.00	0.00	0.86
WB Amphipod Control	3	270	0	270	1.00	0.00	1.00
WB Amphipod Control	4	195	0	195	1.00	0.00	0.72
WB Amphipod Control	5	321	0	321	1.00	0.00	1.19

TABLE C.3. Sea Urchin (*S. purpuratus*) Sperm Cell Test

Sediment Treatment	Rep	Fertilized	Unfertilized	Total
C-1	1	98	2	100
C-1	2	91	9	100
C-1	3	96	4	100
C-1	4	99	1	100
C-1	5	94	6	100
C-2	1	98	2	100
C-2	2	95	5	100
C-2	3	93	7	100
C-2	4	98	2	100
C-2	5	94	6	100
C-5	1	87	13	100
C-5	2	96	4	100
C-5	3	94	6	100
C-5	4	93	7	100
C-5	5	95	5	100
C-6	1	96	4	100
C-6	2	100	0	100
C-6	3	97	3	100
C-6	4	99	1	100
C-6	5	95	5	100
C-7	1	94	6	100
C-7	2	95	5	100
C-7	3	95	5	100
C-7	4	96	4	100
C-7	5	91	9	100
C-12	1	91	9	100
C-12	2	95	5	100
C-12	3	93	7	100
C-12	4	96	4	100
C-12	5	96	4	100
C-13	1	96	4	100
C-13	2	96	4	100
C-13	3	98	2	100
C-13	4	93	7	100
C-13	5	95	5	100

TABLE C.3. (contd)

Sediment Treatment	Rep	Fertilized	Unfertilized	Total
C-14	1	92	8	100
C-14	2	95	5	100
C-14	3	96	4	100
C-14	4	98	2	100
C-14	5	99	1	100
C-15	1	86	14	100
C-15	2	99	1	100
C-15	3	96	4	100
C-15	4	97	3	100
C-15	5	96	4	100
C-19	1	90	10	100
C-19	2	98	2	100
C-19	3	91	9	100
C-19	4	99	1	100
C-19	5	93	7	100
C-20	1	89	11	100
C-20	2	96	4	100
C-20	3	96	4	100
C-20	4	98	2	100
C-20	5	99	1	100
C-Z	1	91	9	100
C-Z	2	98	2	100
C-Z	3	94	6	100
C-Z	4	99	1	100
C-Z	5	89	11	100
$CdCl_2$ (0.27 mg/L)	1	96	4	100
$CdCl_2$ (0.27 mg/L)	2	95	5	100
$CdCl_2$ (0.27 mg/L)	3	98	2	100
$CdCl_2$ (0.27 mg/L)	4	97	3	100
$CdCl_2$ (0.27 mg/L)	5	97	3	100
$CdCl_2$ (1.33 mg/L)	1	88	12	100
$CdCl_2$ (1.33 mg/L)	2	88	12	100
$CdCl_2$ (1.33 mg/L)	3	94	6	100
$CdCl_2$ (1.33 mg/L)	4	85	15	100
$CdCl_2$ (1.33 mg/L)	5	71	29	100

TABLE C.3. (contd)

Sediment Treatment	Rep	Fertilized	Unfertilized	Total
Seawater Only	1	93	7	100
Seawater Only	2	99	1	100
Seawater Only	3	97	3	100
Seawater Only	4	98	2	100
Seawater Only	5	99	1	100
Sequim Bay Reference	1	94	6	100
Sequim Bay Reference	2	97	3	100
Sequim Bay Reference	3	92	8	100
Sequim Bay Reference	4	94	6	100
Sequim Bay Reference	5	91	9	100
For comparison only:				
WB Amphipod Control	1	98	2	100
WB Amphipod Control	2	97	3	100
WB Amphipod Control	3	96	4	100
WB Amphipod Control	4	96	4	100
WB Amphipod Control	5	97	3	100

