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RENTON SEWAGE TREATMENT PLANT PROJECT:
SEAHURST BASELINE STUDY

Q. J. Stober and K. K. Chew, Principal Investigators

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Marine Toxicology

by

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RENTON SEWAGE TREATMENT PLANT PROJECT:
Seahurst Baseline Study
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PREFACE

Expansion of the Renton Sewage Treatment Plant to meet the needs of a growing population in the Seattle metropolitan area was begun in 1982. Since its construction in the mid-1960's, the secondary effluent from this plant has been discharged into the Duwamish River near Tukwila, Washington. The increased capacity of this plant from 36 million gallons per day to 72 mgd would not allow continued compliance with established water quality standards in the Duwamish River. A plan was adopted to construct a pipeline, tunnel and marine outfall system to divert effluent directly to a site due west of the plant in the south central Puget Sound basin at Seahurst.

The Seahurst Baseline Study was designed as a part of the plant expansion plan to obtain pre-discharge ecological data in the marine environment in the East and Colvos Passage areas of south central Puget Sound (Figure 1.1). Data from this Study were utilized to minimize potential impacts of the design, to aid planning efforts, to evaluate several outfall alignments in Seahurst Bay and to assess changes which could occur once effluent was discharged at the site.

The Seahurst Baseline Study was originally designed with three broad objectives: (1) to collect, analyze and interpret the significance of physical, chemical and biological data around the proposed sewage treatment plant outfall at Seahurst to determine the properties and characteristics of the receiving environment; (2) to utilize the information obtained to aid in the siting and design of the outfall pipe and diffuser; and (3) to recommend a post-discharge monitoring plan that would effectively and efficiently determine if the presence of the new outfall significantly changes the receiving water environment (this objective was not fulfilled because Seahurst

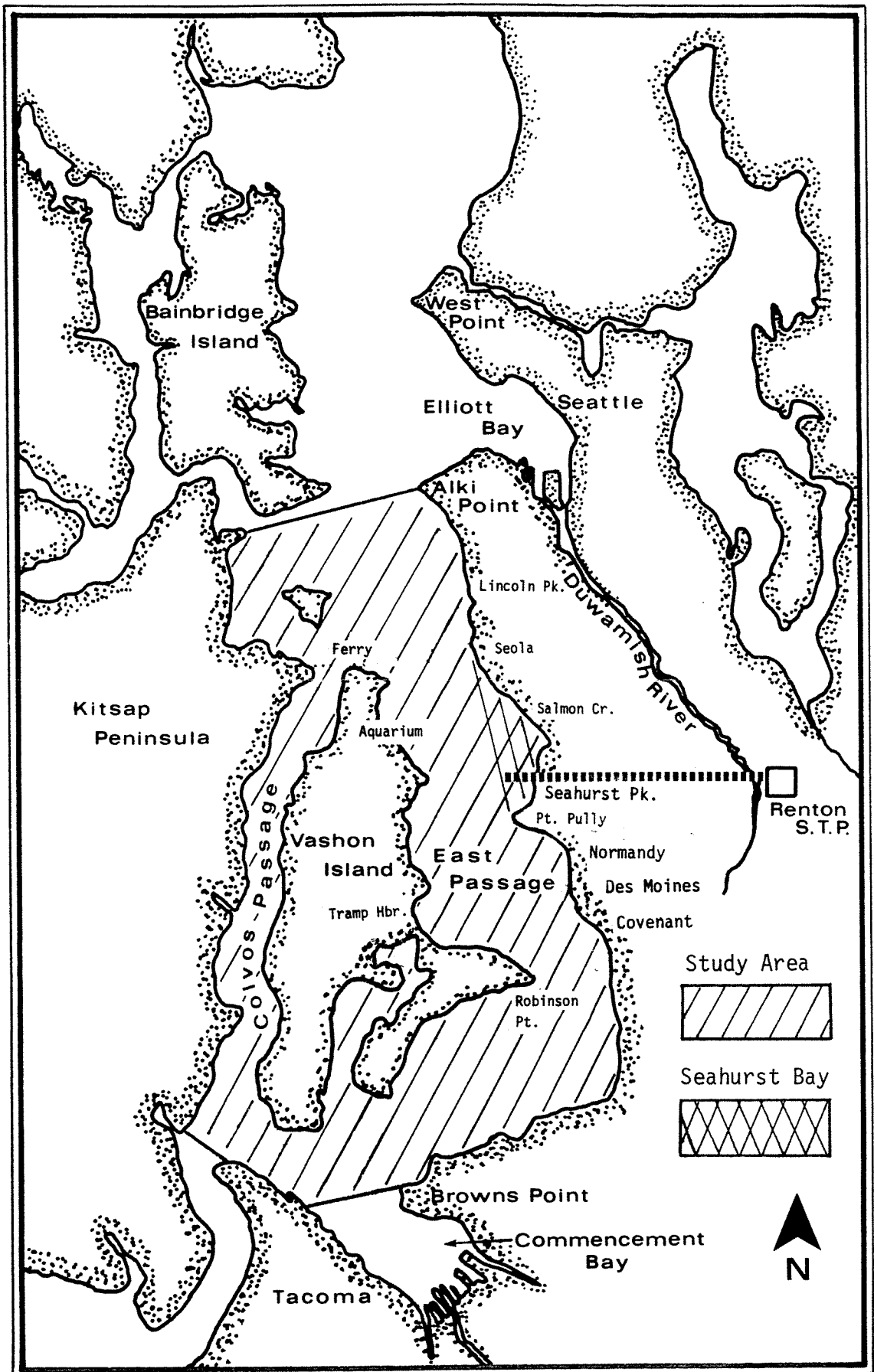


Figure 1.1. The Central Puget Sound basin showing the location of the Renton Sewage Treatment Plant and the Seahurst Baseline study area in the south central basin.

Bay was dismissed from further outfall siting consideration).

The Seahurst Baseline Study was initiated in April 1982. It was to extend for a three year period with field sampling designed to terminate in March 1984 followed by analysis of the data, a final report and a post-effluent discharge monitoring plan in March 1985. In April 1984, after considerable study of new oceanographic, ecological baseline and cost information, the METRO Council decided to move the Renton Treatment Plant outfall site to Duwamish Head in outer Elliott Bay. This final report was produced on a shortened time schedule to document the ecological data collected in Seahurst Bay and the East and Colvos Passages of Puget Sound.

The technical organization of this study is illustrated in Figure 1.2. The major project divisions which routinely interacted were grouped together under water column, environmental health, sediment investigations and chemistry. The studies of the water column included monitoring of the physical/chemical characteristics of the water and the plankton. Sediment investigations included the ecological studies of the intertidal and subtidal benthos and fishes. Environmental health included the measurement of human pathogens (bacteria and viruses), fish pathology (fish health) and marine toxicology (in the water column, sediments and effluent). The chemical analyses were focused on the presence of trace metals in water, biota and sediment, in addition to a variety of support services to the entire project. The METRO Water Quality Laboratory was responsible for the analysis of trace organic compounds in sediments. The organizational structure of this study facilitated efficiency, fiscal control, interdisciplinary integration within and between tasks and ensured that the study scope and schedules were met in a timely manner. This report is similarly organized by task.

Statistical expertise was provided on a regular basis to faculty, staff

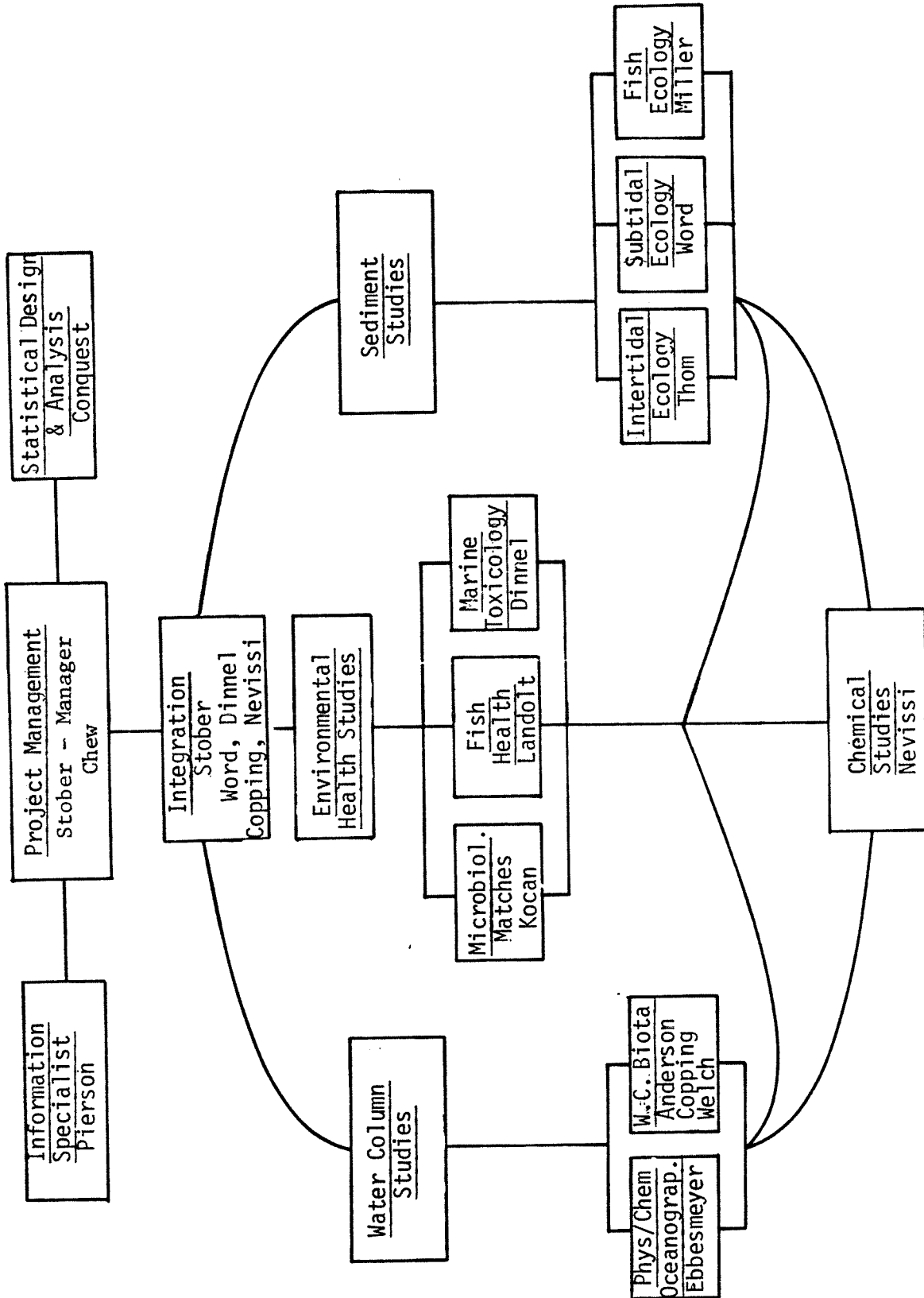


Figure 1.2. Organization of the Renton Sewage Treatment Plant Project, Seahurst baseline study indicating major project divisions, specific tasks, and responsibilities of selected personnel.

and students associated with the project by Dr. Loveday Conquest in the Center for Quantitative Sciences in Forestry, Fisheries and Wildlife. Areas of investigation included computation of required sample sizes in various experimental designs, appropriate transformations of data, use of parametric and nonparametric tests, and general statistical approaches to data analysis.

Organizational and coordination meetings were held routinely throughout the study period within and between task groups to plan sampling and analytical activities and to discuss developing interpretations of the data. The Project Manager and Project Leaders were in daily contact with METRO staff throughout the study in an effort to find solutions to problems and to ensure the maximum performance of all investigators.

The information developed describes the experimental studies conducted and the existing detailed ecological baseline conditions in the south central basin of Puget Sound. These data will have direct applicability in future impact assessments in the area and will provide the basis for long range ecological monitoring of the study area in Puget Sound. The comprehensive and integrated approach used allowed the development of a more complete understanding of the marine ecology and the dispersion of particulates from marine sewage outfalls. The new information gained provides the basis for the design of specific future investigations needed to assess the effects of wastewater management on Puget Sound.

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ABSTRACT

Baseline levels of water column toxicity were measured at eleven stations near Seahurst in East Passage and at one historical station off West Point. Water samples were collected several times per month during summer and fall of 1982 and 1983 at two or three depths per station. The presence of toxicity was based on the results of standard 48-hour oyster embryo survival and abnormal development and sand dollar egg fertilization bioassays.

Measurable background water toxicity was detected at all stations and depths sampled. A high degree of variance dependent on date, location, year and assay type was evident at most stations. Oyster embryo mortality was highest in surface waters and within the Seahurst area of East Passage. Comparison of these results with historical Washington Department of Fisheries data from 1962-76 showed no substantial changes in oyster embryo responses either in magnitude or degree of variability for four similar stations.

Bioassays of five stages of Renton sewage were conducted using a variety of gamete, embryo, and larval stages of common marine animals. The relative sensitivity of the bioassay indices ranked highest for: sand dollar and sea urchin sperm (egg fertilization) followed by oyster and sea urchin embryo abnormality, oyster and sea urchin embryo mortality, and crab zoea mortality. The toxicity of sewage ranked in the following order with chlorinated secondary being most toxic followed by influent, primary, dechlorinated secondary, and unchlorinated secondary. Primary treatment afforded only a slight reduction in acute toxicity over that observed for the influent sewage. Secondary treatment effected a marked reduction in acute toxicity over that observed for primary treated sewage. Unchlorinated and chlorinated-dechlorinated secondary sewage were essentially equal in toxicity.

Chlorinated secondary sewage was more acutely toxic than influent or primary sewage. However, the rapidity of chlorine decay in seawater may "dilute out" the toxic effects of chlorine a short distance from the discharge point. Primary or influent sewage was less toxic than chlorinated secondary sewage. However, the toxicity of these stages may be due to the interaction of a wide variety of pollutants (i.e., metals, xenobiotic organics, petroleum hydrocarbons, pesticides, detergents, etc.) which probably more conservative than chlorine in receiving waters.

The discharge of either unchlorinated or dechlorinated secondary effluent sewage to Puget Sound should not cause any discernable acute toxicity to sensitive marine life stages assuming initial dilution of 100:1 or greater. The discharge of either chlorinated secondary or primary (especially chlorinated primary) sewage could cause acute toxicity on some occasions at initial dilutions less than 200:1.

Ten day amphipod bioassays of sediments from 27 Seahurst area stations were conducted twice each year for two years. Reduced amphipod survival and increased sublethal stress occurred in sediments from the northern East Passage area and were correlated with grain size and toxicant concentration. Seasonal trends in survival were generally not evident and within station variability was not consistently different between seasons and may be associated with patchy chemical distribution. Results suggest that future addition of toxicants to the Seahurst area sediments may result in lower amphipod survival, however, additional types of sediment bioassays should be conducted to refine estimates of sediment toxicity.

12.0 MARINE TOXICOLOGY

P. A. Dinnel, F. S. Ott, and Q. J. Stober

12.1 Introduction

Past toxicological studies by METRO biologists on the real or potential impacts of Renton treatment plant effluent have dealt exclusively with freshwater or anadromous species. The planned move to an estuarine discharge requires a focus on the potential effects of the effluent on marine organisms.

As receiving water for municipal wastes, estuarine systems differ in at least four significant ways from river systems: 1) estuaries are generally larger and able to assimilate greater amounts of waste on a strictly volume-for-volume basis, 2) estuaries are oscillatory, partially recirculating systems, 3) the higher salinity and pH may modify the physical, chemical, and biological interactions of wastes discharged and 4) estuarine/marine waters are inhabited by a much more diverse assemblage of plant and animal life including especially sensitive embryonic/larval stages of many marine animals undergoing development.

These investigations have addressed two specific needs: 1) assessment of present background toxicity in the proposed receiving waters and sediments, and 2) toxicity tests of the five stages of Renton Treatment Plant sewage with sensitive marine life stages to document treatment plant effectiveness in toxicity reduction and prediction of possible zones of toxicity relative to the receiving waters.

Specifically, the following bioassays were conducted during this study:

1. Oyster (Crassostrea gigas) embryo and sand dollar (Dendraster excentricus) sperm bioassays were conducted on sets of receiving water samples collected periodically by the Water Column group.

2. Three sets of bioassays were conducted to measure water toxicity related to phytoplankton metabolites produced in laboratory cultures. Past testing programs have shown that Puget Sound surface waters can be toxic to oyster embryos during periods of phytoplankton blooms.
3. Ten bioassays of the five stages of Renton sewage were conducted to define toxicity to each of the following sensitive marine organism life stages: oyster embryos, sand dollar sperm and eggs, green sea urchin (Strongylocentrotus droebachiensis) sperm, eggs, and embryos, and Dungeness crab (Cancer magister) zoea. Sand dollar and sea urchin sperm bioassays were conducted seasonally (summer/winter) for two years to assess between-season and between-year variability in Renton sewage toxicity.
4. Bioassays of ammonia were conducted to determine its potential toxicity to embryo stages in marine waters.
5. Amphipod (Rhepoxynius abronius) bioassays were conducted on Seahurst baseline sediments from areas identified as "depositional environments" by the Subtidal Biology group.
6. Developmental amphipod/sediment experiments designed to aid interpretation of Baseline bioassay results were conducted to assess the interactive effects of sediment grain size, natural organics, salinity, and toxicants on amphipod survival and behavior. The results of these experiments are the subject of a Ph.D. dissertation and will be reported under separate cover (Ott, in prep.).

The oyster embryo bioassay for toxicants and receiving waters was developed by Woelke (1968, 1972) and has been used by the Washington Department of Fisheries since 1961 as a biomonitoring tool for marine water

quality. Likewise, echinoderm embryos of a variety of species have been extensively used as marine bioassay tools in Europe (Hagstrom and Lonning 1973; 1977), Japan (Kobayashi 1971, 1974; Okubo and Okubo 1962), and the United States (Dinnel et al. 1982; Oshida et al. 1981). Recently, the use of echinoderm gametes in a sperm/fertilization bioassay was developed and refined (Dinnel et al. 1982, 1983) and used to monitor sewage impacts on marine waters (Oshida et al. 1981). Larval crustaceans have also been used for assessing the toxicity of a variety of compounds in seawater and have been found to be especially sensitive to organic compounds including pesticides (Armstrong et al. 1976; Martin et al. 1981).

Bioassays using small crustaceans or juvenile fish are being employed to characterize sediment toxicity from a growing number of areas including Baltimore Harbor, Chesapeake Bay, and Commencement Bay (Ott et al. in prep; Read et al. 1978; Swartz et al. 1982; Tsai et al. 1979). Gammarid amphipods are known to be adversely affected by anthropogenic compounds and are currently being used extensively in sediment bioassay systems (Chapman 1982a, b; McCain, NMFS, Seattle, WA, personal communications; Swartz et al. 1979 and 1982; Levings et al. 1975). Gammarid amphipods are crustaceans with short life cycles and exceedingly high rates of production (Albright and Armstrong 1982). Densities may exceed $60,000/m^2$ and these abundant amphipods constitute a major food source for commercial fish and crabs as well as birds (Albright 1977; Higley and Holton 1975; Smith 1981). Demise of this food source may represent a serious ecological impact.

The infaunal amphipod, Rhepoxynius abronius (used in this study) is available in the minimally contaminated areas of northern Puget Sound. The free-burrowing habits ensure these animals are continuously exposed to contaminants when the animals are in the sediments. It is likely that these

amphipods also ingest contaminants when feeding. R. abronius is reported to feed on infaunal micro-organisms, bacteria, and detritus present in the interstitial waters of sand beaches (Oakden 1981; Oliver et al. 1982).

12.2 Description of the Study Area

The primary study area was located in the South Central Puget Sound basin between Alki Pt. and Brown's Pt. (Figure 1.1, Preface). A single "reference" water column station was located north of this area off West Point; a station for which historical physical/chemical water quality monitoring data exists from previous studies. METRO's plans originally called for the construction of a tunnel from the Renton Sewage Treatment Plant to Puget Sound at Seahurst Park for the offshore discharge of secondary treated effluent.

12.3 Laboratory Facilities

All bioassays were conducted at the University of Washington mobile marine laboratory located at the METRO West Point sewage treatment plant, Seattle, Washington. Seawater from Puget Sound was continuously supplied to the laboratory by 7.5-hp cast-iron pumps. The seawater was pumped from a depth of approximately 7 to 10 m depending on tide level. An 1800-liter fiberglass head-tank supplied seawater to the laboratory via gravity flow. All laboratory plumbing was constructed of polyvinyl chloride (PVC) pipe and fittings conditioned in flowing seawater more than six years.

12.4 Materials and Methods

12.4.1 Test Animal Collection and Handling

12.4.1.1 Oysters

Adult Pacific oysters were collected intertidally from several areas of

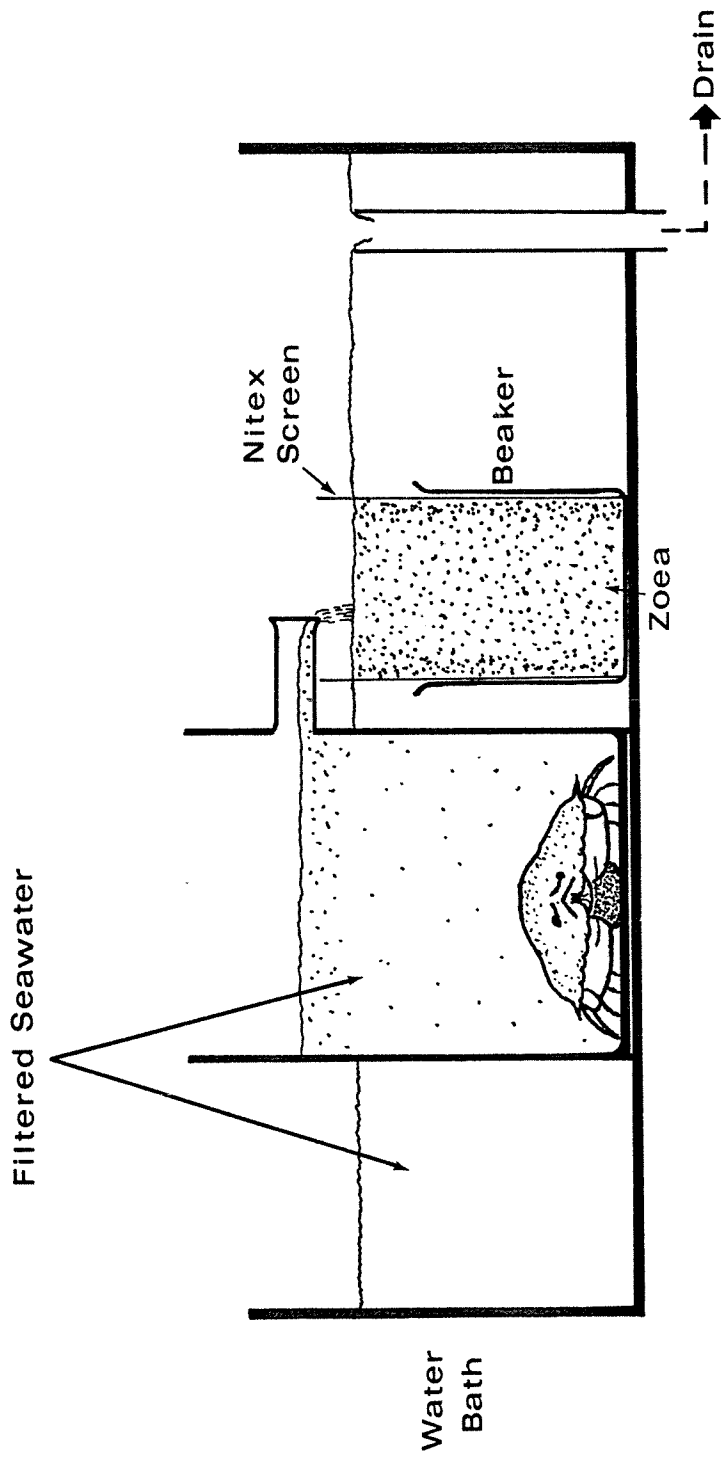


Figure 12.1. System for catching newly hatched crab zoea for use in bioassays.

Puget Sound and Hood Canal (depending on time of the year). Sites of collection of ripe animals included Coast Oyster Company on Quilcene Bay, Hood Canal; Bay Center Mariculture, Willapa Bay; Oakland Bay on South Puget Sound; and Big Beef Creek on Hood Canal. All ripe oysters were transported to the West Point laboratory in a "dry" condition and held a maximum of four weeks in either ambient seawater or in a 20°C flow-through seawater system until used for testing.

For winter tests, oysters from Big Beef Creek were held in a basket at the Manchester dock on Central Puget Sound during summer (the cooler water retarded maturation) and conditioned at Manchester in 20°C seawater for 4-8 weeks. The conditioned oysters were fed phytoplankton cultures daily to insure proper maturation.

12.4.1.2 Sand Dollars

Sand dollars were collected intertidally from Kopachuck State Park on Carr Inlet, South Puget Sound, transported to the lab in ice chests, and maintained in a flow-through tank on a bed of sand at ambient seawater temperature. Ambient planktonic organisms in the seawater and detritus in the sand served as the only food sources.

12.4.1.3 Sea Urchins

Green sea urchins were collected subtidally by diving in the Straits of Juan de Fuca approximately 25 km west of Port Angeles, Washington. The sea urchins were held in ambient West Point flowing seawater and occasionally fed kelp.

12.4.1.4 Dungeness Crab Zoea

Crab zoea were hatched from gravid female crabs collected by the commercial crab boat "Dream" operating out of West Port, Washington. The zoea collection system is diagrammed in Figure 12.1.

12.4.1.5 Amphipods

Amphipods (Rhepoxynius abronius) were collected from either West Beach, Whidbey Island or Bowman Bay, located immediately north of West Beach across Deception Pass. Amphipods were collected subtidally in both locations: at West Beach by towing a benthic sled and at Bowman Bay by operating an airlift hydraulic system designed by P. Plesha (National Marine Fisheries Service, Seattle, Washington). Animals were held 48 to 96-hr prior to use in 20-liter covered plastic buckets containing aerated seawater and one liter of the fraction of native beach sediment which passed through a 1.0-mm screen. Buckets were kept to an ambient temperature flowing seawater trough.

12.4.2 Receiving Water Bioassays

12.4.2.1 Sample Collection, Handling, and Preparation

Receiving water samples were collected several times monthly during the summer by the Water Column group on board METRO's R/V Liberty. Samples were collected by PVC Niskin bottles at three depths (surface, mid-depth, and bottom or surface and bottom for shallow stations) at nine stations in the Seahurst area of East Passage and at one station each off West Point, Browns Point and the north end of Colvos Passage (Figure 12.2). Seahurst stations 4 through 10 were sampled during the first 1-1/2 years and stations 4, 6, 7, 10, 12 and 13 sampled during the last portion of the second year to address a newly established southern alignment for the proposed outfall. Several sampling depths were selected to provide a vertical profile of water column toxicity. The samples were drained into clean, seawater-aged 4-liter polyethylene bottles, packed in ice, and transported to the West Point laboratory upon docking at Shilshole Marina. All receiving water bioassays were started immediately upon reception of the samples at the West Point lab (i.e., less than eight hours after sample collection).

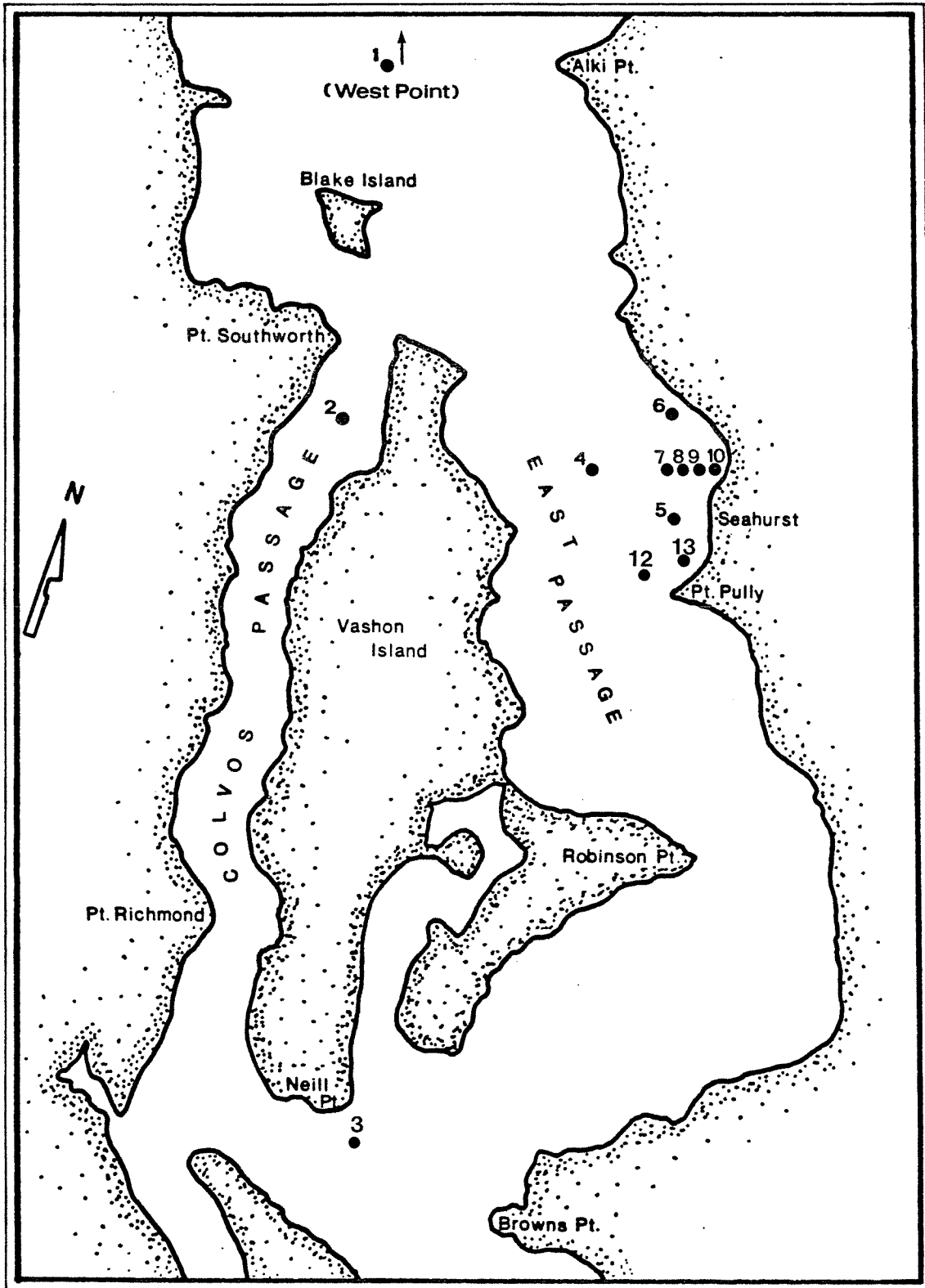


Figure 12.2. Map of south central Puget Sound showing the water column sampling stations.

10.5

Four types of "control" water were tested side-by-side with each set of receiving water samples. The "primary" control water (used as the "standard" for comparing all other water types) was obtained from underground storage tanks at Kincaid Hall (Zoology) at the University of Washington. This water was collected by the truckload once or twice a year from the Anacortes ferry dock at high tide and was used for routine marine embryological work by the Zoology department. This water was selected as the "primary" control water since it was readily available and its quality was thought to be consistent through time. A "carry-along" control sample of Kincaid Hall seawater was sent along with the ice chests during each cruise. This sample was used to monitor any possible sources of sample contamination due to handling in the field. A third "control" sample consisted of West Point filtered (5-um) seawater collected from the seawater system just prior to each bioassay. The fourth "control" sample was seawater previously collected at the Friday Harbor Marine Laboratory on San Juan Island. This water was collected in June, 1982 and May 1983 in several dozen clean polyethylene 4-liter bottles, quickly frozen in a commercial food locker at 0°F, transported back to the University in ice chests, and stored in the Fisheries freezer room at 0°F. The Friday Harbor water should give the best overall results as control water since it came from an area well outside urban/industrial development and the quality should be constant between tests due to quick freezing. This water was not, however, used as the "primary" control during these studies since the effect of freezing on water quality was undetermined.

Test sample preparation was accomplished by rinsing each 1-liter polypropylene (Tri-pour) beaker with the respective sample water and filling three replicate beakers to 1 liter with the sample or control water to be tested with oyster embryos. A 10-ml subsample was removed by pipette from

each beaker and put into borosilicate glass test tubes (16 x 100 mm) for the sand dollar sperm assays. The beakers and test tubes were maintained in water baths during the appropriate exposure periods. Sperm assay tubes were held at ambient seawater temperature and oyster embryo beakers at 20°C.

12.4.2.2 Bioassay Procedures

Oyster Embryo Bioassays

For each embryo bioassay approximately 6-8 sexually ripe adult oysters were spawned by raising the water temperature to 28-30°C for 2-4 hours and then adding sperm from a sacrificed male to the water. Spawning normally occurred in 30-60 minutes in some of the conditioned oysters. The eggs were fertilized and added to the 1-liter test beakers at a density of approximately 25,000-35,000 per beaker within one hour of fertilization. The embryos were incubated at 20°C for 48 hours. At the conclusion of each test a 10-ml subsample was removed from each beaker after thorough mixing and fixed with 10% formalin in glass test tubes.

Each embryo sample was later examined under a compound microscope and tabulated for number of embryos in each sample and number of embryos failing to mature to the normal straight-hinge veliger stage (Woelke 1972).

