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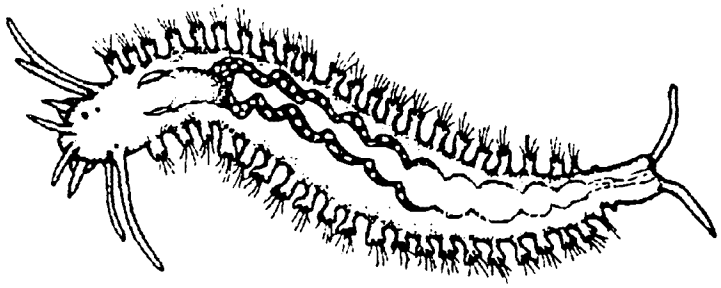
Puget Sound Estuary Program

DEVELOPMENT OF A *NEANTHES* SEDIMENT BIOASSAY FOR USE IN PUGET SOUND



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DEVELOPMENT OF A *NEANTHES* SEDIMENT BIOASSAY FOR USE IN PUGET SOUND

By

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Prepared For

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The following experts participated in the Washington Department of Ecology workshop that resulted in the general experimental design used in the program:

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INTRODUCTION

A draft protocol for a sublethal sediment bioassay using the juvenile stage of *Neanthes* sp. (polychaete) was developed as part of a bioassay test demonstration study conducted for the Seattle District of the U.S. Army Corps of Engineers and the Puget Sound Dredged Disposal Analysis (PSDDA) study (Johns 1988). The study was undertaken to evaluate options in incorporating sublethal tests into the PSDDA dredged material decision-making framework.

The draft protocol for the *Neanthes* sediment bioassay involved measuring survival and change in biomass (total and individual) following exposure to test sediments. Test conditions for the interim bioassay included static renewal of seawater during a 20-day exposure. The five test organisms in each exposure chamber were fed during the bioassay. The results of the test demonstration study suggested the *Neanthes* sublethal bioassay would be a useful test for characterizing sediment quality because of the following factors:

- *Neanthes* juveniles appeared to be sensitive to changes in sediment quality
- *Neanthes* juveniles exhibited consistent responses to a series of sediment exposures
- *Neanthes* sublethal bioassay response criteria (i.e., survival and biomass) are relevant factors in evaluating the potential impacts of contaminated sediments on benthic organisms
- *Neanthes* are easily cultured, and a ready supply of test organisms from a standard stock could be available throughout the year
- *Neanthes* sublethal bioassays can be conducted using a relatively simple static renewal exposure system.

Following completion of the test demonstration study and development of a draft protocol for conducting the *Neanthes* sublethal bioassay, the Washington Department of Ecology conducted an experts workshop on the development of the bioassay to be used as part of the state's marine sediment management program. The general objectives of the workshop were to evaluate the draft protocol for conducting sublethal sediment bioassays using *Neanthes* and to determine the information and research that may be needed to further refine the test development.

As part of the workshop, the experts were asked to categorize and rank research needs obtained during review of the draft protocol to provide guidance on suggested changes to the draft protocol. The workshop resulted in development of an interim protocol (Johns et al. 1989) based on recommended changes that could be made without further testing. The workshop also resulted in the development of research needs for future refinement of the test (Johns et al. 1989).

Research topics identified by the experts were not considered critical to conducting the *Neanthes* bioassay or to establishing an interim protocol, but the experts recommended that results from the research be incorporated into the final protocol to further enhance and extend the usefulness of the test. Research topics identified at the workshop include:

- Determine the optimal number of *Neanthes* that should be placed in each exposure chamber
- Determine the level of food ration that should be provided to each exposure chamber
- Determine if the test can be conducted using a static exposure system
- Determine if the length of the exposure period can be shortened from the current 20 days
- Determine the salinity tolerance limits of the test endpoints
- Determine if sediment grain size has an effect on increases in worm biomass during the exposure period.

Following development of these research recommendations, the workshop participants developed a general experimental approach for each research topic. The research presented in this report results directly from implementation of the recommended studies.

The purpose of this study is to address the research topics identified at the experts workshop and to develop a final *Neanthes* protocol based on the research findings. The final protocol will be published as a separate document and will be included in EPA's *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound.*

METHODS

GENERAL EXPERIMENTAL DESIGN

This study was designed to address the research topics recommended by the experts workshop. The workshop resulted in discrete work elements that were translated into individual experiments as part of the overall study design. However, because the individual experiments do not represent independent study elements, the experiments were not conducted simultaneously. The tests were conducted in a sequential manner so that the results of a particular experiment would be used to modify subsequent experiments, as appropriate. The sequential framework for the six primary experiments is shown in Figure 1. In this series of experiments, the test conditions were maintained according to the interim protocol unless experimental results demonstrated a definite advantage in implementing a modified technique. Experiments 1 through 4 were designed to evaluate the four primary exposure conditions recommended for testing:

- Number of test organisms
- Food ration
- Exposure condition
- Test duration.

If a modified technique was selected in experiments 1 through 4 (Figure 1), the new technique was used in all subsequent experiments.

The six experiments involving sediment exposures were established according to a factorial design involving sediment type and each experimental variable (Table 1). The overall objective of the first four experiments was to evaluate each experiment individually using statistical comparisons to determine whether a modification in the protocol would result in one or more of the following benefits:

1. Increased sensitivity to detect sediment toxicity
2. Simplification of testing procedures
3. Reduced costs.

In experiments 5 and 6, the objective was to determine the applicability of the *Neanthes* bioassay to conditions of reduced salinity or of very fine sediments. An additional test, experiment 7, was used to establish sensitivity of the test organism to a reference toxicant.

SEDIMENT COLLECTION

Sediments used in this study were collected from three sites in Puget Sound and one location in Hood Canal (Figure 2). Within Puget Sound, sampling sites were located in a contaminated embayment (Elliott Bay) and in two relatively uncontaminated areas (Carr Inlet and West Beach). Sediments from Hood Canal were collected from the mouth of the Duckabush River.

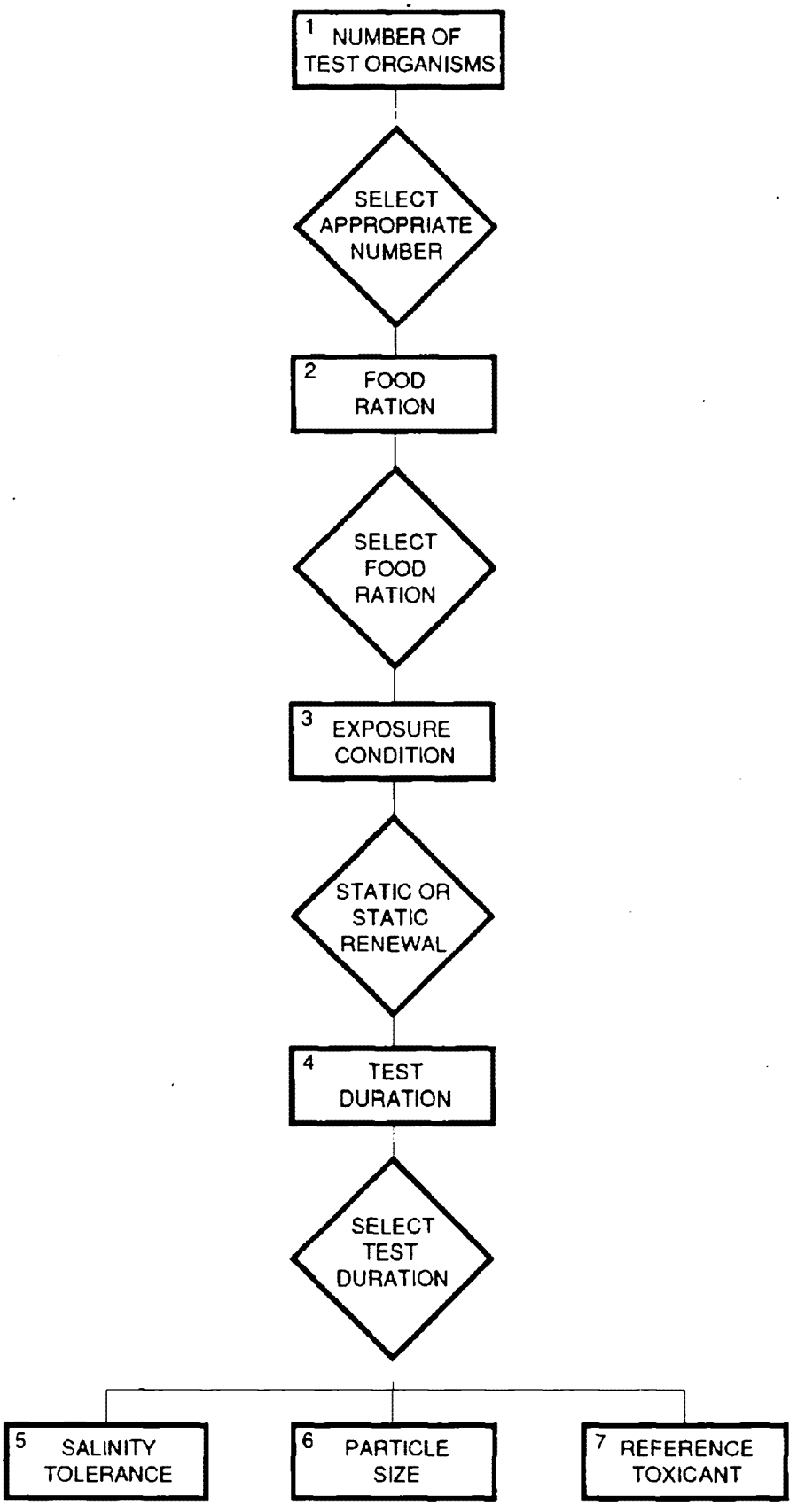

Figure 1. General experimental design showing testing sequence

TABLE 1. EXPERIMENTAL DESIGN FOR SEDIMENT EXPOSURES

Experiment No. 1 - Worm Density

Sediment	Organisms/Exposure Chamber			
	5	10	15	20
Elliott Bay	X	X	X	X
Carr Inlet	X	X	X	X
West Beach	X			

Experiment No. 2 - Food Ration

Sediment	Food Ration (mg)/2 days				
	0	20	40	60	80
Elliott Bay	X	X	X	X	X
Carr Inlet	X	X	X	X	X
West Beach	X		X		X

Experiment No. 3 - Exposure Conditions

Sediment	Exposure Condition	
	Static	Static Renewal
Elliott Bay	X	X
Carr Inlet	X	X

Experiment No. 4 - Test Duration

Sediment	Test Duration (days)		
	10	15	20
Elliott Bay	X	X	X
Carr Inlet	X	X	X
West Beach	X	X	X

Experiment No. 5 - Salinity Tolerance

Sediment	Salinity (ppt)				
	30	28	25	22	19
Duckabush River	X	X	X	X	X
Carr Inlet		X			
West Beach		X			

TABLE 1. (Continued)

Experiment No. 6 - Sediment Grain Size

Sediment	Percent Fines (silt/clay) 2	88-89	93	97
Carr Inlet (CR01)		X		
Carr Inlet (CR02)		X		
Carr Inlet (CR03)				X
Carr Inlet (CR04)		X		
Carr Inlet (CR05)			X	
West Beach	X			

⬚ Shading represents exposure condition in interim protocol.

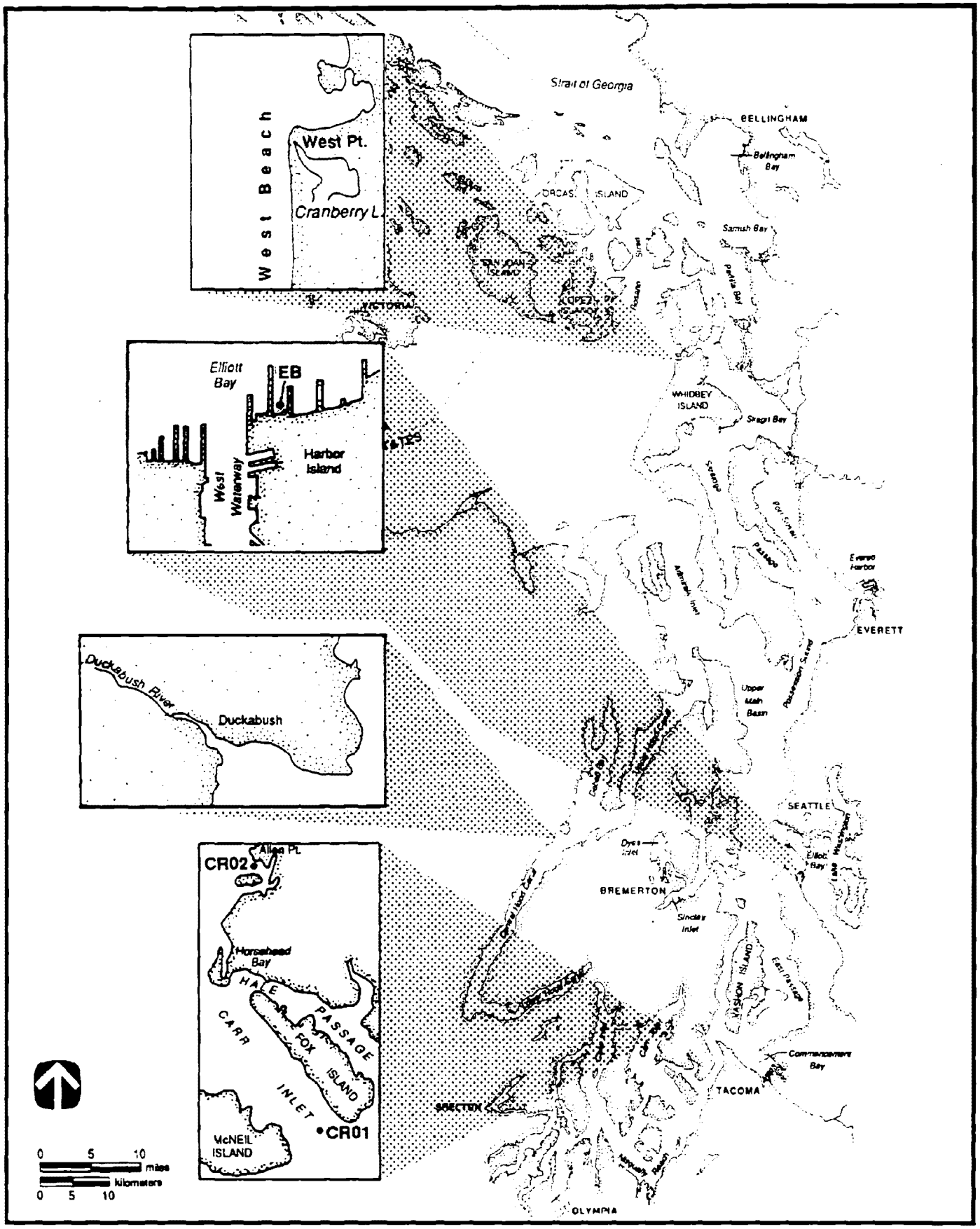

Figure 2. Location of sediment collection sites

Elliott Bay and Carr Inlet Sediments

In Elliott Bay, sediments were collected from a station in Elliott Bay at the north end of Harbor Island (Figure 2). Pastorok and Becker (1989) and Beller et al. (1988) showed that this area of Elliott Bay is contaminated by a complex mixture of chemicals including organic compounds [e.g., polycyclic aromatic hydrocarbons (PAH), pentachlorophenol (PCP), and polychlorinated biphenyls (PCB)] and metals (e.g., arsenic, copper, and mercury). Sediments collected from this station were used as one of the test sediments for the *Neanthes* test demonstration study (Johns 1988) and in a recent bioassay comparison study conducted by Pastorok and Becker (1989).

Of the uncontaminated sediments collected from two stations sampled in Carr Inlet (Figure 2), sediment from station CR01 was used as the reference material for all tests conducted as part of this study. Carr Inlet sediments have been found to be relatively free of contaminants and have been used previously as a reference sediment for bioassays (PTI 1988, 1989). Therefore, sediment from Carr Inlet could serve as a suitable reference sediment for conducting bioassays. Sediment obtained from the second station in Carr Inlet (CR02) was used in a test designed to evaluate the effect of grain size on the survival and growth of *Neanthes* juveniles during a 20-day exposure period.

At both the Elliott Bay and the Carr Inlet stations, approximately 24 liters of sediment were collected using a 0.1-m^2 van Veen bottom grab sampler. To obtain the desired amount of sediment, multiple casts of the sampler were required. All samples from a single station were placed in a plastic bucket and later homogenized in the laboratory. Prior to homogenization, the sediments were press-sieved through a 1.0-mm screen to remove all large infauna and debris.

Following homogenization, sediment from each station was placed in 1-L jars. To preserve the quality of the sediment during the study period, each jar was filled to capacity with sediment and nitrogen was passed over the sediment surface prior to sealing the jar. Sediment samples were maintained at 4° C until needed for a test. Sediment used in each test originated from a full, sealed jar. Jars with unused portions of sediment samples were discarded.

West Beach Sediment

Sediment collected from West Beach on Whidbey Island (Figure 2) was used as the control sediment for all tests. West Beach sediment was found to be a suitable substrate for *Neanthes* juvenile survival and growth by Johns (1988). West Beach sediment was collected within 1 week of beginning each test. Test sediment was obtained from subtidal areas using an epibenthic dredge. In the laboratory, the sediment was sieved through a 0.5-mm screen and stored in plastic containers until needed.

Duckabush River Sediment

Four sediment samples were also collected along a salinity gradient at the mouth of the Duckabush River, which is located south of Brinnon on the western shore of Hood Canal (Figure 2). Prior to obtaining a sediment sample, the pore-water salinity was determined using a portable refractometer. Pore water was obtained by removing a small amount of water-saturated sediment from an intertidal area along the river, then allowing water to collect into the hole. Once

a sampling site was identified as having the appropriate sediment based on pore-water salinity, surficial sediment (i.e., upper 2 cm) was collected using a polypropylene scoop. Sediment collected from each station was placed in a stainless steel bowl, homogenized using the scoop, and placed in 1-L jars. Two liters of sediment were collected at each station. The four Duckabush River stations that were sampled represent sediments with interstitial salinities of 19, 22, 25, and 30 parts per thousand (ppt).