TABLE C.4. Sea Urchin (*S. purpuratus*) Larval Test 1 - Water Quality Monitoring

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/oo) Min	Salinity (°/oo) Max
C-1	1	12.0	12.2	6.3	6.6	7.86	7.99	31.0	31.5
C-1	2	12.0	12.2	6.0	6.3	7.88	8.01	31.5	31.5
C-1	3	12.0	12.7	5.9	6.2	7.88	8.02	31.0	31.5
C-1	4	12.0	12.1	5.9	6.2	7.90	8.00	31.5	31.5
C-1	5	12.1	12.2	6.1	6.3	7.91	8.02	31.0	31.5
C-2	1	12.0	12.5	6.6	6.6	7.89	8.01	31.5	31.5
C-2	2	12.0	12.3	6.6	6.8	7.88	8.01	31.5	31.5
C-2	3	12.0	12.2	6.4	6.7	7.91	8.02	31.5	31.5
C-2	4	12.0	12.5	6.5	6.7	7.92	8.02	31.5	31.5
C-2	5	12.0	12.5	6.4	6.6	7.89	8.01	31.5	31.5
C-5	1	12.0	12.7	6.3	6.6	7.84	7.93	31.5	31.5
C-5	2	12.0	12.3	6.1	6.3	7.84	7.93	31.0	31.5
C-5	3	12.0	12.5	6.3	6.5	7.85	7.98	31.5	31.5
C-5	4	12.0	12.5	5.8	6.2	7.95	8.07	31.0	31.5
C-5	5	12.0	12.3	6.1	6.3	7.90	8.01	31.0	31.5
C-6	1	12.0	12.6	6.6	6.8	7.85	7.99	31.5	31.5
C-6	2	12.0	12.3	6.4	6.7	7.90	8.00	31.0	31.5
C-6	3	12.0	12.8	6.5	6.7	7.89	8.01	31.5	31.5
C-6	4	12.0	12.3	6.3	6.5	7.86	7.90	31.5	31.5
C-6	5	12.0	12.6	6.2	6.5	7.86	7.96	31.5	31.5
C-7	1	12.0	12.2	6.3	6.5	7.90	7.96	31.0	31.5
C-7	2	12.0	12.3	6.2	6.5	7.90	6.00	31.0	31.5
C-7	3	12.0	12.8	6.5	6.7	7.90	8.02	31.5	31.5
C-7	4	12.0	12.6	6.3	6.5	0.08	8.02	31.0	31.5
C-7	5	12.0	12.3	5.9	6.6	7.93	8.00	31.0	31.5
C-12	1	12.0	12.3	6.6	6.7	7.84	7.97	31.0	31.5
C-12	2	12.0	12.3	6.3	6.8	7.84	7.94	31.0	31.5
C-12	3	12.0	12.5	6.2	6.4	7.84	7.96	31.0	31.5
C-12	4	12.0	12.8	6.3	6.7	7.87	8.00	31.5	31.5
C-12	5	12.0	12.8	5.9	6.3	7.87	7.97	31.0	31.5
C-13	1	12.0	12.3	5.3	5.9	7.86	7.93	31.0	31.5
C-13	2	12.0	12.6	5.8	6.0	7.84	7.96	31.5	31.5
C-13	3	12.0	12.8	5.8	6.0	7.83	8.00	31.5	31.5
C-13	4	12.0	12.3	6.2	6.3	7.81	7.93	31.0	31.5
C-13	5	12.0	12.8	5.9	6.3	7.79	7.90	31.0	31.5

TABLE C.4. (contd)