Sand Dollar Sperm Bioassays

For each set of sperm bioassays a male and female sand dollar was spawned separately by injecting approximately 0.5 ml of 0.5-M potassium chloride (KCl) through the oral opening. The eggs were washed several times and adjusted to a density of 2,000/ml. The sperm were mixed, subsampled, killed in 10% acetic acid, counted with a hemacytometer at 400x magnification and the stock solution diluted to a density of 2.0×10^7 /ml. For each bioassay series 0.1 ml of the sperm solution (2.0×10^6 sperm) was added to each test tube and allowed to incubate at ambient seawater temperature ($13.0 \pm 1^\circ\text{C}$) for 60 min

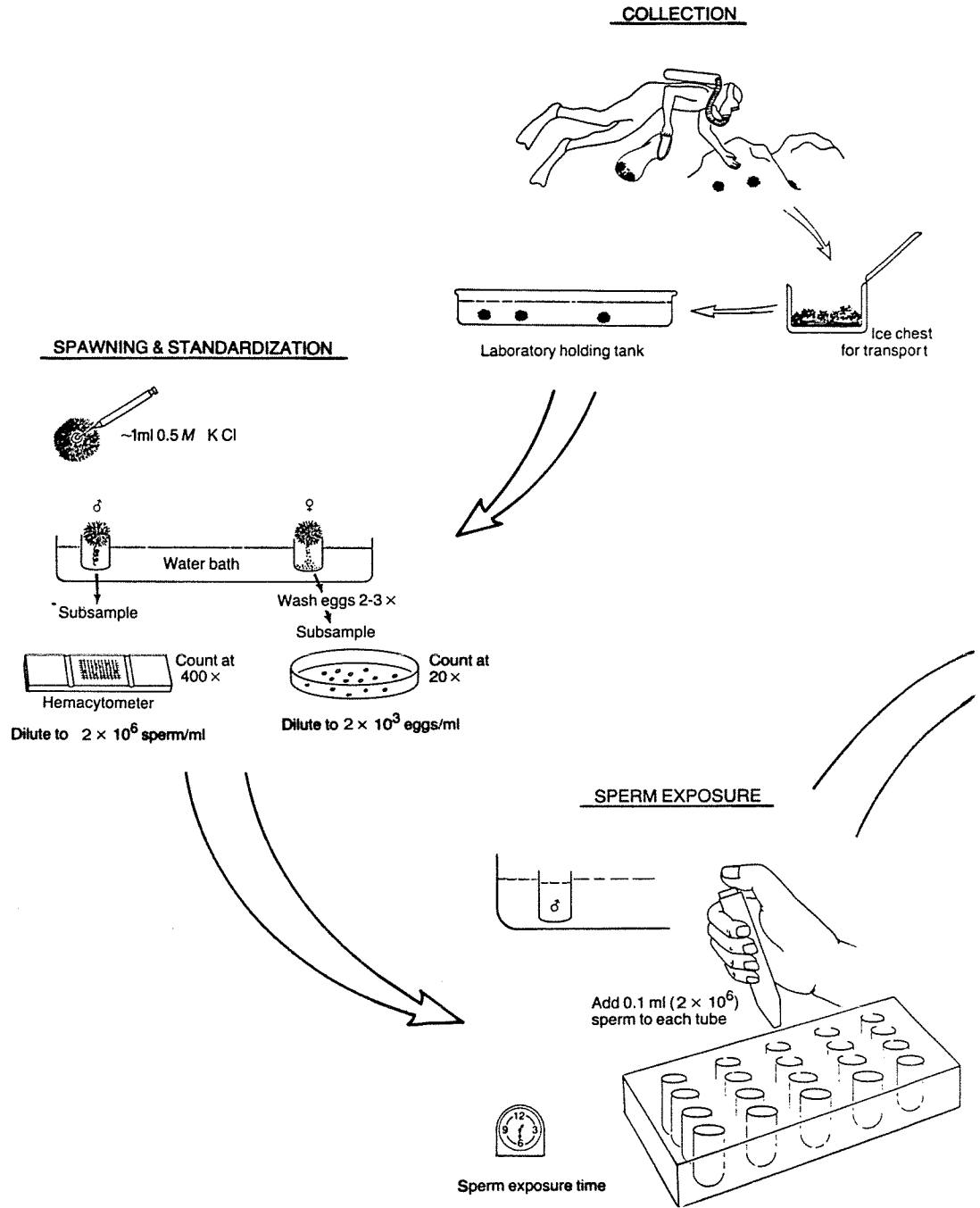


Figure 12.3. Sperm bioassay flow diagram (from Dinnel et al. 1982).

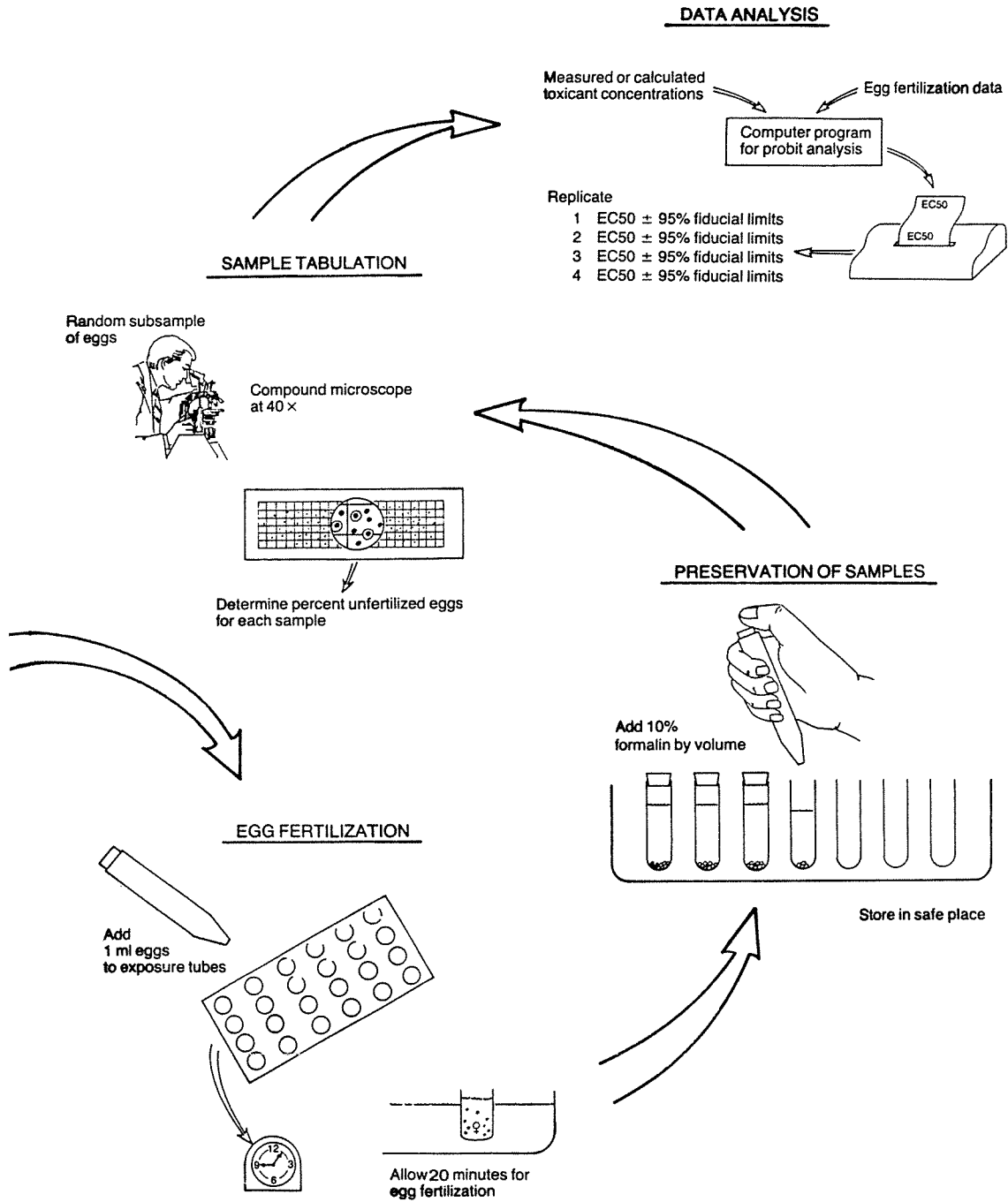


Figure 12.3. (Continued)

followed by addition of 1 ml of the egg solution (2,000 eggs) and further incubated for 20 min to allow fertilization of the eggs. The samples were then fixed with 10% formalin to arrest further fertilization and to preserve the samples until analysis (Figure 12.3).

The exact sperm assay time schedule has been standardized as follows (Dinnel et al. 1983) (time in minutes):

T 0: spawn animals.

T30: mix sperm, subsample for counts.

T30-T150: wash eggs several times and dilute to 2,000/ml.

T90: dilute sperm to 2×10^7 sperm/ml (density is species-dependent).

T120: Add 0.1 ml sperm solution to each test tube at 5-sec intervals.

T180: Add 1 ml of mixed egg solution to each test tube at 5-sec intervals.

T210: Fix each test tube with 10% formalin at 5-sec intervals and cork.

The sperm and egg densities cited above provide for a nominal sperm:egg ratio in each test tube of 1,000:1. For sand dollars this ratio optimizes the sensitivity of the test by assuring enough sperm to fertilize most eggs in the controls, but prevents the introduction of excess sperm which could reduce the test sensitivity (see Dinnel et al. 1983 for specific details regarding refined test methodology).

Later, each sample was tabulated for percentage of unfertilized eggs in the sample, as indicated by the absence of a normal fertilization (vitelline) membrane around each egg in a subsample of 100-200 eggs.

12.4.2.3 Chemical Analyses

Each seawater control and field sample was measured for pH using an Orion model 407A specific ion meter and electrode following calibration with standard pH buffers. Control water salinity was measured by conductivity

bridge at the U.W. School of Oceanography (Paquette 1958). All other receiving water parameters were measured by the Water Column group on board the R.V. Liberty during sample collection. These physical/chemical parameters included: salinity, total ammonia, and chlorophyll "a" for all depths and primary productivity, phytoplankton counts and secchi disk depth for surface waters (see Vol. III, Section 4 - Water Column Ecology for materials and methods).

12.4.2.4 Data Analyses

The biological responses observed in the receiving water samples and the "secondary" control samples (carry-along, West Point filtered, and frozen Friday Harbor seawaters) were adjusted for average net response relative to the "primary" control water from Kincaid Hall.

Two indices were calculated for the oyster embryo assays: average adjusted percent mortality and average adjusted percent abnormality. The average mortality for each set of replicates ($n = 3$) was determined by calculating the arithmetic mean of the survival:

$$\text{Average survival} = \frac{X_1 + X_2 + X_3}{n}$$

where X = number of embryos in each replicate

n = number of replicates

and then determining the percent mortality relative to the average "primary" control survival:

$$\text{Percent Adjusted Oyster Mortality} = 100 - \left(\frac{\text{Average Sample Survival}}{\text{Average Control Survival}} \right) \times 100$$

Positive mortality values indicate higher mortality than the control responses

while negative values are indicative of better survival in the sample water.

The oyster percent abnormality index was determined using a weighted mean:

$$\text{Average Weighted Abnormality} = \frac{(X_1 Y_1) + (X_2 Y_2) + (X_3 Y_3)}{X_1 + X_2 + X_3}$$

where X = number of embryos in each replicate

Y = number of abnormal embryos in each replicate

The weighted mean was used instead of the arithmetic mean as this value tends to vary in relation to the degree of mortality in each set of replicates (Cardwell and Woelke 1979). The average percent abnormality was then determined by:

$$\text{Average Percent Abnormality} = \frac{\text{Average Weighted Abnormality}}{\text{Average Survival}} \times 100$$

Finally, the adjusted percent abnormal response for each set of replicates was established by adjusting for the "primary" control responses using Abbott's formula (Finney 1971):

$$\text{Adjusted Percent Abnormality} = \frac{(\text{Ave. \% Sample Abnormality}) - (\text{Ave. \% Control Abnormality})}{100 - (\text{Ave. \% Control Abnormality})} \times 100$$

Again, the adjusted percent abnormality values may be either positive or negative relative to the "primary" control responses.

The sperm assay responses in the receiving water samples and the "secondary" control samples were also related to the "primary" control responses in much the same way as detailed for the oyster indices above. Specifically, the arithmetic mean was calculated for each set of replicates

(sample and control) to yield an average percent response (percent eggs unfertilized) for each set. These responses were then adjusted to the "primary" control response using Abbott's formula:

$$\text{Adjusted Percent Eggs Unfertilized} = \frac{(\text{Ave. \% Sample Unfert.}) - (\text{Ave. \% Control Unfert.})}{100 - (\text{Ave. \% Control Unfert.})} \times 100$$

to yield either a positive or negative value.

All data summaries, correlations and tests of significance for this report were generated using the control (Kincaid Lab water) adjusted percent responses without applying further transformations. Cardwell and Woelke (1979) have addressed the issue of data transformations for oyster embryo bioassay responses and found that of seven typical data transformations, only one (negative natural log) provided a more powerful discrimination than the untransformed data.

Use of untransformed data assumes that the data are normally distributed and have a constant variance. Initial examination of the control-adjusted responses reflected a normal distribution with a small right skew. The bioassay responses are all-or-none events that possess a binomial distribution. Zar (1974) points out that both binomial and poisson distributions tend to converge to a normal distribution when sample numbers are large.

In their discussion of data transformations, Cardwell and Woelke concluded that even though the normality assumption seems satisfied, variances are not constant; they increase in conjunction with the level of larval response. None-the-less, Cardwell and Woelke completed their analyses using untransformed data because a completely valid model remains unidentified, and any partially effective transformations did not provide benefits overriding

those accruing from working with untransformed data.

12.4.3 Algal Metabolite Bioassays

Algal bioassays designed to assess potential stimulation of phytoplankton growth following the addition of Renton unchlorinated secondary sewage were conducted by the Water Column group (see Section 4.0). The conduct of these assays afforded the chance of using sand dollar sperm and oyster embryo bioassays (as described above) to test for possible algal metabolite toxicity generated in the sewage enriched cultures. Metabolite toxicity was tested for in three sets of algal assays; two tests using the diatom Skeletonema costatum and one test using the dinoflagellate Gymnodinium simplex. In each case culture water was collected from the algal assay flasks at the end of each assay incubation period (6 to 9 days). The results of sand dollar sperm and oyster embryo assays of the culture waters are summarized in Tables 12.8 through 12.10 in conjunction with the chlorophyll "a" maxima values measured in each culture flask.

12.4.4 Renton Sewage Bioassays

12.4.4.1 Sample Collection, Handling and Preparation

Sewage samples were collected on ten separate occasions from the Renton Treatment Plant during the four following periods: summer 1982, winter and summer 1983, and winter 1984. Four-liter grab samples were collected at five steps in the treatment process including influent sewage, primary sewage, secondary sewage, chlorinated secondary sewage, and dechlorinated secondary effluent (Figure 12.4).

Each batch of samples was packed in ice and transported (along with a bottle of Kincaid Hall "control" seawater) to the West Point Laboratory where bioassays were immediately started. A series of 10 or 11 seawater dilutions of each sewage sample was prepared on a volume/volume (0.27 to 20.00% sewage)

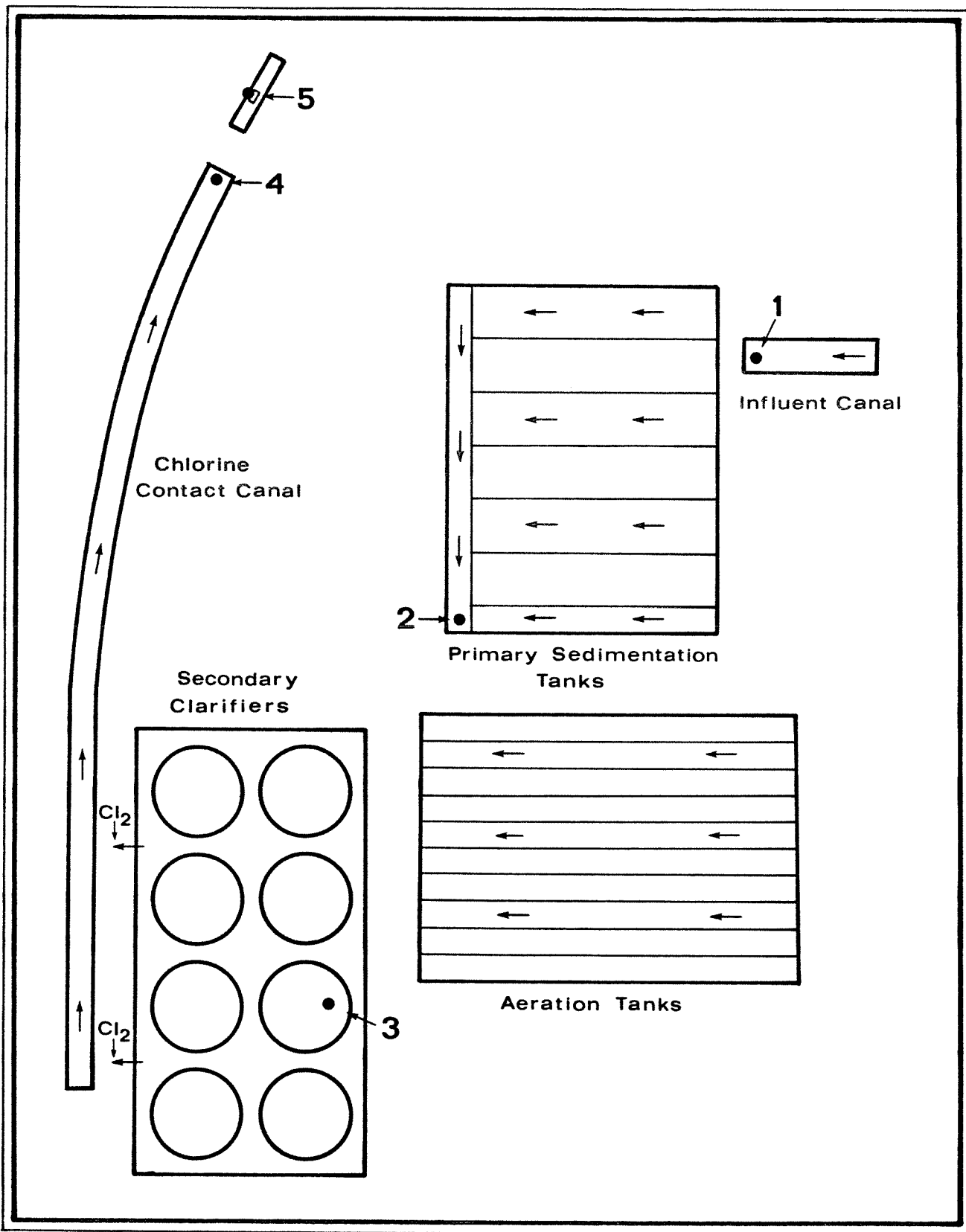


Figure 12.4. Diagrammatic map of the Renton sewage treatment plant showing sampling points for the sewage bioassays. 1 = influent sewage; 2 = primary sewage; 3 = secondary sewage; 4 = chlorinated secondary sewage; 5 = dechlorinated effluent (sampled at the composite sampler).

basis using West Point filtered seawater as the dilutant. One liter Tri-pour plastic beakers were used for the embryo and zoea assays and a 10-ml subsample was removed to glass test tubes for the sperm assays. An additional test series was prepared using deionized freshwater instead of sewage so that the effects of reduced salinity alone could be separated from the overall "toxic" responses observed for each sewage type. Triplicate samples of two types of "control" water were tested with each sewage bioassay (Kincaid Hall and West Point filtered seawater). Each set of beakers and test tubes were incubated for the appropriate exposure times in water baths at the specified test temperature.

12.4.4.2 Sewage Bioassay Procedures

Oyster Embryo Bioassays

Oysters were spawned and eggs fertilized and added to the exposure beakers as described above for the receiving water bioassays (see Section 12.4.2.2). All oyster embryo bioassays of sewage dilutions were conducted at 20°C for 48 hours. Oyster embryo bioassays of sewage were conducted during the summer of 1982 only.

Sand Dollar and Green Sea Urchin Sperm Bioassays

The sperm bioassays were conducted as described above for the receiving water bioassays (Section 12.4.2.2). For sand dollar, a sperm:egg ratio of 1000:1 was used and the summer incubation temperatures (ambient seawater) were 12 to 14°C. Green sea urchin sperm assays required a higher sperm:egg ratio of 1500:1 and the winter ambient seawater incubation temperatures were 8-10°C. Each sperm bioassay was conducted twice to provide a measure of between-year variability in sewage toxicity: sand dollar sperm assays were conducted during the summers of 1982 and 1983 and the sea urchin sperm assays during winters of 1983 and 1984.

Green Sea Urchin Embryo Bioassays

Sexually ripe sea urchins were spawned by injection of 1.0 ml of 0.5-M potassium chloride through the pristomal membrane. The eggs were washed twice in control seawater and fertilized by adding a small amount of sperm. After satisfactory fertilization success, approximately 5,000 eggs (in 1 ml of seawater) were added to 250 ml of Renton sewage dilutions or control seawater. After an exposure period of 96 hours, the experimental beakers were mixed, subsampled (10 ml) and the subsample preserved with formalin. "Mortality" in the test solutions was determined by comparing total embryo counts with control counts. Embryos failing to develop to normal pluteus larvae were considered "abnormal." Sea urchin embryo bioassays of sewage were conducted during the winter of 1983.

Dungeness Crab Zoea Bioassays

Crab zoea bioassays of dilutions of Renton sewage were conducted by adding 25 to 50 newly hatched zoea to plastic beakers containing 250 ml of test or control solution. The beakers were incubated 48 hours at ambient seawater temperature without aeration. At the end of 48 hours, the zoea were inspected for mortality, as defined by the lack of any visible movement at 10 to 20x magnification. Crab zoea bioassays of sewage were conducted during the winter of 1983.

12.4.4.3 Chemical Analyses

Each undiluted sewage sample was measured for pH and total ammonia at the initiation of each set of sewage bioassays. Total residual chlorine was measured in the influent, chlorinated secondary, and dechlorinated sewage samples. Measurements were not made in each sewage dilution as many of the chlorine and ammonia concentrations were below our limits of chemical detection for the dilutions tested. An Orion research model 407A specific ion

meter with appropriate electrodes and standard calibrating solutions was used to measure pH and ammonia. Chlorine in the sewage samples was determined with a titrator based on the design of Andrew and Glass (1974) utilizing dual platinum electrodes, a Keithly 602 electrometer, and a microburet calibrated to 0.1 mg/liter for addition of the titrant (Stober et al. 1978). The back titration method (APHA 1980) was used for determination of total residual chlorine (TRC) in sewage samples.

12.4.4.4 Data Analyses

The sewage bioassays were specifically designed to produce average EC50 values (calculated concentrations of sewage equivalent to a 50 percent effective response) for each type of sewage, based on a series of ten repetitive tests for each bioassay organism. Sewage series tested up to 11 dilutions of sewage: 0.27, 0.41, 0.64, 1.0, 1.5, 2.3, 3.6, 5.5, 8.4, 13.0, and 20.0 percent sewage in West Point seawater. The average organism responses (percent unfertilized eggs for sperm assays; percent mortality or abnormality for embryo assays; percent mortality for crab zoea) were calculated for each sewage type using values corrected for the control responses as outlined in section 12.4.2.4 above. The average adjusted responses were then used to calculate the average EC50's using a computer BMD03S program for probit analysis (a type of non-linear regression analysis; Finney 1971) with an attached FORTRAN program to calculate 95 percent fiducial limits by the method of Litchfield and Wilcoxon (1949).

12.4.5 Ammonia Bioassays

Ammonia is a primary component of sewage which may have a significant toxic effect in sewage bioassays. A bioassay using oyster embryos and sand dollar sperm was conducted to define the possible toxic role of ammonia in the sewage assays.

Ammonium chloride (NH_4Cl) was added to filtered West Point seawater in a concentration series from 0.5 to 100.0 mg/liter (as total ammonia) at a seawater pH of 8.0 ± 0.1 , salinity 30.1 ‰, and an incubation temperature of $12.0 \pm 1.0^\circ\text{C}$. Oyster embryo and sand dollar sperm bioassays were conducted on the ammonia/seawater solutions as described above (section 12.4.2.2). Ammonia in the seawater test solutions was measured as total ammonia using an Orion specific ion meter with an ammonia electrode and appropriate standard solutions. The results were analyzed for EC50's and 95 percent fiducial limits as described for the sewage bioassays.

12.4.6 Sediment Bioassays

12.4.6.1 Sediment Collection, Handling and Preparation

Baseline sediment samples were collected along 10 transects at 27 stations (Figure 12.5) identified as depositional by the Subtidal Task. Sampling was biannual, commencing in October 1982 and terminating in March 1984. In fall 1982, the following stations were sampled: A400E, C400E, C600E, C640, G50W, G780, H75W, H640, I690, J600E, OT-1E and OT-2E (N = 12). For the remaining three sampling periods, G780 was deleted (N = 26 each season). Station D660 was sampled only for use in laboratory tests. Baseline sediment samples were collected with a modified 0.1-m² van Veen grab on board the R.V. Kittiwake. Each sample was collected from the top 2 cm of at least three replicate grabs at each site. The pooled samples were double-bagged, mixed by thorough kneading, transported on ice, and frozen at 0°F until use, a period not exceeding one month.

Baseline bioassays used a "toxic control" sediment in an attempt to standardize test results over time. This sediment was collected from Duwamish South Harbor (Duw SH) a site known to be highly polluted (Malins et al. 1982). Twenty liters of sediment were collected in fall 1982 and again in fall 1983.

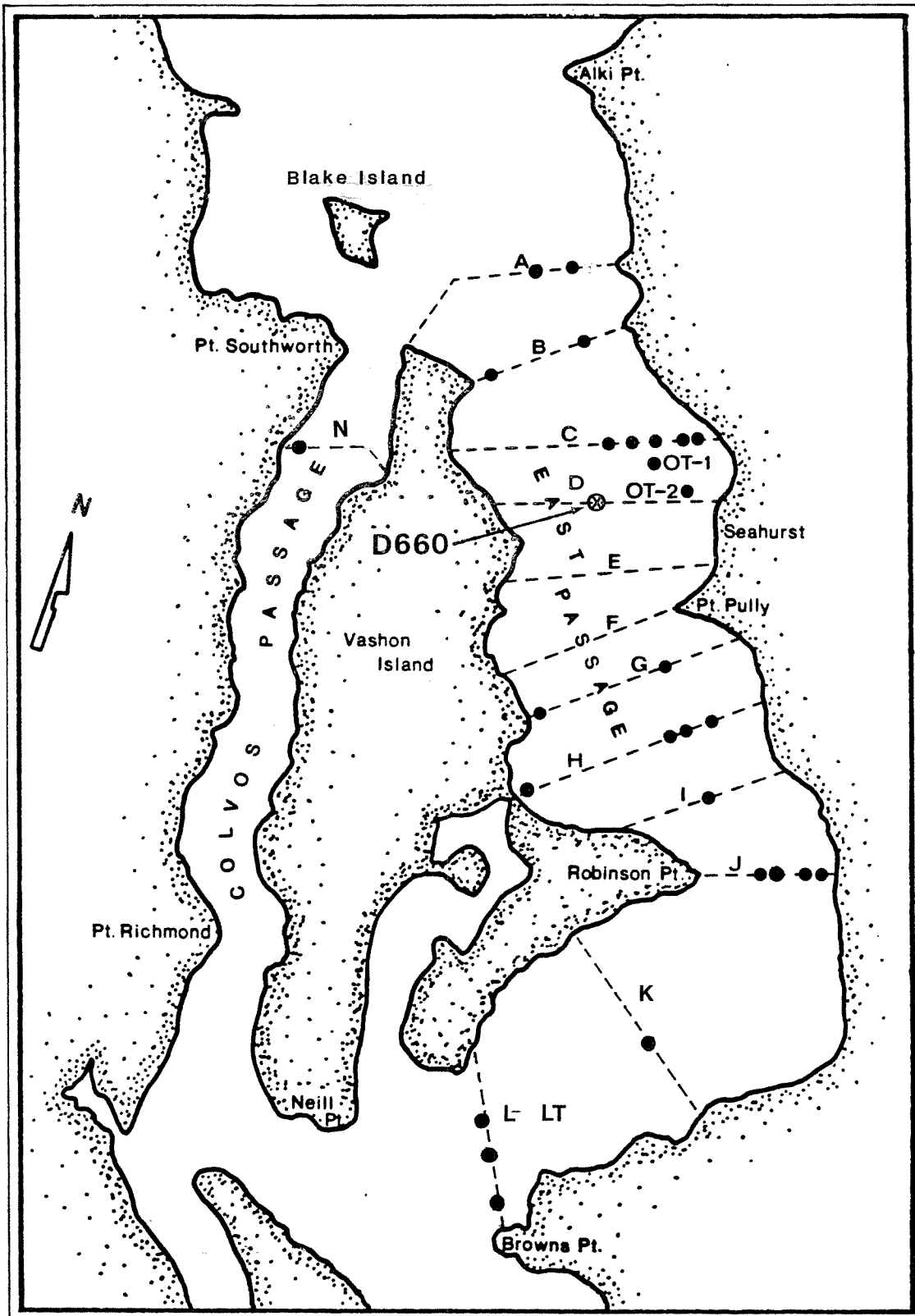


Figure 12.5. Map of south central Puget Sound showing the sites where sediment was collected for the Seahurst sediment bioassays.

The toxic control sediment (top 2 cm from repetitive van Veen grabs) was thoroughly homogenized in a 100-liter PVC tub by hand-stirring with a PVC rod prior to being frozen in plastic bags in 1-liter quantities. Sediment was stored frozen until use, up to one year. Clean control sediment and amphipods were collected simultaneously from West Beach (fall 1982, spring 1983) and Bowman Bay (fall 1983, spring 1984).

12.4.6.2 Bioassay Procedures

Flow-through bioassays were conducted in spring 1983, fall 1983 and spring 1984. Due to limitations in lab capacity, Seahurst sediments and controls were analyzed in two consecutive bioassays (Runs 1 and 2) for each season. In general, sediments north of I-transect were run first, then I-transect and sediments south of I-transect. There is evidence that coarse-grained substrates may affect amphipod survival in a bioassay (Swartz et al. 1982). Sediments from B transect had a high percentage of gravel, so these sediments were screened (2-mm mesh) in a minimum amount of seawater and allowed to settle 3-4 hr prior to use.

In fall 1982, sediments from 12 stations were run simultaneously at 15°C using a recirculating precursor of the flow-through system. Instead of the seawater delivery manifold, the system was aerated by an airline leading into a 50-cm length of 6.3-mm ID PVC pipe, with numerous small holes drilled in it, lying lengthwise down the center of an aquarium and providing a wall of small air bubbles between chambers and continuous movement of water.

Simultaneous with the flow-through bioassays in spring 1984, static bioassays were conducted with 13 Seahurst sediments from: A-transect (2), B75W, C400E, C600E, OT-1E, OT-2E, H-transect (4), I690 and J600E. Each static bioassay was run with appropriate control sediments.

Methodology and control sediment collection sites for corresponding

sampling periods are summarized as follows:

<u>Season</u>	<u>Method</u>	<u>Clean Control</u>	<u>Toxic Control</u>
Fall 1982	Recirculating	West Beach	Duw SH (1982 collection)
Spring 1983	Flow-through	West Beach	Duw SH (1982 collection)
Fall 1983	Flow-through	Bowman Bay	Duw SH (1983 collection)
Spring 1984	Flow-through	Bowman Bay	Duw SH (1983 collection)
Spring 1984	Static (S)	Bowman Bay	Duw SH (1983 collection)

Flow-through System Bioassays

Test chambers were constructed from 10-cm diameter PVC pipe cut to 10-cm lengths (Figure 12.6). Two windows were cut in each 10-cm piece and Nitex screen (500-um mesh) glued with PVC cement around the outer circumference (the screen sandwiched between the outer circumference and a narrow PVC ring). The screen allowed water circulation through the test chambers. Close fitting lids for both ends of the test chambers were manufactured from 10-cm diameter PVC pipe coupling cut into 1.5-cm widths. Nitex screen (500-um) was glued with silicone caulking to lid rings to complete chamber assembly. All parts of the system were aged in flowing seawater two weeks prior to use to leach glue and solvent-related toxicants.