SEDIMENT CHEMISTRY

Chemical analyses of sediment samples collected from Carr Inlet and Elliott Bay were performed for both organic compounds and metals (Table 2). Conventional sediment variables were also measured, including grain size and total organic carbon (TOC) (PSEP 1986). Concentrations of organic compounds were determined following modified U.S. Environmental Protection Agency (EPA) Contract Laboratory Program (CLP) protocols (U.S. EPA 1986a). The analysis of semivolatile compounds, including acid/base/neutral (ABN) extractables, PCBs, and pesticides, followed modified EPA CLP protocols that were consistent with PSEP recommendations for analyses with relatively low detection limits. In particular, modifications included larger sample size (typically 50-100 grams dry weight) and smaller final extract volume for gas chromatography/mass spectroscopy (GC/MS) analyses. Separate sediment subsamples were used for ABN and pesticide/PCB extractions. Ultrasonic extraction was carried out as described by the CLP procedure. Gel permeation chromatography (GPC), an optional cleanup step under CLP, was performed for all sediment ABN extracts to reduce interferences and attain necessary detection limits. Pesticide/PCB analyses were conducted with a slightly modified version of the EPA CLP method. These analyses included extract cleanup by alumina column chromatography and, when necessary, elemental sulfur cleanup, followed by gas chromatography/electron capture detection (GC/ECD) analysis. GC/ECD quantification and confirmation analyses were conducted with fused silica capillary columns rather than the packed columns routinely used in CLP.

For metals, samples were first digested using the total acid digestion technique in the EPA CLP program (U.S. EPA 1986a). Metals in sediment digestates were determined by graphite furnace atomic absorption or by direct-flame atomic absorption spectrometry (except for mercury, which was determined using cold vapor atomic absorption spectrometry).

BIOLOGICAL TESTING

All tests were conducted using juvenile laboratory-cultured *Neanthes* obtained from Dr. Donald Reish at California State University, Long Beach. The bioassay approach used in this study was based on the interim protocol for conducting a sublethal bioassay with juvenile *Neanthes* sp. described by Johns et al. (1989).

The interim protocol calls for the *Neanthes* bioassay to be conducted using a static renewal exposure system (Figure 3). Each exposure system consists of a 1-L jar containing 2 cm of sediment and seawater (at salinities between 28 and 30 ppt). Worms in each exposure chamber are provided with 40 mg of food (TetraMarin®) every second day during the exposure period.

Every third day, one-third of the water volume is exchanged with fresh seawater. Measurements of dissolved oxygen, pH, salinity, and temperature are made prior to each seawater exchange.

TABLE 2. CHEMICALS ANALYZED IN TEST SEDIMENTS

Metals

antimony	copper	nickel
arsenic	lead	silver
cadmium	mercury	zinc

Phenols and Substituted Phenols

phenol	2,4-dimethylphenol
2-methylphenol	pentachlorophenol
4-methylphenol	

Low Molecular Weight Polycyclic Aromatic Hydrocarbons

naphthalene	phenanthrene
acenaphthylene	anthracene
acenaphthene	2-methylnaphthalene
fluorene	

High Molecular Weight Polycyclic Aromatic Hydrocarbons

fluoranthene	benzo(a)pyrene
pyrene	indeno(1,2,3-c,d)pyrene
benz(a)anthracene	dibenzo(a,h)anthracene
chrysene	benzo(g,h,i)perylene
benzofluoranthenes	

Chlorinated Aromatic Hydrocarbons

1,2-dichlorobenzene	1,2,4-trichlorobenzene
1,3-dichlorobenzene	hexachlorobenzene (HCB)
1,4-dichlorobenzene	

Polychlorinated Biphenyls

total PCB (mono- through decachlorobiphenyls)

Chlorinated Aliphatic Hydrocarbons

hexachlorobutadiene	hexachloroethane

Phthalate Esters

dimethyl phthalate	butyl benzyl phthalate
diethyl phthalate	bis(2-ethylhexyl)phthalate
di-n-butyl phthalate	di-n-octyl phthalate

TABLE 2. (Continued)

Miscellaneous Oxygenated Compounds	
benzyl alcohol	benzoic acid
dibenzofuran	
Organonitrogen Compounds	
N-nitrosodiphenylamine	
Pesticides	
total DDTs (p,p')	aldrin
heptachlor	dieldrin
α-chlordane	γ-HCH (lindane)

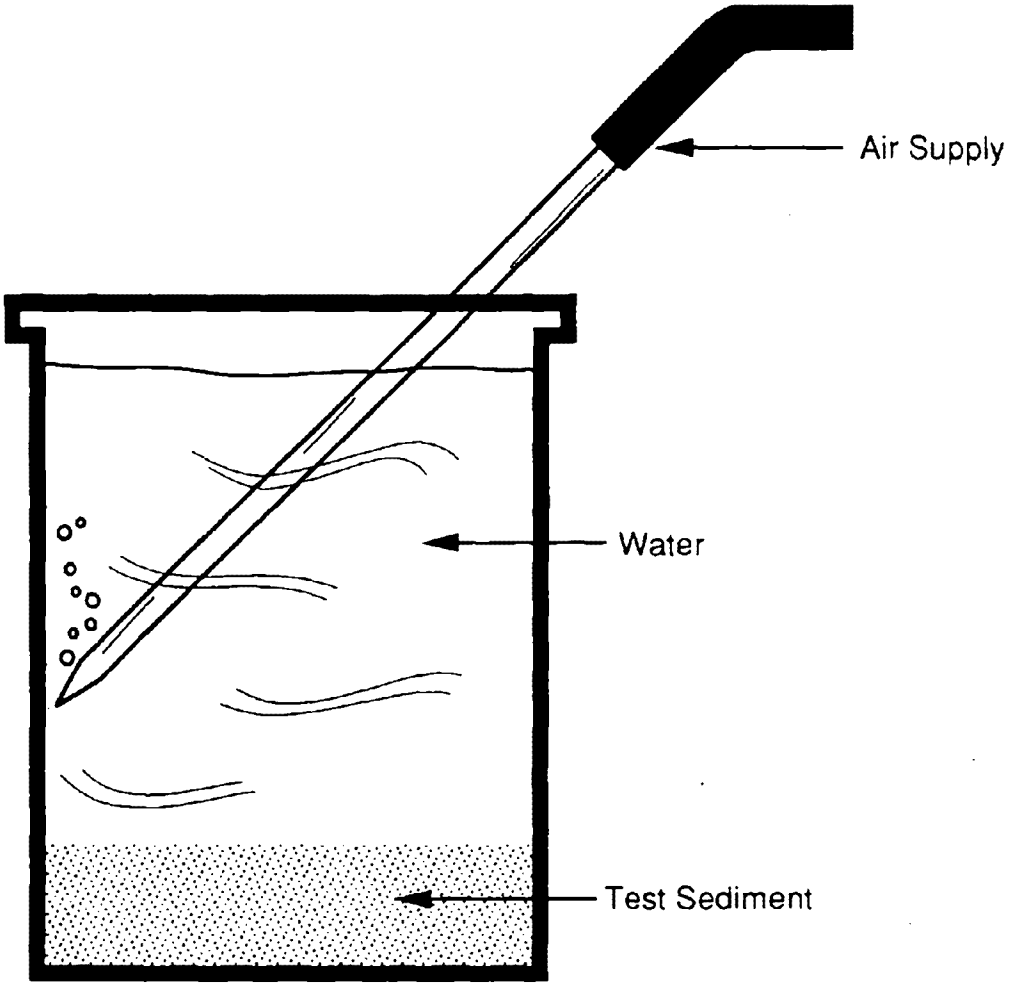

Figure 3. Static exposure system used for the *Neanthes* sublethal bioassay

Following a 20-day exposure period, all surviving worms from each chamber are dried to a constant weight and their biomass is determined. To determine biomass, the worms are quickly rinsed in distilled water, dried at 50° C for 24 hours, and then weighed to the nearest 0.1 mg. Three response criteria are examined: survival, total biomass (as dry weight), and average individual biomass (i.e., total biomass divided by the number of surviving worms).

Because the main focus of this study was to experimentally evaluate recommended changes in the protocol, modifications to the interim test protocol were required. However, in all test series, at least one treatment followed the interim protocol. The following experiments were conducted as part of this study to evaluate recommended changes to the interim protocol.

Worm Density

An experiment was conducted to determine if the number of organisms placed in each exposure chamber should be increased from the presently recommended number of 5 worms per chamber. The test was conducted with four densities of worms: 5, 10, 15, and 20 worms per chamber. Sediments used in the test included samples collected from Elliott Bay, Carr Inlet, and West Beach. All four worm density treatments were tested with the Elliott Bay and Carr Inlet sediments, while only the lowest density treatment was tested with the West Beach sediment (Table 1). There were five replicates of each density/sediment combination.

All treatments were given the same food ration (40 mg of TetraMarin® every other day), regardless of worm density. Other bioassay conditions were held constant for all treatments based on the interim protocol. Following the 20-day exposure period, surviving worms from each treatment were collected and dried to a constant weight and total biomass was determined.

Food Ration

The amount of food that should be provided to each chamber during the exposure period was examined by conducting a food ration experiment. The interim protocol calls for 40 mg of food every second day of exposure. A total of five food ration treatments were tested, ranging from no food to 80 mg (dry weight) of TetraMarin® (i.e., 0, 20, 40, 60, or 80 mg/48 hours). The appropriate food ration was provided to each exposure chamber every second day. The number of worms placed in each exposure chamber was based on test results from the previously described experiment.

Sediments used in this test included samples collected from Elliott Bay, Carr Inlet, and West Beach. All five food ration levels were used with Elliott Bay and Carr Inlet sediments, while only three of the five ration levels (i.e., 0, 40, and 80 mg/48 hours) were used with sediment collected from West Beach (Table 1).

Other bioassay conditions were held constant based on the interim protocol. Following the 20-day exposure period, surviving worms from each treatment were collected and dried to a constant weight and total biomass was determined.

Static vs. Static Renewal Exposure System

An experiment was conducted to determine whether the *Neanthes* sublethal bioassay could be conducted under static exposure conditions. Current protocol recommends the use of a static renewal exposure system in which one-third of the exposure water is exchanged with fresh seawater every third day during the exposure period. A side-by-side comparison of the static and static renewal exposure systems was used to evaluate the need for the static renewal exposure system. The test was conducted with sediments collected from Elliott Bay, Carr Inlet, and West Beach (Table 1).

The number of worms placed in each container and the amount of food provided during the exposure period was based on the results of the previous two experiments. Other bioassay conditions were held constant for all treatments based on the interim protocol. Following the 20-day period, surviving worms from each treatment were collected and dried to a constant weight and total biomass was determined.

During the exposure period, dissolved oxygen, pH, salinity, and temperature were measured every third day just prior to exchanging water in the static renewal exposure system. In addition to measuring the above parameters, water samples were taken periodically to determine the concentration of ammonia and selected contaminants present in the static and static renewal exposure systems. The sampling schedule for water quality is presented in Table 3. Ammonia concentrations were determined for both the static and static renewal exposure systems containing Elliott Bay and Carr Inlet sediments following standard methods published by AMPHA (1985).

Contaminants assayed included PAH (Table 4), copper, and mercury. Both dissolved and total concentrations of copper and mercury were determined. Contaminant concentrations were determined for both the static and static renewal exposure systems, but only for treatments containing Elliott Bay sediment. Water samples for chemical analysis of the selected contaminants were obtained from extra exposure chambers having the same sediment volume and number of juvenile *Neanthes* as all other exposure chambers used in the test. Thirty minutes prior to collecting a water sample, aeration to the exposure chamber was stopped to allow particle settling.

Concentrations of PAH were analyzed by U.S. EPA SW-846 Method 8100 (U.S. EPA 1986b), which employs dual column gas chromatography with flame ionization detection. Total copper was digested by U.S. EPA SW-846 Method 3020 (U.S. EPA 1986b) and analyzed by Method 6010 using furnace atomic absorption spectroscopy. Dissolved copper was determined using Method 6010 after the sample was filtered through a 0.45-μm filter. The concentration of total mercury was determined by Method 7470 using cold vapor atomic absorption spectrometry (U.S. EPA 1986b). Dissolved mercury was determined using Method 7470 after the sample was filtered through a 0.45-μm filter.

Test Duration

An experiment was conducted to determine the optimal exposure period that can be used with the *Neanthes* sublethal bioassay to detect a statistically significant level of organism response. Exposure periods evaluated were durations of 10, 15, and 20 days. Sediments used in this test were collected from Elliott Bay, Carr Inlet, and West Beach (Table 1). Five replicates were used for each treatment and exposure period combination.

TABLE 3. SAMPLING SCHEDULE FOR WATER QUALITY ANALYSIS

Sediment	Exposure System	Water Quality Parameter	Sampling Date (Days) 3	6	12	20
Carr Inlet	Static	Ammonia	--[a]	X	X	X
	Static renewal	Ammonia	X	X	X	X
Elliott Bay	Static	Ammonia	X	X	X	X
		PAH[b]	X	X	X	X
		Metals	X	X	X	X
	Static renewal	Ammonia	--[a]	X	X	X
		PAH	--[a]	X	X	X
		Metals	--[a]	X	X	X

[a] Sample not taken for this exposure condition at these sampling dates.

[b] PAH - polycyclic aromatic hydrocarbons.

TABLE 4. POLYCYCLIC AROMATIC HYDROCARBONS MEASURED IN WATER SAMPLES TAKEN FROM THE STATIC AND STATIC RENEWAL EXPOSURE SYSTEMS CONTAINING ELLIOTT BAY SEDIMENTS

Low Molecular Weight Polycyclic Aromatic Hydrocarbons	
naphthalene	phenanthrene
acenaphthylene	anthracene
acenaphthene	2-methylnaphthalene
fluorene	

High Molecular Weight Polycyclic Aromatic Hydrocarbons	
fluoranthene	benzo(a)pyrene
pyrene	indeno(1,2,3-c,d)pyrene
benz(a)anthracene	dibenzo(a,h)anthracene
chrysene	benzo(g,h,i)perylene
benzofluoranthenes	

The test was conducted using the number of organisms, food ration, and exposure system identified as the most appropriate based upon the results of the previously described experiments. Other bioassay conditions were held constant for all treatments based on the interim protocol. Following exposure periods of 10, 15, and 20 days, surviving worms from the five replicates of each treatment were collected and dried to a constant weight and total biomass was determined.

Salinity Tolerance

Two experiments were conducted to determine the salinity tolerance of *Neanthes*. The first test was an acute bioassay to determine the 96-hour tolerance of juvenile *Neanthes* to low-salinity water. Salinities tested were 10, 15, 20, 25, and 28 ppt.

Water of the appropriate test salinity was prepared by taking field-collected seawater at 28 ppt and diluting samples of the water with distilled water until the desired salinity was attained. Salinity was determined using a hand-held refractometer. The test was conducted without sediment. The worms were not fed during the 96-hour exposure period.

Five worms were placed in 1-L beakers containing 500 mL of water of the appropriate salinity. Water quality conditions (i.e., dissolved oxygen, pH, temperature, and salinity) and the number of surviving worms were determined on a daily basis. Two replicates of each salinity treatment were used in this experiment.

The second salinity tolerance experiment was conducted using sediment samples collected along a salinity gradient on the Duckabush River. Interstitial salinities of the four sediments collected were 30, 25, 22, and 19 ppt. Sediments from Carr Inlet and West Beach were also included in this salinity tolerance experiment. Interstitial salinity of the Carr Inlet and West Beach sediments was between 28 and 30 ppt. The test was conducted following the interim protocol. In all treatments, water overlying the sediment was 28 ppt.

To reduce the amount of initial mixing between the interstitial water and the overlying water, water placed in each exposure chamber was allowed to slowly flow down the inside of the exposure chamber. Following the 20-day exposure period, surviving worms from each treatment were collected and dried to a constant weight and total biomass was determined.

Sensitivity to Sediment Grain Size

The influence of sediment grain size on juvenile *Neanthes* survival and growth was determined following a 20-day exposure to sediments having differing granulometry (expressed as a percentage of the silt/clay fraction in the sediment). In particular, the test was designed to evaluate the potential effects on survival and growth of sediments containing high silt/clay fractions (>88 percent). Six sediment treatments were used in this test, including three field-collected sediments (i.e., two stations in Carr Inlet, stations CR01 and CR02, and one from West Beach). A fourth treatment, CR03, was prepared by collecting that portion of Carr Inlet sediment from station CR02 that passed through a 125-μm screen. The fifth and sixth sediment treatments, designated CR04 and CR05, were prepared by mixing equal weights of sediments from CR01 and CR02, and CR02 and CR03, respectively.

The test was conducted using the interim protocol. Following the 20-day exposure period, surviving worms from each treatment were collected and dried to a constant weight and total biomass was determined.

Sensitivity of *Neanthes* to a Reference Toxicant

Two experiments were conducted to determine the sensitivity of *Neanthes* juveniles to a reference toxicant, cadmium chloride ($CdCl_2$). The first test was a 96-hour toxicity test to determine the range of Cd concentrations to which *Neanthes* is acutely responsive. Concentrations used in the test were 1, 10, 100, and 1,000 parts per million (ppm) Cd.

Based on the results of the first test, a second 96-hour toxicity test was conducted to further define the 96-hour LC_{50} values for *Neanthes* juveniles exposed to Cd as $CdCl_2$. Concentrations used in this test were 10, 18, 32, 56, and 100 ppm Cd. LC_{50} values were determined using probit analyses (Peltier and Weber 1985).

In both experiments, five worms were placed in 1-L jars containing 500 mL of seawater at the appropriate Cd concentration. Three replicates of each Cd concentration were used. The tests were conducted without sediment and the worms were not fed during the 96-hour exposure period. Water quality conditions (i.e., dissolved oxygen, pH, temperature, and salinity) were measured on a daily basis. The number of surviving worms was determined at the end of the 96-hour period.

DATA ANALYSIS

Pairwise statistical comparisons were used to evaluate statistical differences. This approach to statistical testing is typically used to analyze sediment bioassays conducted for environmental regulatory programs and involves pairwise testing of a potentially contaminated sample against a reference sample. To evaluate the recommended changes in the bioassay protocol, pairwise comparisons were made between the relatively uncontaminated Carr Inlet and the contaminated Elliott Bay sediment treatments having similar exposure conditions.

Prior to conducting the pairwise comparisons, treatment pairs were tested for homogeneity of variances using a Cochran's *C*-test. In several treatment pairs, variances were found to be heterogeneous ($P \leq 0.05$). In some cases, variance homogeneity could not be tested because no variance estimate could be made for one or both of the data pairs. This situation arose with survival data when the survival in all treatment replicates was 100 percent.