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/oo) Min	Salinity (°/oo) Max
C-14	1	12.0	12.7	6.3	6.4	7.98	7.99	31.5	31.5
C-14	2	12.0	12.2	6.3	6.7	7.88	8.02	31.5	31.5
C-14	3	12.0	12.6	6.5	6.8	7.87	8.02	31.5	31.5
C-14	4	12.0	12.8	6.4	6.8	7.87	8.02	31.5	32.0
C-14	5	11.9	12.2	6.5	6.6	7.88	8.01	31.5	32.0
C-15	1	12.0	12.2	6.0	6.4	7.81	7.94	31.0	31.5
C-15	2	12.0	12.7	6.0	6.2	7.69	7.87	31.0	31.5
C-15	3	12.0	12.6	5.8	6.0	7.81	7.86	31.0	31.5
C-15	4	12.0	12.3	5.9	6.1	7.84	7.95	31.0	31.5
C-15	5	12.0	12.2	5.8	6.0	7.85	7.92	31.5	31.5
C-19	1	11.9	12.2	6.0	6.2	7.89	8.00	31.5	31.5
C-19	2	12.0	12.6	6.0	6.2	7.91	7.99	31.0	31.5
C-19	3	12.0	12.7	6.1	6.4	7.90	8.02	31.5	31.5
C-19	4	12.0	12.4	6.9	6.1	7.91	8.01	31.0	31.5
C-19	5	11.8	12.2	6.0	6.3	7.94	8.03	31.5	32.0
C-20	1	12.0	12.2	6.4	6.7	7.82	7.95	31.0	31.5
C-20	2	12.0	12.4	6.4	6.5	7.61	7.96	31.0	31.5
C-20	3	12.0	12.7	6.2	6.6	7.84	8.00	31.5	31.5
C-20	4	12.0	12.3	6.2	6.5	7.76	7.94	31.5	31.5
C-20	5	12.0	12.5	6.2	6.5	7.84	7.99	31.5	31.5
C-Z	1	12.0	12.2	5.9	6.2	7.77	7.91	31.5	31.5
C-Z	2	12.0	12.2	5.9	6.2	7.77	7.91	31.0	31.5
C-Z	3	12.0	12.3	5.8	6.0	7.85	7.96	31.0	31.5
C-Z	4	12.0	12.6	5.3	5.5	7.86	7.95	31.0	31.5
C-Z	5	12.0	12.7	5.7	6.1	7.83	7.97	31.0	31.5
$CdCl_2$ (0.27 mg/L)	1	12.0	12.2	7.2	7.8	7.46	7.89	31.0	31.5
$CdCl_2$ (0.27 mg/L)	2	12.0	12.2	7.2	7.8	7.50	7.88	31.5	31.5
$CdCl_2$ (0.27 mg/L)	3	12.0	12.6	7.3	8.5	7.40	7.88	31.0	31.5
$CdCl_2$ (0.27 mg/L)	4	12.0	12.6	7.1	7.8	7.52	7.91	31.5	31.5
$CdCl_2$ (0.27 mg/L)	5	12.0	12.8	7.2	7.9	7.47	7.91	31.5	32.0
$CdCl_2$ (1.33 mg/L)	1	12.0	12.5	7.2	7.8	6.38	6.98	31.5	31.5
$CdCl_2$ (1.33 mg/L)	2	12.0	12.3	7.3	7.8	6.45	7.09	31.5	32.0
$CdCl_2$ (1.33 mg/L)	3	12.0	12.2	7.2	7.8	6.44	7.05	31.5	31.5
$CdCl_2$ (1.33 mg/L)	4	12.0	12.2	7.2	7.8	6.42	7.07	31.5	31.5
$CdCl_2$ (1.33 mg/L)	5	12.0	12.7	7.2	8.0	6.40	7.09	31.5	32.0

TABLE C.4. (contd)