Flow-through bioassays were conducted at ambient seawater temperatures with 4 to 5 replicate exposure chambers per 15-liter glass aquarium (Figure 12.7). Replicate chambers were symmetrically arranged within each aquarium and were set atop 1-cm diameter glass rods to enhance water circulation. Filtered seawater (100-um) was delivered at about 500 ml/min to each aquarium via a

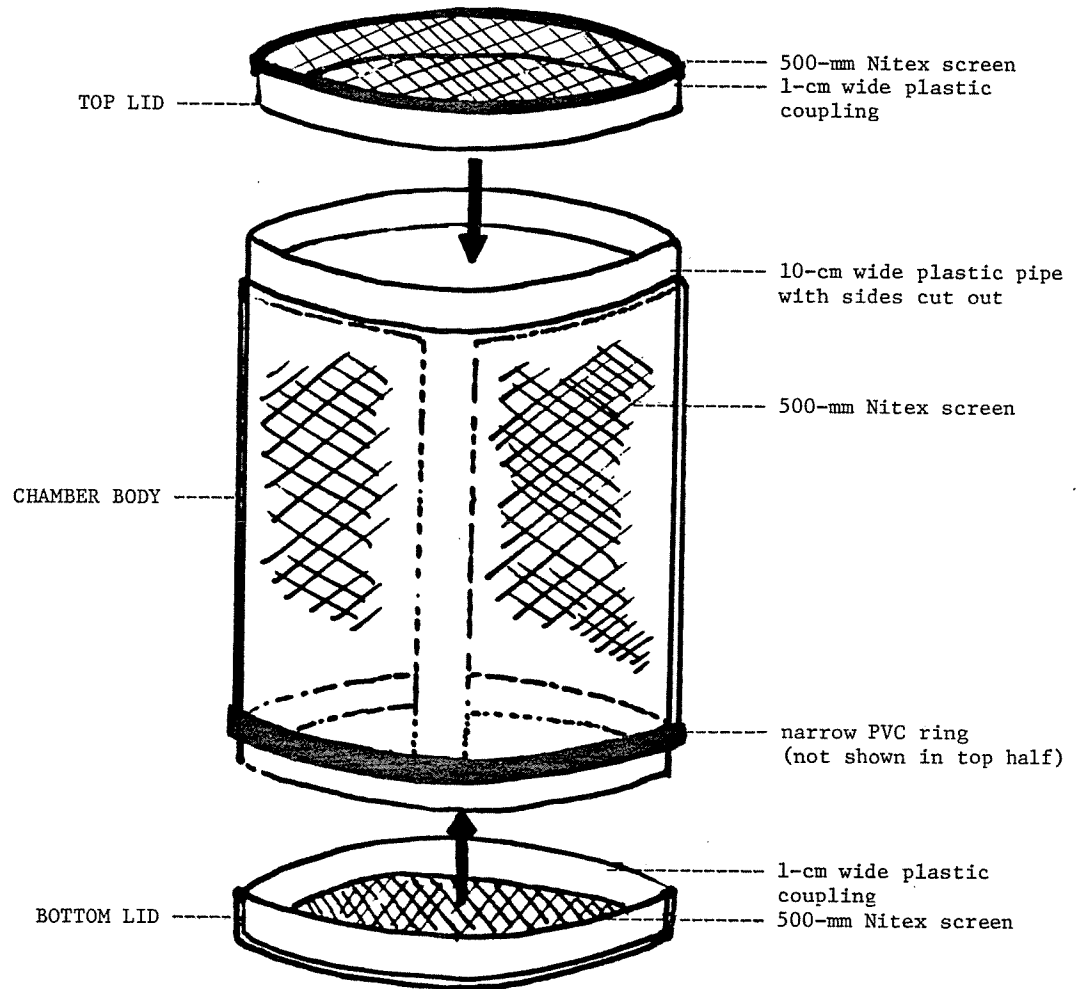


Figure 12.6. Amphipod-sediment bioassay exposure chamber.

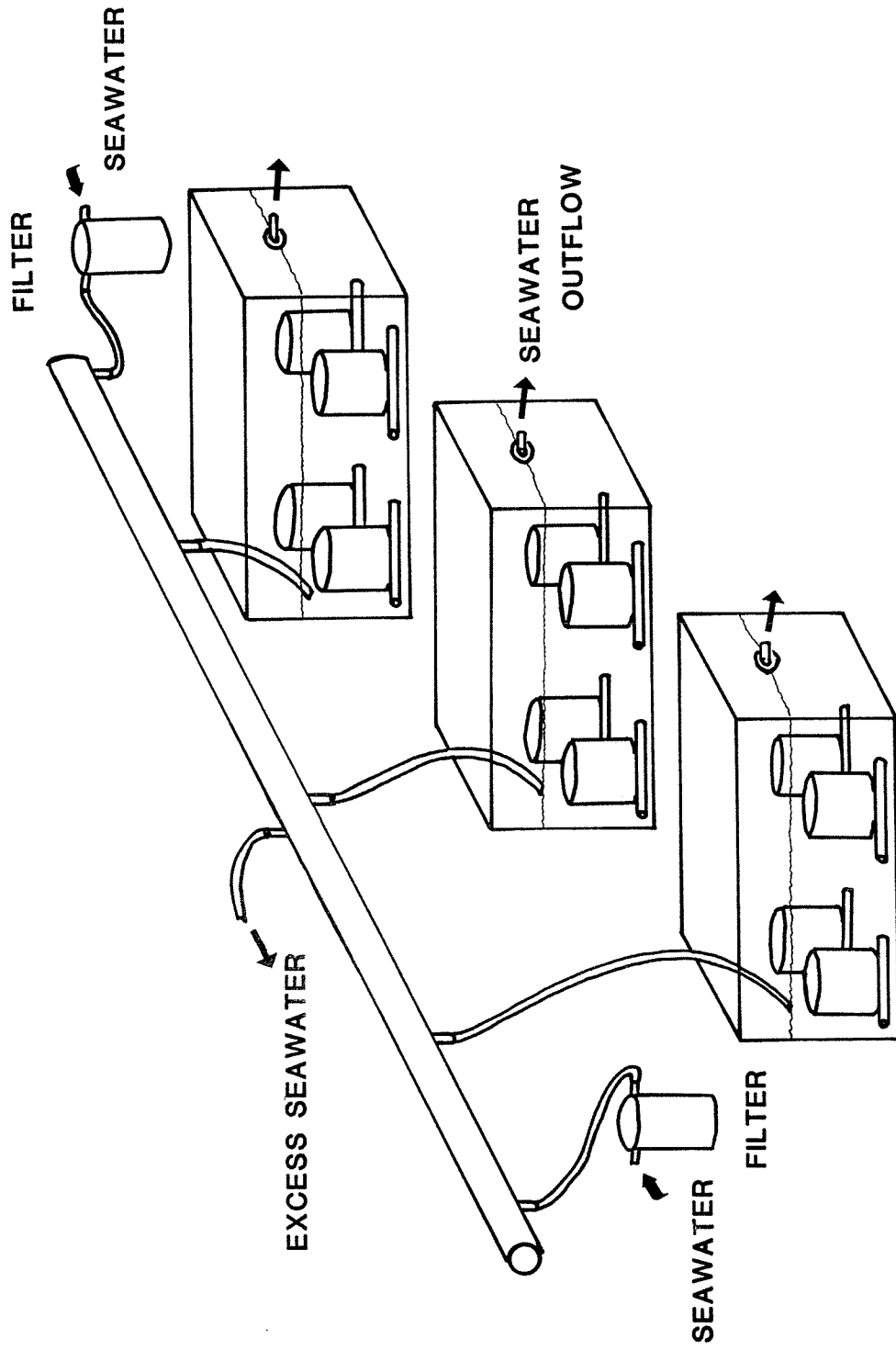


Figure 12.7. Flow-through exposure system for amphipod/sediment bioassays.

manifold consisting of a 10-cm diameter PVC pipe fitted with 1.25-cm OD PVC taps. A length of 1.25-cm ID Tygon tubing ran from the taps to the aquaria and was terminated with a plastic disposable pipet tip which restricted flow. An airlock was prevented by an excess seawater drain tap plumbed into the top of the manifold. Water flowed out each aquarium through a 16-mm hole cut 16 cm above the tank bottom opposite the inflow tube. Aquaria were placed within an ambient temperature flowing seawater trough.

For bioassays, 150-ml of sediment was measured into chambers which were then placed in aquaria and the seawater flow started. Amphipods were screened (1.0-mm mesh) underwater from holding buckets and 20 animals of a selected size range (depending on what was available) were placed in each replicate chamber. Selection of a limited size range insured that all animals were of approximately equal age, thus minimizing some effects from biological variability. Chambers were capped and bioassays run for 10 days under constant light.

Eh and pH were measured on days 2 and 9, or on day 5. The probes, filling solutions, and standards (pH and Zobell solutions) were equilibrated to test temperature prior to use. In anticipation of these measurements, additional chambers had been set up without animals. Lids were removed and the probe inserted directly into the test sediment. Readings were taken immediately and again after the instrument stabilized. If a slow drift upward was observed prior to stabilization, the initial reading was recorded as insertion of the probe into the sediment sometimes allowed overlying seawater (with higher pH and Eh) to mix with sediment.

Static System Bioassays

The static system was slightly modified from the procedure developed by Swartz et al. (1984). One liter plastic Tri-pour beakers were set up with 150

ml of test sediment and 800 ml of 100- μ m filtered seawater 24 hr prior to addition of animals. Beakers were placed in an ambient temperature flowing seawater trough. Air was bubbled into beakers through glass disposable pipettes with tips 3-5 cm from the sediment surface. Beakers were not covered. Bioassays were run for 10 days under constant light. Measurements of pH were taken for all five replicate beakers; Eh was measured only for those beakers which had the highest and lowest pH values.

Termination of Bioassays

At the end of a bioassay, chamber (or beaker) contents were rinsed onto a 1.0-mm mesh screen and amphipods counted. Survivors were placed in petri dishes containing sieved native sediment and ambient temperature seawater. The number of amphipods incapable of burying themselves completely in five minutes, even with gentle prodding, were recorded. This constituted the reburial test.

Between bioassays, aquaria and chambers were washed in detergent, methanol, 0.1-normal nitric acid, and deionized water, sequentially. Plastic beakers were discarded.

Sublethal Stress Tests

Following fall 1982 and spring 1983 bioassays, a post-exposure secondary-stress test was conducted as another measure of sublethal effects. At the end of the 10-day bioassay period, amphipods surviving within a given sediment were pooled and random subsamples of 15 animals were exposed for 48-hr to one of the following stresses: a 5-7°C increase in temperature, a 5 ‰ decrease in salinity, and a combination of both. Mortality was assessed after 48-hr. The post-exposure stress test was discontinued after spring 1983 as it failed to provide additional information over the results of the original 10-day test.

12.4.6.3 Physical/Chemical Sediment Analyses

Percent water, total volatile solids (TVS), biological oxygen demand (BOD), nitrogen, carbon, sulfides, and 15 heavy metals (Ag, As, B, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, V, Zn) were measured in Seahurst sediments by the Marine Chemistry Task, Volume 8, Section 11 of this report where the analytical methods are described. In addition, 130 organic compounds were measured by the METRO Water Quality Lab, Seattle, Washington. Infaunal trophic indices (ITI) are documented in the subtidal ecology section (Volume V, Section 6).

Sediment grain size analyses were conducted by B. Winslow (Fisheries, University of Washington, Seattle, WA) according to the U.S. Geological Survey Procedure (Guy 1969) and the University of Washington's "General Oceanography Laboratory Manual" (Krumbein and Pettijohn 1938). Grain size composition and moment measures (mean, standard deviation, skewness, and kurtosis) were determined in phi units ($\phi = -\log_2$ of the particle diameter in mm). The greater the phi, the finer the sediment. Particle diameters and corresponding phi units associated with each size class are given in Appendix Table 12.13. Hydrogen peroxide was not added to the sediments prior to analysis. As hydrogen peroxide causes particle aggregates to dissociate, these data represent realistic grain sizes in which the amphipods were tested as these sediments aggregate naturally.

12.4.6.4 Data Analysis

Bioassay data were generated as proportions $F1/N$ and $F2/F1$, where N = total number of animals, $F1$ = total number of survivors (number able to rebury plus number unable to rebury), and $F2$ = number unable to rebury. The fractions, which yielded proportion of survivors and proportion unable to rebury, respectively, were arcsine-transformed to best approximate a normal

distribution of the variances (Zar 1974). Data were analyzed by a one-way analysis of variance (ANOVA) to test for differences in response between sediment treatments (Zar 1974). In all statistical tests, the significance level was set at $p = 0.05$. If ANOVA results were significant, data were further analyzed by Dunnett's test (Zar 1974) to determine which treatment mean differed from the mean of the native sediment controls.

Amphipod survival data were compared between seasons by one-way ANOVA for each station. Static and flow-through bioassays from spring 1984 were analyzed for differences in amphipod survival by a two-way ANOVA (Zar 1974). Sediment quality, heavy metal and organic data were analyzed separately using principal component analysis (Daling and Tamura 1970). Arcsine-transformed survival data were compared to both original (unreduced) data sets and principal components using Pearson correlation coefficients (Kleinbaum and Kupper 1978). Cluster analyses (Gauch 1982) were conducted on the reduced sediment quality and heavy metal data sets with the following variables "transformed" to make their range compatible with the other variables in the data set: Ag (x 10), Cd, Hg, Mn, Zn (x 0.1), carbon (x 10) and BOD (x 0.01). Principal components of the organic data set were summed to create 11 factors for cluster analysis at each station. Canberra (Lance and Williams 1966, 1967) dissimilarity coefficients were calculated with a flexible beta = -0.25. Results of the principal components and cluster analyses for sediment quality, heavy metal and organic "toxicant" data were then compared with amphipod survival in an attempt to discern what primary factors were associated with reduced amphipod survival.

12.5 Results

12.5.1 Receiving Water Bioassays

The results of the receiving water bioassays are presented graphically in

Figures 12.8A through 12.8J (1982) and 12.9A through 12.9L (1983) and average responses are summarized by station and depth in Tables 12.1 through 12.3 for 1982, 1983 and 1982 & 1983 combined, respectively. Also included in these three tables is supporting physical/chemical data summarized as average values for each time period. A more complete summary of the biological response data (mean, standard deviation, number of samples, 95 percent confidence limits and ANOVA summary) is presented by station by depth, and by year in Appendix Tables 12.1 through 12.3.

The oyster embryo "primary" (Kincaid Lab water) control responses (percent mortality and abnormality) are summarized by test date for 1982 and 1983 tests in Tables 12.4 and 12.5, respectively. By proposed ASTM (1980) standards, oyster embryo bioassay results should be discarded if control mortality exceeds 30% or abnormality exceeds 10% over a 48-hr incubation period. For the purposes of this study test results were accepted as valid if neither the control mortality or abnormality exceeded 50 percent for reasons discussed below. All oyster embryo assays which failed to meet ASTM proposed standards are flagged with an asterisk in Tables 12.4 and 12.5.

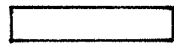
12.5.2 Algal Metabolite Bioassays

The first algal assay using Skeletonema costatum was tested for metabolite toxicity using the sperm assay only (Table 12.6). The results (adjusted for the Kincaid control water response) of this test showed reduced fertilization success in the cultures which were grown in the presence of 2.0% Renton secondary sewage. These same samples also registered the highest chlorophyll "a" maxima values. However, no toxicity was observed in S. costatum stock culture water which had been incubated for up to four weeks.

The second algal assay using S. costatum was tested for metabolite toxicity using both sperm and oyster embryo assays (Table 12.7). Results of

Figures 12.8 A through 12.8 J. Results of the 1982 Seahurst receiving water bioassays by station, by depth, and by bioassay response.

Legend:



% oyster embryo mortality



% oyster embryo abnormal

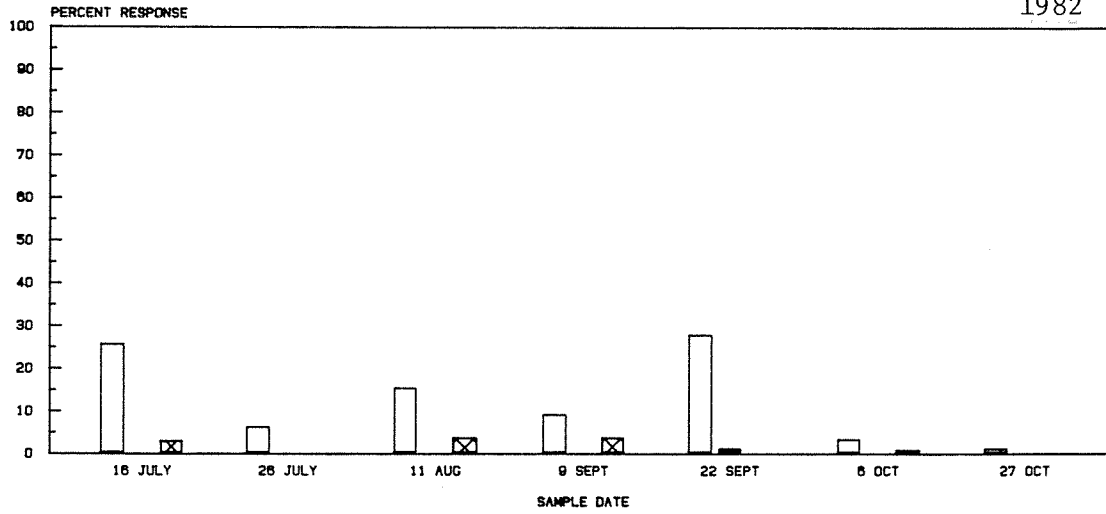


% sand dollar eggs unfertilized

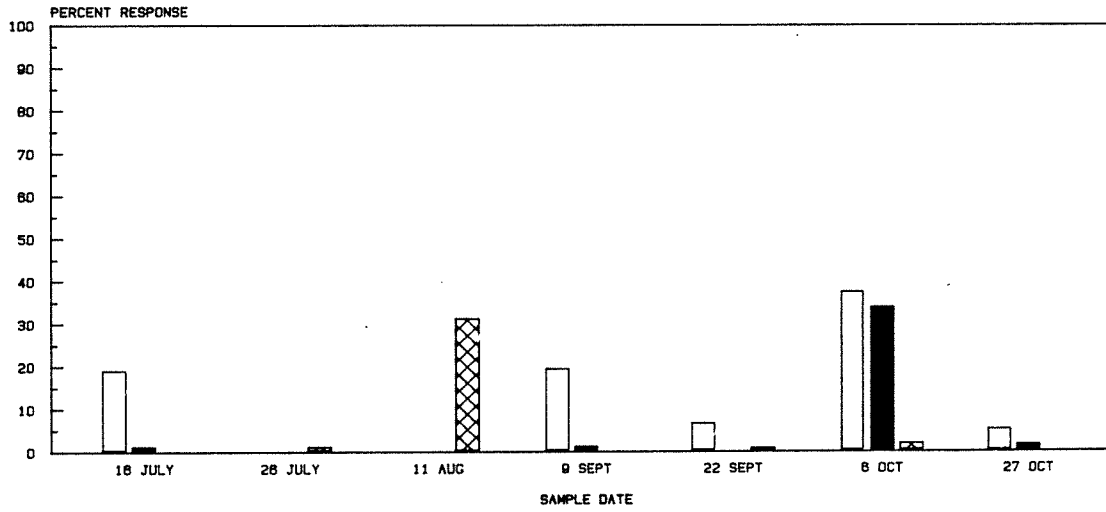
N = not tested (or results discarded due to unacceptably high oyster embryo control mortality or abnormality).

34
STATION 1 -- 0 METERS

1982



STATION 1 -- 100 METERS



STATION 1 -- 200 METERS

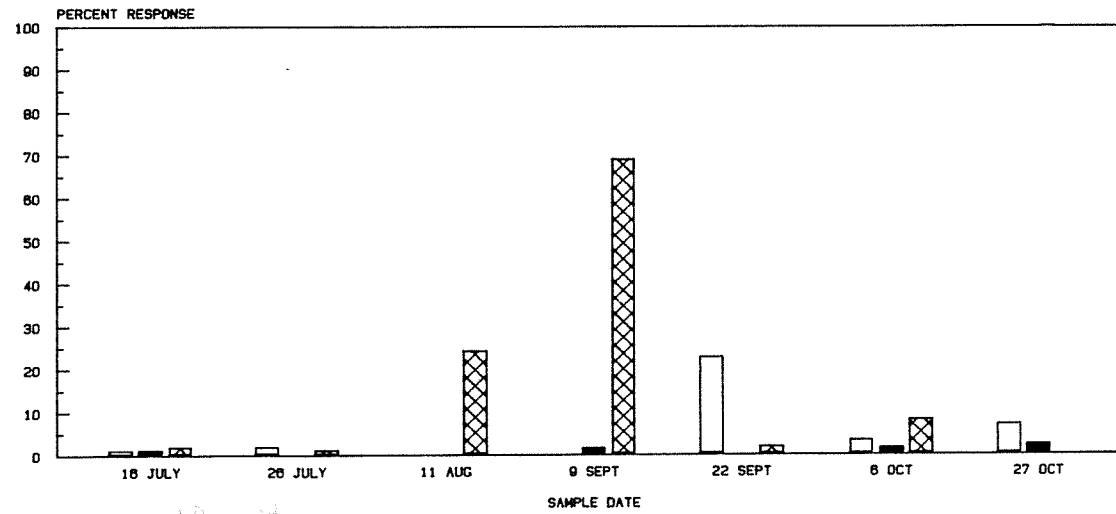
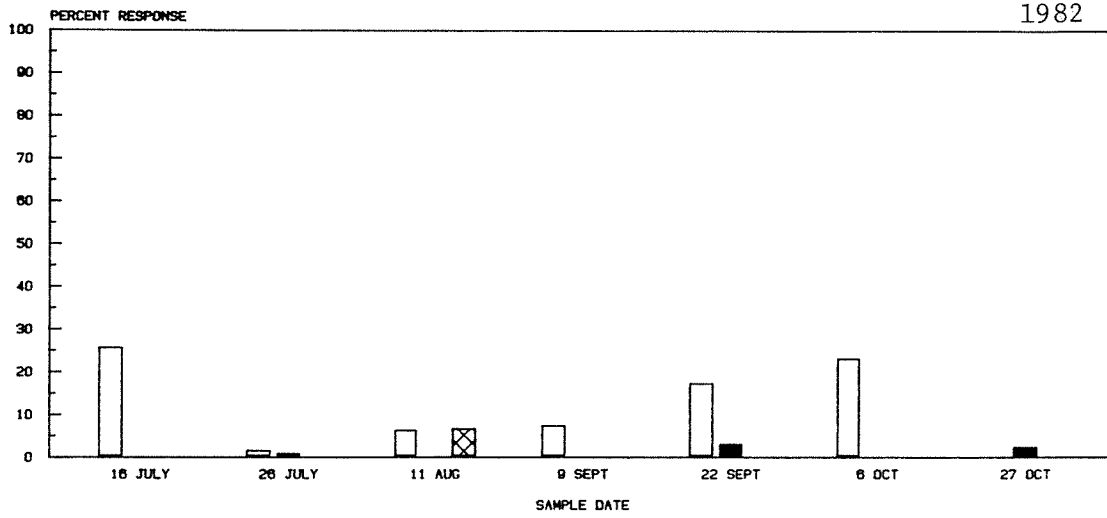
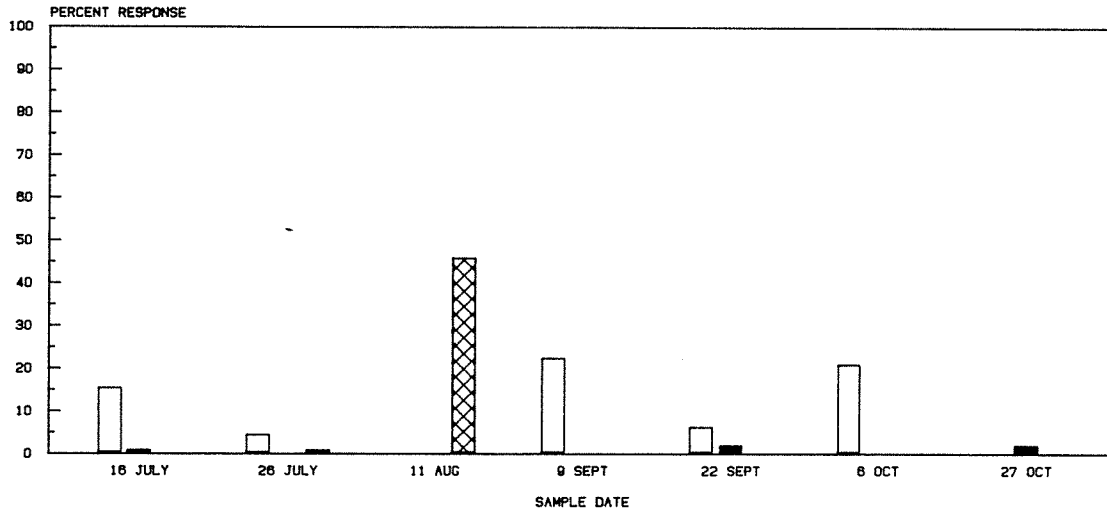


Figure 12.8A.

STATION 2 -- 0 METERS



STATION 2 -- 50 METERS



STATION 2 -- 100 METERS

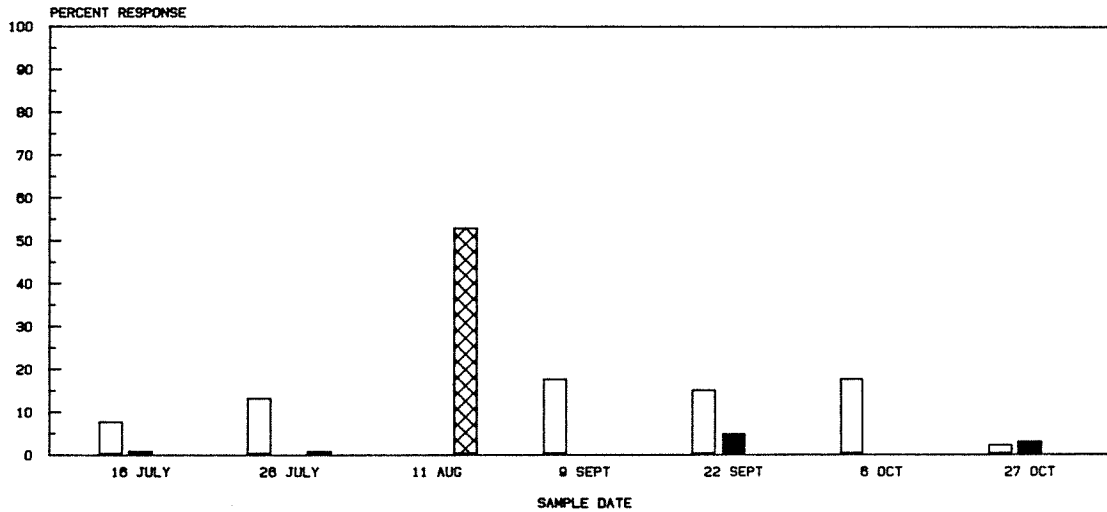
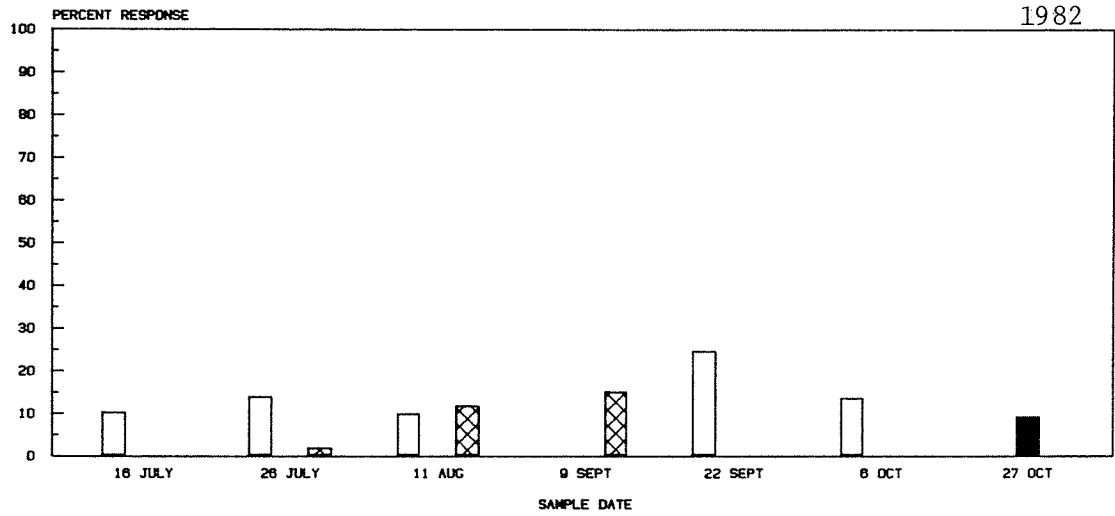
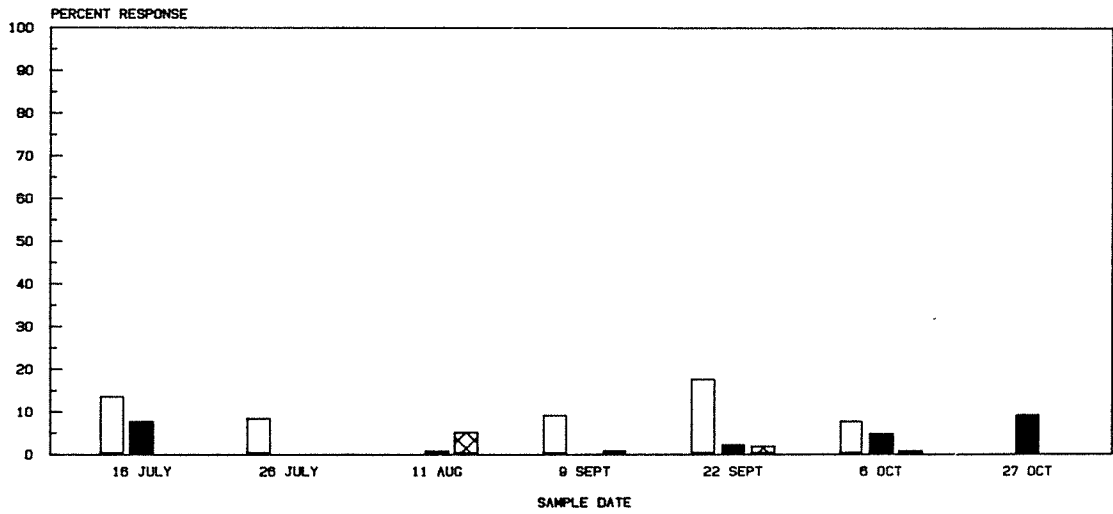


Figure 12.8B.