Approximately 30 percent of the pairwise comparisons evaluated for this study failed to meet the variance assumption required when using the more common parametric tests [e.g., *t*-test and one-way analysis of variance (ANOVA)]. Therefore, all pairwise comparisons were made using a nonparametric test, the Mann-Whitney *U*-test.

In some experiments, additional statistical evaluations of the test data were conducted using ANOVA across all treatments having the same sediment. If significant differences were found, a Student-Newman-Keuls procedure was conducted to identify nonsignificant subsets of treatments.

Statistical power analysis was used to evaluate the experimental results for the number of test organisms and test duration. Statistical power analyses were conducted using a one-way ANOVA

model (Scheffe 1959; Cohen 1977). For these analyses, the within-group mean square for the ANOVA was used as an estimate of residual variance. The results of comparisons of contaminated vs. reference sediments among different numbers of test organisms and exposure times were evaluated as minimum detectable difference (MDD) and corresponding power for a fixed design [i.e., replicate number, treatment number, and significance level (α)]. The predicted MDD between the mean response to a contaminated sample and the mean response to a reference sample at a specified power was used as a measure of the bioassay performance among alternative numbers of organisms and alternative exposure times.

RESULTS AND DISCUSSION

SEDIMENT CHEMISTRY

This section provides an overview of chemical concentrations found in each sediment and of general sediment quality based on comparisons to 1988 Puget Sound apparent effects threshold (AET) values normalized to sediment dry weight (Barrick et al. 1988). AET values provide an estimate of the concentration of each chemical that may be associated with adverse effects in Puget Sound. Data on the chemical concentrations and conventional variables measured in sediments collected from both Carr Inlet stations (i.e., stations CR01 and CR02) and Elliott Bay are presented in Appendix A.

Sediments from both Carr Inlet stations are relatively fine-grained (>88 percent silt/clay) and contain levels of TOC (1.3 and 0.93 percent for Carr Inlet stations CR01 and CR02, respectively) commonly found in Puget Sound sediments.

Sediments from Carr Inlet contain relatively low concentrations of organic compounds and metals. Several PAH compounds were detected at Carr Inlet, including phenanthrene, anthracene, and chrysene, but all concentrations were below 100 parts per billion (ppb). None of the organic contaminants detected in sediments collected from Carr Inlet exceeded any of the 1988 bioassay AET values (i.e., amphipod bioassay, oyster larvae bioassay, and Microtox). Arsenic, cadmium, copper, lead, and mercury were detected in both Carr Inlet sediments; however, none of the concentrations exceeded the 1988 bioassay AET values.

Sediment from the Elliott Bay station is moderately fine-grained (> 47 percent silt/clay) and has a TOC content of 2.1 percent. This sediment was highly contaminated with several organic compounds and metals. Organic contaminants exceeding one or more of the 1988 bioassay AET values include low molecular weight PAH, high molecular weight PAH, phthalates, 2-methylphenol, PCP, and PCBs (Table 5). Copper and mercury exceeded all three bioassay AET values (i.e., amphipod bioassay, oyster larvae bioassay, and Microtox). Zinc and arsenic exceeded only the amphipod AET.

BIOLOGICAL TESTING

Worm Density

Mean survival of *Neanthes* juveniles following a 20-day exposure to Elliott Bay, Carr Inlet, and West Beach sediments ranged from a high of 100 percent to a low of 36 percent (Table 6; Figure 4). Survival was greater than 95 percent for all treatments containing Carr Inlet and West Beach sediments, regardless of density, and for Elliott Bay sediment where the density was five worms per chamber. The coefficient of variation (mean value divided by the standard deviation) for the survival data for these treatments was low, ranging from 0 to 9 percent.

TABLE 5. CHEMICAL CONTAMINANTS IN ELLIOTT BAY SEDIMENT EXCEEDING 1988 BIOASSAY AET

Chemical	Elliott Bay Sediment Concentration[a]	AET Exceedances[b]
Metals		
Arsenic	112	A
Copper	1,490	A,M,O
Mercury	3.5	A,M,O
Zinc	1,010	A
Organic Compounds		
LPAH		
Acenaphthene	780	M,O
Fluorene	790	M,O
Phenanthrene	4,800	M,O
Anthracene	1,900	M,O
HPAH		
Fluoranthene	8,100	M,O
Pyrene	12,000	M,O
Benz(a)anthracene	4,000	M,O
Chrysene	3,300	M,O
Benzofluoranthenes	10,000	A,M,O
Benzo(a)pyrene	8,900	A,M,O
Indeno(1,2,3-c,d)pyrene	1,600	M,O
Dibenzo(a,h)anthracene	710	A,M,O
Benzo(g,h,i)perylene	3,600	A,M,O
Phthalates		
Dimethyl phthalate	110	M
Butyl benzyl phthalate	320	M
Bis(2-ethylhexyl)phthalate	6,100	M,O
Phenols		
2-methylphenol	78	A,M,O
Pentachlorophenol	1,900	A,M,O
Total PCB	1,460	M,O

[a] Metals concentrations are reported in mg/kg dry weight. Concentrations of organic compounds are reported in μg/kg dry weight.

[b] A - amphipod mortality test
M - Microtox test (saline extract)
O - oyster larvae abnormality.

TABLE 6. SURVIVAL, TOTAL BIOMASS, AND AVERAGE INDIVIDUAL BIOMASS DATA FOR THE WORM DENSITY EXPERIMENT

Sediment	Worm Density (no. worms/ chamber)	Survival[a]	Total Biomass[a] (mg dry weight)	Average Individual Biomass[a,b] (mg dry weight)
West Beach	5	95.0 ± 4.5	22.6 ± 6.2	4.6 ± 0.7
Carr Inlet	5	96.0 ± 4.0	79.9 ± 6.1	16.7 ± 1.2
	10	98.0 ± 2.0	111.6 ± 7.1	11.9 ± 0.8
	15	98.6 ± 1.4	134.4 ± 10.2	9.0 ± 0.6
	20	97.0 ± 2.0	147.5 ± 9.5	7.6 ± 0.6
Elliott Bay	5	100 ± 0.0	17.7 ± 4.4	3.5 ± 0.9
	10	72.0 ± 9.2	24.5 ± 6.4	3.3 ± 0.6
	15	41.2 ± 14.3	17.4 ± 8.9	1.9 ± 0.7
	20	36 ± 12.1	21.8 ± 10.0	2.1 ± 0.7

[a] Value reported as mean ± standard error.

[b] Biomass of worms at test initiation was 1.0 ± 0.2 mg (dry weight).

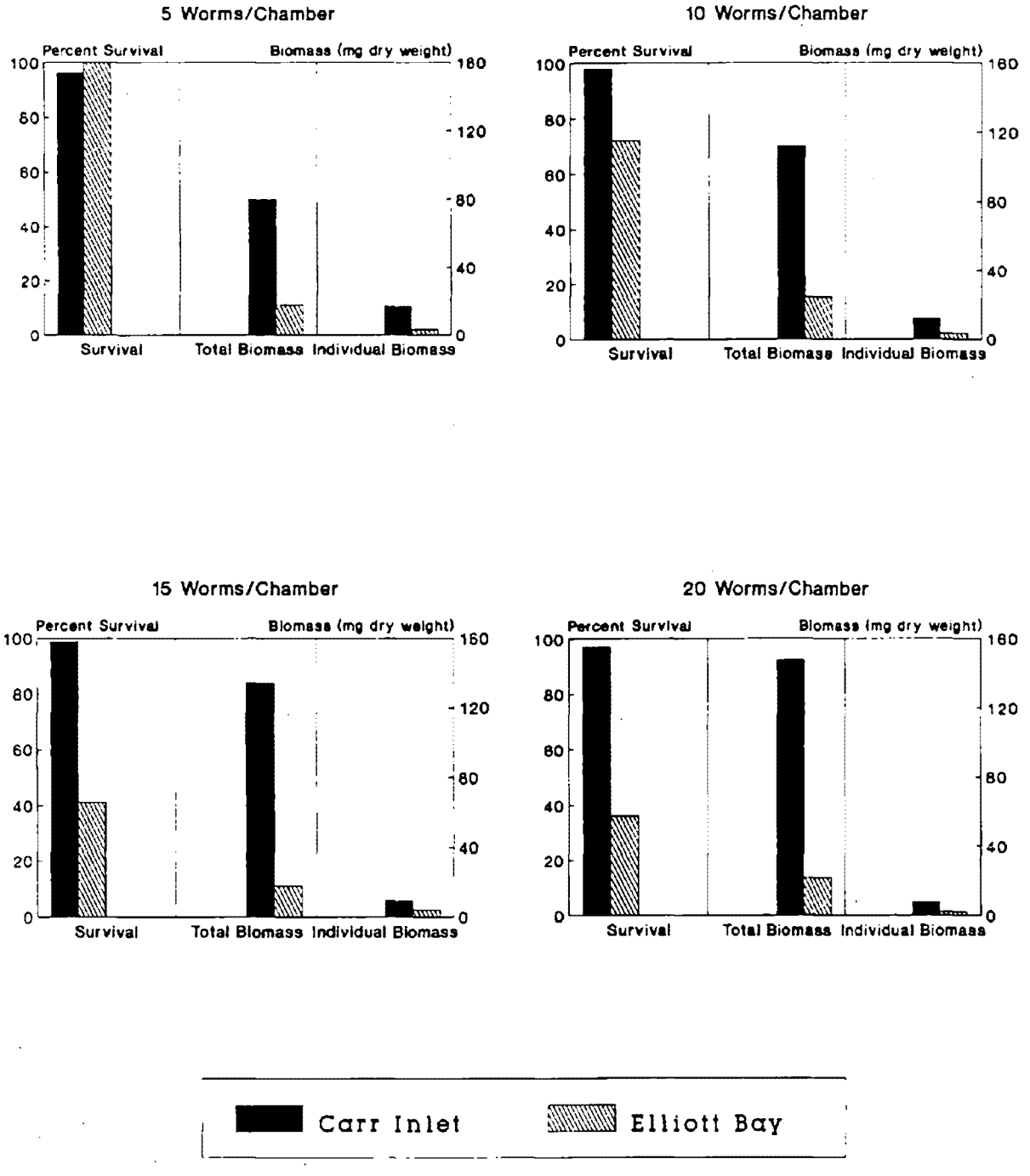

Figure 4. Survival and biomass of juvenile worms from the worm density experiment

In contrast to the Carr Inlet sediment exposures, survival was dependent upon worm density in the Elliott Bay sediments. The lowest survival rates were found in Elliott Bay treatments containing 10, 15, and 20 worms per exposure chamber. In all cases, survival was not greater than 72 percent and decreased with increasing worm density (Table 6). In contrast to the treatments exhibiting high survival rates, the coefficient of variation for treatments with low survival rates was high, ranging from 21 to 38 percent.

A pairwise comparison of survival rates between Carr Inlet and Elliott Bay treatments with the same worm densities showed that significant differences in survival rate existed for some treatment pairs. No significant differences were found in survival between the Carr Inlet and Elliott Bay sediments having a worm density of five worms per chamber. However, significant differences were noted at all other worm densities (Table 7).

For both the Carr Inlet and Elliott Bay sediment treatments, the total biomass of tissue collected from each treatment increased with the number of worms remaining in the replicate chamber (Table 6; Figure 4). Highest total biomass values at a given worm density were found for worms collected from the Carr Inlet sediment. Significant differences were noted in total biomass for all Carr Inlet and Elliott Bay treatment pairs having the same worm density (Table 7). In all cases, Carr Inlet treatments exhibited significantly higher total biomass than did Elliott Bay treatments.

An ANOVA of the data for treatments containing Carr Inlet sediment indicates significant differences in total biomass among the worm density treatments. An *a posteriori* analysis showed that total biomass was lowest at a density of five worms, with other worm densities forming two overlapping, nonsignificant subsets (Table 8). A similar comparison for the Elliott Bay sediment indicated that no significant differences were found in total biomass among the worm density treatments.

Average individual biomass (total biomass divided by the number of surviving worms per exposure chamber) decreased with the number of surviving worms in each exposure chamber (Table 6). Highest average individual biomass values were found for worms collected from the Carr Inlet sediment. Significant differences were noted in average individual biomass for all Carr Inlet and Elliott Bay treatment pairs having the same worm density (Table 7). In all cases, Carr Inlet treatments exhibited significantly higher average individual biomass than the Elliott Bay treatments.

The greatest numerical difference between treatment pairs occurred at a density of five worms. At this density, average worm growth in Elliott Bay sediment was only about 21 percent of the growth in Carr Inlet sediments. This results from two factors. First, average worm growth in Carr Inlet sediment decreased with increasing worm density, probably resulting from food limitation and possibly from aggressive interaction among worms. Second, the average individual biomass in contaminated Elliott Bay sediments was consistently low and did not change as a function of worm density.

An ANOVA of the individual biomass data for treatments containing Carr Inlet sediment indicates significant differences among the treatments (Table 8). An *a posteriori* analyses indicated that higher individual biomass was measured at a density of 5 worms. Lowest individual biomass occurred at densities of 15 and 20 worms and these data were not distinguishable from each other. No significant differences were noted in average individual biomass among the worm density treatments in the Elliott Bay sediment.

TABLE 7. PAIRWISE STATISTICAL COMPARISON OF *NEANTHES* BIOASSAY RESPONSES FOR THE WORM DENSITY EXPERIMENT

Worm Density (no. worms/ chamber)[a]	Treatment Comparison[b]	Response	Comparison Result[c]
5	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*
10	EB vs. CI	Percent survival	*
		Total biomass	*
		Individual biomass	*
15	EB vs. CI	Percent survival	*
		Total biomass	*
		Individual biomass	*
20	EB vs. CI	Percent survival	*
		Total biomass	*
		Individual biomass	*

[a] Worm density treatment based on the initial number of worms placed in each exposure chamber.

[b] EB - Elliott Bay
CI - Carr Inlet.

[c] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

TABLE 8. RESULTS OF ONE-WAY ANALYSIS OF VARIANCE FOR BIOMASS DATA FROM THE WORM DENSITY EXPERIMENT

Sediment Type	Response Parameter	ANOVA Results[a]	Treatment Grouping[b]			
Carr Inlet	Total biomass	*	CI5	CI10	CI15	CI20
	Individual biomass	*	CI5	CI10	CI15	CI20
Elliott Bay	Total biomass	ns				
	Individual biomass	ns				

[a] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

[b] Treatments grouped by the same line are not significantly different based on an *a posteriori* analysis.

A power analysis model was used to estimate the ability to statistically discriminate differences in average individual biomass between treatments containing Carr Inlet and Elliott Bay sediments. At all four worm densities, the ability to detect significant differences between the two sediments increases as the difference in average biomass increases (Figure 5). Treatments containing 5, 10, or 20 worms per chamber displayed similar levels of statistical power to detect a given difference in biomass between sediment types. For example, at three densities (5, 10, and 20) there is a power of about 0.8 to detect a 40-50 percent difference in the overall mean. At a density of 15 worms, the power was considerably less, resulting in a power of only 0.4 to detect a 50 percent difference in overall mean individual biomass.

Based on the results of this experiment, it was concluded that the *Neanthes* sublethal bioassay can be successfully conducted with worm densities ranging from 5 to 20 worms per exposure chamber. Worms survived in all treatment combinations, and sufficient tissue was available at the end of the 20-day exposure period to determine total and individual biomass.

Because the primary response criterion for the *Neanthes* sublethal bioassay is a change in biomass, the recommended worm density at which the bioassay should be conducted should be one at which worm survival is maximized. Maximization of survival will ensure the presence of sufficient tissue to determine biomass at the conclusion of the exposure period. Although survival was high at all worm densities for treatments containing West Beach and Carr Inlet sediments, survival decreased as worm density increased in the contaminated sediment from Elliott Bay. Only at a worm density of five worms per chamber was the survival in the Elliott Bay sediments greater than 80 percent.

Results of the power analysis indicate that at the lower worm density, relatively small changes in biomass can be statistically detected. Increasing the number of worms per exposure chamber results in no increased ability to detect significant effects. Due to the observed high survival rate, the cost savings using only five worms, and because sufficient statistical power can be achieved at the density of five worms per chamber, it is recommended that all *Neanthes* sublethal bioassays be conducted using this density. Therefore, all subsequent experiments in this study were conducted using five worms per exposure chamber.

Food Ration

Mean survival of *Neanthes* juveniles following the 20-day exposure to Elliott Bay, Carr Inlet, and West Beach sediments ranged from a high of 100 percent to a low of 0 percent (Table 9; Figure 6). Survival was consistently high (≥92 percent) for all treatments containing Carr Inlet and West Beach sediments and for the Elliott Bay sediment in which the food ration was 80 mg/ 48 hours. The coefficient of variation for the survival data for these treatments was low to moderate, ranging from 0 to 18 percent.

The lowest survival rates were found in treatments containing Elliott Bay sediment and a food ration of less than 80 mg/48 hours. In all cases, survival was lower than 76 percent, with mortality increasing as food rations decreased (Table 9). Unlike the data for treatments exhibiting high survival rates, the coefficient of variation for treatments with low survival rates was moderate to high, ranging from 17 to 43 percent.