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/oo) Min	Salinity (°/oo) Max
Sequim Bay Reference	1	12.0	12.9	6.3	6.6	7.93	8.06	31.5	31.5
Sequim Bay Reference	2	12.0	12.6	6.4	6.6	7.91	8.02	31.5	31.5
Sequim Bay Reference	3	12.0	12.5	6.1	6.6	7.92	8.02	31.0	31.5
Sequim Bay Reference	4	11.9	12.2	6.1	6.4	7.94	8.02	31.5	31.5
Sequim Bay Reference	5	12.0	12.2	6.3	6.6	7.94	8.07	31.5	31.5
Seawater Only	1	11.9	12.3	7.1	7.8	7.97	8.10	31.5	31.5
Seawater Only	2	12.0	12.8	7.1	7.7	7.96	8.12	31.0	31.5
Seawater Only	3	12.0	12.9	7.2	7.7	7.97	8.12	31.5	31.5
Seawater Only	4	12.0	12.8	7.2	7.7	7.97	8.11	31.5	31.5
Seawater Only	5	12.0	12.6	7.2	7.7	7.96	8.11	31.0	31.5
West Beach Reference	1	12.0	12.3	7.0	7.4	7.95	8.07	31.5	31.5
West Beach Reference	2	12.0	12.3	6.7	7.3	7.95	8.06	31.5	31.5
West Beach Reference	3	11.8	12.2	6.8	7.3	7.96	8.06	31.5	31.5
West Beach Reference	4	12.0	12.4	7.0	7.5	7.95	8.07	31.0	31.5
West Beach Reference	5	12.0	12.3	6.9	7.2	7.95	8.07	31.5	31.5

TABLE C.5. Sea Urchin (*S. purpuratus*) Larval Test 2 - Water Quality Monitoring

Sediment Treatment	Rep	Temperature (°C) Min	Temperature (°C) Max	Dissolved Oxygen (mg/L) Min	Dissolved Oxygen (mg/L) Max	pH Min	pH Max	Salinity (°/oo) Min	Salinity (°/oo) Max
C-17	1	11.8	12.1	5.5	7.2	8.00	8.12	31.0	32.0
C-17	2	11.8	12.1	5.6	7.2	8.00	8.14	31.5	32.0
C-17	3	11.8	12.1	5.8	7.2	8.00	8.16	31.5	32.0
C-17	4	11.8	12.1	6.3	7.3	7.93	8.16	31.0	32.0
C-17	5	11.8	12.1	5.8	7.2	7.97	8.17	31.5	32.0
$CdCl_2$ (1.33 mg/L)	1	11.8	12.1	7.4	8.9	6.19	6.88	31.5	32.0
$CdCl_2$ (1.33 mg/L)	2	11.8	12.1	7.5	9.0	6.19	6.82	31.0	32.0
$CdCl_2$ (1.33 mg/L)	3	11.8	12.1	7.6	8.9	6.14	6.90	31.5	32.0
$CdCl_2$ (1.33 mg/L)	4	11.8	12.1	7.5	9.0	6.22	6.89	31.5	31.5
$CdCl_2$ (1.33 mg/L)	5	11.8	12.1	7.4	9.0	6.21	6.84	31.5	31.5
Seawater Only	1	11.8	12.1	7.5	8.9	7.96	8.24	31.5	32.0
Seawater Only	2	11.8	12.1	7.3	8.9	8.04	8.24	32.0	32.0
Seawater Only	3	11.8	12.1	7.4	9.0	8.03	8.23	31.5	32.0
Seawater Only	4	11.8	12.1	7.4	9.0	7.96	8.24	31.5	31.5
Seawater Only	5	11.8	12.1	7.4	8.8	8.03	8.24	31.0	32.0
Sequim Bay Reference	1	11.8	12.1	5.8	6.8	7.99	8.16	31.5	32.0
Sequim Bay Reference	2	11.8	12.1	5.6	7.0	7.96	8.14	31.5	32.0
Sequim Bay Reference	3	11.8	12.1	5.4	6.9	8.03	8.16	31.5	32.0
Sequim Bay Reference	4	11.8	12.1	5.7	6.7	8.03	8.16	31.5	32.0
Sequim Bay Reference	5	11.8	12.1	5.8	7.1	8.03	8.15	31.5	32.0
West Beach Reference	1	11.8	12.1	6.6	8.1	8.04	8.18	31.5	32.5
West Beach Reference	2	11.8	12.1	6.3	7.9	8.03	8.18	31.5	32.0
West Beach Reference	3	11.8	12.1	6.6	8.1	7.97	8.18	31.0	32.0
West Beach Reference	4	11.8	12.1	6.7	8.1	8.03	8.19	32.0	32.5
West Beach Reference	5	11.8	12.1	6.7	8.3	8.03	8.19	32.0	32.0

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