STATION 3 -- 0 METERS



STATION 3 -- 75 METERS



STATION 3 -- 150 METERS

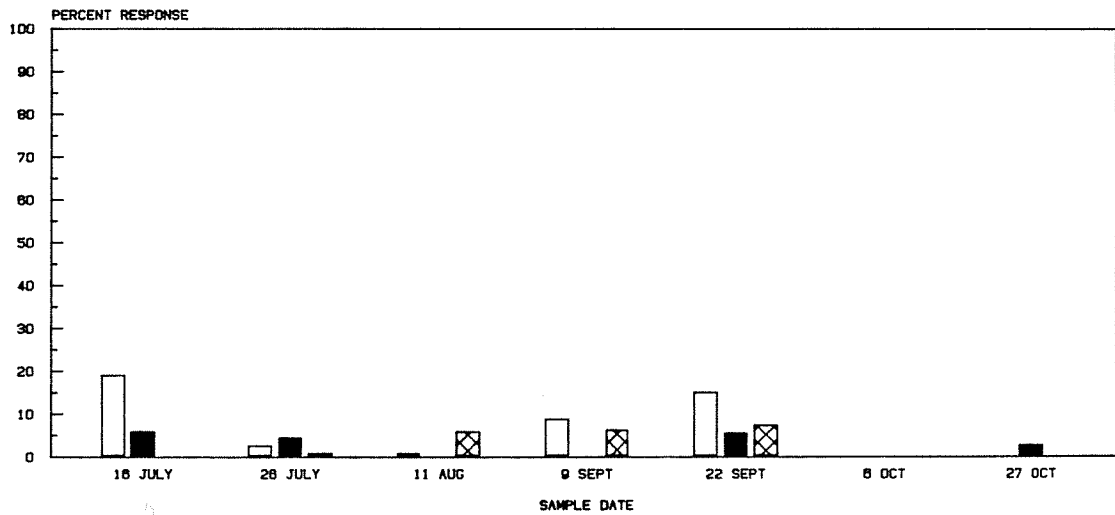
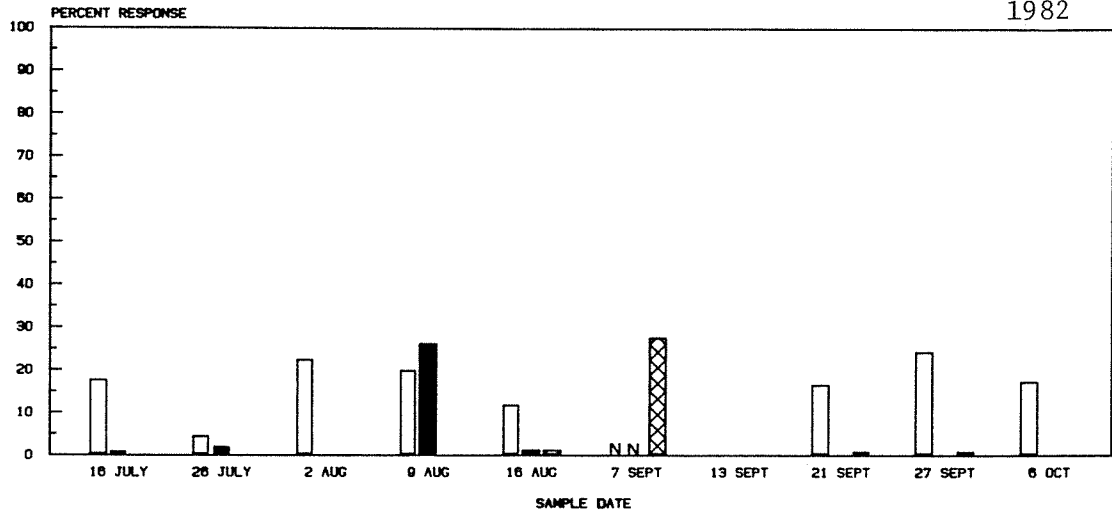


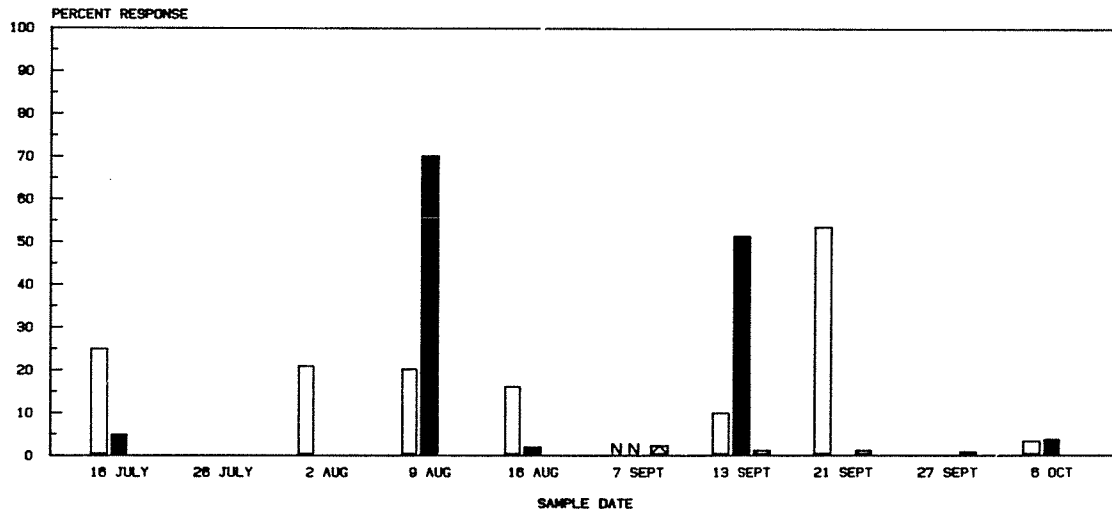
Figure 12.8C:

STATION 4 -- 0 METERS

1982



STATION 4 -- 75 METERS



STATION 4 -- 150 METERS

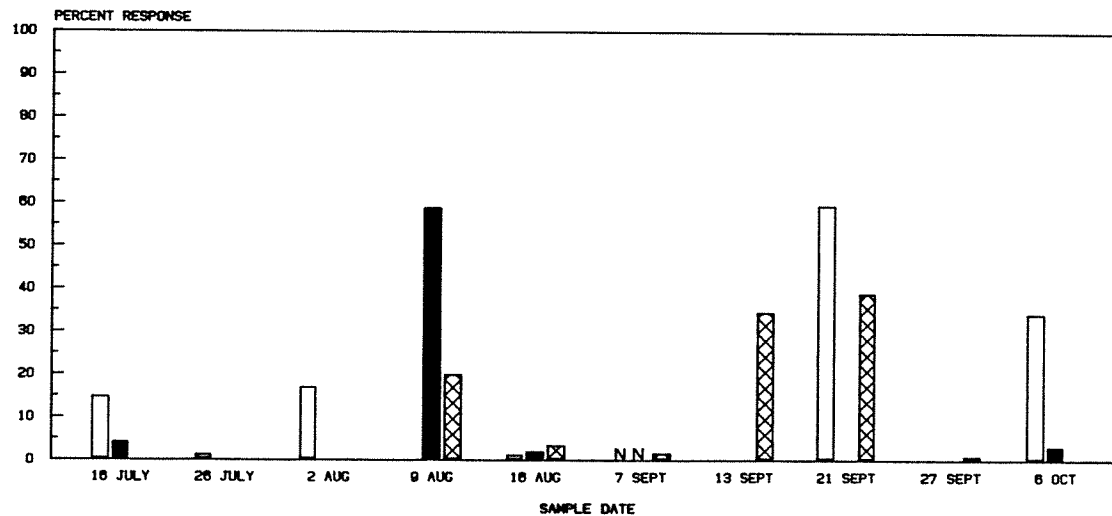
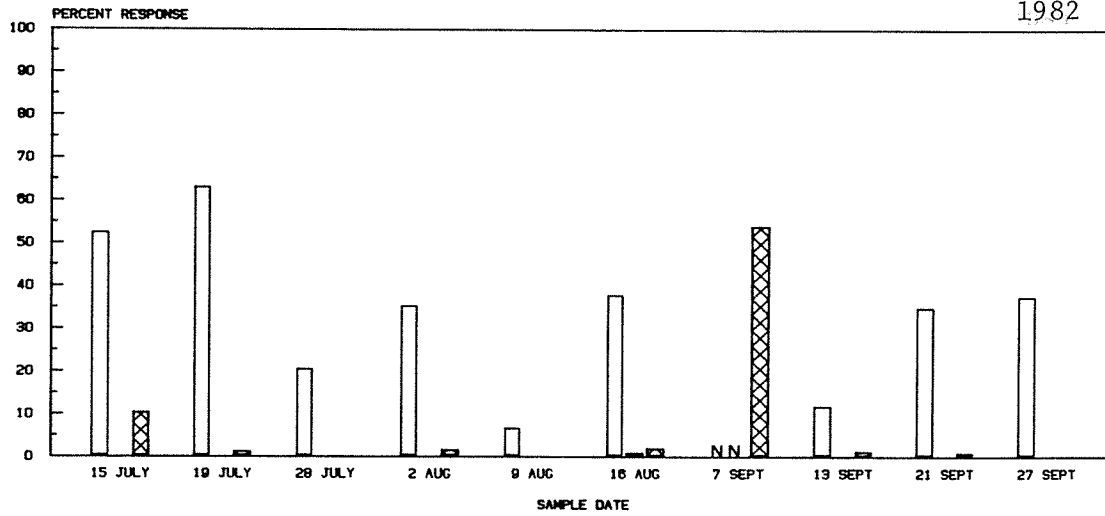


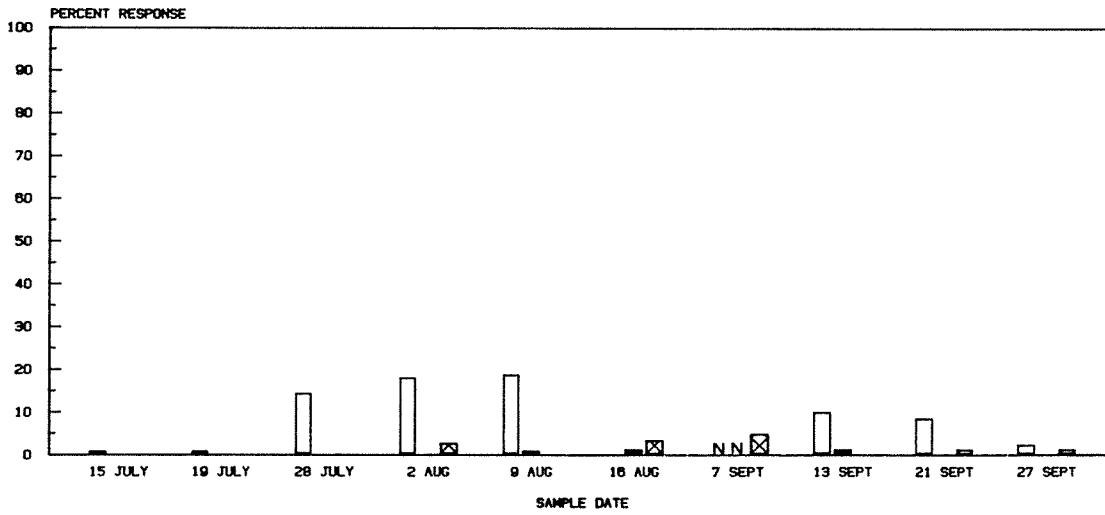
Figure 12.8D

38
 STATION 5 -- 0 METERS

1982



STATION 5 -- 50 METERS



STATION 5 -- 100 METERS

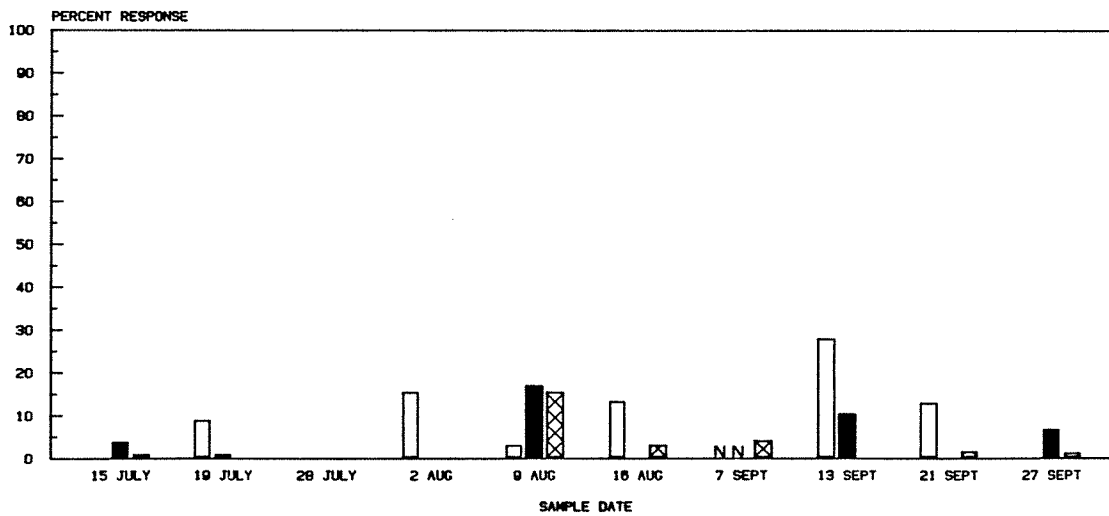
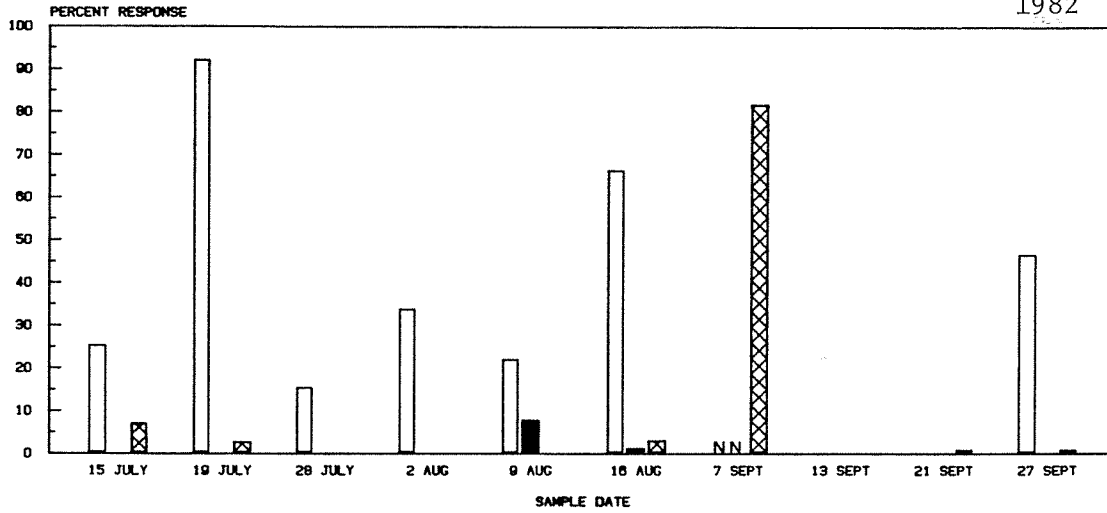


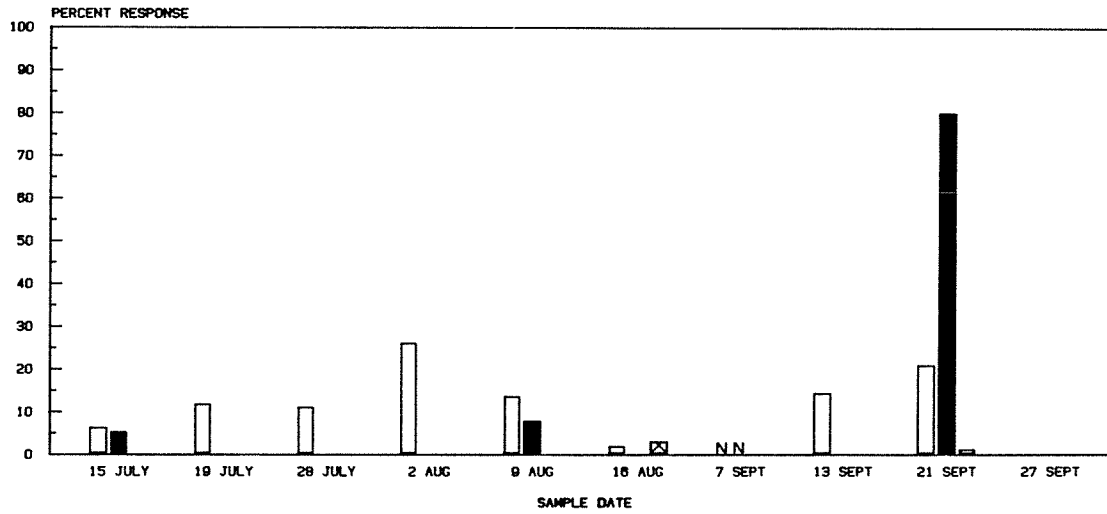
Figure 12.8E.

STATION 6 -- 0 METERS

1982



STATION 6 -- 50 METERS



STATION 6 -- 100 METERS

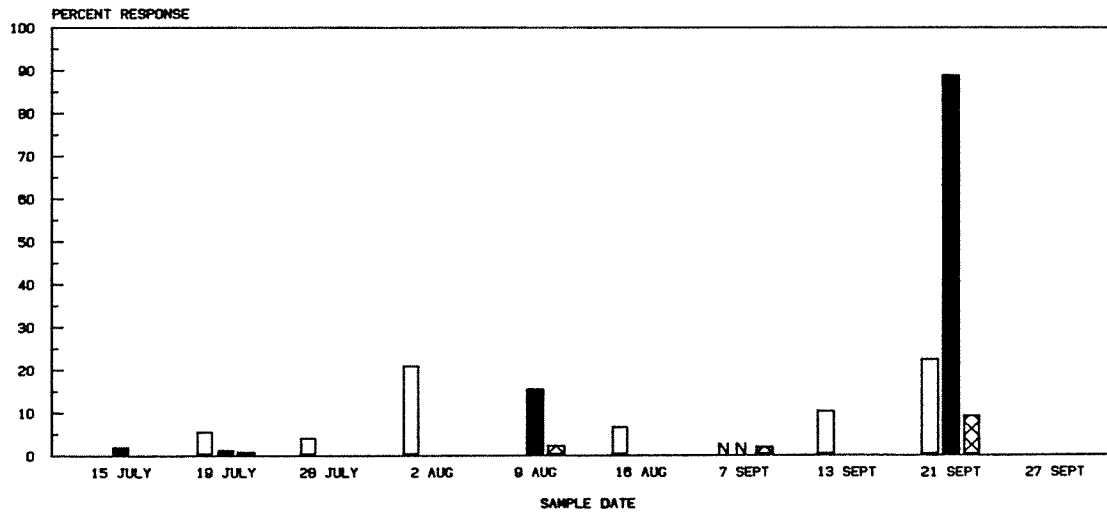
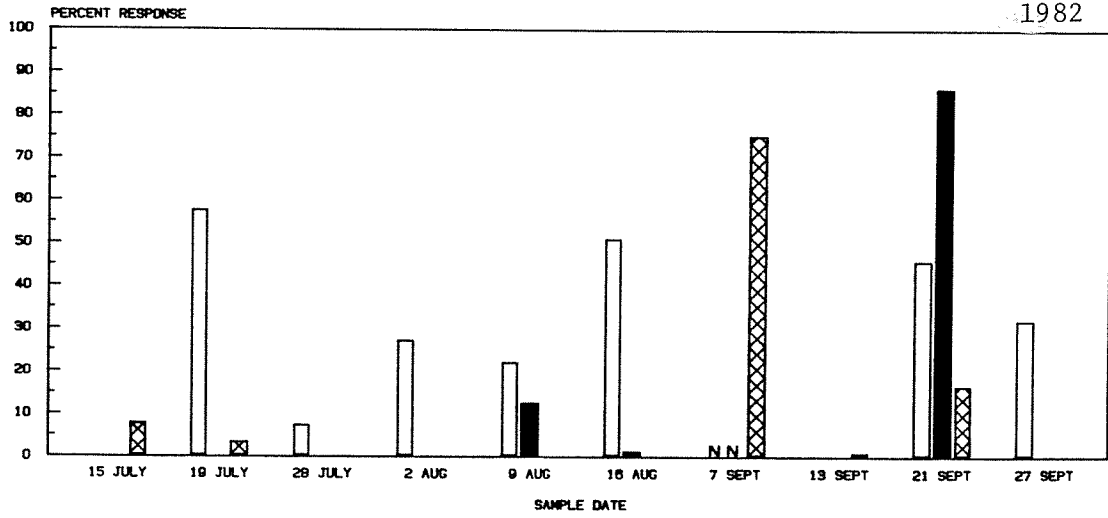
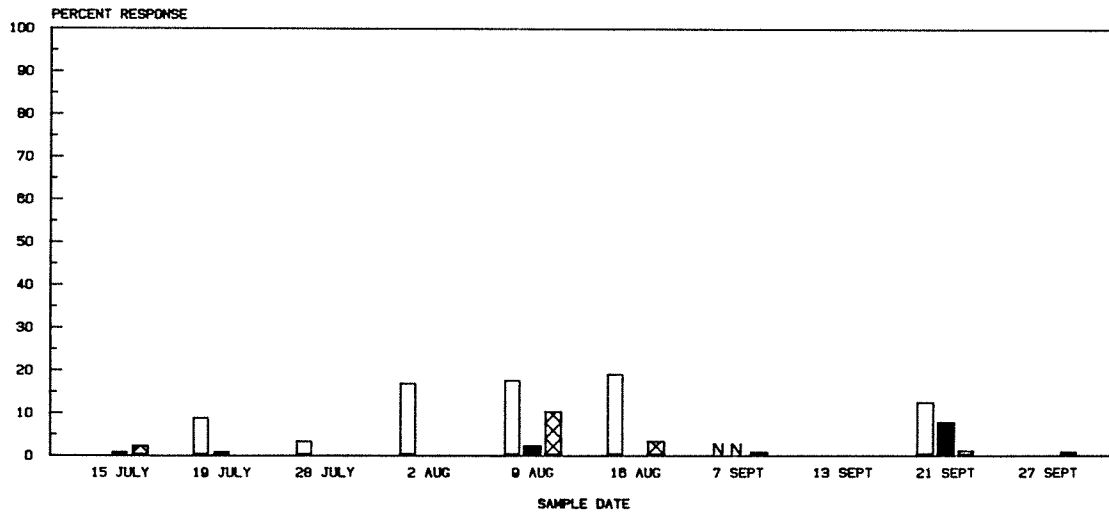


Figure 12.8F.

STATION 7 -- 0 METERS



STATION 7 -- 50 METERS



STATION 7 -- 75 METERS

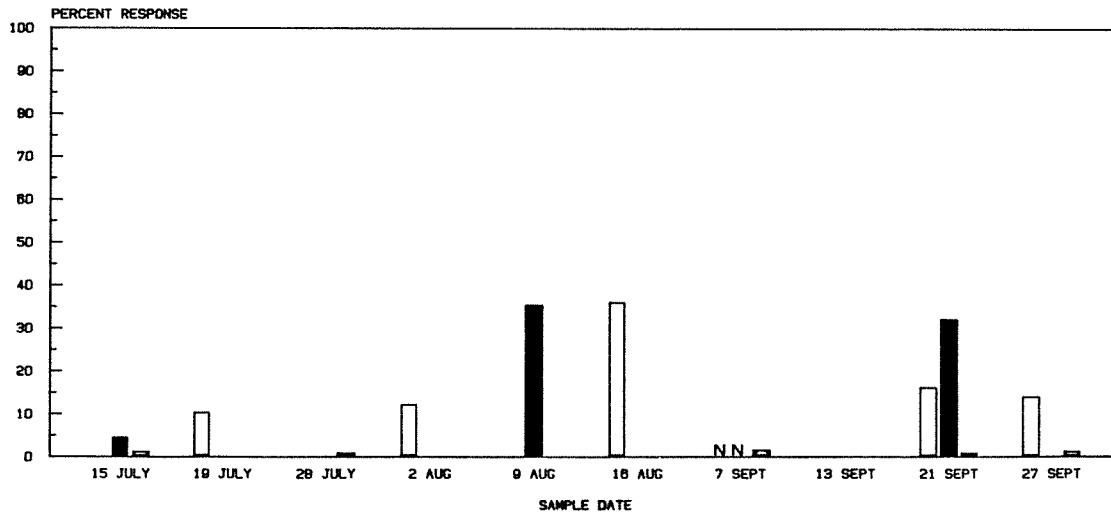
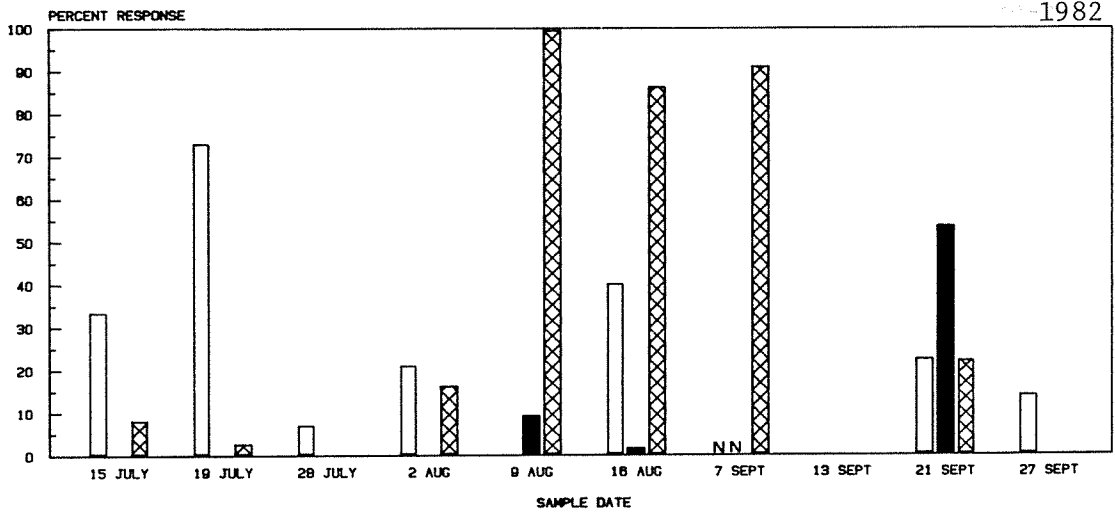


Figure 12.8G.

STATION 8 -- 0 METERS



STATION 8 -- 50 METERS

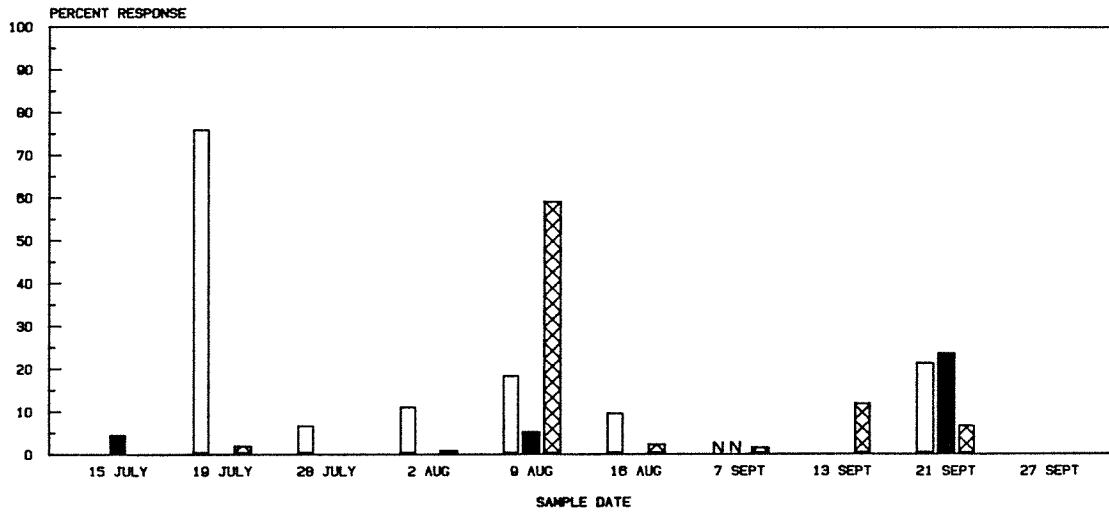
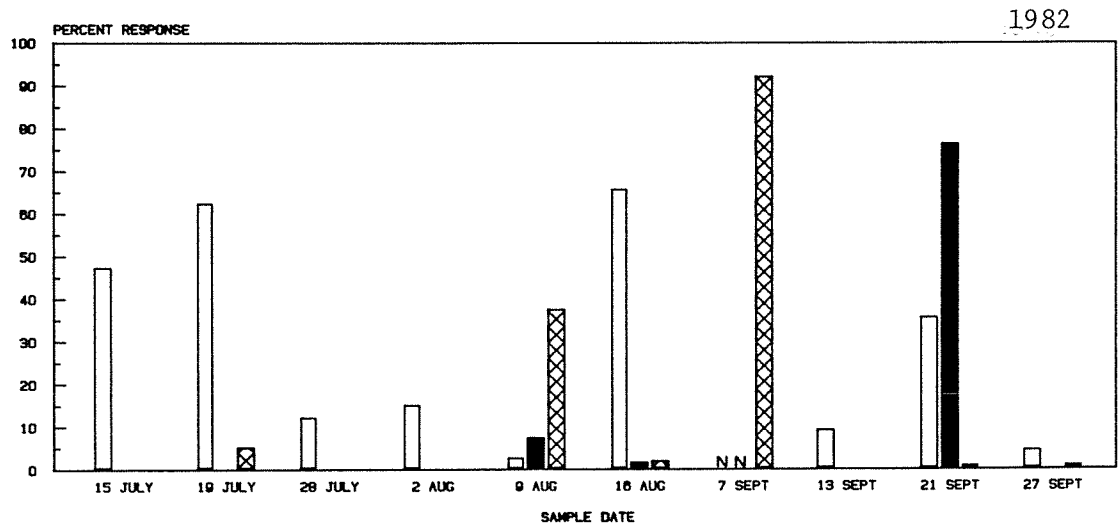


Figure 12.8H.

STATION 9 -- 0 METERS



STATION 9 -- 25 METERS

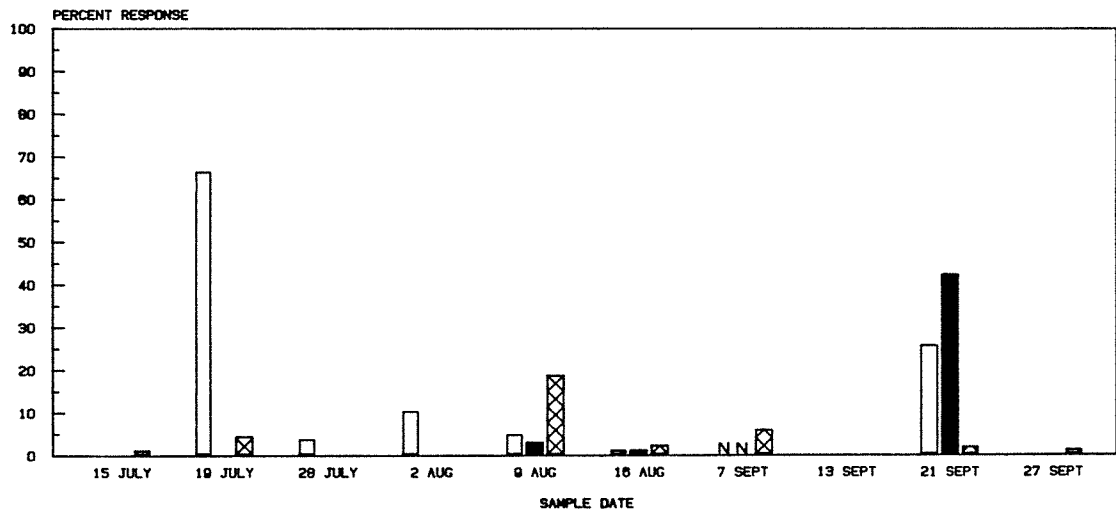
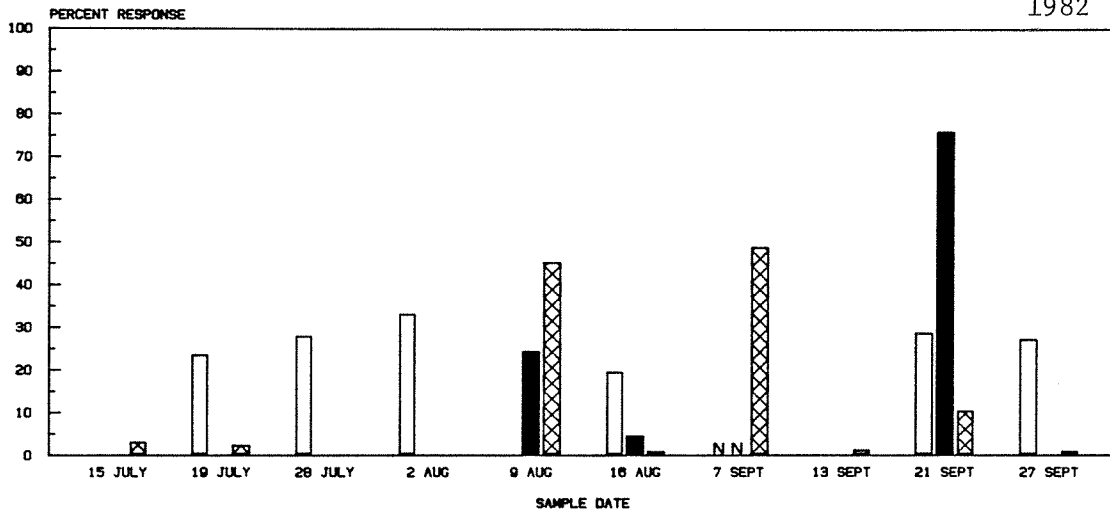


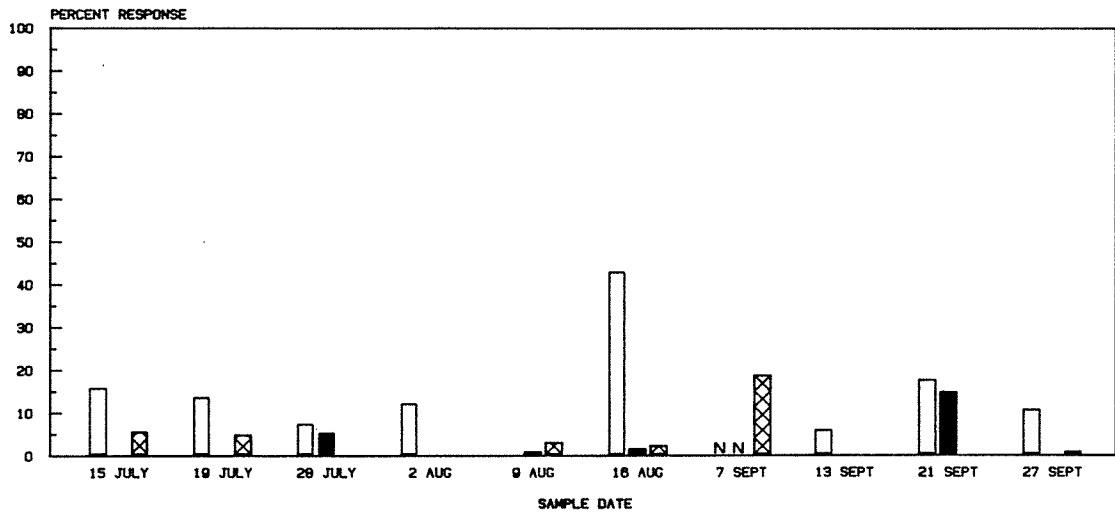
Figure 12.8I.

STATION 10 -- 0 METERS

1982



STATION 10 -- 10 METERS



12.8
Figure 12.8J.

12.9
12.9

Figures 12.9 A through 12.9 L. Results of the 1983 Seahurst receiving water bioassays by station, by depth, and by bioassay response.