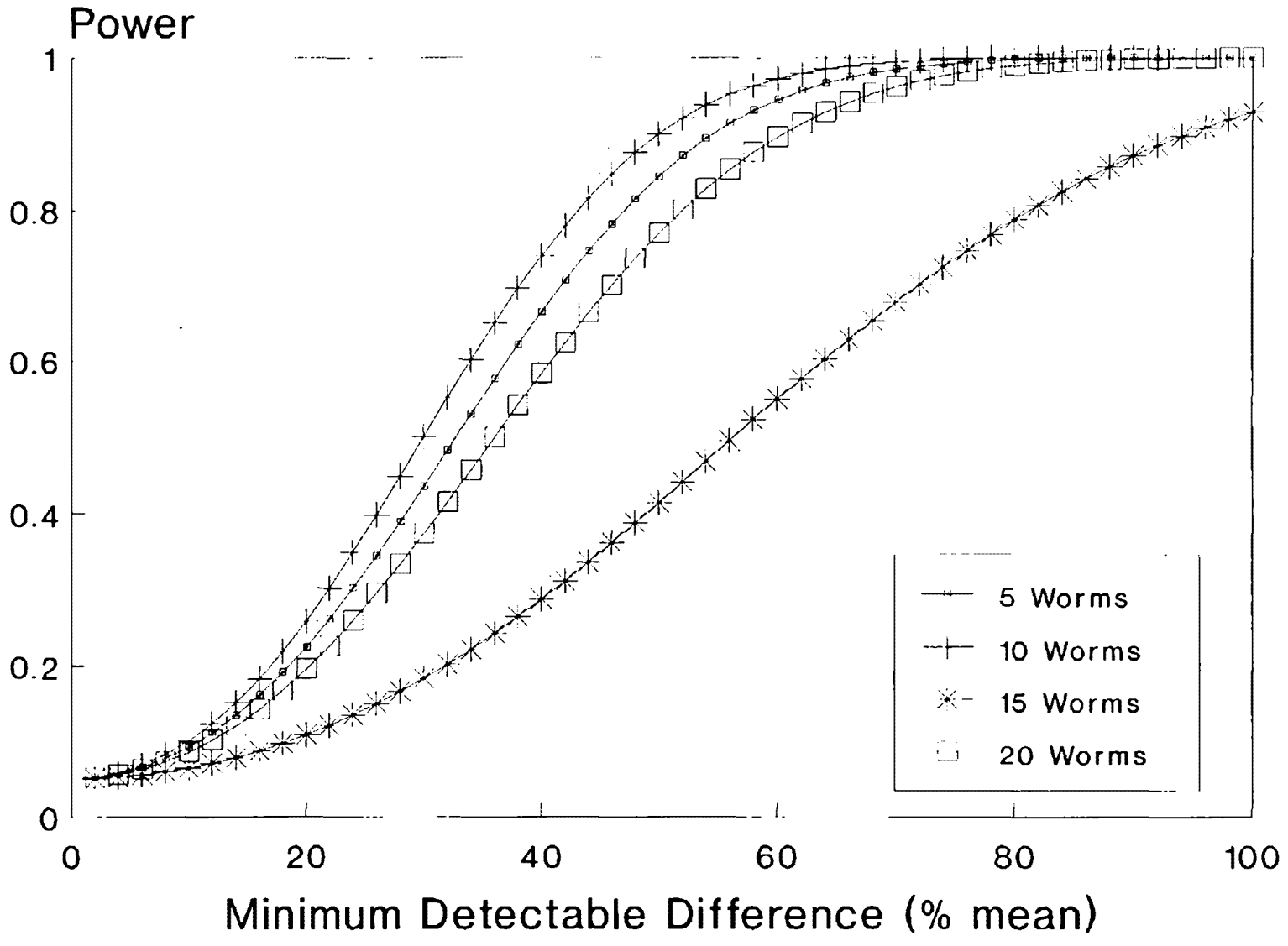

Figure 5. Statistical power vs. minimum detectable difference for total biomass in the worm density experiment

TABLE 9. SURVIVAL, TOTAL BIOMASS, AND AVERAGE INDIVIDUAL BIOMASS DATA FOR THE FOOD RATION EXPERIMENT

Sediment	Food Ration[a]	Survival[b]	Total Biomass[b] (mg dry weight)	Average Individual Biomass[b,c] (mg dry weight)
West Beach	0	100 ± 0.0	6.5 ± 0.6	1.3 ± 0.1
	40	96.0 ± 4.0	106.1 ± 5.9	22.1 ± 1.7
	80	92.0 ± 8.0	109.8 ± 9.5	23.0 ± 2.0
Carr Inlet	0	92.0 ± 4.9	2.8 ± 0.3	0.6 ± 0.1
	20	88.0 ± 4.9	65.1 ± 8.9	14.6 ± 1.3
	40	96.0 ± 4.0	112.5 ± 10.9	23.2 ± 1.6
	60	92.0 ± 4.9	124.5 ± 19.2	27.2 ± 4.6
	80	96.0 ± 4.0	163.8 ± 8.4	34.4 ± 2.3
Elliott Bay	0	0.0 ± 0.0	0.0 ± 0.0	--[d]
	20	16.0 ± 7.5	2.5 ± 1.7	4.1 ± 2.6
	40	76.0 ± 11.7	18.1 ± 3.7	4.8 ± 0.7
	60	76.0 ± 19.4	27.6 ± 7.7	7.3 ± 0.8
	80	92.0 ± 8.0	46.5 ± 11.7	9.8 ± 2.1

[a] Value presented as mg of food provided every 48 hours.

[b] Value reported as mean ± standard error.

[c] Biomass of worms at test initiation was 0.8 ± 0.1 mg (dry weight).

[d] Individual biomass could not be determined.

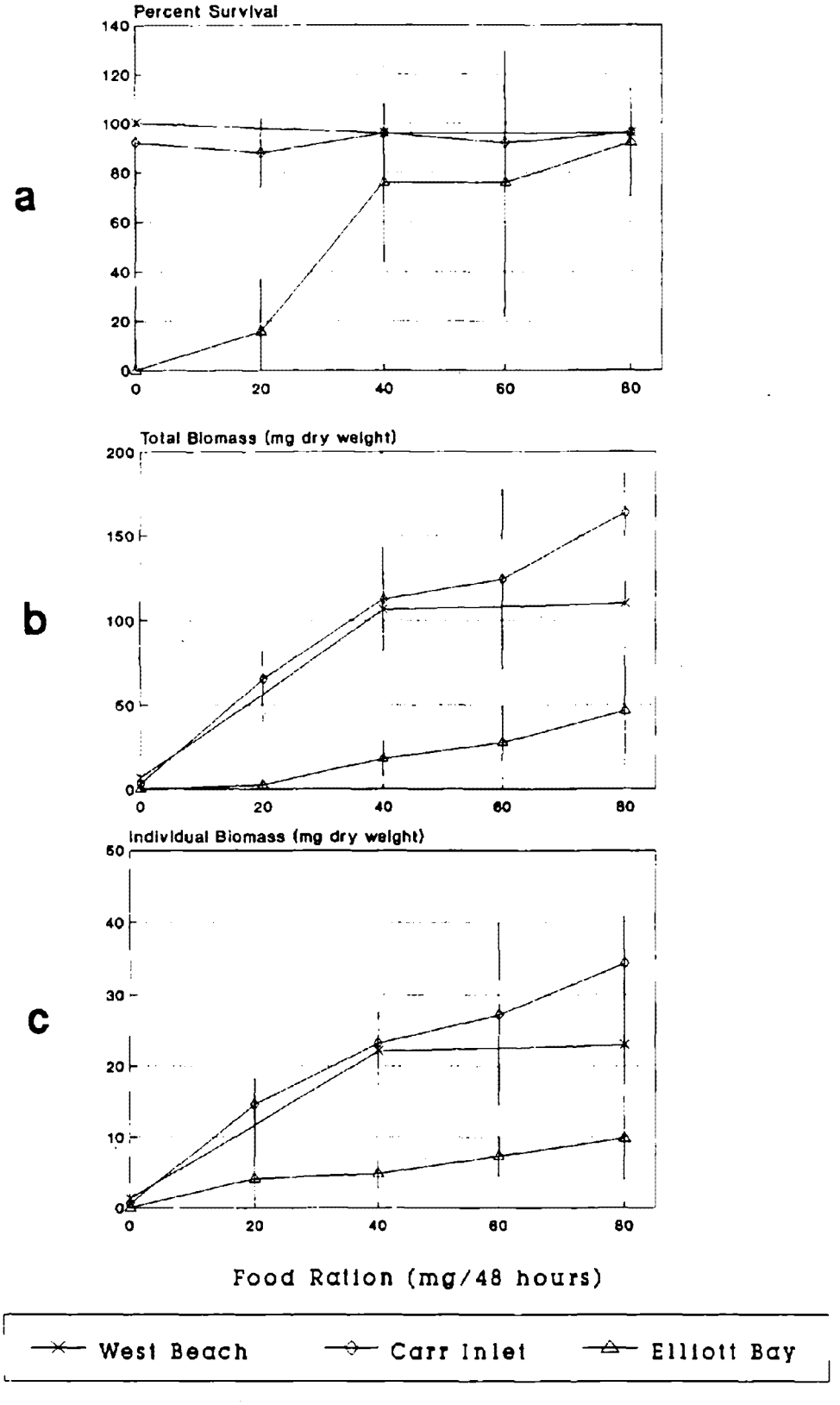

Figure 6. Survival (a), biomass (b), and individual biomass (c) of juvenile worms from the food ration experiment. Bars represent standard error.

The addition of food appeared to ameliorate the lethal effects of Elliott Bay sediments. With no added food, there was a 100 percent mortality of test organisms in Elliott Bay sediments. The addition of increasing amounts of food resulted in corresponding increases in the survival of test organisms. A pairwise comparison of survival rates between Carr Inlet and Elliott Bay treatments having a similar food ration showed that significant differences in survival rate existed only at the lower food levels (i.e., no food and 20 mg/48 hours) (Table 10).

For both Carr Inlet and Elliott Bay sediment treatments, the total biomass of tissue collected from each treatment increased with the number of worms remaining in each replicate chamber (Table 9; Figure 6). Highest total biomass values were found for worms collected from the Carr Inlet sediment. Significant differences were noted in total biomass for all Carr Inlet and Elliott Bay treatment pairs having the same worm density (Table 10). In all cases, Carr Inlet treatments exhibited significantly higher biomass values than did Elliott Bay treatments.

An ANOVA of the total biomass data for treatments containing Carr Inlet sediment indicates significant differences among the treatments (Table 11). The treatment with the highest food ration (i.e., 80 mg/48 hours) exhibited a higher total biomass than all other treatments. Significant differences in total biomass were also noted between the other treatments. An ANOVA of the total biomass data for the Elliott Bay sediment also indicates significant differences among the treatments (Table 11). Differences are due to the higher food ration treatments (i.e., 60 and 80 mg/48 hours) having a higher total biomass than treatments provided with lower food rations.

Average individual biomass increased as food rations increased (Table 9; Figure 6). Highest individual biomass values were found for worms collected from the Carr Inlet sediment. Lowest individual biomass values were observed for worms recovered from the Elliott Bay sediment.

For both biomass endpoints (total and individual), growth appeared to increase as food rations increased up to the maximum feeding rate (Figure 6). With the exception of individual biomass in the Elliott Bay treatment, the statistical results do not indicate the existence of a food saturation level up to a ration of 80 mg/48 hours. However, the relative increases in biomass with increasing food ration were generally lower from 40-80 mg/48 hours than from 0-40 mg/48 hours.

Although biomass increased as food rations increased for all three sediments tested, the Elliott Bay treatments with food rations of 60 and 80 mg/48 hours had excess food and fungal growth on the sediment surface of several exposure chambers. Excess food was also observed at the sediment surface of one replicate of the 80 mg/48 hours Carr Inlet treatments.

Because of lower survival rates in toxic sediments at food rations below 40 mg/48 hours and the fact that excess food and fungal growth were observed in treatments containing greater than 60 mg of food every 48 hours, it is recommended that a ration of 40 mg TetraMarin® be provided every 48 hours to each exposure chamber when conducting the *Neanthes* sublethal bioassay. Although the addition of no food during testing would result in a cost savings, it is not recommended because of the very low growth rate observed in all sediments. It should be noted that TOC could be used as a food source by *Neanthes* if the carbon is in a labile form and is present in high concentrations. Worms did not appear to obtain any significant nutritional value from the sediments used in this study (based on the results from the treatments containing no added food source).

TABLE 10. PAIRWISE STATISTICAL COMPARISON OF *NEANTHES* BIOASSAY RESPONSES BETWEEN ELLIOTT BAY AND CARR INLET TREATMENTS FOR THE FOOD RATION EXPERIMENT

Food Ration[a]	Treatment Comparison[b]	Response	Comparison Result[c]
No food	EB vs. CI	Percent survival	*
		Total biomass	*
		Individual biomass	*
20 mg/48 hours	EB vs. CI	Percent survival	*
		Total biomass	*
		Individual biomass	*
40 mg/48 hours	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*
60 mg/48 hours	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*
80 mg/48 hours	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*

[a] *Neanthes* were provided a level of TetraMarin® as a food source during the 20-day exposure period.

[b] EB - Elliott Bay
CI - Carr Inlet.

[c] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

TABLE 11. RESULTS OF ONE-WAY ANALYSIS OF VARIANCE FOR BIOMASS DATA FROM THE FOOD RATION EXPERIMENT

Sediment Type	Response Parameter	ANOVA Results[a]	Treatment Grouping[b]				
Carr Inlet	Total biomass	*	CI0	CI20	CI40	CI60	CI80
	Individual biomass	*	CI0	CI20	CI40	CI60	CI80
Elliott Bay	Total biomass	*	EB0	EB20	EB40	EB60	EB80
	Individual biomass	ns					

[a] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

[b] Treatments grouped by the same line are not significantly different based on an *a posteriori* analysis.

Static vs. Static Renewal Exposure System

With the exception of the Elliott Bay treatment employing the static exposure system, mean survival in all treatments was greater than 92 percent (Table 12; Figure 7). In the Elliott Bay treatment employing the static system, mean survival of the five replicates was 60 percent. The coefficient of variation for all treatments exhibiting high survival was low, ranging from 0 to 11 percent. The coefficient of variation in the one treatment with a low survival rate was 37 percent, resulting from a range of 20 to 100 percent survival in the individual replicates. A pairwise comparison of survival data between the static and static renewal exposure systems for Elliott Bay and Carr Inlet treatments showed that no significant differences ($P>0.05$) in survival rate existed (Table 13).

For each sediment type, worm growth was similar under static and static renewal conditions. No significant differences in total biomass were detected between worms exposed in the static and in the static renewal exposure systems for a given sediment type (Table 13). Significant differences in total biomass were detected, however, between worms exposed to the different sediments. Regardless of the type of exposure system employed, the total biomass of worms exposed to the Elliott Bay sediment was significantly lower than the total biomass of worms exposed to either the Carr Inlet or the West Beach sediments (Table 12).

As observed with total biomass, no significant differences in average individual biomass were detected within each sediment between worms exposed in the static and the static renewal exposure systems (Table 13). Significant differences in individual biomass were detected, however, between worms exposed to the different sediments (Table 12). The individual biomass of worms exposed to the Elliott Bay sediment in both exposure systems was significantly lower than the individual biomass of worms exposed to either the Carr Inlet or the West Beach sediments.

Monitoring of water quality during the test identified differences between the static and static renewal exposure systems. For both the Carr Inlet and Elliott Bay sediments, ammonia levels tended to be higher in exposure chambers using the static exposure system than chambers having the static renewal exposure system (Table 14).

For treatments containing Elliott Bay sediment, concentrations of copper were similar in water samples taken from the static and static renewal systems (Table 15). Total copper concentrations in the static exposure system ranged from 30 to 53 μg/L during the exposure period, with dissolved copper concentrations ranging from 18 to 41 μg/L. Overall, the dissolved fraction of copper accounted for 60-93 percent of the concentration of copper in the static exposure system water. Total copper concentrations in the static renewal exposure system ranged from 35 to 61 μg/L during the exposure period. Dissolved copper, which accounted for 61 to 94 percent of the copper concentration, ranged from 22 to 39 μg/L.

Mercury was not detected (detection limit of 0.1 μg/L) in any of the water samples taken from treatments for either the static or the static renewal exposure systems. PAH was also not detected (detection limit of 1 μg/L) in any of the water samples taken from the static and static renewal exposure systems.

Contaminants present in the water were periodically removed from the exposure chamber when using the static renewal system. However, analysis of the contaminant mass that was removed relative to the mass present in the sediment indicates that the loss was minimal (Table 16). For copper, the only contaminant consistently detected in the water samples, less than 1 percent

TABLE 12. SURVIVAL, TOTAL BIOMASS, AND AVERAGE INDIVIDUAL BIOMASS DATA FOR THE EXPOSURE SYSTEM EXPERIMENT

Sediment	Exposure System	Survival[a]	Total Biomass[a] (mg dry weight)	Average Individual Biomass[a,b] (mg dry weight)
West Beach	Static	100 ± 0.0	118.9 ± 7.9	23.8 ± 1.6
	Static renewal	100 ± 0.0	112.9 ± 10.9	22.6 ± 2.2
Carr Inlet	Static	96.0 ± 4.0	90.8 ± 14.6	18.5 ± 2.6
	Static renewal	100 ± 0.0	105.8 ± 6.8	21.2 ± 1.3
Elliott Bay	Static	60.0 ± 16.7	11.5 ± 5.3	3.6 ± 1.1
	Static renewal	92.0 ± 4.9	11.1 ± 1.8	2.5 ± 0.4

[a] Value reported as mean ± standard error.

[b] Biomass of worms at test initiation was 1.0 ± 0.1 mg (dry weight).