Legend:



% oyster embryo mortality



% oyster embryo abnormal

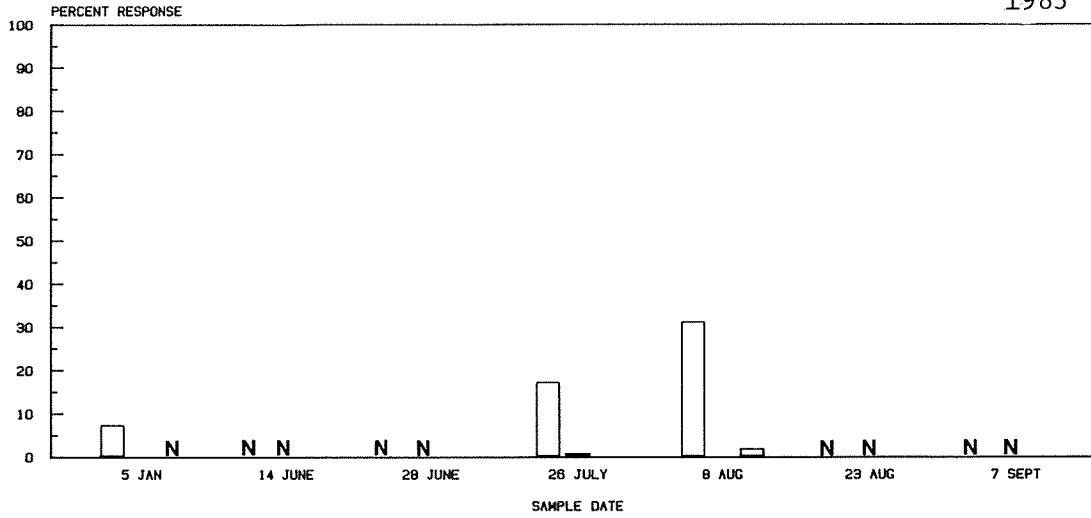


% sand dollar eggs unfertilized

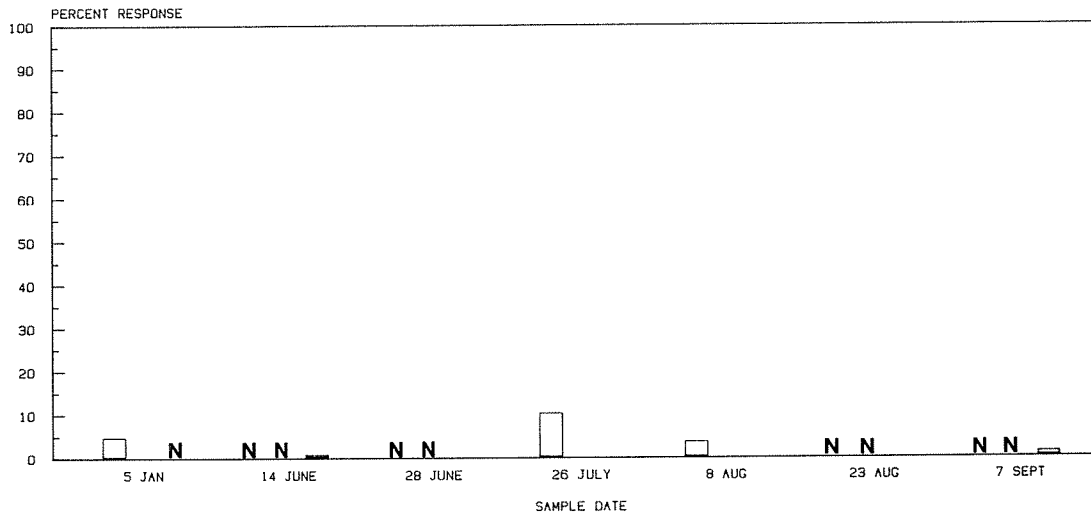
N = not tested (or results discarded due to unacceptably high oyster embryo control mortality or abnormality)

STATION 1 -- 0 METERS

1983



STATION 1 -- 100 METERS



STATION 1 -- 200 METERS

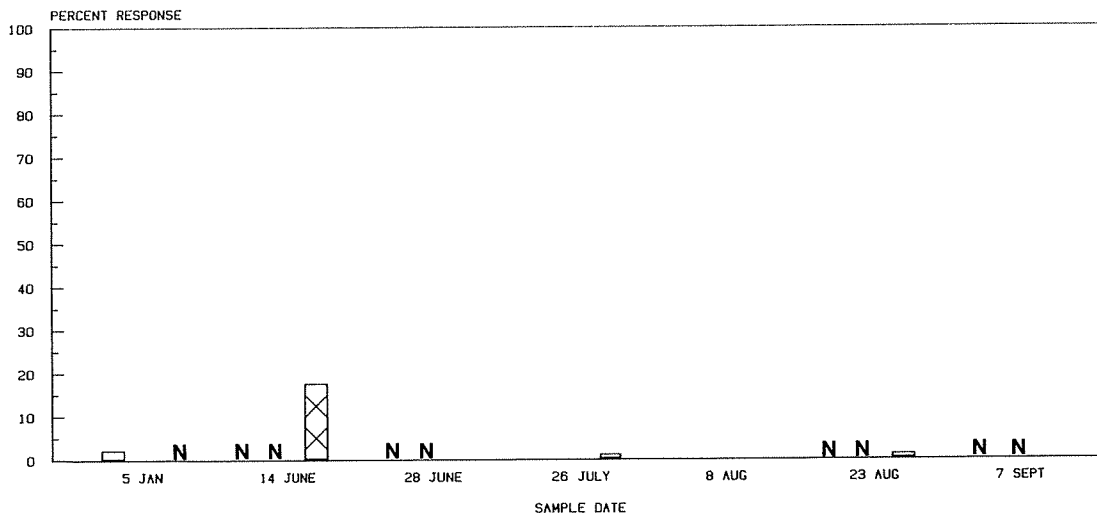
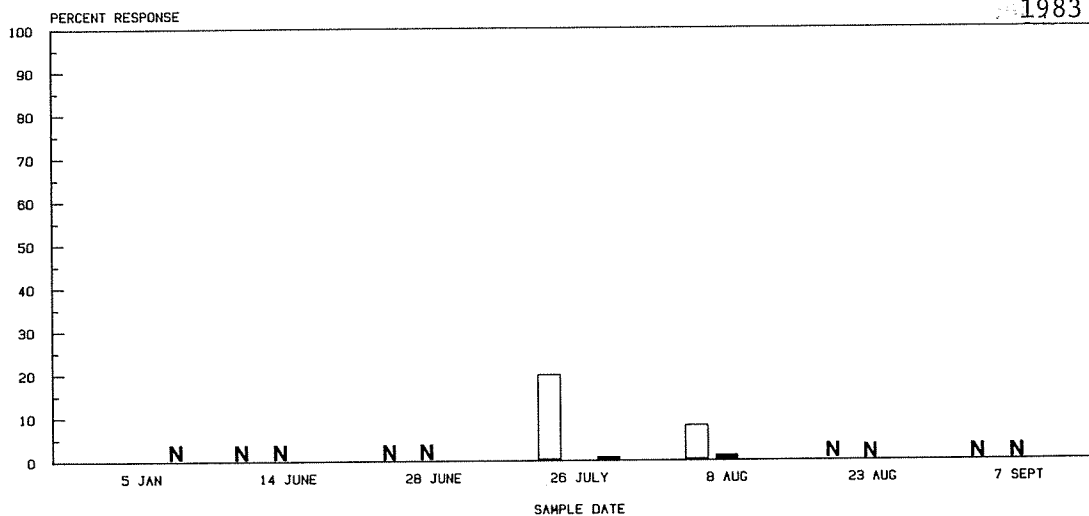


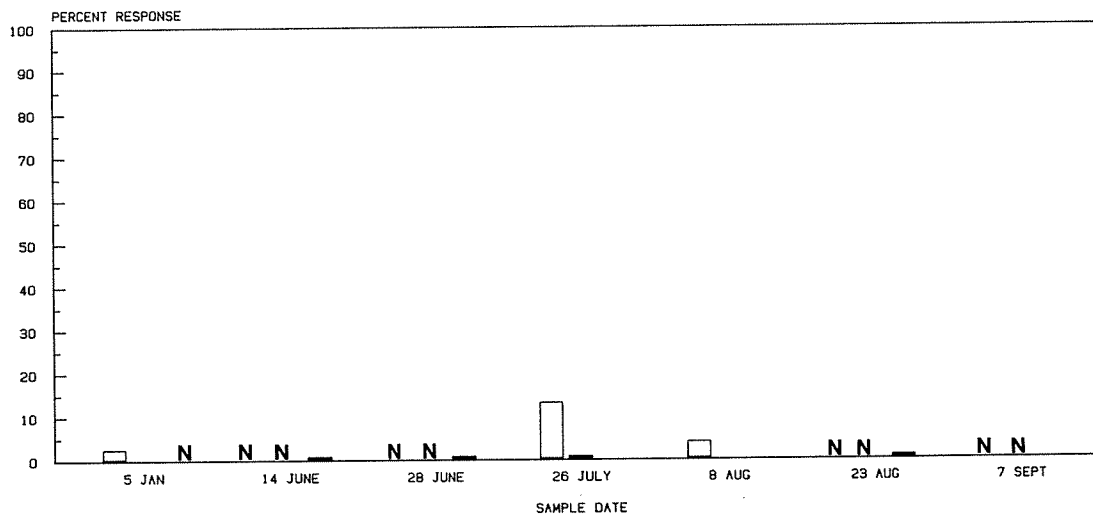
Figure 12.9 A.

STATION 2 -- 0 METERS

1983



STATION 2 -- 50 METERS



STATION 2 -- 100 METERS

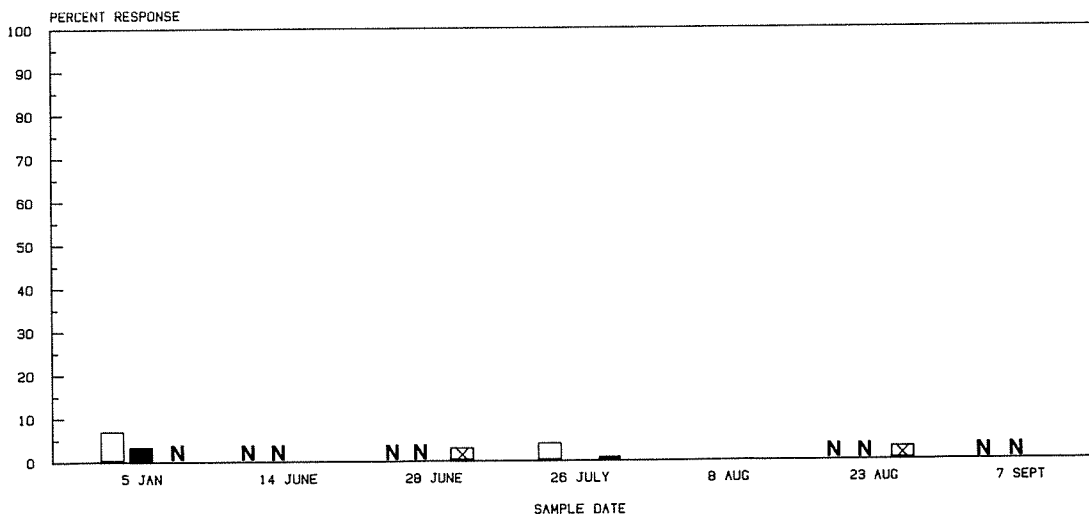
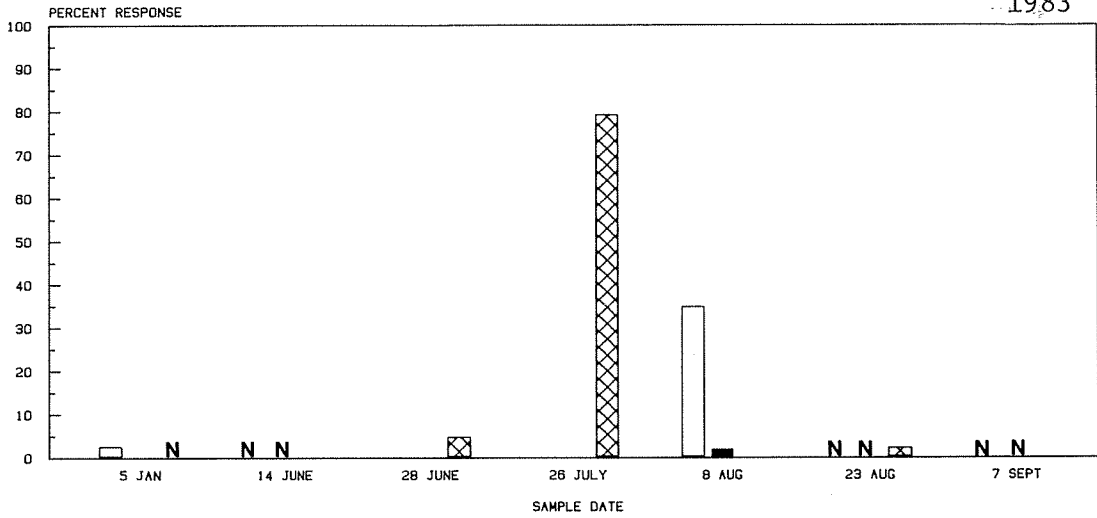


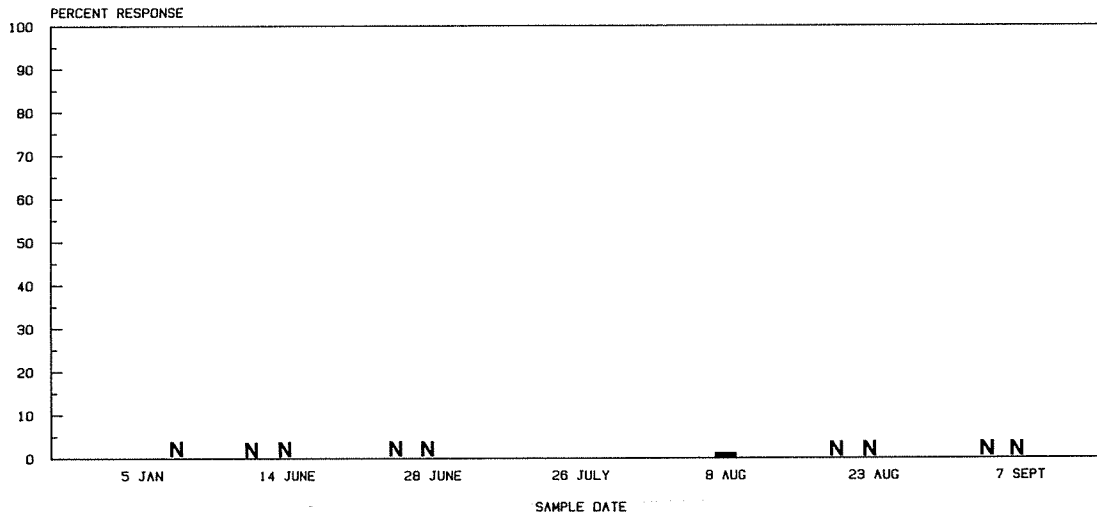
Figure 12.9 B.

STATION 3 -- 0 METERS

1983



STATION 3 -- 75 METERS



STATION 3 -- 150 METERS

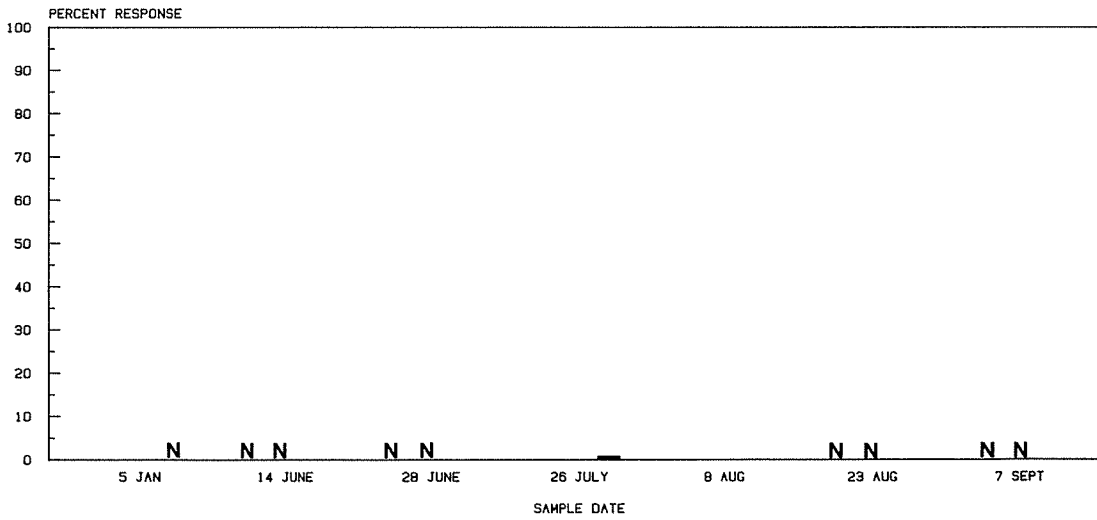
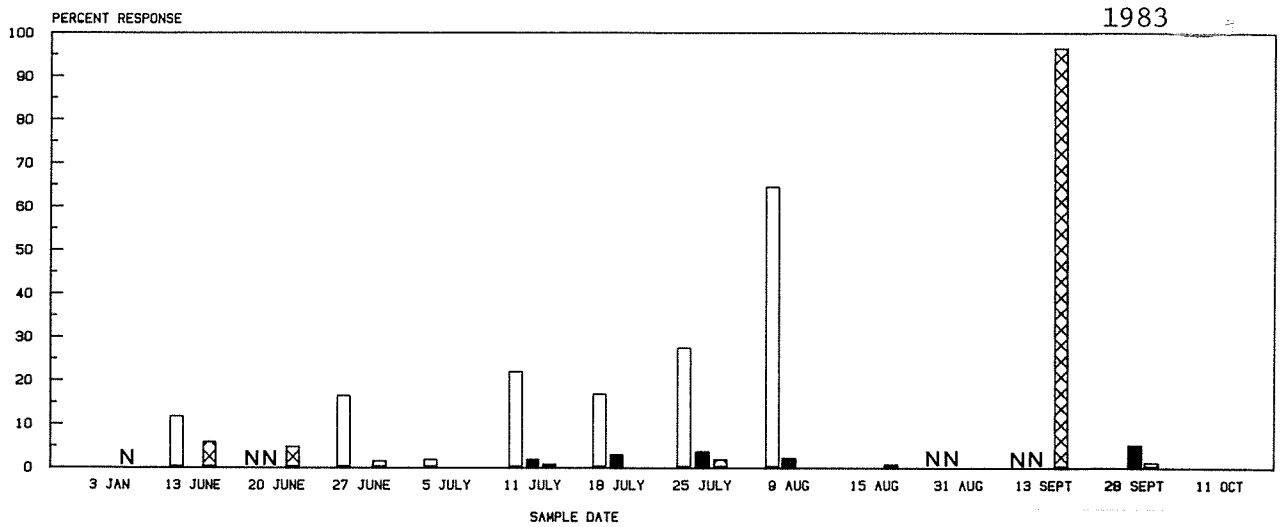
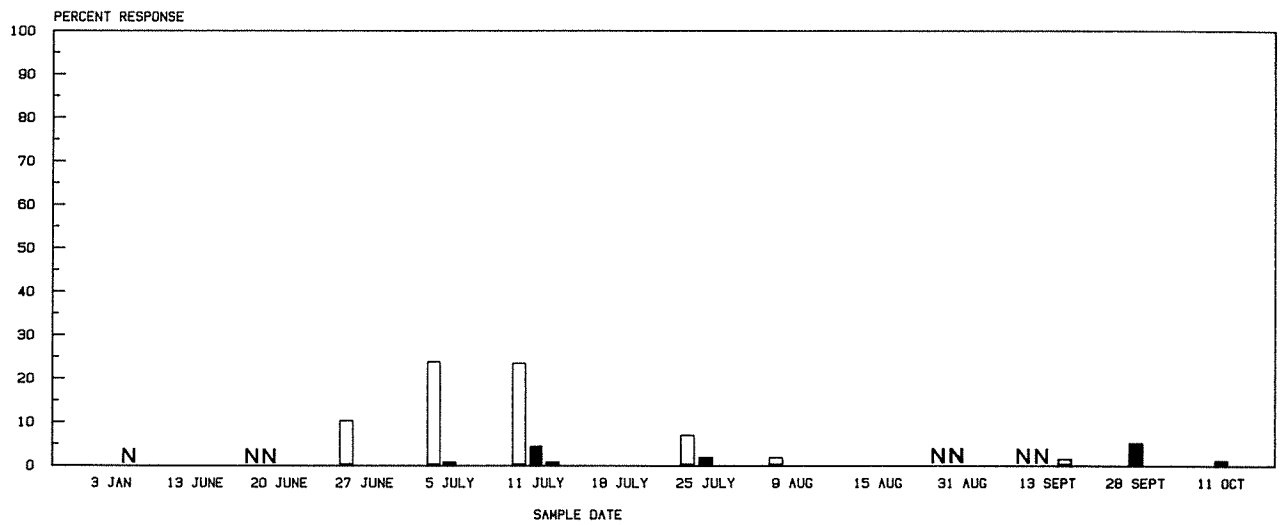


Figure 12.9 C.

STATION 4 -- 0 METERS



STATION 4 -- 75 METERS



STATION 4 -- 150 METERS

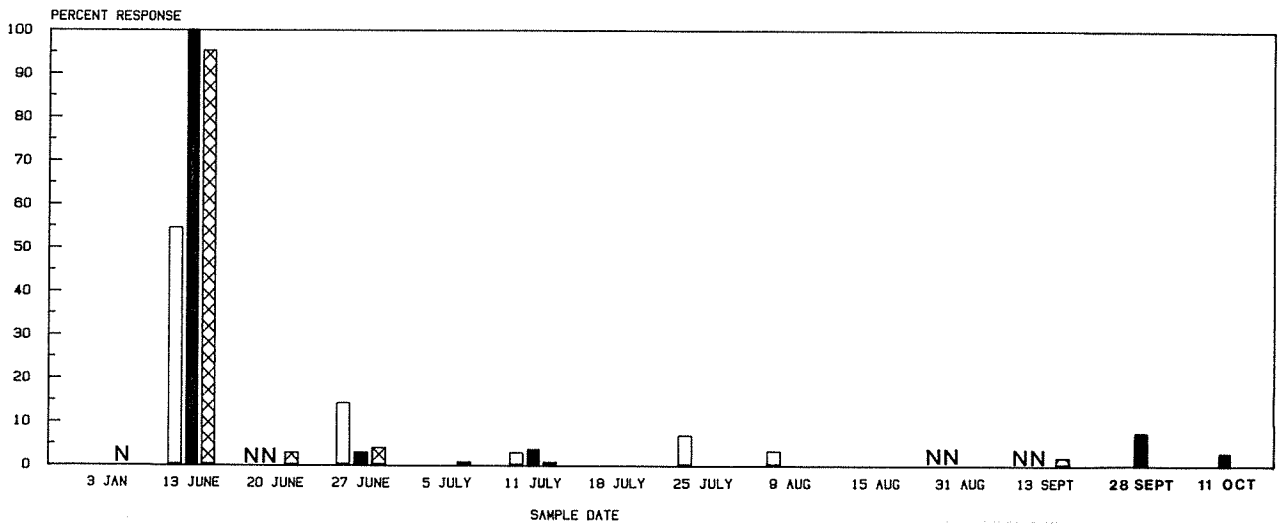
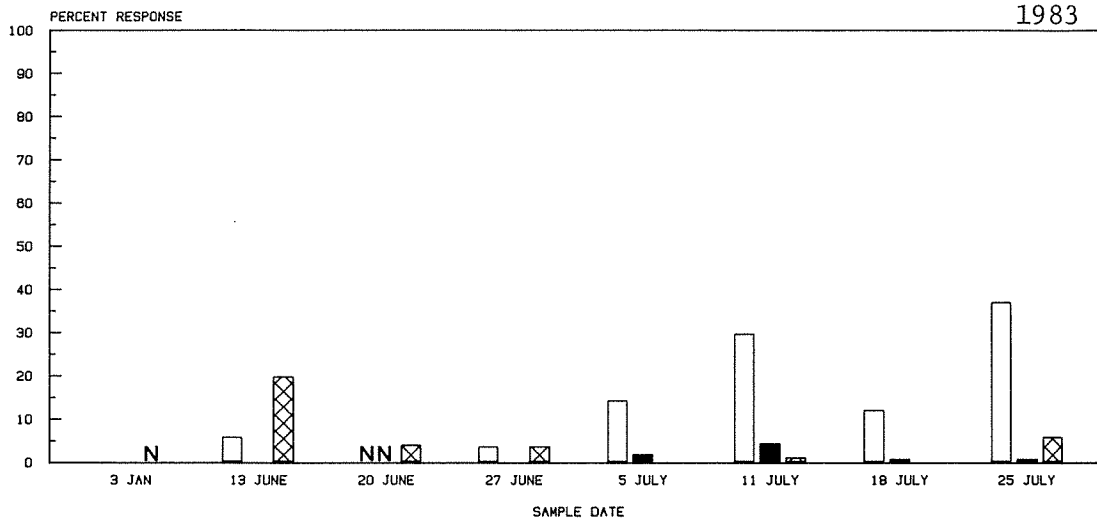
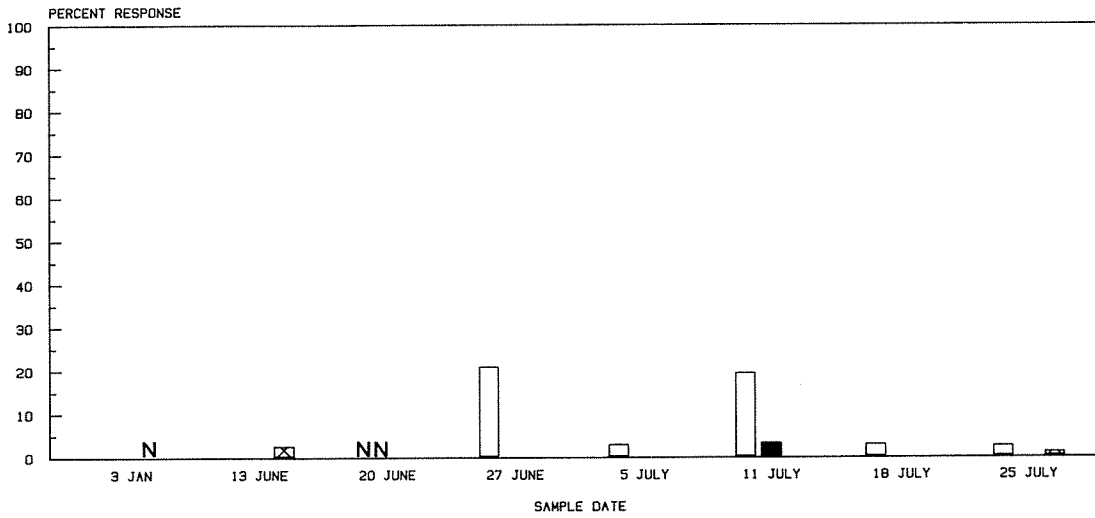


Figure 12.9 D.

STATION 5 -- 0 METERS



STATION 5 -- 50 METERS



STATION 5 -- 100 METERS

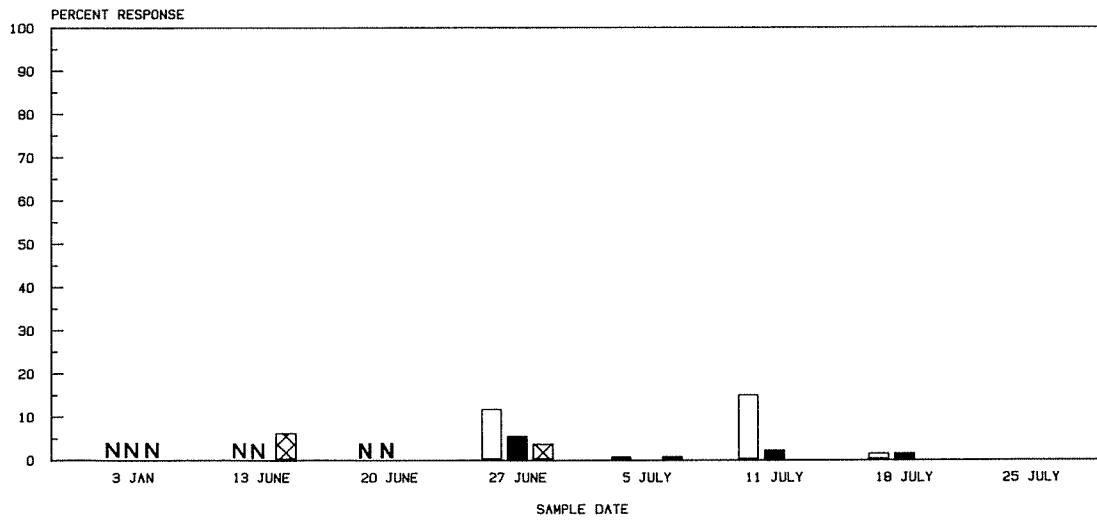
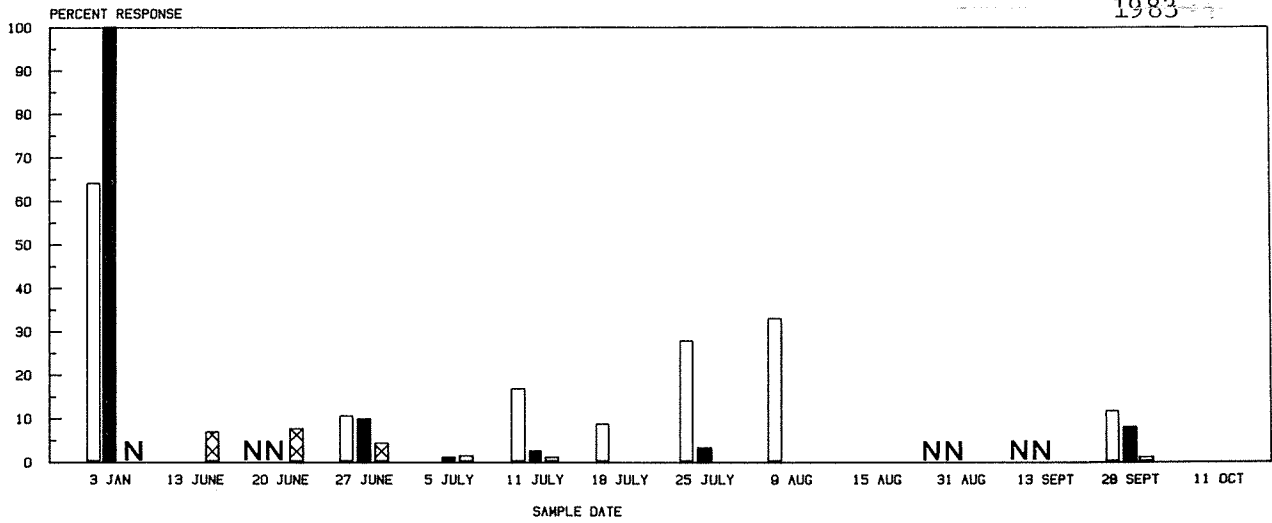


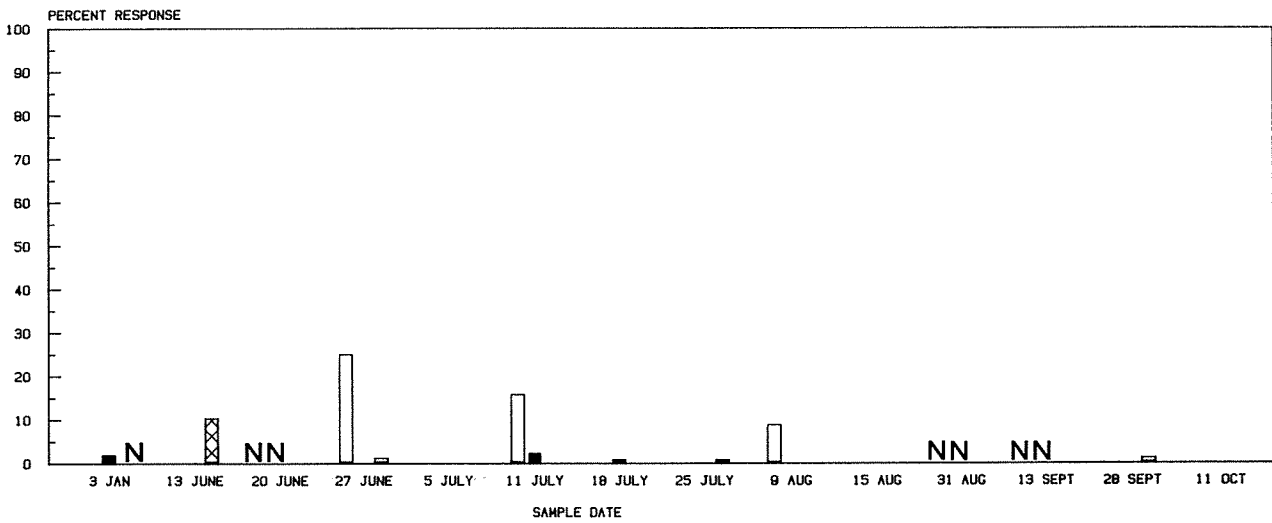
Figure 12.9 E.

STATION 6 -- 0 METERS

1983



STATION 6 -- 50 METERS



STATION 6 -- 100 METERS

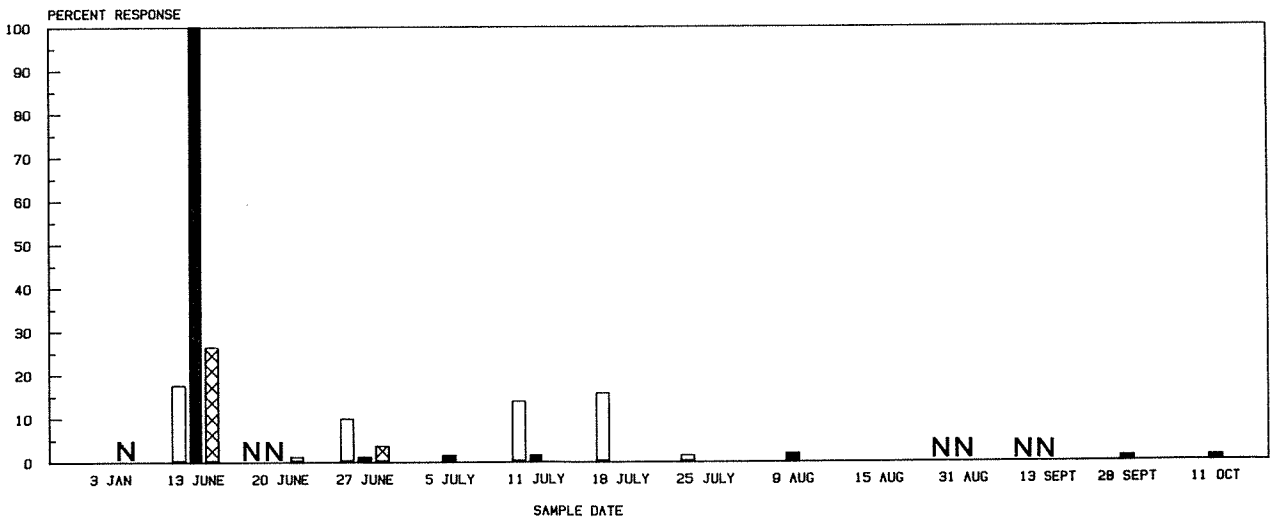
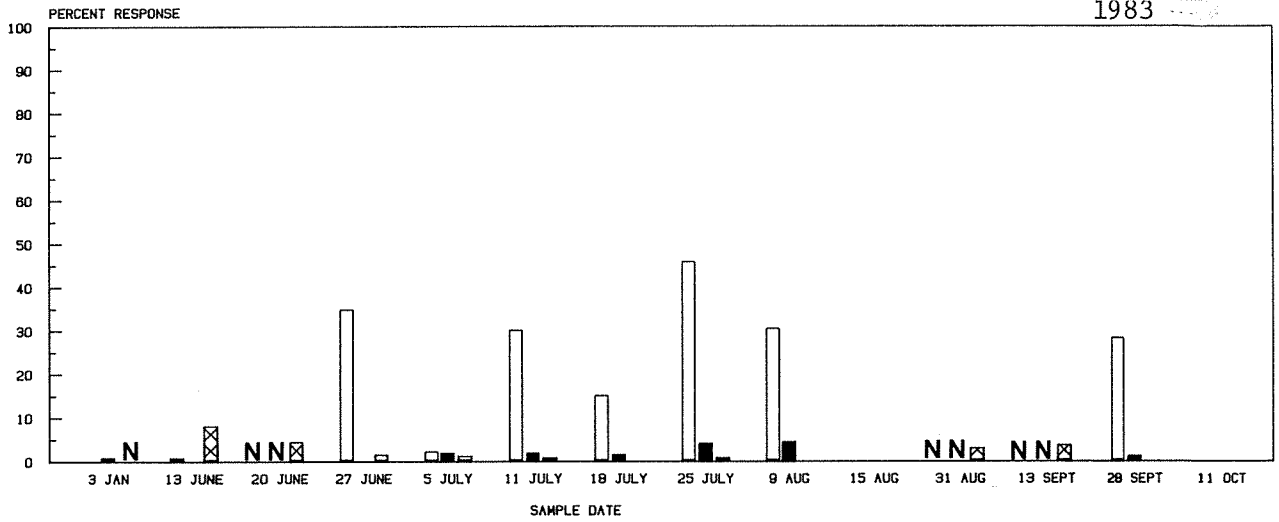
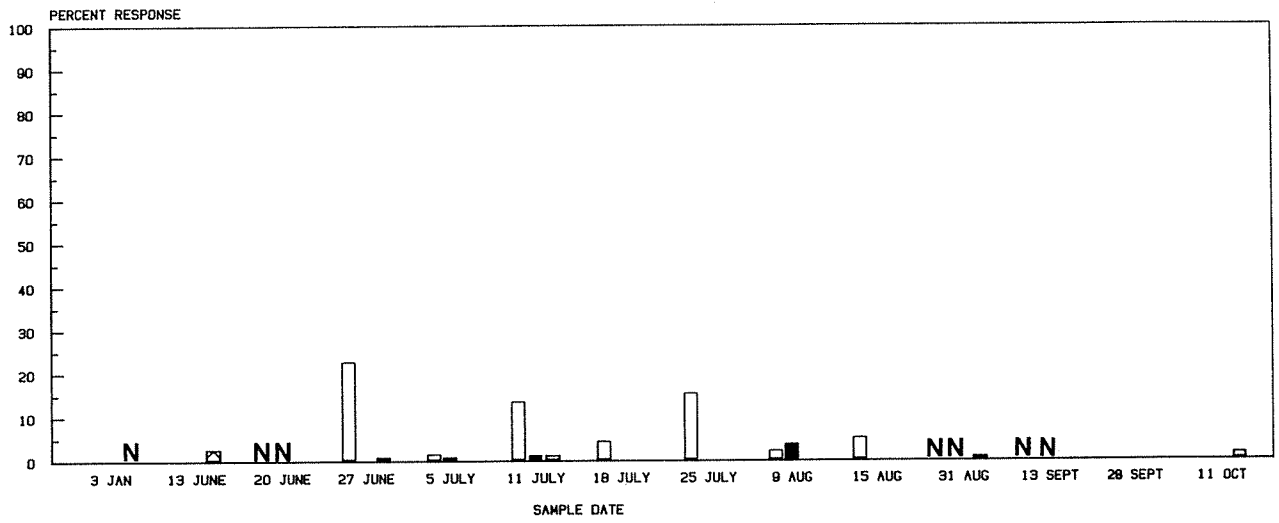


Figure 12.9 F.

STATION 7 -- 0 METERS



STATION 7 -- 50 METERS



STATION 7 -- 75 METERS

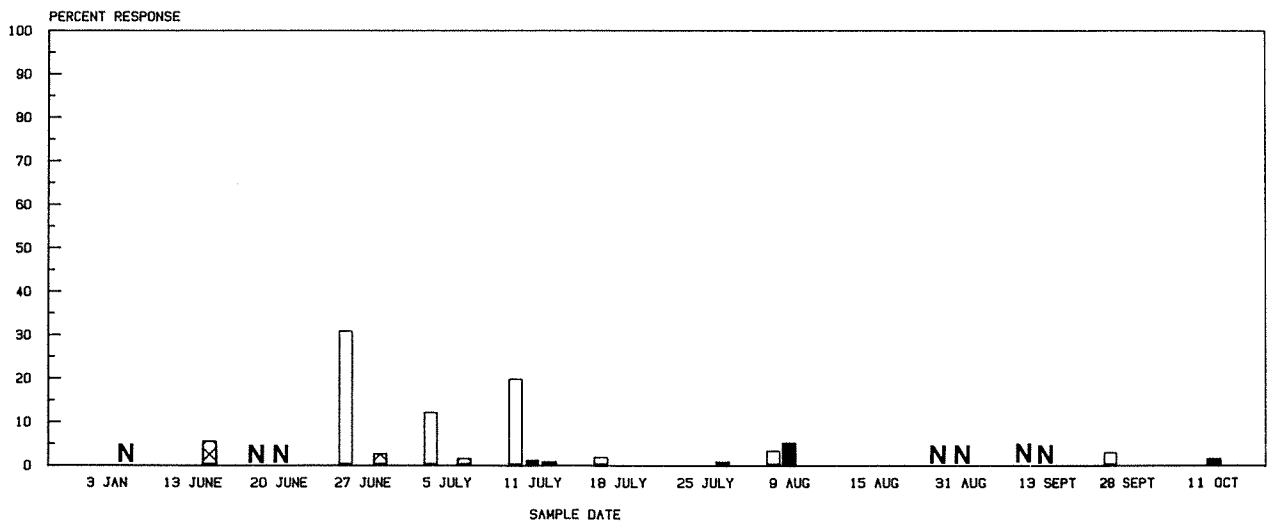
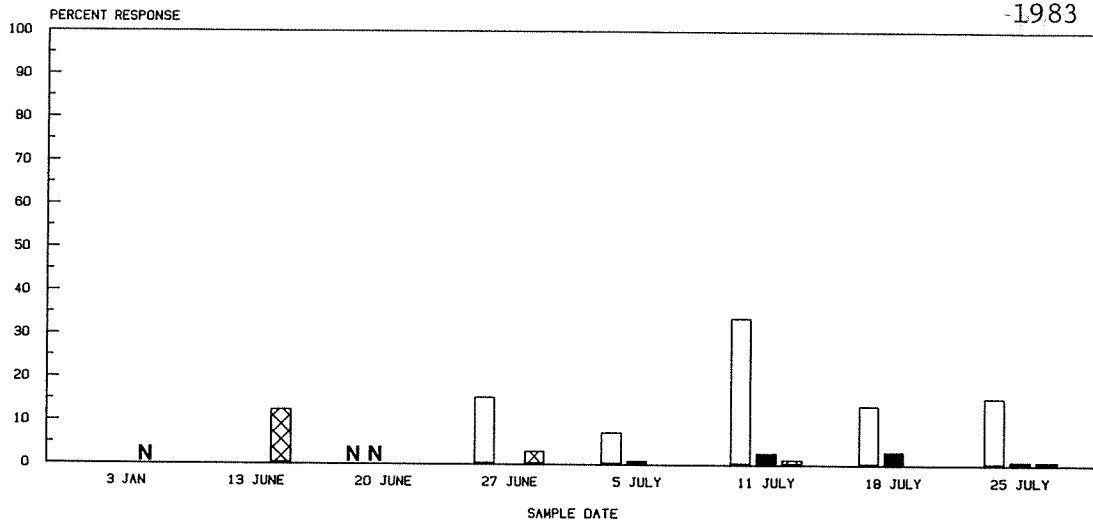


Figure 12.9 G.

STATION 8 -- 0 METERS



STATION 8 -- 50 METERS

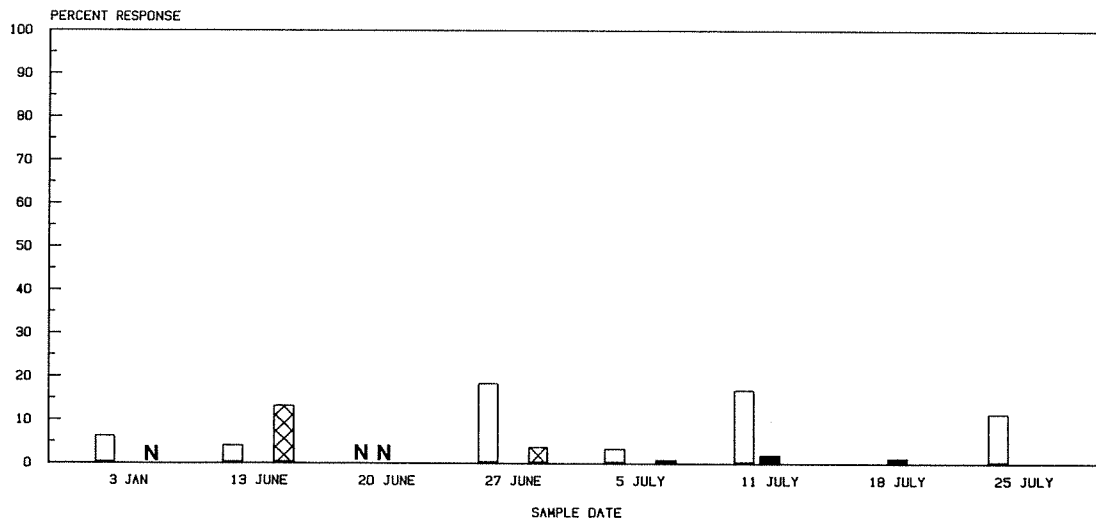
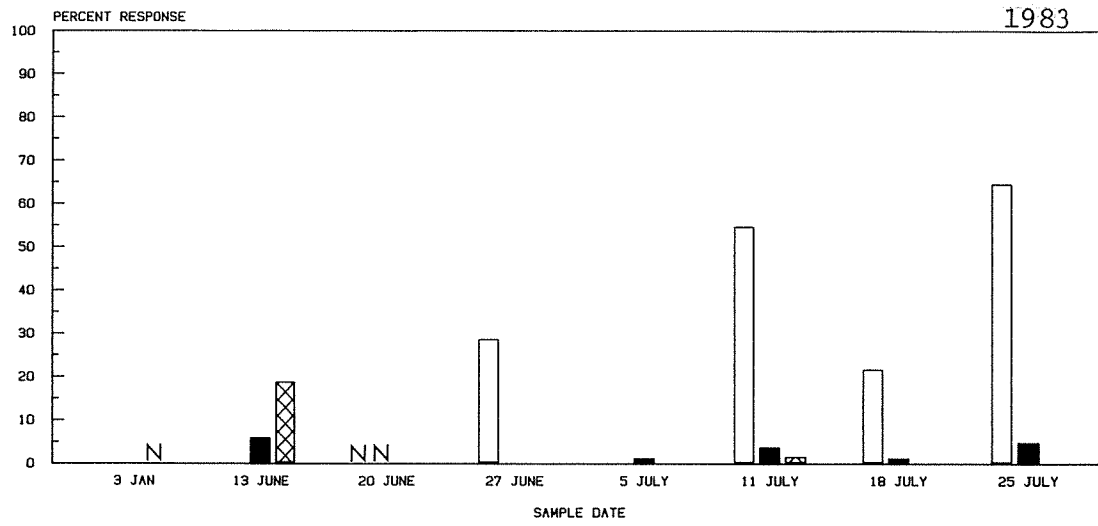


Figure 12.9 H.

STATION 9 -- 0 METERS



STATION 9 -- 25 METERS

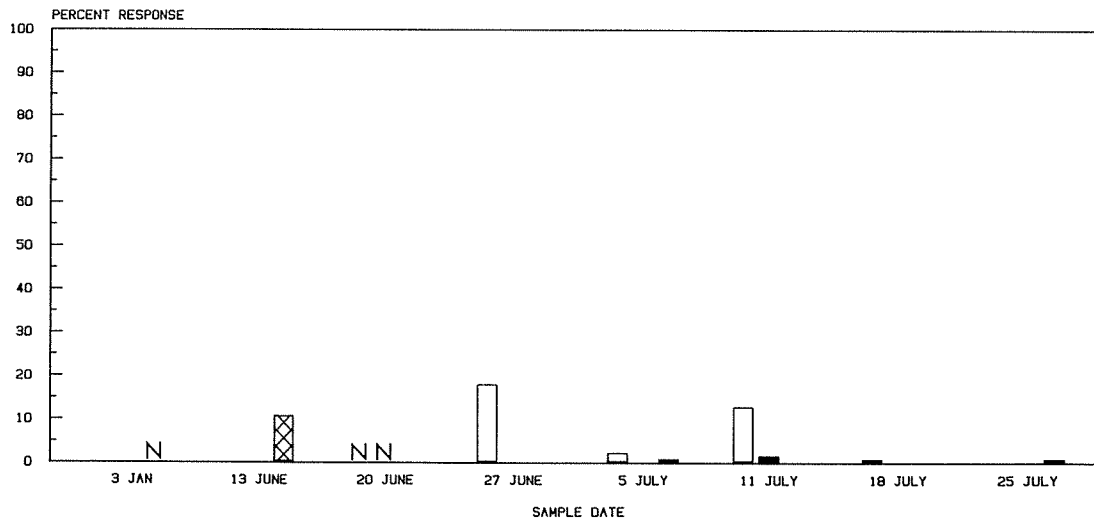
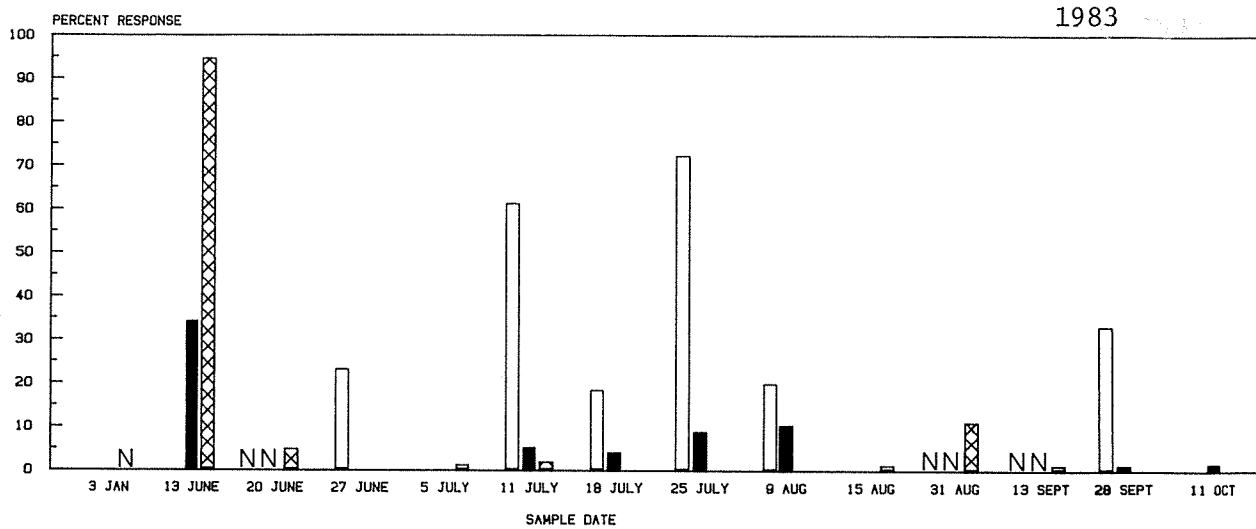


Figure 12.9 I.

STATION 10 -- 0 METERS



STATION 10 -- 10 METERS

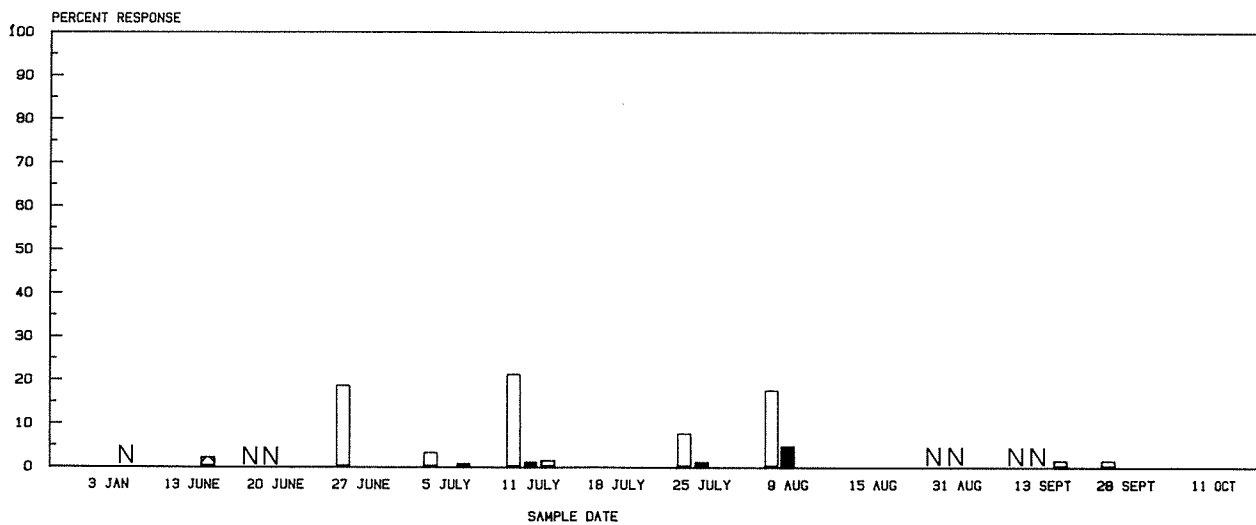
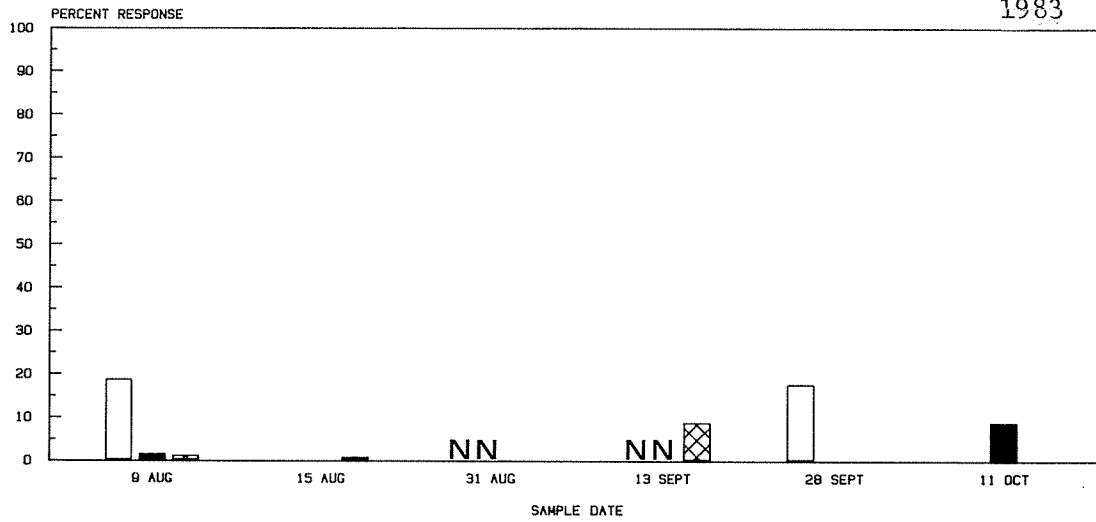


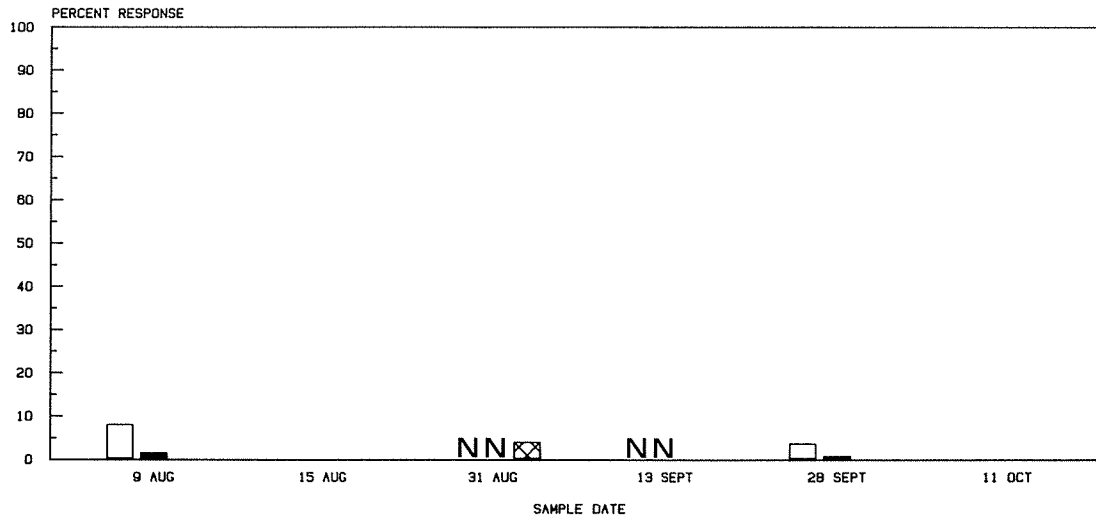
Figure 12.9 J.

STATION 12 -- 0 METERS

1983



STATION 12 -- 100 METERS



STATION 12 -- 200 METERS

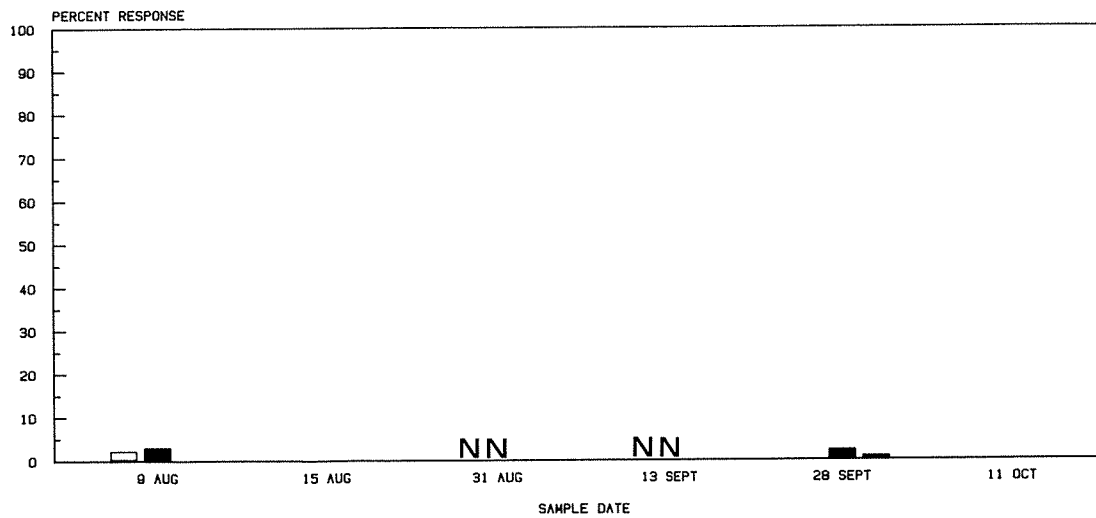
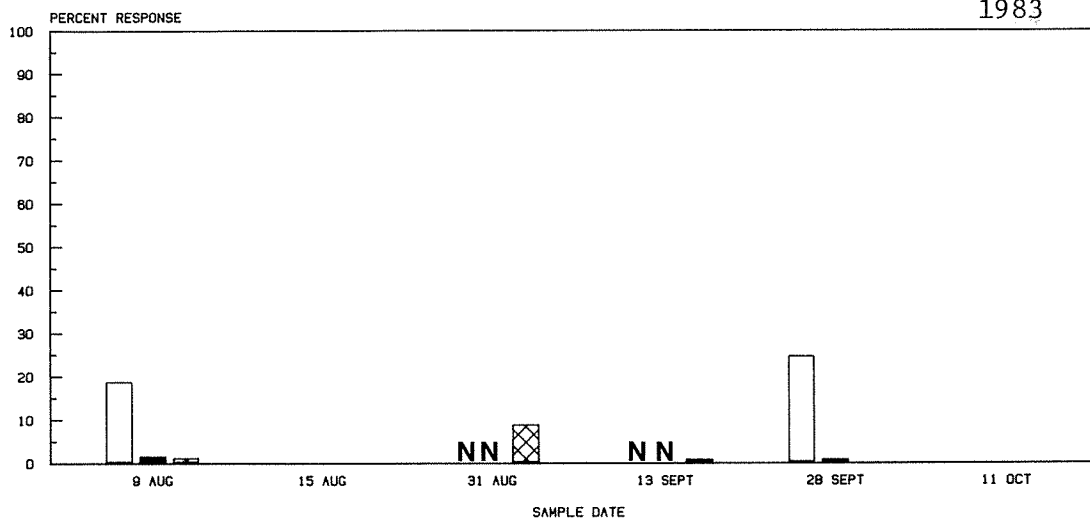


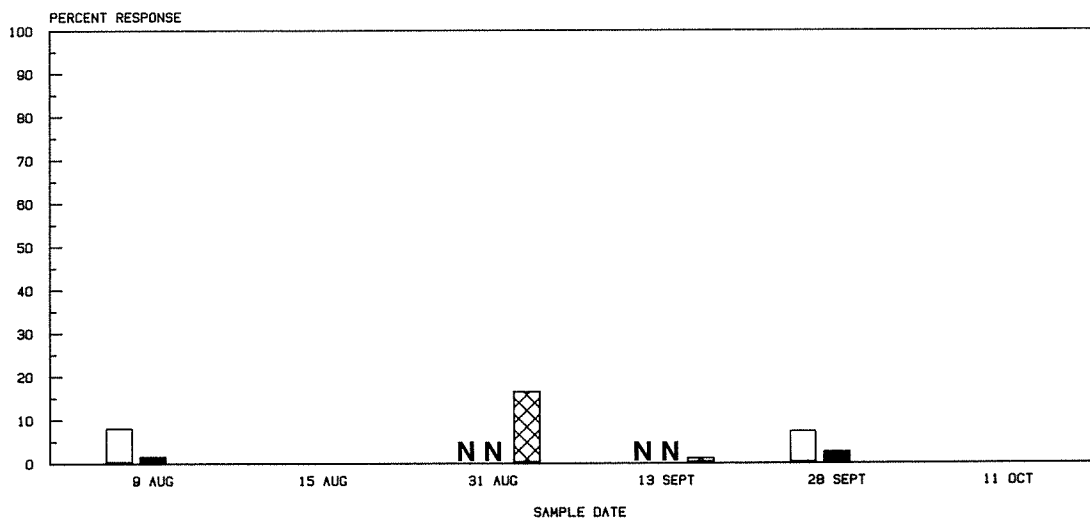
Figure 12.9 K.

STATION 13 -- 0 METERS

1983



STATION 13 -- 40 METERS



STATION 13 -- 60 METERS

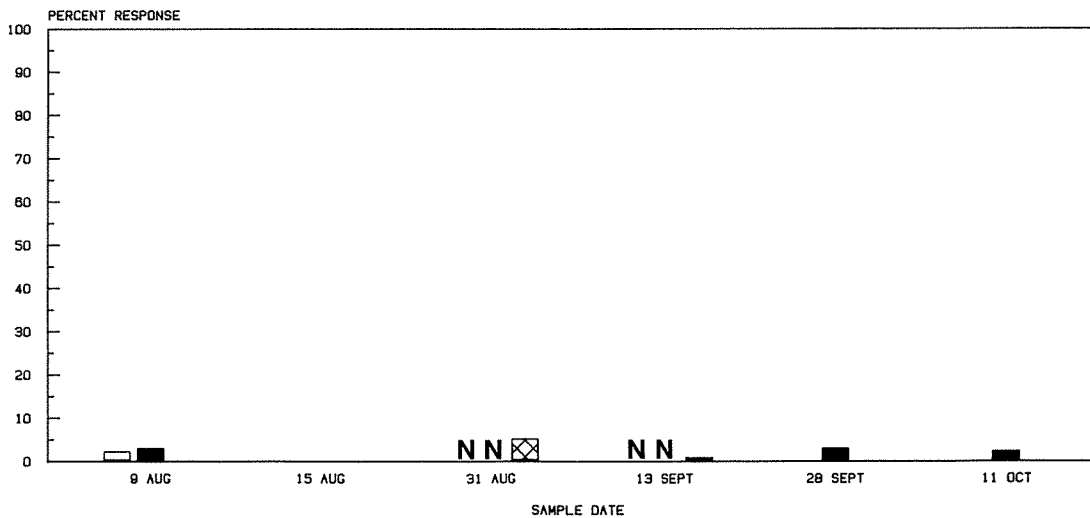


Figure 12.9 L.

Table 12.1. Breakdown of 1982 average biological assay responses to control and Seahurst area receiving waters (adjusted for Kincaid Lab control water responses) and average physical/chemical data for these same samples. Dashes mean not measured.

CALCULATED AVERAGE VALUES

1982	Oyster Embryo		Sand Dollar		Salinity ‰	pH	Total Ammonia ($\mu\text{g-at/l}$)	Chlorophyll "a" ($\mu\text{g/l}$)	Primary production ($\text{mg Carbon/m}^2/\text{day}$)	Diatom counts ($\times 10^3$)	Dinoflagellate counts ($\times 10^3$)	Other phyto- plankton counts ($\times 10^3$)	Secchi disk depth (meters)
	% mortality	% abnormal	% Eggs unfertilized	% Eggs fertilized									
Control Seawater:													
Kincaid Lab	0	0	0	0	29.7	7.7	--	--	--	--	--	--	--
Kincaid carry- along	1.7	-2.2	0.9	0.9	29.7	7.7	--	--	--	--	--	--	--
West Point	11.7	-1.4	-0.2	-0.2	29.5	7.8	--	--	--	--	--	--	--
Friday Harbor (frozen)	-0.2	-8.4	-1.0	-1.0	29.5	7.9	--	--	--	--	--	--	--
By Station:													
1	9.6	1.3	6.6	6.6	29.4	7.8	1.83	1.16	--	168.7	50.29	371.9	9.5
2	9.9	-0.5	4.4	4.4	29.6	7.8	1.54	1.61	--	--	--	--	9.9
3	6.7	1.5	2.2	2.2	29.3	7.8	1.07	1.74	--	--	--	--	5.5
4	13.8	0.3	4.1	4.1	29.9	7.9	1.58	1.13	20.55	79.5	13.11	229.4	11.0
5	15.7	-7.1	2.7	2.7	29.8	7.9	1.35	1.99	--	--	--	--	9.4
6	15.9	-0.8	2.7	2.7	29.7	8.0	1.03	2.30	--	--	--	--	9.4
7	9.5	-4.1	3.7	3.7	29.7	8.0	1.38	2.74	55.75	355.8	16.33	444.2	8.7
8	16.1	-5.9	19.8	19.8	29.5	8.0	1.42	3.02	--	--	--	--	8.8
9	18.7	5.4	8.1	8.1	29.4	8.0	1.85	3.81	--	--	--	--	8.6
10	11.2	-3.3	6.9	6.9	29.4	8.0	1.07	4.38	89.30	656.6	37.59	687.4	8.8
By Depth:													
0	21.1*	-5.3	9.3	9.3	29.2	8.0	1.41	5.01	45.60	332.3	29.94	464.1	9.05
10	8.0	-6.8	3.2	3.2	29.5	8.0	1.21	3.47	--	--	--	--	--
25	9.2	-6.6	3.3	3.3	29.6	8.0	1.84	1.42	--	--	--	--	--
50	8.9	-4.2	2.8+	2.8+	29.7	7.9	1.41	0.76	--	--	--	--	--
75	6.0	4.3	0.3	0.3	29.9	7.8	1.15	0.34	--	--	--	--	--
100	7.8	3.2	3.1	3.1	29.9	7.8	1.28	0.46	--	--	--	--	--
150	7.8	0.6	6.8	6.8	30.1	7.8	1.58	0.20	--	--	--	--	--
200	4.4	0.3	14.8	14.8	29.8	7.8	2.05	0.27	--	--	--	--	--

*Significantly ($p < 0.05$) greater than population mean response.

+Significantly ($p < 0.05$) lower than population mean response.

Table 12.2. Breakdown of 1983 average biological assay responses to control and Seahurst area receiving waters (adjusted for the Kincaid Lab control water responses) and average physical/chemical data for these same samples. Dashes mean not measured.