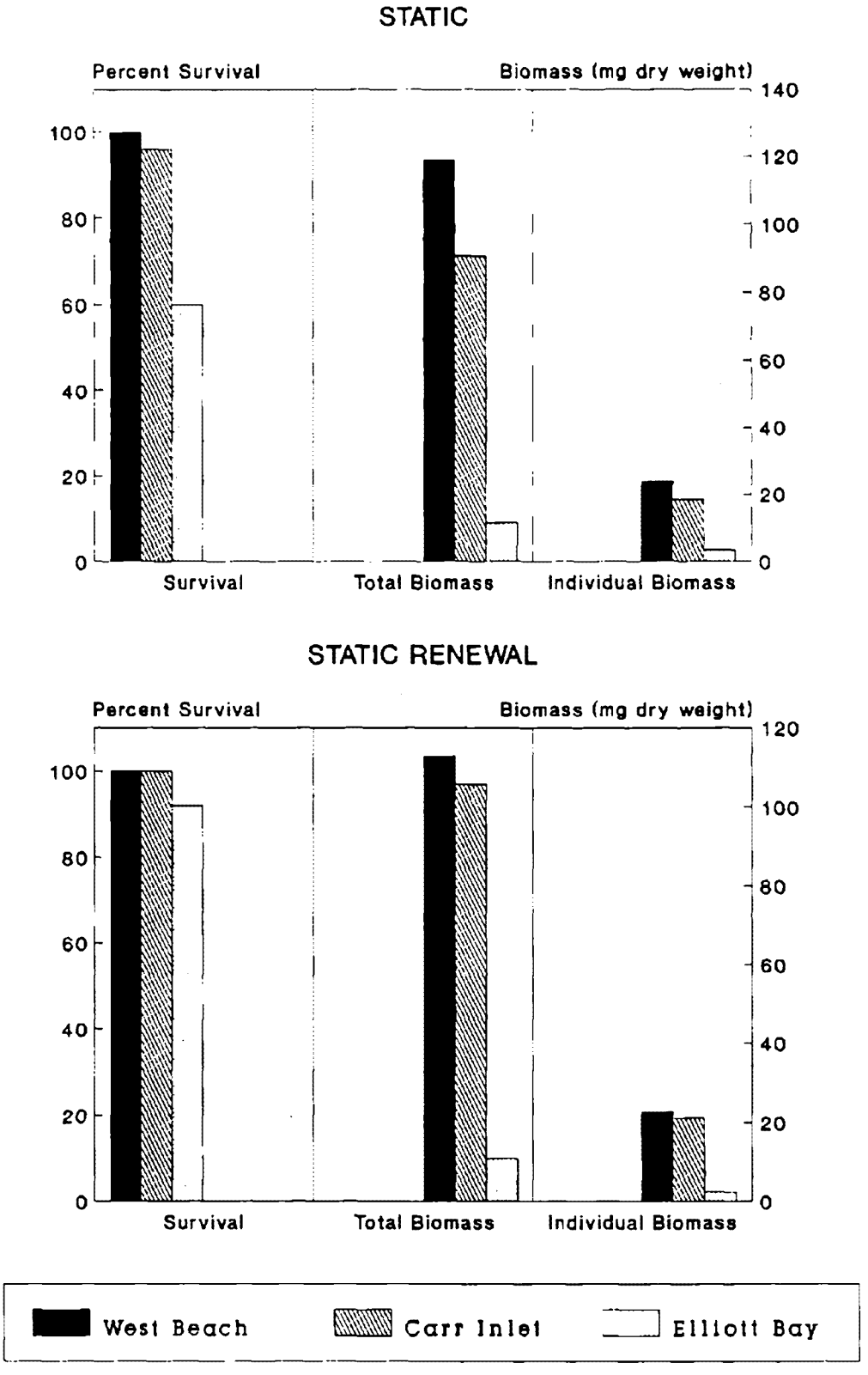

Figure 7. Survival and biomass of juvenile worms from the exposure system experiment

TABLE 13. PAIRWISE STATISTICAL COMPARISON OF *NEANTHES* BIOASSAY RESPONSES FOR THE EXPOSURE SYSTEM EXPERIMENT

Exposure System	Treatment Comparison[a]	Response	Comparison Result[b]
Static[c]	EB vs. CI	Percent survival	ns
		Total biomass	*
		Percent biomass	*
Static renewal[d]	EB vs. CI	Percent survival	ns
		Total biomass	*
		Percent biomass	*

[a] EB - Elliott Bay
CI - Carr Inlet.

[b] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

[c] In the static exposure system, no water changes were made during the 20-day exposure period.

[d] In the static renewal exposure system, one-third of the water volume was exchanged with fresh seawater every third day.

TABLE 14. AMMONIA CONCENTRATIONS FROM STATIC AND STATIC RENEWAL EXPOSURE SYSTEMS DURING A 20-DAY EXPOSURE PERIOD

Sediment	Exposure System	Ammonia Concentration (mg/L)			
		Day 3	Day 6	Day 12	Day 20
Carr Inlet	Static	--[a]	3.20	0.62	0.12
	Static renewal	1.20	2.90	0.33	0.11
Elliott Bay	Static	1.60	4.48	4.63	0.29
	Static renewal	--[a]	3.51	3.04	0.08

[a] Sample not collected for this treatment.

TABLE 15. CONTAMINANT CONCENTRATIONS FROM STATIC AND STATIC RENEWAL EXPOSURE SYSTEMS CONTAINING ELLIOTT BAY SEDIMENT

Exposure system	Contaminant	Contaminant Concentration[a] (μg/L) Day 3	Day 6	Day 12	Day 20
Static	PAH[b]	1U	1U	1U	1U
	Total copper	30	53Z	43Z	44Z
	Dissolved copper	18	32Z	33Z	41Z
	Total mercury	0.1U	0.1U	0.1U	0.1U
	Dissolved mercury	0.1U	0.1U	0.1U	0.1U
Static renewal	PAH	--[c]	1U	1U	1U
	Total copper	--	61Z	35Z	35Z
	Dissolved copper	--	39Z	22Z	32Z
	Total mercury	--	0.1U	0.1U	0.1U
	Dissolved mercury	--	0.1U	0.1U	0.1U

[a] Qualifier codes:

U - undetected at detection limit shown
Z - blank-corrected, still above detection limit.

[b] Polycyclic aromatic hydrocarbons (PAH) analyzed as part of this study are reported in Table 4.

[c] Sample not collected.

TABLE 16. MASS LOSS OF CONTAMINANTS IN THE STATIC RENEWAL EXPOSURE SYSTEM CONTAINING ELLIOTT BAY SEDIMENT

Compound	Sediment Mass (μg)	Water Concentration (μg/L)	Mass Load Removed with with Water Exchange[a] (μg)	Percent Loss of Contaminant[b]	Percent Contaminant Remaining in Sediment
PAH					
2-Methylnaphthalene	30	<1	<1.8	<6.0	>94
Pyrene	1,392	<1	<1.8	<0.1	>99.9
Copper	17,284	44	79.2	0.5	99.5
Mercury	406	<0.1	<0.2	<0.05	>99.95

[a] Mass determined as the product of average concentration of chemical and total water volume removed during the entire exposure period.

[b] Percentage loss determined by dividing chemical mass removed by the sediment concentration of the contaminant.

of the mass present in the sediment was removed during the 20-day exposure period. Although mercury and PAH were not detected in the water samples, the maximum potential loss of these compounds can be approximated by assuming the concentrations were at the detection limits of 0.1 μg/L and 1 μg/L for mercury and PAH, respectively. For mercury, the maximum potential loss relative to the sediment mass was less than 1 percent. For PAH, the maximum potential loss ranged from less than 1 percent for pyrene to 6 percent for 2-methylnaphthalene.

Although there are potential cost savings in using a static exposure system, it was concluded that the *Neanthes* sublethal bioassay should continue to be conducted with the static renewal exposure system. Overall water quality appeared to be better in the static renewal system than in the static system. Survival of worms to 20 days was high in all sediment types when the static renewal system was employed. In contrast, low survival rates were observed in the static exposure system for worms exposed to Elliott Bay sediment. Elliott Bay sediment has a higher organic content (2.1 percent TOC) than the other test sediments. Furthermore, use of the static renewal exposure system does not appear to result in significant loss of contaminants from the exposure system.

Test Duration

The survival rate at the end of all three exposure periods was greater than 76 percent in all three sediment treatments (Table 17; Figure 8). No mortality was observed in the West Beach control, while the lowest survival rate observed in the Carr Inlet sediment was 96 percent for the 10-day exposure period. Survival in the Elliott Bay sediment was lower than in the other sediments and ranged from 76 to 84 percent. A pairwise comparison of survival rates between Carr Inlet and Elliott Bay treatments for the three exposure times showed no significant difference in survival rates (Table 18). Worms maintained in the West Beach and Carr Inlet sediments exhibited increases in total and average individual biomass with increasing exposure periods (Figure 8). Growth curves for worms from both the Carr Inlet and the West Beach sediments indicated that a significant proportion of the biomass gained during the 20-day exposure period occurred between days 10 and 20. Total biomass increases between test initiation and day 10 ranged from 20 to 24 mg (dry weight). Total biomass increases between day 10 and day 20 ranged from 44 to 55 mg (dry weight) (Table 17).

Worms maintained in the Elliott Bay sediments exhibited little change in total biomass and average individual biomass between the 10-day and the 20-day exposure periods (Table 17). For these worms, average biomass of individual worms ranged from 1.1 to 1.4 mg (dry weight) per worm. Total biomass was also relatively stable during the 20-day exposure period. During this same period, the biomass of individual worms maintained in West Beach and Carr Inlet sediments increased by approximately 2.9 times.

A pairwise comparison of the biomass data for worms from the Elliott Bay sediment to worms from the Carr Inlet sediment indicated that significant differences in both total biomass and average individual biomass existed at all three exposure periods (Table 18).

A power analysis model was used to determine the change in the ability to detect a statistically significant difference in total biomass between worms maintained in Elliott Bay sediment and those maintained in Carr Inlet sediment with increasing exposure periods. At all three exposure periods, the ability to discriminate between the Elliott Bay and the Carr Inlet sediments increases as the difference in mean biomass increases (Figure 9). However, much smaller differences in

TABLE 17. SURVIVAL, TOTAL BIOMASS, AND AVERAGE INDIVIDUAL BIOMASS DATA FOR THE TEST DURATION EXPERIMENT

Sediment	Exposure Period (days)	Survival[a]	Total Biomass[a] (mg dry weight)	Average Individual Biomass[a,b] (mg dry weight)
West Beach	10	100 ± 0.0	23.9 ± 4.7	4.8 ± 1.0
	15	100 ± 0.0	39.9 ± 1.5	8.0 ± 0.4
	20	100 ± 0.0	67.8 ± 4.9	13.5 ± 1.0
Carr Inlet	10	96.0 ± 4.0	27.9 ± 4.0	5.7 ± 0.7
	15	100 ± 0.0	55.1 ± 6.7	11.0 ± 1.4
	20	100 ± 0.0	83.0 ± 2.8	16.6 ± 0.6
Elliott Bay	10	80.0 ± 6.3	5.8 ± 1.0	1.4 ± 0.2
	15	76.0 ± 11.7	4.2 ± 0.9	1.1 ± 0.1
	20	84.0 ± 7.5	4.5 ± 0.8	1.1 ± 0.1

[a] Value reported as mean ± standard error.

[b] Biomass of worms at test initiation was 0.8 ± 0.1 mg (dry weight).

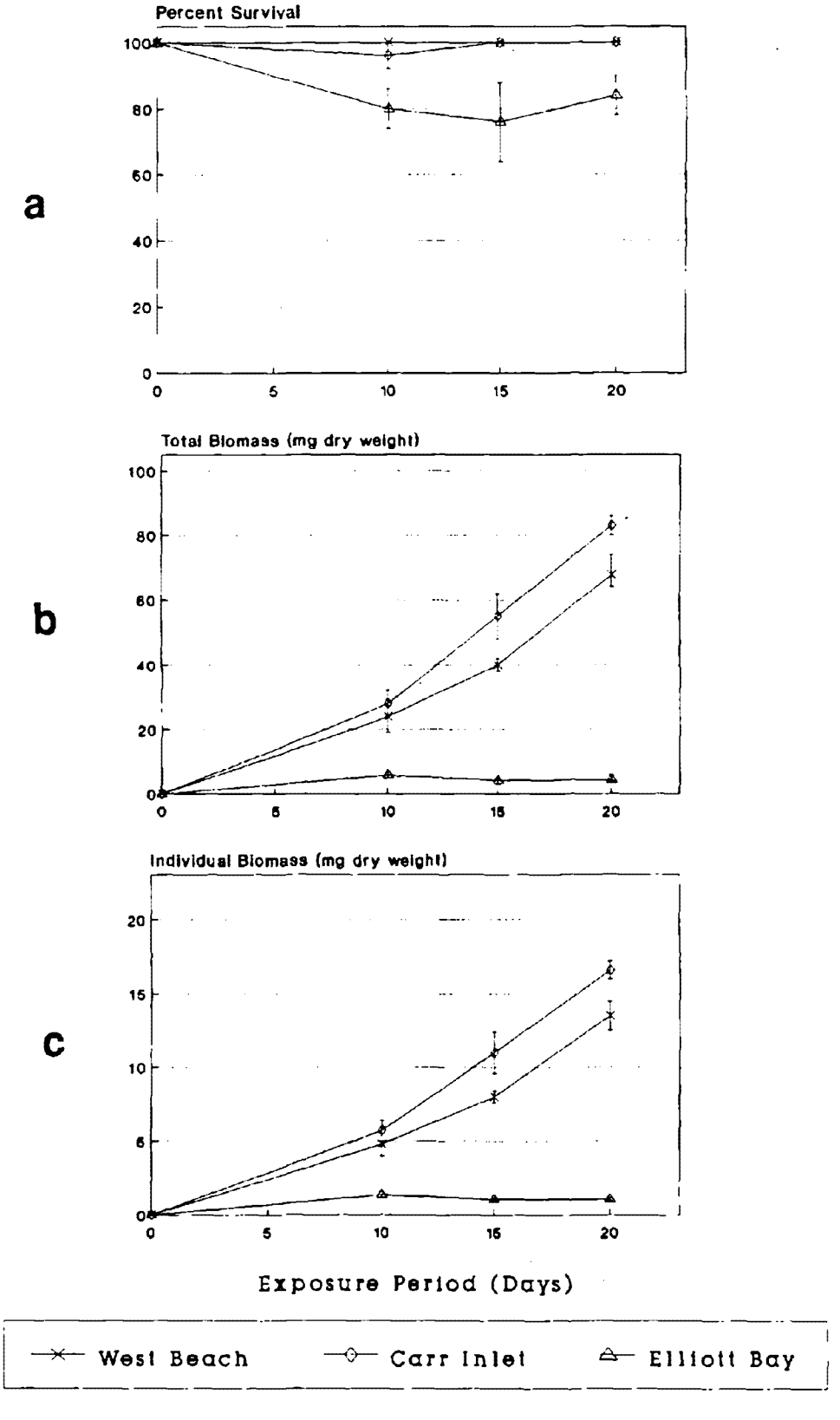

Figure 8. Survival (a), total biomass (b), and individual biomass (c) of juvenile worms from the test duration experiment. Bars represent standard error.

TABLE 18. PAIRWISE STATISTICAL COMPARISON OF *NEANTHES* BIOASSAY RESPONSES FOR THE TEST DURATION EXPERIMENT

Exposure Period (days)	Treatment Comparison[a]	Response	Comparison Result[b]
10	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*
15	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*
20	EB vs. CI	Percent survival	ns
		Total biomass	*
		Individual biomass	*

[a] EB - Elliott Bay
CI - Carr Inlet.

[b] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

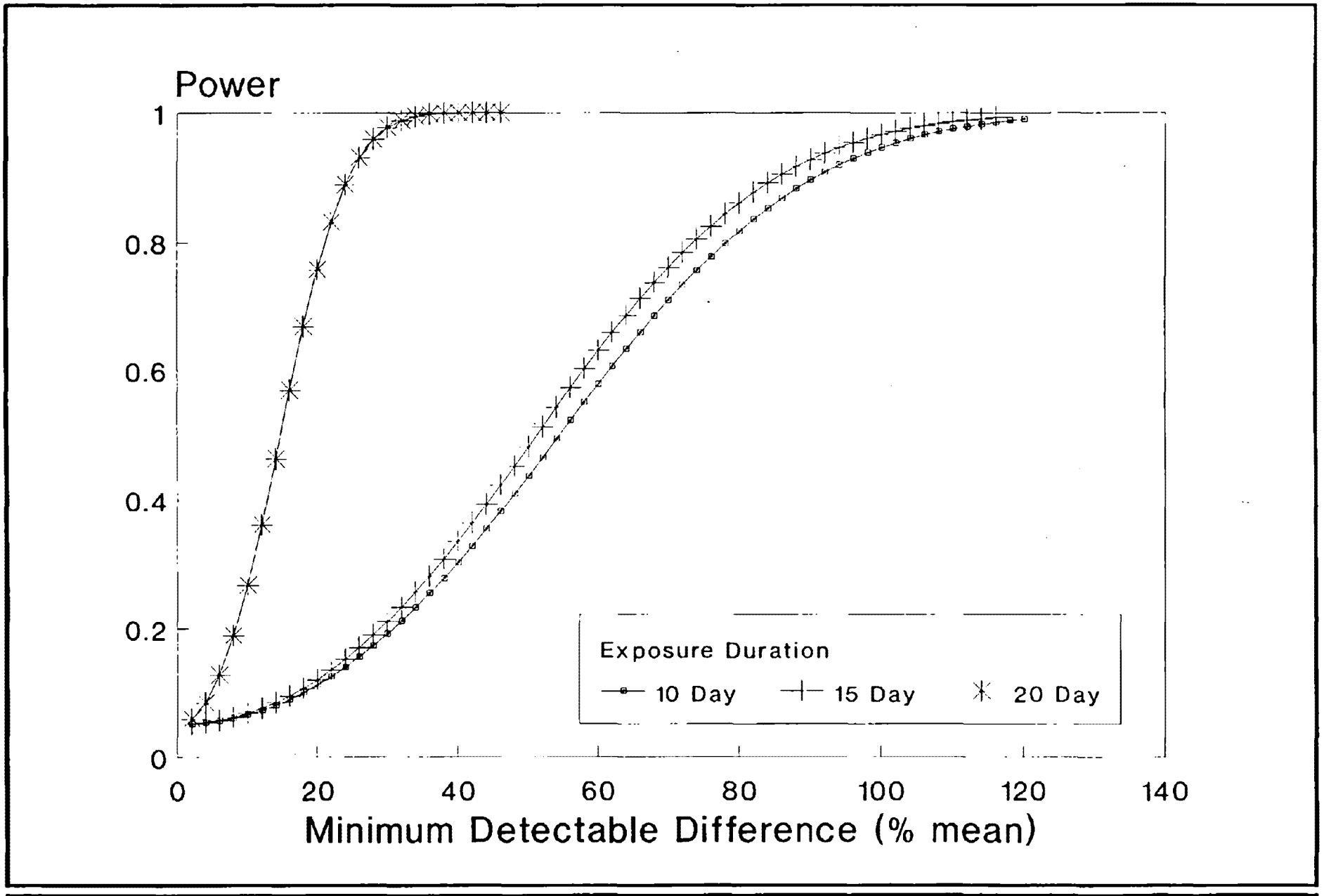

Figure 9. Statistical power vs. minimum detectable difference for total biomass in the test duration experiment

mean values can be discriminated following a 20-day exposure than following 10- and 15-day exposure periods. For example, at a power of 0.8, the MDD in mean values that can be detected following 20 days is 21 percent, while the MDD is 78 and 74 percent following 10- and 15-day exposure periods, respectively. Therefore, although statistical differences were detected between Carr Inlet and Elliott Bay treatments for all three exposure periods, there is a substantially higher probability of detecting effects at the longer exposure period.