CALCULATED AVERAGE VALUES

1983	Oyster mortality %	Embryo abnormal %	Sand Dollar % Eggs unfertilized	Salinity ‰	pH	Total Ammonia (ug-at/l)	Chlorophyll "a" (µg/l)	Primary production (mg Carbon/m ² /day)	Diatom counts (X 10 ³)	Dinoflagellate counts (X 10 ³)	Other phyto-plankton counts (X 10 ³)	Secchi disk depth (meters)
Control Seawater:												
Kincaid Lab	0	0	0	29.6	8.0	--	--	--	--	--	--	--
Kincaid carry-along	-3.4	1.0	-0.1	29.6	8.1	--	--	--	--	--	--	--
West Point	0.1	-0.4	0.1	29.2	8.2	--	--	--	--	--	--	--
Friday Harbor (frozen)	3.6	3.7	3.4	26.0	8.3	--	--	--	--	--	--	--
By Station:												
1	6.5	-0.3	1.0	29.5	8.2	0.78	3.89	--	51.5	1.41	34.7	7.4
2	6.0	0.3	0.1	29.3	8.2	0.50	3.56	--	--	--	--	8.9
3	0.4	0.2	3.8	29.2	8.2	0.27	6.53	--	--	--	--	5.0
4	1.8	2.7	5.0	29.6	8.2	0.31	5.82	37.36	115.4	9.53	81.7	8.5
5	7.3	-0.4	2.1	29.3	8.2	0.33	5.09	--	--	--	--	6.9
6	3.2	5.8	1.2	29.5	8.2	0.31	5.06	--	--	--	--	8.5
7	4.4	-1.1	0.7	29.4	8.2	0.33	4.93	43.15	107.3	2.72	23.2	8.7
8	8.2	-0.7	2.5	29.0	8.2	0.34	2.73	--	--	--	--	7.1
9	12.0	0.4	2.0	28.9	8.3	0.60	4.59	--	--	--	--	6.8
10	8.5	1.9	4.3	29.1	8.3	0.71	11.02	84.78	244.6	5.00	43.1	8.6
12	-3.4	-0.6	-0.1	29.8	8.2	0.28	6.55	--	--	--	--	9.6
13	-3.7	-0.2	1.3	29.6	8.2	0.87	5.62	--	--	--	--	10.3
By Depth (m):												
0	12.5*	1.6	4.0	28.7	8.4	0.51	14.85	50.91	154.1	6.62	61.3	8.1
10	1.3	-0.5	-0.1	28.9	8.3	0.82	5.68	--	--	--	--	--
25	2.7	-0.5	1.6	29.3	8.2	0.79	1.63	--	--	--	--	--
40	-6.8	0.2	2.2	29.3	8.2	0.62	1.18	--	--	--	--	--
50	1.5	-1.0	0.6	29.5	8.1	0.36	1.39	--	--	--	--	--
60	-9.8	0.1	0.2	29.8	8.2	0.44	0.53	--	--	--	--	--
75	-1.4	-0.7	-0.4+	29.7	8.1	0.23	0.67	--	--	--	--	--
100	1.2	4.1	1.0	29.8	8.1	0.27	0.86	--	--	--	--	--
150	-2.0	7.4	4.8	30.0	8.1	0.25	0.59	--	--	--	--	--
200	-11.1	-0.7	1.0	30.1	8.1	0.90	0.38	--	--	--	--	--

* = Significantly (p < 0.05) greater than population mean responses.
+ = Significantly (p < 0.05) lower than population mean responses.

Table 12.3. Breakdown of combined (1982 and 1983) average biological assay responses to control and Seahurst area receiving waters (adjusted for the Kincaid Lab control water responses) and average physical/chemical data for these same samples. Dashes mean not measured. (Note: stations 12 and 13 and depths 40 and 60 m reflect only data collected in 1983.)

CALCULATED AVERAGE VALUES

1982 & 1983 Combined Data	Oyster Embryo % mortality	Embryo % abnormal	Sand Dollar % Eggs unfertilized	Salinity ‰	pH	Total Ammonia (ug-at/l)	Chlorophyll "a" (ug/l)	Primary production (mg Carbon/m ² /day)	Diatom counts (X 10 ³)	Dinoflagellate counts (X 10 ³)	Other phyto- plankton counts (X 10 ³)	Secchi disk depth (meters)
Control Seawater:												
Kincaid Lab	0	0	0	29.6	8.0	--	--	--	--	--	--	--
Kincaid carry- along	-0.6	-0.7	0.4	29.6	8.0	--	--	--	--	--	--	--
West Point	6.3	-1.0	-0.1	29.3	8.0	--	--	--	--	--	--	--
Friday Harbor (frozen)	1.6	-2.8	1.5	27.5	8.2	--	--	--	--	--	--	--
By Station:												
1	8.7	0.8	4.1	29.4	8.0	1.30	2.52	--	116.3	28.4	221.0	8.4
2	8.7	-0.2	2.4	29.4	8.0	1.02	2.61	--	--	--	--	9.4
3	4.8	1.1	3.0	29.2	8.0	0.67	4.20	--	--	--	--	5.2
4	7.2	1.7	4.6	29.7	8.1	0.83	3.87	30.27	100.2	11.0	14.4	9.5
5	12.2	-4.3	2.5	29.5	8.1	0.88	3.33	--	--	--	--	8.3
6	8.9	2.8	1.8	29.6	8.1	0.60	3.91	--	--	--	--	8.9
7	6.7	-2.4	2.0	29.6	8.1	0.75	4.02	48.71	227.5	9.3	226.9	8.7
8	12.6	-3.6	12.7	29.2	8.1	0.94	2.89	--	--	--	--	8.1
9	15.8	-2.9	5.6	29.2	8.1	1.28	4.16	--	--	--	--	7.8
10	9.7	-0.5	5.4 ⁺	29.2	8.2	0.84	8.25	--	461.4	22.1	382.2	8.7
12	-3.4	-0.6	-0.1	29.8	8.2	0.28	6.55	--	--	--	--	9.6
13	-3.7	-0.2	1.3	29.6	8.2	0.87	5.62	--	--	--	--	10.3
By Depth (m):												
0	16.8*	-1.9	6.5	29.0	8.2	0.89	9.31	48.44	245.2	18.5	267.3	8.5
10	4.3	-3.3	1.3	29.4	8.2	0.96	5.28	--	--	--	--	--
25	6.4	-3.9	2.6	29.4	8.1	1.37	1.68	--	--	--	--	--
40	-6.8	0.2	2.2	29.7	8.2	0.61	2.34	--	--	--	--	--
50	5.4	-2.7	1.7	29.6	8.0	0.85	1.02	--	--	--	--	--
60	-9.8	0.1	0.2	29.8	8.2	0.44	1.65	--	--	--	--	--
75	2.3	1.8	-0.1 ⁺	29.8	8.0	0.62	0.64	--	--	--	--	--
100	4.9	3.6	2.0	29.8	8.0	0.72	0.67	--	--	--	--	--
150	3.2	3.8	5.7	30.0	8.0	0.84	0.42	--	--	--	--	--
200	-3.3	-0.2	6.1	30.0	8.0	1.30	0.53	--	--	--	--	--

* = Significantly (p < 0.05) greater than population mean response.

+ = Significantly (p < 0.05) lower than population mean response.

Table 12.4 . Unadjusted "Primary" (Kincaid Lab water) control mortalities and abnormalities for each oyster embryo test set for both receiving water and sewage bioassays conducted during 1982.

Date	Percent control mortality	Percent control abnormal
<u>Receiving water bioassays</u>		
*15 July	49.4	20.7
16	16.4	1.1
*19	31.3	8.8
*26	40.9	15.7
*28	33.8	13.9
* 2 Aug	40.2	23.6
* 9	47.8	11.9
11	14.7	3.4
16	14.0	2.6
30	> 80 (discarded)	--
7 Sept	> 80 (discarded)	--
9	0.0	7.4
*13	21.6	44.9
*21	34.8	27.2
22	0.0	6.0
27	19.2	5.7
* 6 Oct	45.4	16.2
27	12.0	5.3
<u>Sewage bioassays</u>		
*21 July	14.5	15.0
23	25.7	5.2
*30	23.5	27.3
4 Aug	23.8	6.0
* 6	30.6	20.5
*13	29.6	13.7
* 3 Sept	25.4	13.3
15	> 80 (discarded)	--
20	> 80 (discarded)	--
28	> 80 (discarded)	--

* = Control mortality and/or abnormality does not meet ASTM (1980) standards.

Table 12.5 . Unadjusted "primary" (Kincaid Lab water) control mortalities and abnormalities for each oyster embryo receiving water bioassay conducted during 1983.

Date	Percent Control Mortality	Percent Control Abnormal
3 Jan.	8.9	0.2
5 Jan.	11.4	1.7
*13 June	38.9	12.1
14 June	67.7 (discarded)	75.7
20 June	84.1 (discarded)	-
*27 June	43.6	12.2
28 June	53.9 (discarded)	50.6
5 July	4.2	1.0
11 July	0.0	0.3
18 July	3.3	0.4
25 July	14.9	0.6
26 July	3.1	1.5
8 Aug.	5.0	0.7
9 Aug.	5.0	0.7
*15 Aug.	16.9	10.7
*28 Sept.	44.6	1.0
*11 Oct.	35.9	5.4

* = control mortality and/or abnormality does not meet ASTM (1980) proposed standards.

Table 12.6. Results of a sand dollar sperm bioassay of seawater from a *Skeletonema costatum*/nutrient bioassay (6 July-14 July, 1983) utilizing water from Seahurst stations 4 and 10 enriched with either a NO₃ spike or 0.25 to 2.0% (vol/vol) dilutions of Renton unchlorinated secondary sewage. The sperm bioassay tested water from the end of an 8-day algal incubation period. Chlorophyll "a" values are the maximum values recorded during the algal assay period. Dashes mean not measured.

Sample	Chlorophyll "a" maxima (mg/m ³)	Sperm bioassay % eggs unfertilized
<u>Station 4</u>		
Control water	12.41	0.3
150 mg/l NO ₃	27.10	0.0
0.25% Sewage	24.86	0.3
0.5% Sewage	-	-
1.0% Sewage	27.20	3.0
2.0% Sewage	42.27	38.6
<u>Station 10</u>		
Control water	27.84	0.0
150 mg/l NO ₃	28.34	0.6
0.25% Sewage	25.47	10.7
0.5% Sewage	34.15	9.1
1.0% Sewage	40.69	6.0
2.0% Sewage	56.02	65.5
Stock IMR Culture Water		5.6
Water from culture started 19 June		-0.7
Water from culture started 28 June		0.0
West Point control		-0.4
Kincaid control		0.0

Table 12.7 Results of sand dollar sperm and oyster embryo bioassays of seawater from a *Skeletonema costatum*/nutrient bioassay (19-25 July, 1983) utilizing water from Seahurst stations 4 and 10 enriched with either a NO₃ spike or 0.25 to 2.0% (vol/vol) dilutions of Renton unchlorinated secondary sewage. The sperm and embryo bioassays tested water from the end of a 6-day algal incubation period. Chlorophyll "a" values are the maximum values recorded during the algal assay period. Dashes mean not measured.

Sample	Chlorophyll "a" maxima (mg/m ³)	Sperm Bioassay	Oyster Bioassay*	
		Average adjusted % eggs unfertilized	Adjusted % mortality	Adjusted % abnormal
<u>Station 4</u>				
Control water	17.48	0.0	54.5	68.1
150 mg/1 NO ₃	18.08	0.0	55.3	100.0
0.25% sewage	20.65	0.4	51.3	100.0
0.5% sewage	18.60	0.0	28.7	79.7
1.0% sewage	25.13	0.0	24.7	92.5
2.0% sewage	26.70	0.0	100.0	-
<u>Station 10</u>				
Control water	12.38	0.7	28.3	0.1
150 mg/1 NO ₃	12.48	-0.3	NT	NT
0.25% sewage	14.20	0.7	29.1	86.9
0.5% sewage	16.33	0.4	31.9	92.3
1.0% sewage	12.80	0.0	12.6	15.7
2.0% sewage	17.80	0.0	71.8	98.6
West Pt. Control		0.0	0.0	0.0
Kincaid Control		0.0	-	-

*Control oyster mortality = 0.0%

the sperm bioassay showed essentially control level of fertilization in culture water from all algal assay test flasks. The chlorophyll "a" maxima values, however, were all substantially less than the 42-56 mg/m³ associated with the reduced fertilization success observed in 2% sewage from the first assay. Results of the oyster embryo assay showed several trends:

1. Elevated embryo mortality in both station 4 and 10 control water with approximately twice the response occurring in station 4 water as compared to station 10.
2. Highest embryo mortality was observed in the 2% sewage-spiked cultures. These samples also registered the highest chlorophyll "a" maxima values for each respective station.
3. Control water from station 10 produced no abnormal embryos while station 4 control water caused a 68% abnormal response. A graph of embryo abnormality vs. chlorophyll "a" maxima values yields a plot which suggests a threshold value of approximately 13 mg/m³ chlorophyll "a"; values above this threshold are associated with a high level of oyster embryo abnormality (Figure 12.10).

The third algal bioassay using the dinoflagellate Gymnodinium simplex was tested for metabolite toxicity using only the sperm assay (Table 12.8). Only two samples showed significant toxicity: the station 10 water with 2% sewage sample caused the highest reduction in fertilization success and is again correlated with the highest chlorophyll "a" maxima value. The NO₃-spiked water from station 4 was the other high response and occurred at a chlorophyll "a" value less than those which produced essentially control fertilization. Hence, no reason is evident for the NO₃-spiked sample response.

12.5.3 Renton Sewage Bioassays

The fifty-percent effective concentrations (EC50's) were determined for

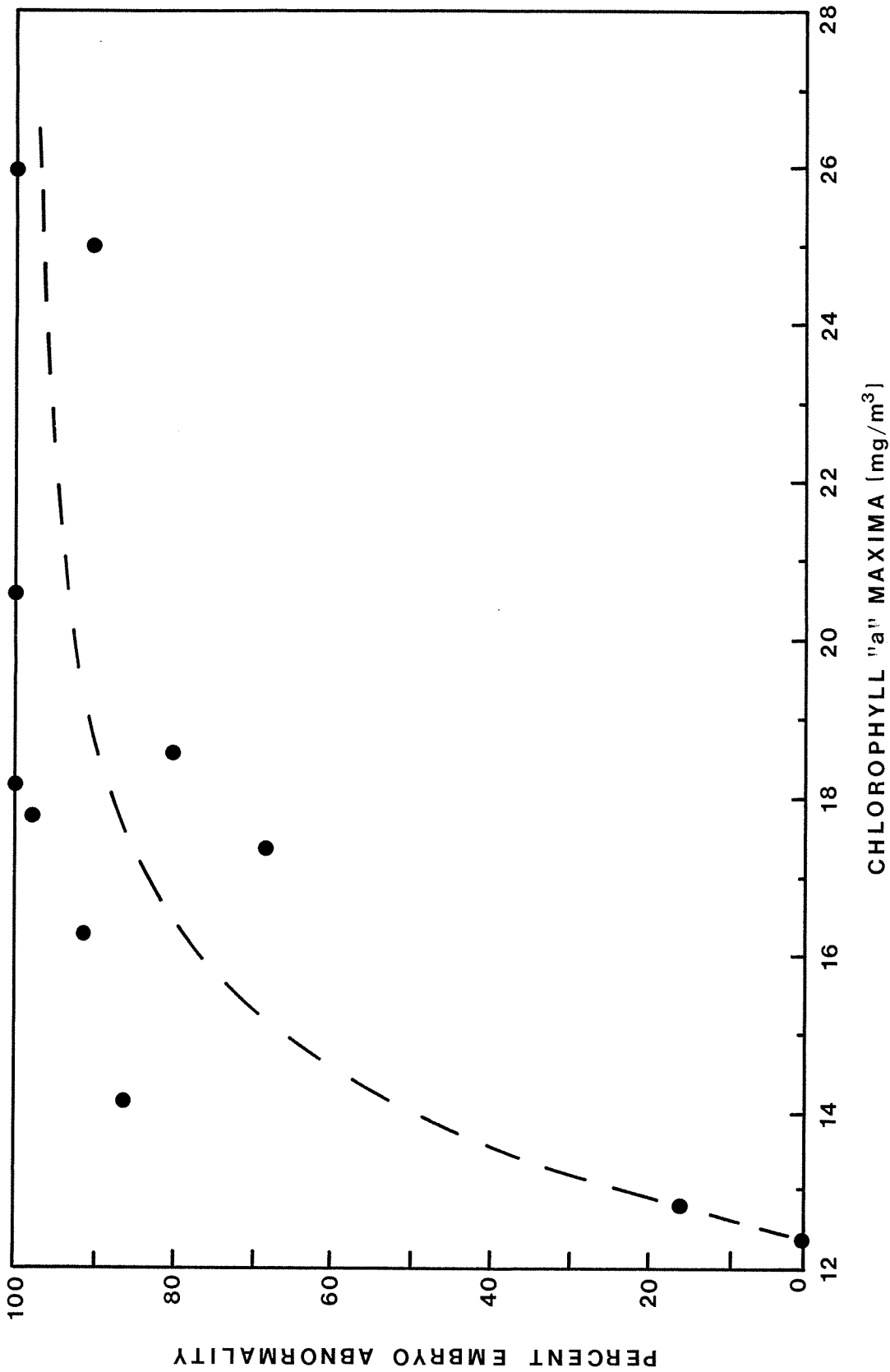


Figure 12.10. Percent oyster embryo abnormality following 48-hr exposures to seawater from diatom (*Skeletonema costatum*) growth bioassays in sewage enriched seawater. Chlorophyll "a" maxims were measured from the algal assay prior to the oyster embryo assay. The regression line is eye-fitted. The control and no₃-spiked sample responses are included in this plot.

Table 12.8. Results of a sand dollar sperm bioassay of seawater from a *Gymnodinium simplex*/nutrient bioassay (1-10 Aug., 1983) utilizing water from Seahurst stations 4 and 10 enriched with either a NO₃ spike or 0.25 to 2.0% (vol/vol) dilutions of Renton unchlorinated secondary sewage. The sperm bioassay tested water from the end of a 9-day algal incubation period. Chlorophyll "a" values are the maximum values recorded during the algal assay period.

Sample	Chlorophyll "a" maxima (mg/m ³)	Sperm bioassay % eggs unfertilized
<u>Station 4</u>		
Control water	32.09	2.0
150 mg/l NO ₃	39.05	74.6
0.25% Sewage	24.90	0.4
0.5% Sewage	22.67	4.0
1.0% Sewage	38.76	1.0
2.0% Sewage	55.31	0.7
<u>Station 10</u>		
Control water	24.80	0.4
150 mg/l NO ₃	37.84	-0.3
0.25% Sewage	22.72	1.0
0.5% Sewage	36.57	2.7
1.0% Sewage	43.57	1.7
2.0% Sewage	63.66	84.7
West Pt. Control		3.0
Kincaid Control		0.0

each life stage for all four sets of sewage bioassays (Table 12.9). Toxicity for the various stages of Renton sewage was found to be: chlorinated secondary > influent > primary > dechlorinated secondary > unchlorinated secondary > freshwater. Primary sewage was slightly more toxic than the influent during the summer 1983 sperm tests, however.

Test organism sensitivity was found to be: 60-min sand dollar sperm assay > 48-hr oyster embryo abnormal index > 48-hr oyster embryo mortality index for summer tests and 60-min sea urchin sperm assay > 96-hr sea urchin embryo abnormal index > sea urchin embryo mortality index > crab zoea mortality for winter tests. Direct organism sensitivity comparisons for all stages was not possible as different samples of sewage were used for summer and winter tests. The 95% fiducial limits for each EC50 are recorded in Appendix Table 12.4.

Sixty-min sperm bioassays are as sensitive (or more sensitive) indicators of sewage-related acute toxicity in seawater than the other life stages tested for greater exposure times. Toxicity declined during the winter 1983 tests from the summer 1982 tests assuming that sand dollar and sea urchin sperm are equally sensitive to sewage. Renton sewage was less toxic during the summer of 1983 than during the summer of 1982 based on the sand dollar sperm assays. Based on sea urchin sperm bioassays, sewage toxicity was similar for both winter periods. Influent and primary sewage are equally toxic. Toxicity is reduced following secondary treatment. A high level of toxicity is generated by chlorination, but is largely mitigated by the dechlorination process.

Average pH, ammonia, and residual chlorine values of Renton sewage samples for each bioassay set are recorded in Table 12.10 and detailed for each individual bioassay in Appendix Tables 12.5 through 12.8. Likewise, average salinity, pH, and temperature for each bioassay control water are recorded in Table 12.11 and detailed by season in Appendix Tables 12.9 through

Table 12.9. Comparison of 50 percent effective concentrations (EC50's) for all bioassays of stages of Renton sewage (and equivalent freshwater) dilutions in seawater conducted during four different periods of time. The EC50's are based on the average results of 7 to 10 separate bioassays of each sewage stage with each test stage.

Renton Sewage Stage	Summer 1982		Winter 1983			Summer 1983	Winter 1984
	Sand Dollar Sperm Assay	Oyster Embryo Abnormal Mortality	Crab zoea Mortality	Sperm Assay	Green Sea Urchin Embryo Abnormal Mortality	Sand Dollar Sperm Assay	Green Urchin Sperm Assay
Influent	1.88	4.21	5.68	NT	NT	12.95	10.54
Primary	2.90	5.90	7.31	>20	10.54	15.85	10.71
Secondary	17.62	>20	>20	>20	>20	18.08	>20
Chlorinated	1.02	2.26	3.24	>20*	0.43	8.67	2.26
Dechlorinated	7.13 †	>20	>20	>20	>20	17.40	>20
Freshwater	>20	>20	>20	>20	>20	>20	NT

NT = not tested

*Many moribund zoea noted in 20.0 and 13.0% chlorinated sewage.

†Effluent was only partially dechlorinated during this test period with an average measured residual oxidant value of 0.06 mg/liter versus <0.01 mg/liter for the other test periods when a new dechlorination system was on-line (see appendix tables 12.5 through 12.8 for measured chlorine values).

Table 12.10 Average pH, ammonia and total residual chlorine measured in ten bioassay sewage samples (undiluted) during the test periods indicated.

Parameter	Sewage Sample Period			
	Summer 1982	Winter 1983	Summer 1983	Winter 1984
<u>pH</u>				
Influent	6.84	NT ¹	6.82	6.84
Primary	6.99	7.01	6.79	6.88
Secondary	7.22	7.04	6.90	6.89
Chlorinated	7.10	7.04	6.88	6.80
Dechlorinated	7.17	7.10	6.94	6.84
<u>Ammonia (mg/l)</u>				
Influent	20.0	NT	23.2	15.9
Primary	18.4	12.5	21.8	12.4
Secondary	11.6	10.7	20.5	10.4
Chlorinated	12.1	10.6	19.8	10.3
Dechlorinated	11.4	11.2	19.3	10.2
<u>Residual Chlorine (mg/l)</u>				
Chlorinated	1.655	2.001	NM ²	0.940
Dechlorinated	0.059	0.000	NM	<0.010

¹NT = not tested

²NM = not measured due to broken precision buret.

Table 12.11 Average salinity, pH, and temperature of two sources of seawater used as controls for sewage bioassays during the seasons indicated. Each value is the average of ten measurements.

Parameter	Sewage Bioassay Period			
	Summer 1982	Winter 1983	Summer 1983	Winter 1984
<u>West Pt. Control Water</u>				
Salinity (°/oo)	29.49	28.83	29.57	28.88
pH	7.62	7.96	8.08	7.91
Temperature (°C)	12.6	8.9	13.4	9.08
<u>Kincaid Control Water</u>				
Salinity (°/oo)	29.71	29.71	29.59	29.59
pH	7.84	8.01	8.21	7.89

12.12.

12.5.4 Ammonia Bioassays

The bioassays of ammonia in seawater produced the following EC50's as total ammonia: oyster embryo abnormality index = 11.7 (10.2-13.4) mg/liter, oyster embryo mortality index 21.6 (18.5-25.4) mg/liter, and the sand dollar sperm assay index = 35.0 (30.2-40.5) mg/liter.

12.5.5 Baseline Sediment Bioassays

12.5.5.1 Toxicity by Geographic Area

Amphipod mean survival patterns for fall 1982 through spring 1984 are shown in Figure 12.11. In general, amphipod survival in the Seahurst baseline sediments was high compared to the Duwamish South Harbor (Duw SH) toxic control. Consistently reduced survival occurred north of Seahurst along Transects A, B, C and at site OT-1E, and some reduced survival occurred along Transect H. There was no apparent temporal differences in mean survival; variability tends to increase with mean sediment toxicity year round.

Using the bioassay replicate design of N = 4, significant differences between control and treatment survival can only be detected if the means differ by approximately 15%. The average West Beach control survival was 94.6 ± 8.3% (N = 28). No difference in amphipod survival in Seahurst sediments could be statistically detected for means ranging between 80-100%. Comparisons of station means versus the native sand control for each bioassay within a season are detailed in Appendix Table 12.14.

Most survival means in Seahurst sediments were above 80% survival.

Sediments that resulted in mean survival below 80% included:

A400E	B200E	OT-1E	*Duw SH
*A600E	C400E	H400E	
B75W	C640E	J400E	

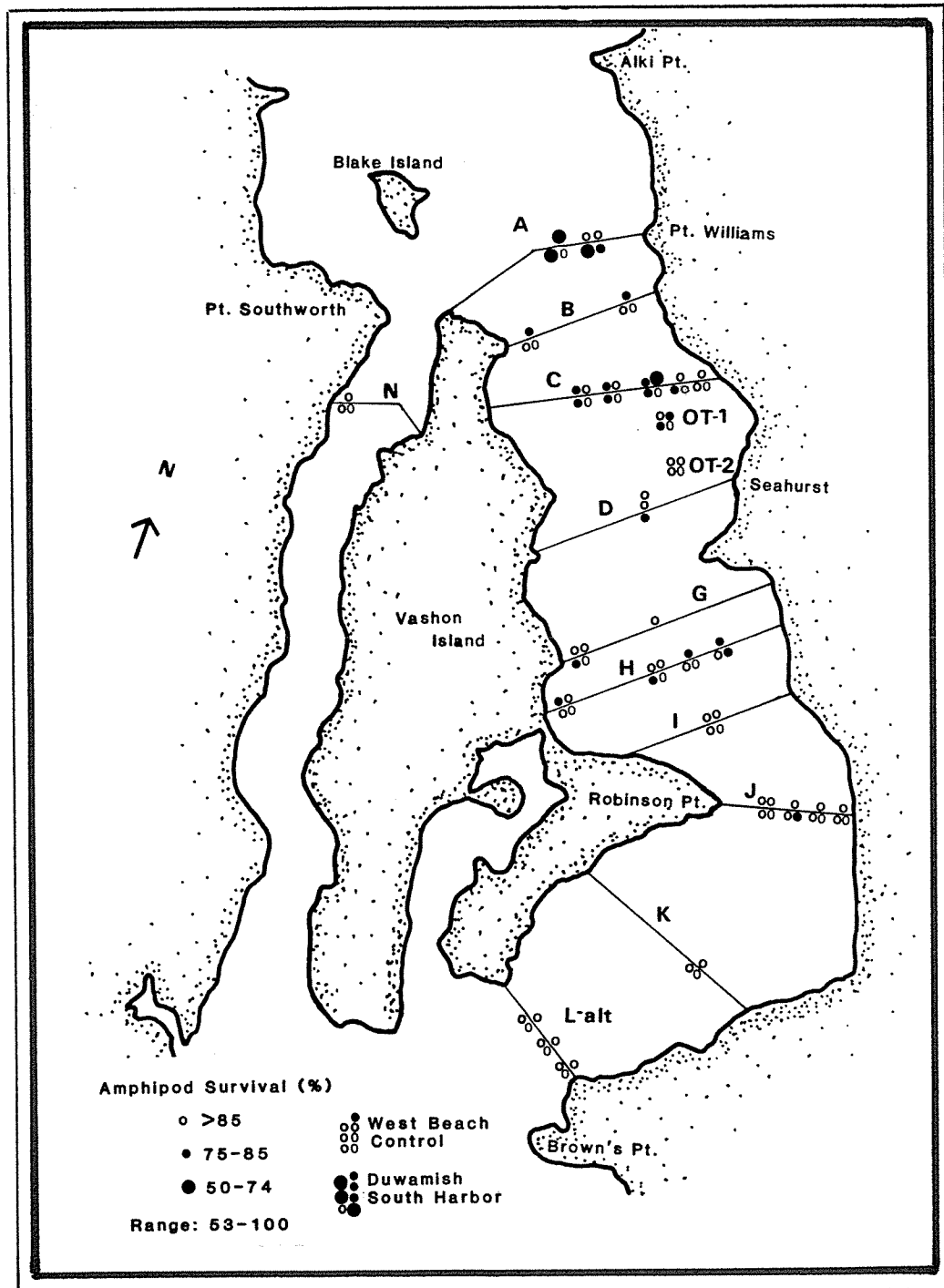


Fig. 12.11. Amphipod mean survival in Seahurst Baseline sediments for fall 1982, spring 1983, fall 1983, and spring 1984 (flow-through) bioassays. Dot clusters represent stations with fall 1982 in upper left and proceeding clockwise. Control dot clusters are stacked with run 1 depicted above run 2.

where * indicates that at least 2 means were less than 80%.

The sublethal reburial test for Seahurst sediments indicated that most survivors were able to rebury themselves following a 10-day bioassay. Sediments which resulted in greater than 4% of the survivors unable to rebury included:

*A400E H600E Duw SH
*A600E I690

where * indicates at least 2 means were above 4%. Sediments which caused consistent lethal and sublethal toxicity were: A400E, A600E, and Duw SH.

12.5.5.2 Toxicity by Season

Average survival was significantly higher in spring 1984 than for any other season ($F_{26,333} = 2.466$).

<u>Season</u>	<u>N</u>	<u>System</u>	<u>Mean + Std. dev.</u>
Fall 1982	48	Recirculating	17.52 <u>+2.06</u>
Spring 1983	104	Flow-through	17.05 <u>+ 2.14</u>
Fall 1983	104	Flow-through	17.56 <u>+ 2.18</u>
Spring 1984	104	Flow-through	18.38 <u>+ 1.67</u>

Mean survival in the other three seasons were not statistically different although spring 1983 had the lowest survival. Control survival in native sand was significantly lower in run 1, spring 1983, relative to the mean from other runs. Duw SH toxic control survival was not significantly different from the clean controls in spring 1983.

Comparisons of mean survival within each station between seasons showed that no differences were detected for 13 of the 26 Seahurst stations and the controls (Table 12.12). The trend toward higher survival in spring 1984 was evident in 10 of the remaining 13 stations. Within station variability was not associated with grain size or mean sediment toxicity.

Table 12.12. Statistical comparison of amphipod survival over time (fall 1982 through spring 1984) in Seahurst baseline sediments using a flow-through system. NS indicates non-significant F value; *, significant, with a one-way ANOVA ($p = 0.05$). Differences in seasonal survival, as indicated by < symbol, are significant with Student-Newman-Keuls test; seasons separated by commas or / are not significantly different ($p = 0.05$). Seasons are arranged in ascending order of survival. Season code: 1 = fall 1982; 2 = spring 1983; 3 = fall 1983; 4 = spring 1984.

<u>Station</u>	<u>F value</u>		<u>Student-Newman-Keuls Test</u>	
			<u>ambiguous</u>	<u>distinct</u>
N50 W	0.353	NS		
A400 E	1.415	NS		
A600 E	8.599	*		2, 3 < 4
B75 W	11.955	*		2 < 3 < 4
B200 E	25.577	*		2, 4 < 3
C75 E	9.132	*		3, 2 < 4
C200 E	12.541	*		3, 2 < 4
C400 E	4.405	*	2,1,3/1,3,4	
C600 E	0.709	NS		
C640	2.912	NS		
OT-1 E	1.638	NS		
OT-2 E	5.395	*		1, 3, 2 < 4
G50 W	6.120	*	3,2/2,4,1	
H75 W	5.421	*	1,2/2,4,3	
H400 E	7.640	*		4, 2 < 3
H600 E	3.351	NS		
H640	0.458	NS		
I690	0.793	NS		
J50 E	7.210	*		2, 3, < 4
J75 E	28.276	*		2 < 4, 3
J400 E	37.563	*		4 < 3 < 2
J600 E	2.004	NS		
K400 E	0.752	NS		
L-alt 50E	0.447	NS		
L-alt 200E	0.411	NS		
L-alt 400E	0.733	NS		
<u>Controls</u>				
Bowman Bay	2.413	NS		
Duwamish	0.572	NS		

12.5.5.3 Toxicity by Method

In spring 1984 static bioassays, survival was significantly lower than Bowman Bay controls for the majority of sediments tested (Appendix Table 12.15). When flow-through and static results were compared with a two-way ANOVA, the interaction term was significant for both runs 1 and 2 indicating that survival within a sediment type depended on method. Greater variability in survival between flow-through and static systems occurred for sediments causing greater toxicity (below 80-85% survival) from the northern Seahurst transects (Figure 12.12).