The growth characteristics of *Neanthes* juveniles appear to follow the pattern generally observed in marine invertebrates in which the life cycle is characterized by periods of rapid growth (Rossi and Anderson 1976; Petrich and Reish 1979). Typically, these periods of rapid growth occur prior to attaining sexual maturity. In *Neanthes*, development time to sexual maturity is approximately 5-6 weeks. Thus, the 20-day exposure period originally used with the *Neanthes* sublethal bioassay was designed to incorporate the effects of being exposed during a significant portion of the growth-critical juvenile life stage. The 20-day exposure period accounts for between 48 to 57 percent of the juvenile life stage in *Neanthes* (Reish 1980).

The results of this experiment indicate that statistical differences between growth patterns in worms exposed to clean and contaminated sediments can be detected following exposure periods of 10 or 15 days. As exposure time increases to 20 days, however, the power to detect statistical differences increases substantially. Of the three exposure periods examined in this experiment, statistical power is the greatest following a 20-day exposure period. In this experiment, statistical power was similar for the 10- and 15-day exposures. The similarity of these results was caused by the relatively high variance associated with the 15-day exposure. To reduce the influence of this possible experimental artifact, the power analyses were recalculated using an average of the within-group mean squares for the individual ANOVAs. This value was used as an estimate of the overall average residual variance and is independent of any specific results associated with individual experiments. Power analyses using this average error estimate (Figure 10) show that a 15-day exposure would result in a statistical power intermediate between the 10- and 20-day exposures. The results of the statistical power analysis of the biomass data indicate that the 20-day exposure period recommended in the interim protocol is the most suitable period for evaluating the effects of exposure to contaminated sediments on the growth of juvenile *Neanthes*.

Although a potential cost savings could be achieved by using a shorter exposure period, it is recommended that the exposure period for the *Neanthes* sublethal bioassay remain at 20 days. Because a 20-day exposure period covers a significant portion of the juvenile life stage, care must be taken to ensure that the worms are only 2-3 weeks postemergence at the initiation of the bioassay (Johns et al. 1989). If worms older than 3 weeks are used, test data may not be valid because of the marked decrease in tissue production observed as *Neanthes* reach sexual maturity (Johns and Ginn 1990).

Salinity Tolerance

Neanthes juveniles were acutely sensitive to low salinity waters based on a 96-hour exposure to salinities ranging from 10 to 28 ppt (Table 19). No mortalities were observed at salinities above 20 ppt. Below 20 ppt, however, mortality rate increases as salinity decreases, with the LC_{50} value approximated at 15 ppt.

In the second salinity experiment, mean survival rates were high in all sediment treatments, ranging from 88 to 100 percent (Table 20). *Neanthes* growth was similar in all sediment types in

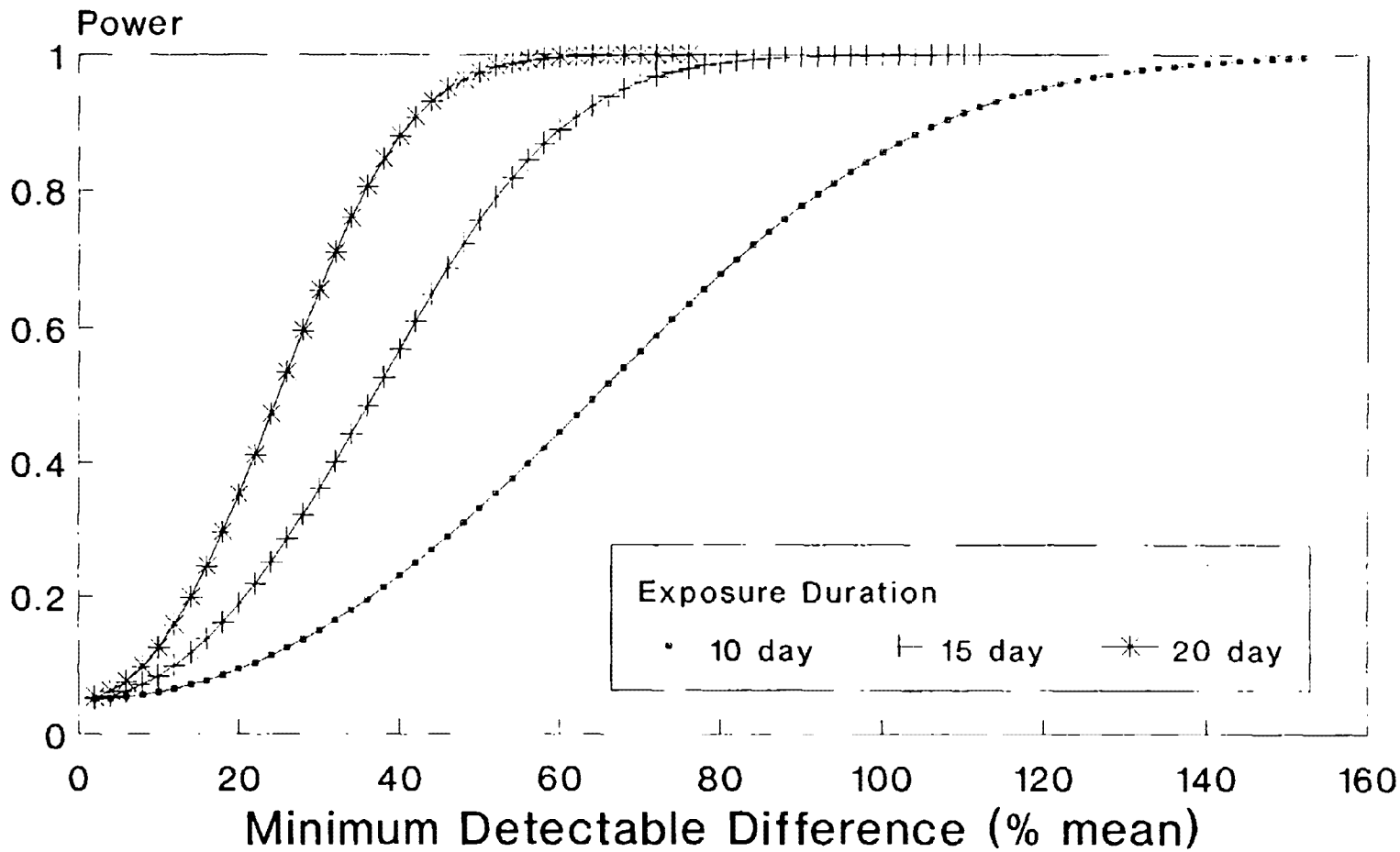

Figure 10. Statistical power vs. minimum detectable difference for total biomass in the test duration experiment calculated using average mean square error.

TABLE 19. SURVIVAL DATA FOR THE 96-HOUR SALINITY TOLERANCE EXPERIMENT

Salinity (ppt)	Percent Survival[a]
28	100 ± 0.0
25	100 ± 0.0
20	100 ± 0.0
15	50 ± 10
10	0.0 ± 0.0

[a] Value reported as mean ± standard error.

TABLE 20. SURVIVAL, TOTAL BIOMASS, AND AVERAGE INDIVIDUAL BIOMASS DATA FOR THE *NEANTHES* SALINITY TOLERANCE EXPERIMENT

Sediment	Initial Interstitial Salinity (ppt)	Survival[a]	Total Biomass (mg dry weight)[a]	Average Individual Biomass (mg dry weight)[a,b]
West Beach	28	96.0±4.0	47.7±5.8	9.9±1.0
Carr Inlet	28	100.0±0.0	51.3±3.8	10.3±0.8
Duckabush River	30	100.0±0.0	49.9±5.3	10.0±2.4
	25	88.0±4.9	39.0±7.4	9.0±2.1
	22	100.0±0.0	59.9±15.4	12.0±3.1
	19	100.0±0.0	42.7±7.7	8.5±0.4

[a] Value reported as mean ± standard error.

[b] Biomass of worms at test initiation was 0.4 ± 0.1 mg (dry weight).

this experiment (Table 20). No significant differences (P>0.05) in total biomass or average individual biomass were detected among the treatments.

Although no significant differences in worm response were noted following a 20-day exposure to sediments having initially low salinity interstitial water, it should be noted that *Neanthes* is acutely sensitive to salinity exposures below 20 ppt. Thus, caution should be used when performing and interpreting the results of *Neanthes* bioassays conducted with sediments collected from low salinity areas. It should be recognized that this experimental design did not allow evaluation of worm response to interstitial waters below a salinity of 19 ppt. Further testing may be required to define the effects of low interstitial salinity on worm survival and growth.

Differences between the results observed in the 96-hour seawater test and the 20-day sediment test may be attributable to behavioral modifications and to the effects of overlying water in the exposure chamber. In the sediment exposure experiment, worms placed in treatments containing low salinity sediments could effectively avoid the low salinity by remaining close to the sediment surface where the overlying salinity was 28 ppt. Burrowing activity could also facilitate the mixing of interstitial water with overlying water, thus increasing the interstitial salinity. Based on the test data for the two salinity experiments, *Neanthes* bioassays should be conducted with interstitial salinities greater than 20 ppt when possible. If the bioassay is conducted with salinities lower than 20 ppt, care should be taken in ascribing changes in *Neanthes* response solely to factors other than salinity.

Sensitivity to Sediment Grain Size

Mean survival was consistently high (96-100 percent) for *Neanthes* juveniles exposed to uncontaminated sediments having silt/clay fractions between 53 and 97 percent (Table 21). In addition, no mortality was observed in the West Beach control, which is primarily sand (silt/clay fraction = 2 percent). A pairwise comparison of survival data for the various grain-size treatments to data for Carr Inlet showed that no significant differences in survival rate exist.

Neanthes growth was also very similar among the ranges of particle sizes tested (Table 21). Total biomass at 20 days ranged from 65.7 to 72.9 mg over the range of sediment types tested. Statistical analysis by ANOVA revealed no significant difference (P>0.05) in total or individual biomass.

In this experiment, the response criteria used in the *Neanthes* sublethal bioassay (survival, total biomass, and average individual biomass) do not appear to be sensitive to changes in sediment grain size, even when it ranges from very coarse-grained sands to silty material. During sieving procedures conducted at the end of the experiment, it appeared that surviving worms were able to construct and maintain tubes in all sediment types. In their natural habitat, *Neanthes* live in subsurface tubes constructed of mucus and material collected from the surrounding environment. If not offered sediment in the laboratory, *Neanthes* will build a mucus tube on the container surface incorporating food particles, fecal material, and available debris into the tube matrix.

The results of this experiment indicate that *Neanthes* is able to survive and grow in a wide range of sediment types. Sediment type, based on the silt and clay composition, should not affect the suitability of the *Neanthes* sublethal bioassay for assessing most marine sediments. It should be noted, however, that statistical differences in worm growth were noted between worms exposed to West Beach and Carr Inlet sediments in three of the experiments (i.e., worm density, test

TABLE 21. SURVIVAL, TOTAL BIOMASS, AND AVERAGE INDIVIDUAL BIOMASS FOR THE SEDIMENT GRAIN-SIZE EXPERIMENT

Sediment Type	Grain Size[a]	Survival[b]	Total Biomass (mg dry weight)[b]	Average Individual Biomass (mg dry weight)[b,c]
West Beach	2	100±0.0	65.7±6.4	13.2±1.3
CR01	53	100±0.0	73.2±7.1	14.6±1.4
CR04	71	100±0.0	65.9±5.3	13.2±1.1
CR02	89	96.0±4.0	75.4±9.7	15.5±1.6
CR05	93	100±0.0	62.7±6.8	12.5±1.4
CR03	97	96.0±4.0	72.9±7.1	15.5±2.1

[a] Grain size reported as percent silt/clay.

[b] Value reported as mean ± standard error.

[c] Biomass of worms at test initiation was 0.5 ± 0.2 mg (dry weight).

duration, and food ration at 80 mg/48 hours) conducted as part of this study. Because of the potential for statistically detecting differences in growth for worms exposed to clean sediment of widely differing grain size, the reference sediment used in *Neanthes* bioassays should have a similar grain size to the test sediments to avoid potential differences in organism response related to the physical characteristics of the sediment.

Sensitivity of *Neanthes* to a Reference Toxicant

Neanthes juveniles were found to be acutely sensitive to cadmium exposure. The 96-hour LC_{50} value for cadmium is 22 ppm (Table 22). This value is similar to the LC_{50} value (12 ppm) reported by Reish (1984) for adult *Neanthes*.

It is recommended that a reference toxicant control using $CdCl_2$ be required when any *Neanthes* sublethal bioassay is performed for an environmental regulatory program. Data from the toxicant control can be used to determine the relative sensitivity of the particular test organisms in each bioassay series to ensure comparability of test results.

Response of the Biomass Endpoint and Statistical Power

A comparison of the relative percent reduction in biomass between worms maintained in Carr Inlet and Elliott Bay sediments was made to evaluate the consistency of the biomass response endpoint. Data for all treatment conditions from the six experiments in which Carr Inlet, West Beach, and Elliott Bay sediments were tested are included in this comparison. For the interim protocol exposure conditions, the range in percent reduction (Elliott Bay vs. Carr Inlet) observed for the total biomass endpoint was from 78 to 95 percent (Table 23). The total biomass response for Elliott Bay and Carr Inlet treatments was also relatively consistent among the different exposure conditions evaluated in this study. For the total range of test conditions examined, the percent reduction in total biomass in Elliott Bay exposures ranged from 72 to 96 percent (Table 23). These results show a relatively high degree of consistency in the response of *Neanthes* when exposed to the same sediments over a period of 4 months.

Table 23 also contains a compilation of all two-sample comparisons of the total biomass in Elliott Bay and West Beach exposures with the total biomass in reference sediment exposures from Carr Inlet. Two measures of percent difference are compiled: the percent change from reference biomass and the percent change from the overall mean biomass. The two-sample statistical results conducted for this study show the lowest differences that were found to be statistically significant ($P \leq 0.05$) were 18 and 10 percent relative to the reference and mean values, respectively. Differences greater than 33 percent (from the reference) and 20 percent (from the mean) were always significant in the test results.

The actual statistical results presented in Table 23 agree well with the power analysis results presented in Figures 5, 9, and 10. Depending on individual experimental variances, the power analyses predict a moderate (~ 50 percent) to high (~ 90 percent) statistical probability of detecting a 30 percent change relative to the overall mean. The power analyses indicate that there would be a low probability of detecting differences of less than 10 percent from the mean biomass. In the test results, all differences from the mean biomass of less than 10 percent were nonsignificant ($P > 0.05$).

TABLE 22. SURVIVAL DATA FOR 96-HOUR LC_{50} TEST CONDUCTED WITH *NEANTHES* JUVENILES USING CADMIUM CHLORIDE

Test Series	Cadmium Concentration (ppm)	Survival[a]
Test I	0	100 ± 0.0
	1	100 ± 0.0
	10	100 ± 0.0
	100	0.0 ± 0.0
	1,000	0.0 ± 0.0
Test II	0	100 ± 0.0
	10	100 ± 0.0
	18	80 ± 0.0
	32	0.0 ± 0.0
	56	0.0 ± 0.0
	100	0.0 ± 0.0

[a] Value reported as mean ± standard error.

TABLE 23. RESULTS OF STATISTICAL COMPARISONS AND PERCENT DIFFERENCES BETWEEN TEST SITES FOR *NEANTHES* BIOASSAYS

Test[a]	Comparison (test, reference)[b]	Statistical Results[c]	Percent Difference	
			From Reference[d]	From Overall Mean[e]
WD-5	EB, CI	*	78	64
WD-5	WB, CI	*	72	56
WD-10	EB, CI	*	78	64
WD-15	EB, CI	*	87	77
WD-20	EB, CI	*	85	74
FR-20	EB, CI	*	96	93
FR-40	EB, CI	*	84	72
FR-40	WB, CI	ns	6	3
FR-60	EB, CI	*	78	64
FR-80	EB, CI	*	72	56
FR-80	WB, CI	*	33	20
S	EB, CI	*	87	78
S	WB, CI	ns	31	13
SR	EB, CI	*	90	81
SR	WB, CI	ns	7	3
TD-20	EB, CI	*	95	90
TD-20	WB, CI	*	18	10
TD-15	EB, CI	*	92	86
TD-15	WB, CI	ns	28	16
TD-10	EB, CI	*	79	66
TD-10	WB, CI	ns	14	8
GS	WB, CI(01)	ns	11	5
S	WB, CI	ns	7	4

[a] WD - worm density (organisms per exposure chamber)
FR - food ration (mg/48 hours)
S - static exposure
SR - static renewal exposure
TD - test duration (days)
GS - grain size
S - salinity.

[b] EB - Elliott Bay
CI - Carr Inlet
WB - West Beach.

[c] * - significant, $P \leq 0.05$
ns - not significant, $P > 0.05$.

[d] $100 \left(1 - \frac{\text{test}}{\text{reference}}\right)$

[e] $100 \left[1 - \left(\frac{\text{test}}{\frac{\text{test}+\text{reference}}{2}}\right)\right]$

SUMMARY

Based on the results presented in this report, the interim protocol for conducting the sublethal *Neanthes* bioassay described by Johns et al. (1989) can be finalized with little revision. The results of experiments indicate that the test parameters identified in the interim protocol were suitable for test refinement. Specifically, the experiments showed that:

- A worm density of five worms per exposure chamber results in sufficient biomass to statistically detect differences among treatments. Increasing worm density did not result in an increased ability to detect significant effects.
- A food ration of 40 mg/48 hours is sufficient to promote adequate growth in *Neanthes* juveniles during a 20-day exposure period. Although the experimental results did not indicate the existence of a food saturation level up to a ration of 80 mg/48 hours, increasing the food ration above 40 mg/48 hours resulted in excess food and fungal growth on the sediment surface in some exposure conditions. Food rations below 40 mg/48 hours resulted in lowered survival rates in the exposure chambers containing contaminated sediment.
- The static renewal exposure system should be employed in all *Neanthes* sublethal bioassays conducted with sediment. Use of the static renewal exposure system resulted in high survival in all sediment types, and overall water quality appeared to be better in the static renewal system than in the static exposure system. In addition, use of the static renewal exposure system does not appear to result in significant loss of contaminants.
- Test duration for the *Neanthes* sublethal bioassay should remain at 20 days. Although significant differences in growth between treatments can be detected at exposure times of less than 20 days, the experiment results indicated that statistical power is greatest following a 20-day exposure period.
- Based on the test data for the two salinity experiments, *Neanthes* bioassays should be conducted with interstitial salinities greater than 20 ppt. If the bioassay is going to be conducted with sediment containing interstitial salinities lower than 20 ppt, care should be taken in ascribing changes in *Neanthes* response to factors other than salinity. In all cases, the salinity of the overlying water in the exposure chamber should be 28 ppt.
- Sediment grain size does not appear to affect the suitability of the *Neanthes* sublethal bioassay for assessing marine sediments. Because differences in growth could occur due to sediment grain size or TOC, the reference sediment should be similar to the test sediment for these two factors.
- Comparisons of the relative percent reduction in biomass between worms maintained in Carr Inlet and in Elliott Bay sediments show a high degree of consistency in the response of *Neanthes*.

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APPENDIX A

Sediment Chemistry Data

TABLE A-1. CONCENTRATIONS OF CHEMICALS OF CONCERN IN CARR INLET AND ELLIOTT BAY SEDIMENTS

Compound	Carr Inlet (CR01)[a]	Carr Inlet (CR02)[a]	Elliott Bay[a]
METALS (mg/kg dry weight; ppm)			
Antimony	1.4G	0.14G	10.3G
Arsenic	18.1	4.4	112
Cadmium	0.8E	0.7E	1.4E
Copper	62.3	28.5	1,490
Lead	37.5	13.3	384
Mercury	0.14	0.05	3.5
Nickel	36.7	35.8	50.5
Silver	0.39E	0.14E	1.2E
Zinc	111	61.6	1,010
ORGANICS (ug/kg dry weight; ppb)			
Low Molecular Weight Polycyclic Aromatic Hydrocarbons (PAH)			
Naphthalene	14U	11U	390
Acenaphthylene	14U	11U	440
Acenaphthene	14U	11U	780
Fluorene	14U	11U	790
Phenanthrene	100	9E	4,800
Anthracene	38	11U	1,900
2-Methylnaphthalene	14U	11U	260
High Molecular Weight PAH			
Fluoranthene	170E	32E	8,100
Pyrene	170E	27E	12,000
Benz(a)anthracene	83E	10E	4,000E
Chrysene	100	20	3,300
Benzofluoranthenes	150E	28E	10,000
Benzo(a)pyrene	90E	11U	8,900
Indeno(1,2,3,-c,d)pyrene	50E	11U	1,600
Dibenzo(a,h)anthracene	14U	11U	910
Benzo(g,h,i)perylene	56E	11U	3,600
Chlorinated Hydrocarbons			
1,3-Dichlorobenzene	14U	11U	13U
1,4-Dichlorobenzene	14U	11U	14U
1,2-Dichlorobenzene	14U	11U	13U
Hexachlorobenzene	14U	11U	13U
Phthalates			
Dimethyl phthalate	14U	11U	110
Diethyl phthalate	14U	11U	13U
Di-n-butyl phthalate	14U	11U	140
Butyl benzyl phthalate	14U	11U	320E
Bis(2-ethylhexyl)phthalate	64	11U	6100
Di-n-octyl phthalate	14U	11U	130U

TABLE A-1. (Continued)

Compound	Carr Inlet (CR01)[a]	Carr Inlet (CR02)[a]	Elliott Bay[a]
Polychlorinated Biphenyls			
Total PCB	8.2K	4.2K	1460
Phenols			
Phenol	27U	22U	170
2-Methylphenol	14U	11U	78E
4-Methylphenol	14U	11U	180E
2,4-Dimethylphenol	33U	26U	31U
Pentachlorophenol	22U	17U	1,900
Miscellaneous Extractables			
Benzyl alcohol	68U	54U	64U
Benzoic acid	140U	108U	128U
Dibenzofuran	14U	11U	110
Hexachloroethane	41U	33U	39U
Hexachlorobutadiene	14U	11U	13U
N-Nitrosodiphenylamine	14U	11U	13U
Pesticides			
Total DDT	2U	2U	34
Aldrin	1U	1U	1U
Chlordane	1.5U	1.5U	1.5U
Dieldrin	2U	2U	2U
Heptachlor	1U	1U	1U
Lindane	1U	1U	1U

[a] Qualifier codes used:

U - Undetected at detection limit shown
E - Estimate
G - Estimate is greater than value shown
K - Detected at less than detection limit shown.

APPENDIX B

Bioassay Test Data

TABLE B-1. BIOASSAY TEST DATA FOR THE WORM DENSITY EXPERIMENT

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
West Beach-5	1	4	12.8	3.2
	2	5	18.9	3.8
	3	5	29.9	6.0
	4	0	--	--
	5	5	28.6	5.7
Carr Inlet-5	1	5	70.2	14.0
	2	5	103.5	20.7
	3	5	74.4	14.9
	4	4	71.8	18.0
	5	5	79.6	15.9
Carr Inlet-10	1	10	113.8	11.4
	2	10	135.3	13.5
	3	10	114.0	14.0
	4	10	93.0	9.3
	5	9	101.8	11.3
Carr Inlet-15	1	15	160.7	10.7
	2	14	108.1	7.7
	3	15	144.5	9.6
	4	15	146.1	9.7
	5	15	112.8	7.5
Carr Inlet-20	1	18	157.7	8.8
	2	19	152.3	8.0
	3	20	168.8	8.4
	4	20	145.9	7.3
	5	20	112.7	5.6
Elliott Bay-5	1	5	22.1	4.4
	2	5	11.8	2.4
	3	5	32.2	6.4
	4	5	6.5	1.3
	5	5	15.9	3.2
Elliott Bay-10	1	9	46.5	5.2
	2	9	29.2	3.2
	3	4	13.6	3.4
	4	7	21.9	3.1
	5	7	11.2	1.6
Elliott Bay-15	1	0	0.0	--
	2	5	4.6	0.9
	3	5	7.8	1.6
	4	13	47.7	3.7
	5	8	27.1	3.4

TABLE B-1. (Continued)

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
Elliott Bay-20	1	12	47.6	4.0
	2	5	10.9	2.2
	3	15	44.3	3.0
	4	4	6.1	1.5
	5	0	0.0	--

[a] 5 = 5 worms/chamber
10 = 10 worms/chamber
15 = 15 worms/chamber
20 = 20 worms/chamber.

TABLE B-2. BIOASSAY TEST DATA
FOR THE FOOD RATION EXPERIMENT

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
West Beach-0	1	5	6.9	1.4
	2	5	6.6	1.3
	3	5	6.6	1.3
	4	5	4.5	0.9
	5	5	8.1	1.6
West Beach-40	1	5	101.0	20.2
	2	5	123.6	24.7
	3	4	107.3	26.8
	4	5	87.9	16.8
	5	5	110.7	22.1
West Beach-80	1	5	141.2	28.2
	2	4	103.1	25.8
	3	5	88.3	17.7
	4	5	95.9	19.1
	5	5	120.6	24.1
Carr Inlet-0	1	4	2.4	0.6
	2	5	2.8	0.6
	3	5	3.5	0.7
	4	5	3.2	0.6
	5	4	2.1	0.5
Carr Inlet-20	1	5	91.1	18.2
	2	5	78.6	15.7
	3	4	57.0	14.3
	4	4	58.5	14.6
	5	4	40.3	10.1
Carr Inlet-40	1	5	124.0	24.8
	2	5	137.4	27.5
	3	5	118.7	23.7
	4	5	109.6	21.9
	5	4	72.6	18.2
Carr Inlet-60	1	5	160.1	32.0
	2	5	103.6	20.7
	3	4	168.5	42.1
	4	5	127.0	25.4
	5	4	63.4	15.9
Carr Inlet-80	1	4	163.0	40.8
	2	5	173.6	34.7
	3	5	188.5	37.7
	4	5	155.1	31.0
	5	5	138.7	27.7

TABLE B-2. (Continued)

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
Elliott Bay-0	1	0	0.0	--
	2	0	0.0	--
	3	0	0.0	--
	4	0	0.0	--
	5	0	0.0	--
Elliott Bay-20	1	1	9.0	9.0
	2	0	0.0	--
	3	1	3.2	3.2
	4	0	0.0	--
	5	2	0.3	0.2
Elliott Bay-40	1	4	21.0	5.3
	2	5	22.9	4.6
	3	5	27.0	5.4
	4	3	6.5	2.2
	5	2	13.2	6.6
Elliott Bay-60	1	5	23.8	4.8
	2	5	44.3	8.9
	3	4	31.4	7.9
	4	0	0.0	--
	5	5	38.4	7.7
Elliott Bay-80	1	5	26.0	5.2
	2	5	62.0	12.4
	3	5	42.9	8.6
	4	5	82.8	16.6
	5	3	18.8	6.3

[a] 0 = no food
20 = 20 mg/48 hours
40 = 40 mg/48 hours
60 = 60 mg/48 hours
80 = 80 mg/48 hours.

TABLE B-3. BIOASSAY TEST DATA
FOR THE EXPOSURE SYSTEM EXPERIMENT

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
West Beach - S	1	5	137.2	27.4
	2	5	102.4	20.5
	3	5	138.8	27.8
	4	5	109.2	21.8
	5	5	106.7	21.3
West Beach - R	1	5	116.1	23.2
	2	5	119.7	23.9
	3	5	147.8	29.6
	4	5	84.4	16.9
	5	5	96.3	19.3
Carr Inlet - S	1	5	123.0	24.6
	2	5	96.0	19.2
	3	5	105.6	21.1
	4	4	36.2	9.1
	5	5	93.3	18.7
Carr Inlet - R	1	5	110.6	22.1
	2	5	128.5	25.7
	3	5	106.1	21.2
	4	5	93.4	18.7
	5	5	90.4	18.1
Elliott Bay - S	1	1	2.3	2.3
	2	1	2.9	2.9
	3	4	11.4	2.9
	4	4	31.5	7.9
	5	5	9.7	1.9
Elliott Bay - R	1	4	12.7	3.2
	2	5	16.6	3.3
	3	4	10.9	2.7
	4	5	5.8	1.2
	5	5	9.4	1.9

[a] S = static exposure system
R = static renewal exposure system.

TABLE B-4. BIOASSAY TEST DATA FOR THE TEST DURATION EXPERIMENT

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
West Beach-10	1	5	10.4	2.1
	2	5	30.9	6.2
	3	5	24.2	4.8
	4	5	30.1	6.0
	5	--[b]	--	--
West Beach-15	1	5	43.5	8.7
	2	5	34.7	6.9
	3	5	40.4	8.1
	4	5	40.0	8.0
	5	5	40.9	8.2
West Beach-20	1	5	56.1	11.2
	2	5	80.4	16.1
	3	5	75.7	15.1
	4	5	57.7	11.5
	5	5	69.0	13.8
Carr Inlet-10	1	5	27.4	5.5
	2	5	30.7	6.1
	3	5	37.2	7.4
	4	5	31.0	6.2
	5	4	13.3	3.3
Carr Inlet-15	1	5	78.9	15.8
	2	5	39.5	7.9
	3	5	53.8	10.8
	4	5	45.6	9.1
	5	5	57.5	11.5
Carr Inlet-20	1	5	76.2	15.2
	2	5	87.1	17.4
	3	5	89.2	17.8
	4	5	76.6	15.3
	5	5	86.1	17.2
Elliott Bay-10	1	4	7.5	1.9
	2	4	6.7	1.7
	3	5	6.6	1.3
	4	3	1.7	0.6
	5	4	6.6	1.7
Elliott Bay-15	1	5	7.0	1.4
	2	4	5.1	1.3
	3	5	4.2	0.8
	4	2	1.5	0.8
	5	3	3.2	1.1

TABLE B-4. (Continued)

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
Elliott Bay-20	1	4	3.5	0.9
	2	4	4.8	1.2
	3	5	3.3	0.7
	4	5	7.6	1.5
	5	3	3.1	1.0

[a] 10 = 10 day exposure
15 = 15 day exposure
20 = 20 day exposure.

[b] Replicate dropped in handling.

TABLE B-5. BIOASSAY TEST DATA
FOR THE SEDIMENT GRAIN-SIZE EXPERIMENT

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
West Beach-1	1	5	81.4	16.3
	2	5	60.3	12.1
	3	5	80.3	16.1
	4	5	51.9	10.4
	5	5	54.4	10.9
Carr Inlet-2 (CR01)	1	5	84.7	16.9
	2	5	55.3	11.1
	3	5	92.0	18.4
	4	5	59.4	11.9
	5	5	74.5	14.9
Carr Inlet-3 (CR02)	1	5	84.7	16.9
	2	5	94.5	18.9
	3	4	45.2	11.3
	4	5	60.6	12.1
	5	5	92.2	18.4
Carr Inlet-4 (CR03)	1	5	64.8	13.0
	2	5	87.3	17.5
	3	5	67.3	13.5
	4	5	53.7	10.7
	5	4	91.5	22.8
Carr Inlet-5 (CR04)	1	5	77.7	15.5
	2	5	71.3	14.3
	3	5	52.1	10.4
	4	5	54.4	10.9
	5	5	74.1	14.8
Carr Inlet-6 (CR05)	1	5	63.4	12.7
	2	5	52.9	10.6
	3	5	85.0	9.0
	4	5	66.9	17.0
	5	5	77.7	13.4

[a] 1 = 2 percent fines
2 = 88 percent fines
3 = 89 percent fines
4 = 97 percent fines
5 = 88.5 percent fines
6 = 93 percent fines.

TABLE B-6. BIOASSAY TEST DATA FOR THE SALINITY EXPERIMENT

Treatment[a]	Replicate	Number Surviving	Total Biomass (mg dry weight)	Average Biomass (mg dry weight)
West Beach-28	1	5	48.2	9.6
	2	5	42.4	8.5
	3	5	68.0	13.6
	4	5	32.2	8.1
	5	4	47.5	9.5
Carr Inlet-28	1	5	39.9	8.0
	2	5	57.2	11.4
	3	5	47.6	9.5
	4	5	61.4	12.3
	5	5	50.4	10.1
Duckabush River-30	1	5	68.0	13.6
	2	5	35.6	7.1
	3	5	47.1	9.4
	4	5	46.7	9.3
	5	5	52.3	10.5
Duckabush River-25	1	4	39.5	9.9
	2	5	38.3	7.7
	3	4	28.1	7.0
	4	4	48.9	12.2
	5	5	40.3	8.1
Duckabush River-22	1	5	76.9	15.4
	2	5	39.6	7.9
	3	5	49.2	9.8
	4	5	70.8	14.2
	5	5	62.8	12.6
Duckabush River-15	1	5	43.4	8.7
	2	5	39.7	7.9
	3	5	42.4	8.5
	4	5	33.5	6.7
	5	5	54.7	10.9

[a] 30 = 30 ppt salinity
28 = 28 ppt salinity
25 = 25 ppt salinity
22 = 22 ppt salinity
15 = 15 ppt salinity.

APPENDIX C

Water Quality Monitoring Data

TABLE C-1. WATER QUALITY MONITORING DATA FOR THE WORM DENSITY EXPERIMENT

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15
West Beach-5	2	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	30	28	28	31	32
		Dissolved oxygen (mg/L)	7.9	7.1	7.1	6.8	7.0	8.2
		pH	8.1	8.1	8.0	8.1	8.0	8.0
	4	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	30	30	28	28	31	32
		Dissolved oxygen (mg/L)	7.9	6.9	6.8	6.9	7.0	8.2
		pH	8.1	8.1	7.9	8.1	8.0	8.0
Carr Inlet-5	1	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	30	30	28	30	32
		Dissolved oxygen (mg/L)	7.7	7.0	7.2	6.6	6.8	8.3
		pH	8.1	8.2	8.1	8.0	7.9	8.0
	4	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	30	30	28	30	32
		Dissolved oxygen (mg/L)	7.7	7.1	6.8	7.2	6.8	8.0
		pH	8.1	8.0	7.4	8.2	7.9	8.0
Carr Inlet-10	1	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	28	30	32
		Dissolved oxygen (mg/L)	7.8	6.8	7.6	6.6	7.0	8.4
		pH	8.1	8.1	8.1	8.0	8.0	8.1
	3	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	28	30	32
		Dissolved oxygen (mg/L)	7.7	6.8	7.7	6.9	7.0	8.4
		pH	8.1	8.2	8.2	8.0	8.0	8.1
Carr Inlet-15	3	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	30	30	28	32	32
		Dissolved oxygen (mg/L)	7.7	6.9	7.7	7.2	7.2	8.0
		pH	8.1	8.2	8.3	8.2	8.1	8.1
	5	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	30	30	30	28	32	32
		Dissolved oxygen (mg/L)	7.7	7.0	7.8	7.2	7.1	7.9
		pH	8.0	8.2	8.1	8.0	8.1	8.1
Carr Inlet-20	3	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	28	30	30	28	30	32
		Dissolved oxygen (mg/L)	7.7	7.0	7.7	7.0	7.1	8.0
		pH	8.1	8.1	8.1	8.1	7.9	8.1
	5	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	28	28	30	28	30	32
		Dissolved oxygen (mg/L)	7.7	7.0	7.8	7.0	7.3	7.9
		pH	8.1	8.1	8.2	8.1	7.9	8.1

TABLE C-1. (Continued)

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15
Elliott Bay-5	2	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	30	28	30	28	29	32
		Dissolved oxygen (mg/L)	7.7	6.2	7.2	6.8	7.0	8.1
		pH	8.0	8.2	8.0	8.1	7.9	8.1
	4	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	28	29	32
		Dissolved oxygen (mg/L)	7.7	6.4	7.4	6.7	7.1	7.9
		pH	8.1	8.0	8.1	8.1	7.9	8.1
Elliott Bay-10	3	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	28	29	32
		Dissolved oxygen (mg/L)	7.8	6.4	7.4	6.6	6.8	8.2
		pH	8.1	8.1	8.1	8.0	7.9	8.0
	5	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	28	29	32
		Dissolved oxygen (mg/L)	7.8	6.6	7.1	6.6	6.8	8.0
		pH	8.1	8.1	7.9	8.0	7.9	8.0
Elliott Bay-15	2	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	30	28	30	30	28	31
		Dissolved oxygen (mg/L)	7.8	6.6	7.1	7.1	6.9	8.0
		pH	8.1	81	8.0	8.1	8:1	7.6
	4	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	30	31	32
		Dissolved oxygen (mg/L)	7.7	6.5	7.8	7.1	6.9	8.2
		pH	8.0	7.9	8.0	8.1	8.1	7.6
Elliott Bay-20	1	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	29	28	30	28	32	32
		Dissolved oxygen (mg/L)	7.8	6.3	7.4	6.8	7.0	8.4
		pH	8.1	8.0	7.9	7.9	7.9	8.0
	4	Temperature (°C)	18	18	19	20	22	22
		Salinity (ppt)	28	30	30	28	32	32
		Dissolved oxygen (mg/L)	7.7	6.7	7.4	6.7	7.1	8.0
		pH	8.1	8.0	8.0	8.0	8.0	8.1

[a] 5 = 5 worms/chamber
10 = 10 worms/chamber
15 = 15 worms/chamber
20 = 20 worms/chamber.

TABLE C-2. WATER QUALITY MONITORING DATA FOR THE FOOD RATION EXPERIMENT

Treatment[a]	Replicate	Variable	Test Date (days) 2	5	8	11	14	17	20
West Beach-0	2	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.2	7.6	7.0	7.3	7.6	7.0
		pH	8.1	8.1	8.1	8.2	8.1	8.2	8.1
	5	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.2	8.3	7.4	7.8	7.3	7.5	7.4
		pH	7.9	8.1	8.1	8.2	8.2	8.1	8.1
West Beach-40	3	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	29	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.1	7.4	6.7	7.5	7.3	7.4
		pH	8.1	8.0	8.1	8.1	8.1	7.9	8.0
	4	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	29	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.4	7.1	7.3	7.5	7.0
		pH	8.1	8.0	8.1	8.2	8.1	8.0	8.0
West Beach-80	2	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.4	6.8	7.3	7.5	7.0
		pH	8.0	8.1	8.1	8.3	8.2	8.2	8.0
	4	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.6	7.1	7.4	7.6	7.3
		pH	8.0	8.1	8.2	8.3	8.2	8.2	8.0
Carr Inlet-0	1	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.6	8.3	7.4	7.0	7.4	5.9	7.2
		pH	8.1	8.3	8.4	8.5	8.3	8.2	8.2
	3	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.6	7.0	7.4	7.7	7.5
		pH	7.9	8.1	8.1	8.6	8.3	8.4	8.2
Carr Inlet-20	4	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.6	6.9	7.4	7.4	7.7
		pH	8.0	8.1	8.3	8.4	8.3	8.3	8.4
	5	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.6	6.8	7.4	7.9	7.5
		pH	8.1	8.1	8.3	8.5	8.4	8.4	8.2

TABLE C-2. (Continued)

Treatment[a]	Replicate	Variable	Test Date (days) 2	5	8	11	14	17	20
Carr Inlet-40	2	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.6	8.3	7.6	6.8	7.4	7.9	7.5
		pH	8.1	8.1	8.3	8.5	8.4	8.4	8.2
	5	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.6	7.0	7.3	7.9	7.5
		pH	8.0	8.1	8.3	8.4	8.3	8.3	8.3
Carr Inlet-60	3	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.5	8.2	7.6	7.1	7.3	7.2	7.5
		pH	8.1	8.1	8.3	8.4	8.2	8.4	8.2
	5	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	32	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.3	7.7	7.0	2.7[b]	7.6	7.7
		pH	8.1	8.2	8.3	8.3	8.0	8.2	8.3
Carr Inlet-80	1	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.2	8.4	7.1	7.0	1.0[b]	7.2	7.4
		pH	8.0	8.0	8.2	8.2	7.8	8.2	8.3
	5	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.3	8.3	7.4	6.8	6.7	7.3	7.4
		pH	8.0	8.0	8.2	8.2	8.1	8.2	8.1
Elliott Bay-0	1	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	28	30	30	30
		Dissolved oxygen (mg/L)	7.5	8.1	7.6	6.9	7.3	7.5	7.5
		pH	8.1	8.0	8.2	8.3	8.2	8.3	8.2
	4	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.2	7.6	6.9	7.3	7.5	7.7
		pH	8.1	8.1	8.2	8.4	8.2	8.4	8.4
Elliott Bay-20	2	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.4	8.1	7.5	7.2	7.1	7.6	7.2
		pH	8.1	8.0	8.2	8.5	8.1	8.4	8.3
	3	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.3	8.1	7.5	7.1	6.9	7.7	7.4
		pH	8.1	8.0	8.2	8.1	8.3	8.1	8.2

TABLE C-2. (Continued)

Treatment[a]	Replicate	Variable	Test Date (days) 2	5	8	11	14	17	20
Elliott Bay-40	1	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	32	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	6.9	7.1	7.5	7.4	8.0	5.1	7.4
		pH	8.0	8.0	8.2	8.0	7.4	7.7	7.9
	3	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	6.8	7.1	7.5	7.4	7.9	7.3	7.4
		pH	8.2	8.0	8.2	8.1	7.9	8.2	8.0
Elliott Bay-60	1	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.2	8.0	7.5	7.2	7.1	7.4	7.2
		pH	8.1	8.0	8.2	8.2	8.1	8.1	8.1
	5	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	5.4	8.1	7.3	7.4	7.1	7.3	7.5
		pH	7.5	8.0	8.1	8.3	8.2	8.2	8.0
Elliott Bay-80	4	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.3	8.2	7.3	7.1	7.3	7.7	7.5
		pH	7.9	7.9	8.2	8.2	8.2	8.0	8.1
	4	Temperature (°C)	21	19	19.5	20	21	20	19
		Salinity (ppt)	30	26	26	30	30	30	30
		Dissolved oxygen (mg/L)	7.2	8.2	8.4	6.9	7.3	7.3	7.4
		pH	8.1	7.9	8.1	8.2	8.1	8.1	8.0

[a] 0 = no food
20 = 20 mg/48 hours
40 = 40 mg/48 hours
60 = 60 mg/48 hours
80 = 80 mg/48 hours.

[b] Aeration to the exposure chamber was not on.

TABLE C-3. WATER QUALITY MONITORING DATA FOR THE TEST DURATION EXPERIMENT

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15	18	20
West Beach-10	3	Temperature (°C)	19	19	20	19	--	--	--	--
		Salinity (ppt)	28	28	29	28	--	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.5	7.2	7.7	--	--	--	--
		pH	8.1	8.1	8.1	8.1	--	--	--	--
	4	Temperature (°C)	19	19	20	19	--	--	--	--
		Salinity (ppt)	28	28	28	28	--	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.5	7.2	7.7	--	--	--	--
		pH	8.1	8.1	8.1	8.1	--	--	--	--
West Beach-15	1	Temperature (°C)	19	19	20	19	19	--	--	--
		Salinity (ppt)	28	29	29	29	28	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.5	7.0	7.5	8.0	--	--	--
		pH	8.1	8.0	8.1	8.0	8.0	--	--	--
	5	Temperature (°C)	19	19	20	19	19	--	--	--
		Salinity (ppt)	28	30	29	28	28	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.5	7.3	7.7	7.8	--	--	--
		pH	8.1	8.1	8.1	8.1	8.1	--	--	--
West Beach-20	2	Temperature (°C)	19	19	20	19	19	19	19	19
		Salinity (ppt)	28	28	28	28	28	28	28	30
		Dissolved oxygen (mg/L)	7.5	7.9	7.3	7.7	7.9	7.5	7.3	6.8
		pH	8.1	8.0	8.0	8.1	8.1	8.2	8.1	8.2
	4	Temperature (°C)	19	19	20	19	19	18	19	19
		Salinity (ppt)	28	28	28	28	28	28	28	30
		Dissolved oxygen (mg/L)	7.5	7.7	7.4	7.7	8.1	7.5	7.1	7.0
		pH	8.1	8.2	8.2	8.2	8.2	8.3	8.2	8.2
Carr Inlet-10	3	Temperature (°C)	19	19	20	19	--	--	--	--
		Salinity (ppt)	28	28	29	28	--	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.7	7.3	7.7	--	--	--	--
		pH	8.1	8.1	8.2	8.2	--	--	--	--
	2	Temperature (°C)	19	19	20	19	--	--	--	--
		Salinity (ppt)	28	28	28	29	--	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.7	7.1	7.6	--	--	--	--
		pH	8.1	8.1	8.3	8.3	--	--	--	--
Carr Inlet-15	3	Temperature (°C)	19	19	20	19	19	18	--	--
		Salinity (ppt)	28	30	30	29	28	28	--	--
		Dissolved oxygen (mg/L)	7.5	7.7	7.4	7.7	7.9	7.5	--	--
		pH	8.1	8.2	8.1	8.3	8.3	8.3	--	--
	5	Temperature (°C)	19	19	20	19	19	18	--	--
		Salinity (ppt)	28	30	29	28	28	28	--	--
		Dissolved oxygen (mg/L)	7.5	7.7	7.3	7.7	8.0	7.5	--	--
		pH	8.0	8.2	8.3	8.4	8.2	8.1	--	--

TABLE C-3. (Continued)

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15	18	20
Carr Inlet-20	2	Temperature (°C)	19	19	20	19	19	18	19	19
		Salinity (ppt)	28	28	28	28	28	28	29	30
		Dissolved oxygen (mg/L)	7.5	7.7	7.3	7.7	7.8	7.5	7.2	7.0
		pH	8.0	8.0	8.3	8.1	8.2	8.2	8.3	8.3
	3	Temperature (°C)	19	19	20	19	19	18	19	19
		Salinity (ppt)	28	28	29	28	28	28	29	30
		Dissolved oxygen (mg/L)	7.5	7.7	7.4	7.7	7.8	7.5	7.3	7.0
		pH	8.0	8.1	8.0	8.3	8.3	8.4	8.3	8.4
Elliott Bay-10	1	Temperature (°C)	19	19	20	19	--	--	--	--
		Salinity (ppt)	28	28	29	28	--	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.9	7.4	7.7	--	--	--	--
		pH	8.1	8.2	8.1	8.3	--	--	--	--
	3	Temperature (°C)	19	19	20	19	--	--	--	--
		Salinity (ppt)	28	28	28	28	--	--	--	--
		Dissolved oxygen (mg/L)	7.5	7.9	7.3	7.7	--	--	--	--
		pH	8.1	8.2	8.2	8.2	--	--	--	--
Elliott Bay-15	4	Temperature (°C)	19	19	20	19	19	18	--	--
		Salinity (ppt)	28	28	28	28	28	28	--	--
		Dissolved oxygen (mg/L)	7.7	7.9	7.4	7.7	7.8	7.5	--	--
		pH	8.1	8.1	8.1	8.1	8.1	8.3	--	--
	5	Temperature (°C)	19	19	20	19	19	18	--	--
		Salinity (ppt)	28	28	28	28	28	28	--	--
		Dissolved oxygen (mg/L)	7.5	7.7	7.2	7.7	7.7	7.5	--	--
		pH	8.1	8.1	8.1	8.1	8.2	8.3	--	--
Elliott Bay-20	3	Temperature (°C)	19	19	20	19	19	18	19	19
		Salinity (ppt)	28	28	28	30	28	28	28	28
		Dissolved oxygen (mg/L)	7.9	7.5	7.2	6.8	7.5	7.7	7.3	7.7
		pH	8.2	8.4	8.4	8.3	8.1	8.2	8.4	8.3
	4	Temperature (°C)	19	19	20	19	19	18	19	19
		Salinity (ppt)	28	22	28	30	28	28	28	28
		Dissolved oxygen (mg/L)	7.9	7.5	7.2	7.0	7.5	7.7	7.3	7.7
		pH	8.0	8.2	8.3	8.2	8.1	8.2	8.2	8.2

[a] 10 = 10-day exposure
15 = 15-day exposure
20 = 20-day exposure.

TABLE C-4. WATER QUALITY MONITORING DATA FOR THE SEDIMENT GRAIN-SIZE EXPERIMENT

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15	18	20
West Beach-1	3	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	29	29	28	29	30	30
		Dissolved oxygen (mg/L)	7.2	6.5	8.3	7.3	7.1	7.8	7.9	7.7
		pH	8.2	8.1	8.2	8.0	8.0	8.3	8.2	8.1
	5	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	30	30	30	29	30	30
		Dissolved Oxygen (mg/L)	7.2	6.6	8.3	7.6	7.3	7.8	8.0	7.7
		pH	8.1	8.1	8.2	8.2	8.0	8.2	8.1	8.0
Carr Inlet-2 (CR01)	1	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	31	30	31	31
		Dissolved Oxygen (mg/L)	7.2	6.3	8.3	7.5	7.3	7.8	8.0	7.7
		pH	8.0	8.1	8.3	8.3	8.2	8.3	8.4	8.4
	4	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	30	29	30	30	32	32
		Dissolved Oxygen (mg/L)	7.0	6.5	8.2	7.7	7.5	7.7	7.9	7.7
		pH	8.3	8.1	8.3	8.4	8.3	8.5	8.5	8.14
Carr Inlet-3 (CR02)	3	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	29	29	30	30	30	24
		Dissolved oxygen (mg/L)	7.2	6.4	7.9	7.8	7.5	7.9	7.9	7.7
		pH	8.2	7.9	8.2	8.2	8.1	8.3	8.2	8.2
	4	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	29	29	30	28
		Dissolved oxygen (mg/L)	7.2	6.6	8.1	7.2	7.5	7.7	7.9	7.6
		pH	8.2	8.0	8.1	7.9	7.8	8.0	8.0	8.0
Carr Inlet-4 (CR03)	2	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	29	29	30	29
		Dissolved oxygen (mg/L)	6.2	6.6	8.3	7.2	6.9	7.7	7.9	7.5
		pH	8.1	8.1	8.2	7.9	8.3	8.2	8.2	8.1
	4	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	28	29	30	28
		Dissolved oxygen (mg/L)	6.8	6.7	8.3	7.5	7.3	7.7	7.9	7.5
		pH	8.3	8.1	8.1	8.1	8.0	8.2	8.1	8.1
Carr Inlet-5 (CR04)	2	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	30	29	30	29
		Dissolved oxygen (mg/L)	7.0	6.3	8.3	7.3	7.3	7.7	7.9	7.5
		pH	8.2	8.0	8.1	8.1	8.0	8.2	8.2	8.0
	3	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	28	29	30	29
		Dissolved oxygen (mg/L)	7.0	6.5	8.3	7.4	7.3	7.9	8.0	7.7
		pH	8.2	8.1	8.2	8.1	8.0	8.3	8.3	8.2

TABLE C-4. (Continued)

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15	18	20
Carr Inlet-6 (CR05)	1	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	30	29	30	29
		Dissolved oxygen (mg/L)	7.0	6.3	8.3	7.3	7.3	7.7	7.9	7.5
		pH	8.2	8.0	8.1	8.1	8.0	8.2	8.0	8.0
	3	Temperature (°C)	18	24	18	18	20	18	19	19
		Salinity (ppt)	30	29	28	29	28	29	30	29
		Dissolved oxygen (mg/L)	7.0	6.6	8.1	7.4	6.6	7.7	7.9	7.5
		pH	8.2	8.1	8.1	8.0	8.0	8.3	8.3	8.2

[a] 1 = 2 percent fines
2 = 88 percent fines
3 = 89 percent fines
4 = 97 percent fines
5 = 88.5 percent fines
6 = 93 percent fines.

TABLE C-5. WATER QUALITY MONITORING DATA FOR THE SALINITY EXPERIMENT

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15	18	20
West Beach-28	1	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	27	28	28	27	28	28
		Dissolved oxygen (mg/L)	8.7	7.2	7.1	7.9	7.9	7.3	7.1	7.3
		pH	7.8	7.9	7.9	8.3	8.3	8.3	8.1	7.9
	2	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	27	27	28	28	28	28
		Dissolved oxygen (mg/L)	8.7	7.5	7.5	7.9	7.9	7.3	6.9	7.1
		pH	8.1	8.1	8.0	8.2	8.2	8.2	7.9	8.0
Carr Inlet-28	1	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	29	30	30	30	30	29
		Dissolved oxygen (mg/L)	8.7	7.8	7.7	7.9	7.9	7.2	7.2	7.3
		pH	8.2	8.2	8.1	8.4	8.4	8.4	8.2	8.3
	4	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	27	28	28	28	29	29
		Dissolved oxygen (mg/L)	8.7	7.1	7.4	7.9	7.9	7.2	7.2	7.3
		pH	8.1	8.1	8.0	8.4	8.4	8.4	8.2	8.3
Duckabush River-30	1	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	27	28	28	28	28	26
		Dissolved oxygen (mg/L)	7.5	7.7	7.9	7.7	6.9	7.1	7.3	7.4
		pH	8.1	8.0	7.9	8.2	8.2	8.1	8.0	8.2
	3	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	28	28	28	27	28	28
		Dissolved oxygen (mg/L)	7.7	7.7	8.5	7.9	7.7	7.1	7.0	7.3
		pH	8.1	8.1	8.0	8.2	8.2	8.1	7.7	7.9
Duckabush River-25	1	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	27	27	28	27	28	26
		Dissolved oxygen (mg/L)	8.7	6.8	6.4	7.9	7.7	7.1	6.9	7.1
		pH	7.9	7.7	7.6	8.2	8.2	8.1	7.9	8.0
	5	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	27	28	28	27	28	26
		Dissolved oxygen (mg/L)	8.7	7.7	7.7	7.9	7.9	7.1	7.6	5.5
		pH	8.2	8.1	8.0	8.2	8.2	8.2	7.5	7.6
Duckabush River-22	2	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	28	28	28	27	28	27
		Dissolved oxygen (mg/L)	8.7	7.6	7.7	7.9	8.0	7.2	6.9	7.1
		pH	8.2	8.1	8.0	8.2	8.2	8.1	7.7	7.9
	4	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	26	26	27	27	27	26
		Dissolved oxygen (mg/L)	8.7	7.6	7.7	7.9	8.0	7.2	7.1	7.2
		pH	8.1	8.1	8.0	8.1	8.0	7.9	7.7	7.8

TABLE C-5. (Continued)

Treatment[a]	Replicate	Variable	Test Date (days) 0	3	6	9	12	15	18	20
Duckabush River-15	3	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	26	27	27	29	28	27
		Dissolved oxygen (mg/L)	8.7	7.7	7.7	7.9	7.9	7.3	7.0	7.3
		pH	8.2	8.2	8.1	8.4	8.3	8.2	8.0	8.1
	5	Temperature (°C)	18	17.5	20	18	19	19	19	18.5
		Salinity (ppt)	26	26	26	27	27	27	27	26
		Dissolved oxygen (mg/L)	8.7	7.8	7.7	7.9	7.9	7.3	7.1	7.3
		pH	8.2	8.2	8.1	8.2	8.1	8.0	7.9	8.0

[a] 30 = 30 ppt salinity
28 = 28 ppt salinity
25 = 25 ppt salinity
22 = 22 ppt salinity
15 = 15 ppt salinity.