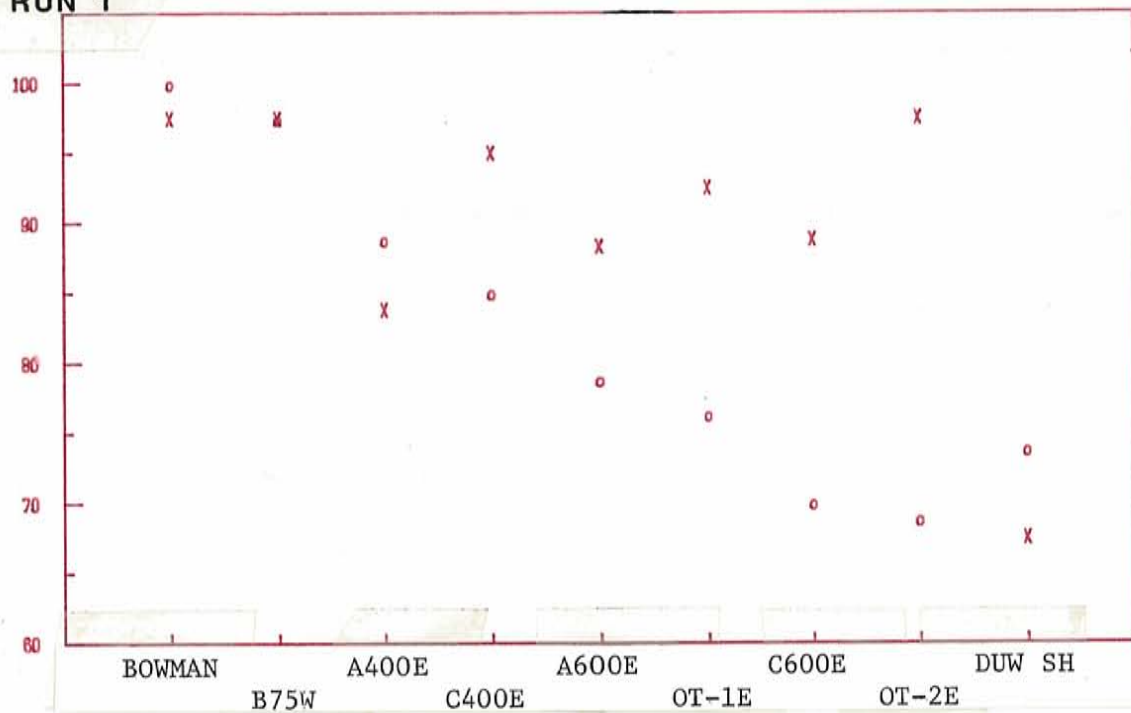
12.5.5.4 Toxicity by Sediment Quality

Fifty Seahurst sediments from fall 1982 and spring 1983 were clustered on grain size composition (Appendix Table 12.16), percent water, total volatile solids (TVS), carbon, and BOD measurements. Four stations (G50W, G780, H75W, J400E) were missing data from one season, so that 23 stations paired into fall and spring components. The resulting dendograms divide the stations into three major groups (I-III) each with two subgroups (Figure 12.13). Groups were ranked by decreasing grain size (mean phi) and basic statistics for associated stations presented in Table 12.13. Groups 1-4 are predominately sands (75, 94, 66, and 42%, respectively), while groups 5-6 are silt-clays.

Variability in sediment parameters between fall-spring station pairs increased with depth. All eight stations which were less than 400 feet deep (with the exception of N50W), clustered as pairs within groups 1-3. Three of the five 400 feet deep stations clustered separately and four of these (C640, OT-1E, OT-2E, and J600E) were divided between Groups II and III (sands and silt-clays, respectively).

Although mean survival between groups was significantly different, means could not be distinguished statistically in either the flow-through or static

RUN 1



RUN 2

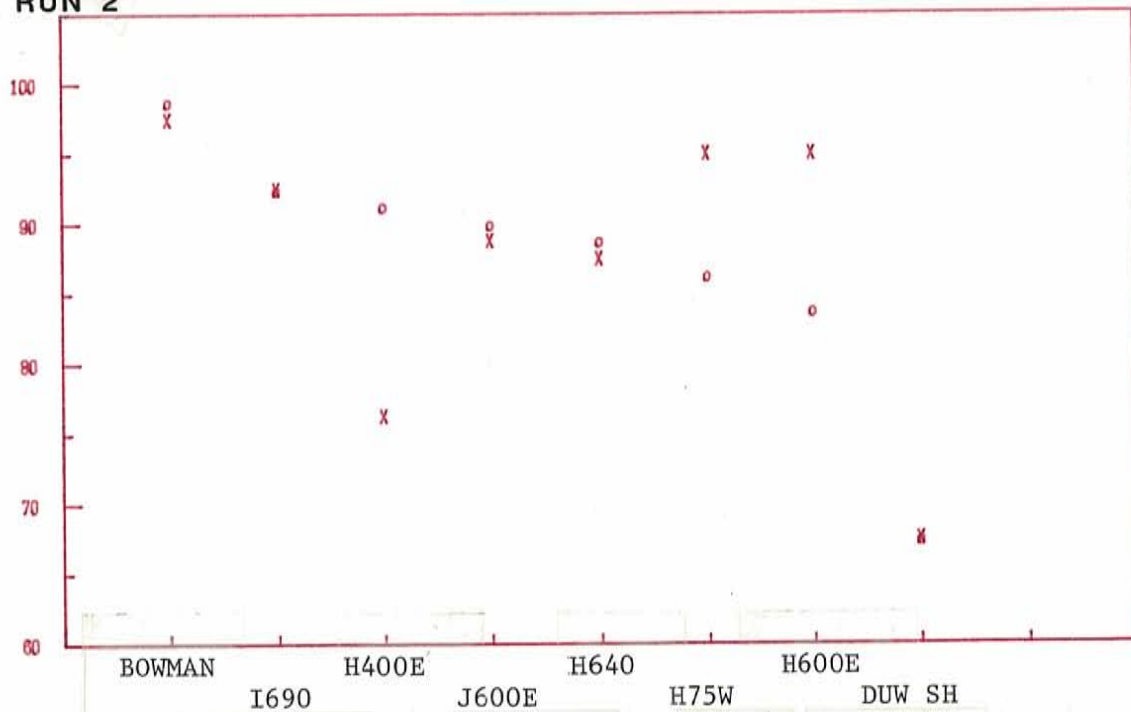


Figure 12.12. Amphipod mean survival in static (o) and flow-through (x) bioassays conducted in spring 1984 using Seahurst sediments. N = 4 with 20 animals per replicate.

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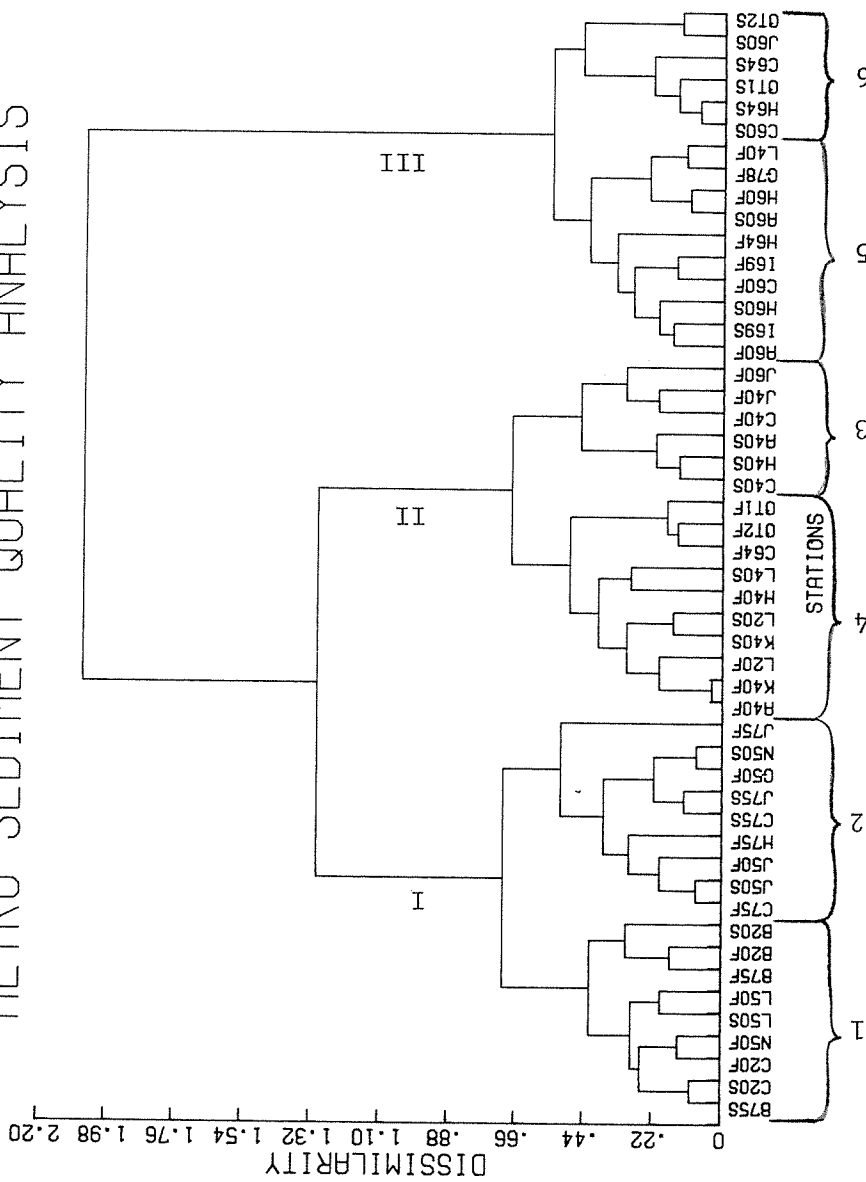


Figure 12.13, Dendrogram showing six major groupings of stations ranked by decreasing grain size. S = spring, F = fall.

Table 12.13. Sediment quality characteristics and ranking of six major groups of stations by decreasing grain size corresponding to the Canberra cluster analysis.

Group 1 N=9 N50F, B75F, B75S, B200F, B200S, C200F, C200S, L-alt50S, L-alt50F					Group 2 N=9 N50S, C75F, C75S, G50F, H75F, J50F, J50S, J75F, J75S				
	Mean	S.D.	Skew	Kurt		Mean	S.D.	Skew	Kurt
Gravel	15.9	14.2	0.3	-1.5	Gravel	0.1	0.1	0.2	-1.8
Sand	49.7	11.1	-1.3	1.8	Sand	47.0	17.2	-1.0	2.4
Fine sand	25.5	11.3	0.2	0.4	Fine sand	47.4	21.2	-0.6	2.8
Silt	3.0	1.9	0.6	-1.1	Silt	1.8	2.8	2.9	8.3
Clay	5.8	2.3	1.6	3.4	Clay	3.7	4.1	2.8	8.1
Mean phi	1.6	0.6	-0.7	0.7	Mean phi	2.4	0.4	1.5	0.7
% Water	25.8	4.1	2.3	6.2	% Water	25.0	1.0	-1.0	0.8
Carbon	3.9	4.4	2.9	8.5	Carbon	1.0	0.3	1.6	0.7
TVS	1.5	0.5	0.4	-1.8	TVS	0.4	0.4	0.5	-1.3
BOD	282.3	93.9	-0.2	-2.0	BOD	3023.4	4037.4	1.0	-1.2

Group 3 N=6 A400S, C400F, C400S, H400S, J400F, J600F					Group 4 N=10 A400F, C640F, OT-1F, OT-2F, H400F, K400F, K400S, L200F, L200S, L400S				
	Mean	S.D.	Skew	Kurt		Mean	S.D.	Skew	Kurt
Gravel	5.7	5.8	0.4	-2.5	Gravel	0.9	1.0	0.8	-0.7
Sand	28.8	10.5	0.2	-1.0	Sand	22.3	7.5	-0.7	0.7
Fine sand	37.1	10.3	-0.0	-2.0	Fine sand	19.8	4.9	0.2	-2.1
Silt	9.7	3.2	-1.2	0.6	Silt	25.6	9.1	0.3	-0.8
Clay	18.8	8.2	1.2	1.3	Clay	31.4	4.4	0.7	-0.2
Mean phi	3.8	0.8	1.5	3.0	Mean phi	5.5	0.6	0.2	0.0
% Water	45.0	14.7	0.7	2.0	% Water	58.0	11.2	0.2	-1.9
Carbon	10.5	3.1	-0.1	0.3	Carbon	12.5	4.9	0.7	-0.7
TVS	4.5	3.1	1.6	2.5	TVS	5.8	2.7	0.5	-1.7
BOD	831.5	550.2	1.6	3.2	BOD	1239.3	668.5	1.2	1.1

Group 5 N=10 A600F, A600S, C600F, G780F, H400F, H600F, H640F, I690F, I690S, L400F					Group 6 N=6 C600S, C640S, OT-1S, OT-2S, H640S, J600S				
	Mean	S.D.	Skew	Kurt		Mean	S.D.	Skew	Kurt
Gravel	0	0	0	0	Gravel	0.1	0.2	1.0	-1.8
Sand	5.5	4.6	1.3	2.1	Sand	1.3	1.2	1.5	2.4
Fine sand	7.8	4.9	1.2	0.2	Fine sand	4.6	0.9	-0.7	0.5
Silt	36.4	4.7	-0.6	0.4	Silt	38.5	4.4	0.5	0.0
Clay	50.7	6.3	-0.5	-1.0	Clay	55.4	3.5	-0.7	-1.0
Mean phi	7.7	0.6	-1.2	0.6	Mean phi	8.2	0.2	-0.1	1.1
% Water	67.5	5.3	-1.3	1.0	% Water	69.1	1.5	-0.4	0.9
Carbon	19.8	5.9	-1.2	0.2	Carbon	22.8	4.0	0.4	-1.5
TVS	7.6	1.9	-0.0	-1.9	TVS	34.5	40.4	1.0	-1.9
BOD	1684.8	498.7	-0.8	-1.3	BOD	1972.3	595.7	-0.3	-0.9

system (Table 12.14). No trends were apparent within the data for the flow-through system. With a static system, survival was optimal in group 2 (highest percent sand, lowest percent TVS) and decreased with decreasing sand composition and increasing TVS.

A summary of significant Pearson correlation coefficients (r) between survival and selected sediment quality parameters shows that correlations with survival were strongest with a static bioassay system and for water, TVS, and mean phi (highest column and row sums, respectively; Table 12.15). Correlations were lowest with sulfides and nitrogen, but this may reflect inaccurate measurements of these parameters (J. Word, Fisheries, University of Washington, Seattle, WA, personal communication). In general, linear correlations were poor ($r^2 < 0.05$), although statistically significant, and may indicate that non-linear relationships may be more appropriate.

Values for ITI were generally highest at the OT stations and decreased to the north while remaining fairly constant to the south (J. Word, personal communication). The lower-than-normal ITI values in the northern stations may reflect increased deposition, TVS, and carbon in these sediments (Word et al., Vol. V, Section 6).

12.5.5.5 Toxicity by Heavy Metals

Twenty-five Seahurst sediments from the fall 1982 collection were clustered on 15 heavy metals: Ag, Bs, B, Ba, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, V, Zn (Appendix Table 12.17). (Three stations were missing: H75W, J400E, Lalt 400E.) The resulting dendograms divide the stations into four groups (Figure 12.14). Groups were ranked by increasing metal content and basic statistics for associated stations presented in Table 12.16.

Based on the metal data, the stations also clustered by depth, implying that the metals aggregated by depth. A principle component analysis of the

Table 12.14. Rearrangement and comparison of the same mean amphipod survival data in Seahurst sediments grouped by Canberra cluster analysis using three different data sets. Means are based on N with 20 animals per replicate; FT = flow-through system, S = static system. ANOVA results are significant (*) or nonsignificant (NS) at $p = 0.05$; a,b,c = cell means grouped by Student-Neuman-Keuls test at $p = 0.05$.

Data set	Survival						F	
	1	2	3	4	5	6		
Sediment quality	FT	92.4 ^a (76)	89.9 ^{a,b} (52)	83.5 ^c (52)	88.6 ^{b,c} (68)	85.0 ^{b,c} (64)	88.9 ^{b,c} (48)	5.987* (359)
	S	83.3 ^{a,b,c} (4)	97.5 ^a (4)	88.3 ^{a,b} (12)	--	86.0 ^{b,c} (16)	76.3 ^c (16)	5.297* (52)
Organic factors	FT	91.0 ^a (64)	90.5 ^a (88)	88.4 ^b (104)	89.3 ^{a,b} (64)			5.004* (320)
	S	87.5 (12)	79.0 (8)	87.0 (20)	76.3 (4)			2.006 NS (44)
Heavy metals	FT	93.2 ^a (52)	89.9 ^b (60)	85.4 ^c (72)	87.0 ^{b,c} (148)			9.314* (332)
	S	--	97.5 ^a (4)	83.3 ^b (12)	83.0 ^b (32)			5.405* (48)

Table 12.15. Summary of significant ($p = 0.10$) Pearson correlation coefficients (r) for amphipod survival by season and method with selected sediment quality variables. R = recirculating, FT = flow-through, and S = static system. Column and row sums the absolute values of r .

Season (Method)	N	Percent Water	Carbon	TVS	BOD	Mean phi	Row Sum.
Fall 1982 (R)	48	0.338	-0.275	-0.262	-0.233	-0.209	1.317
Spring 1983 (FT)	104	--	--	--	--	--	0
Fall 1983 (FT)	104	0.373	-0.143	-0.338	-0.359	-0.340	1.553
Spring 1984 (FT)	104	0.225	-0.218	--	-0.155	-0.235	0.833
Spring 1984 (S)	48	0.478	-0.496	-0.528	-0.386	-0.477	2.365
Column Sum		1.414	1.132	1.128	1.133	1.261	

FLEXIBLE (BETA = -0.25)
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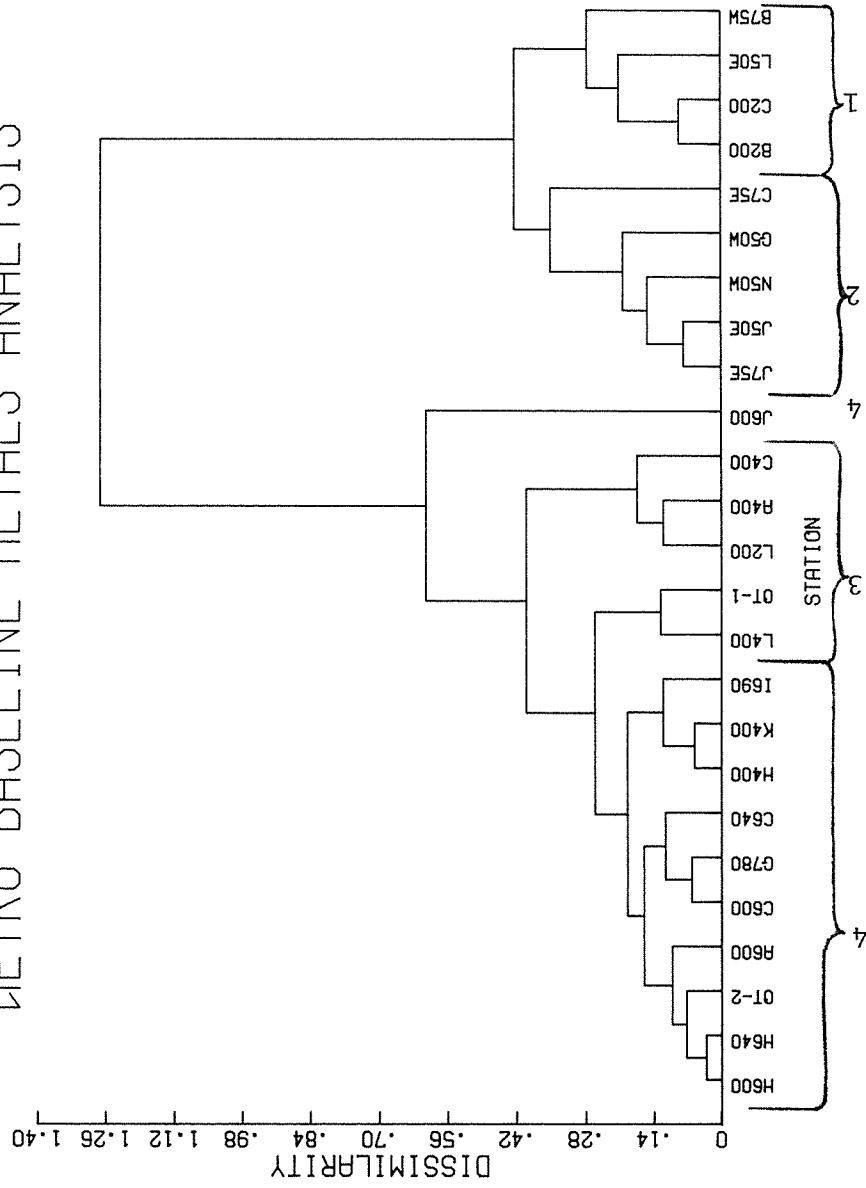


Figure 12.14. Dendrogram showing four major groupings of stations from lowest to highest level of heavy metals.

12.17

Table 12.16. Heavy metal characteristics and ranking of four major groups of stations by increasing concentration based on the Canberra cluster analysis.

Group 1 N=4 B75W, B200E, C200E, L-alt 50E					Group 2 N=5 N50W, C75E G50W, J50E, J75E				
	Mean	S.D.	Skew	Kurt		Mean	S.D.	Skew	Kurt
Ag	9.3	1.6	-0.7	1.5	Ag	12.5	8.1	2.0	4.2
As	4.3	1.4	0.1	1.5	As	6.6	3.0	-1.4	2.6
B	7.0	2.8	-0.2	1.5	B	10.6	6.0	-1.1	1.8
Ba	17.8	4.5	1.6	2.9	Ba	25.4	5.4	0.4	0.6
Be	2.2	0.2	-0.7	1.3	Be	2.6	0.4	-1.5	2.2
Cd	6.7	1.7	1.1	0.9	Cd	18.3	8.1	-0.8	-1.8
Co	6.7	0.7	0.1	0.8	Co	8.9	1.6	-1.1	-0.0
Cr	30.4	4.2	-0.0	-5.7	Cr	26.1	4.1	-0.9	-1.5
Cu	5.5	2.9	1.3	1.1	Cu	11.4	6.1	-0.6	1.3
Hg	1.4	0.1	1.5	1.8	Hg	2.9	2.5	1.2	2.8
Mn	20.0	4.9	0.8	1.3	Mn	69.4	84.0	2.0	4.3
Ni	17.5	9.4	0.3	-3.7	Ni	21.5	5.7	0.4	-1.8
Pb	5.1	1.9	-1.0	-0.2	Pb	6.5	3.3	-1.3	1.8
V	26.2	2.1	-0.2	0.6	V	29.0	2.3	-1.8	3.1
Zn	2.8	0.4	1.6	2.9	Zn	3.1	0.7	-1.6	2.7

Group 3 N=5 A400E, C400E, OT-1E L-alt 200E, L-alt 400E					Group 4 N=11 A600E, C600E, C640, OT-2E, G780, H400E, H600E, H640, I690, J600E, K400E				
	Mean	S.D.	Skew	Kurt		Mean	S.D.	Skew	Kurt
Ag	20.6	4.5	-1.0	2.1	Ag	25.1	5.1	1.0	0.4
As	9.2	4.3	1.7	2.1	As	23.6	6.4	0.0	-0.1
B	16.9	5.3	0.3	-2.6	B	21.5	9.5	-0.6	0.1
Ba	61.1	20.7	1.2	1.0	Ba	81.1	14.6	-0.7	0.1
Be	5.4	1.0	-0.0	1.3	Be	6.6	0.4	0.5	-0.2
Cd	28.9	8.3	-0.8	1.8	Cd	27.9	9.6	0.7	-0.2
Co	17.2	2.3	-0.2	-2.2	Co	20.5	2.7	2.3	6.5
Cr	51.4	11.9	1.5	2.6	Cr	74.8	11.5	1.4	2.2
Cu	49.6	17.3	-0.6	-1.9	Cu	57.1	12.5	-0.3	-1.1
Hg	23.5	9.9	-0.1	-0.9	Hg	26.4	8.7	0.0	-0.5
Mn	42.4	12.9	0.3	1.6	Mn	71.1	19.7	0.9	-0.2
Ni	34.6	7.2	0.6	0.2	Ni	42.5	5.3	0.9	0.9
Pb	26.2	14.6	1.8	3.1	Pb	39.1	5.6	-1.1	0.4
V	60.7	11.5	-0.8	1.4	V	72.8	6.9	-0.5	-0.7
Zn	7.7	2.2	1.3	1.8	Zn	11.1	1.2	0.1	-0.8

heavy metal data showed close association between all metals except Mn. In benthic environments, Mn behaves differently than other metals as the level of Mn is controlled by sediment pH and Eh. As sediment tends toward anoxic conditions (with increasing depths), Mn is solubilized with Fe^{+2} and Ca^{+2} while Cu, Pb, and Zn become increasingly enriched in sediments (Riley 1971). This relationship between Mn, other metals and depth was evident from the cluster analysis.

Mean survival between groups decreased with increasing metal concentration (Table 12.14). This trend was stronger with a static system. A summary of significant Pearson correlation coefficients (r) between survival and heavy metals shows strongest correlations, based on row and column sums, for a static system and for Cd, Cu, Hg, Pb, and V (Table 12.17). Mean concentrations (and range) for these metals are: Cd 225.9 ug/l (51 - 462); Cu, 38.2 mg/l (3.8 - 76.2); Hg, 167.3 ug/l (1 - 411); Pb, 23.9 mg/l (1.1 - 44.9); and V, 54.1 ug/l (23.6 - 80.8). All correlations were negative except for Mn with a static system.

12.5.5.6 Toxicity by Organic Chemicals

A principal components analysis based on dry weight sediment concentrations of 65 organic compounds from 29 Seahurst sediments (all 1982 collections) yielded 11 major factors (Table 12.18). Of the 27 stations, four stations (I690, H400E, H600E, and H640) had two sets of measurements (designated "A" and "B") and two stations were missing (A400E, A600E). The concentrations of each compound within each factor were summed for each station. These 27 stations were then clustered on the sums of the 11 organic factors. The resulting dendograms divide the stations into four groups (Figure 12.15). Groups were ranked by increasing organic concentration and basic statistics for associated stations presented in Table 12.19.

Table 12.17 Summary of significant ($p = 0.05$) Pearson correlation coefficients (r) for amphipod survival by season and method with heavy metals. R = recirculating, FT = flow-through, and S = static system. Column and row sums the absolute value of r .

	Season (method)					Row sum
	Fall 1982 (R)	Spring 1983 (FT)	Fall 1983 (FT)	Spring 1984 (FT)	Spring 1984 (S)	
N	44	92	92	92	48	
Ag		-0.215	-0.295	-0.300		0.810
As	-0.252			-0.255	-0.244	0.751
B			-0.200	-0.251	-0.310	0.761
Ba			-0.348	-0.255	-0.418	1.021
Be				-0.187		0.187
Cd	-0.329		-0.429	-0.201	-0.260	1.219
Co			-0.270	-0.401		0.671
Cr			-0.250	-0.295		0.545
Cu	-0.259		-0.327	-0.349	-0.478	1.413
Hg	-0.411		-0.346	-0.263	-0.725	1.745
Mn		-0.214			+0.511	0.725
Ni			-0.373	-0.319	-0.386	1.078
Pb	-0.287		-0.302	-0.241	-0.534	1.364
V	-0.260		-0.270	-0.323	-0.406	1.259
Zn			-0.296	-0.316	-0.378	0.990
Column sum	1.798	0.419	3.411	3.656	4.650	

Table 12.18. Organic chemical composition of the 11 principal components from the Seahurst sediment analysis. LPAH (or HPAH) = low (or high) molecular weight polycyclic aromatic hydrocarbons, PCB = polychlorinated biphenols, PHTH = phthalates, MISC = miscellaneous.

Factor	N	Class	Organic chemical composition
1	12	LPAH HPAH PHTH	Anthracene, phenanthrene Benzo(a)anthracene, fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, pyrene, benzo(a)pyrene Dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate
2	7	MISC	Hexachlorobutadiene, dieldrin, endosulfan sulfate, 1,1-dichloroethylene, 1,2-trans-dichloroethylene, trichloroethylene, toluene
3	8	LPAH HPAH PHTH MISC	Acenaphthene Dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, benzo(g,h,i)perylene Di-n-butyl phthalate, di-octyl phthalate Carbon tetrachloride, nicotine
4	8	PCB MISC	Aroclor 1242, aroclor 1248, aroclor 1254, aroclor 1260 4,4-DDE, phenol, n-nitrosodimethylamine, isophorene
5	3	LPAH MISC	Fluorene 1,1,2-trichloroethane, tetrachloroethylene
6	2	LPAH	Naphthalene, acenaphthylene
7	2	MISC	Methylene chloride, benzene
8	3	MISC	Chloroform, dichlorobromomethane, trichlorofluoromethane
9	3	MISC	Pentachlorophenol, chlorobenzene, bromoform
10	1	MISC	Ethylbenzene
11	1	MISC	1,4-dichlorobenzene

FLEXIBLE (BETA = -0.25)

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METRO ORGANIC FACTORS ANALYSIS

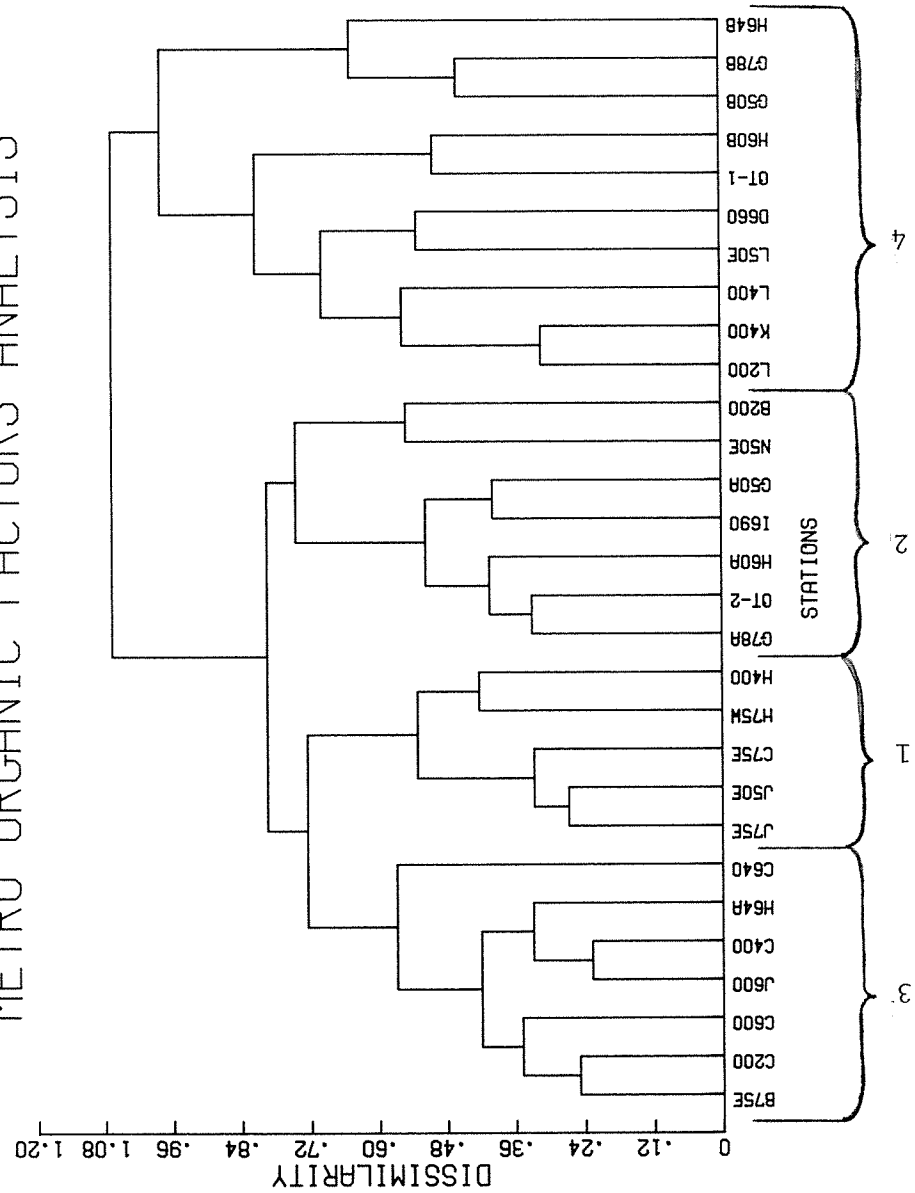


Figure 12.15. Dendrogram showing four major groupings of stations ranked from lowest to highest level of organic chemicals.

Table 12.19. Organic chemical factor characteristics and ranking of four major groups of stations by increasing concentration based on the Canberra cluster analysis.

Group 1 N=5 C75E, H75E, H400E, J50E, J75E					Group 2 N=7 N50W, B200E, OT-2E, G50W, G780, H600E, I690				
<u>Factor</u>	<u>Mean</u>	<u>S.D.</u>	<u>Skew</u>	<u>Kurt</u>	<u>Factor</u>	<u>Mean</u>	<u>S.D.</u>	<u>Skew</u>	<u>Kurt</u>
1	55.4	81.0	2.0	4.1	1	296.3	270.9	0.2	-2.1
2	0.1	0.0	1.9	3.8	2	3.4	5.3	2.3	5.6
3	37.2	26.8	-0.3	-2.6	3	111.1	103.0	0.5	-1.3
4	45.4	45.1	1.7	2.6	4	179.3	281.7	2.5	6.4
5	34.1	76.1	2.2	5.0	5	2.0	2.6	1.0	-1.0
6	0	0	0	0	6	0	0	0	0
7	0.2	0.2	0.6	-3.1	7	1.0	0.6	-0.1	-1.9
8	0.3	0.2	-0.3	-2.7	8	0.2	0.2	1.2	0.3
9	5.0	8.4	2.2	4.8	9	2.6	2.8	0.4	-2.1
10	0.0	0	0	0	10	0.0	0.0	1.6	4.3
11	0.1	0.2	2.2	5.0	11	7.3	9.4	1.5	1.0

Group 3 N=7 B75W, C200E, C400E, C600E, C640, H640, J600E					Group 4 N=6 OT-1E, D660, K400E, L-alt 50E, L-alt 200E, L-alt 400E				
<u>Factor</u>	<u>Mean</u>	<u>S.D.</u>	<u>Skew</u>	<u>Kurt</u>	<u>Factor</u>	<u>Mean</u>	<u>S.D.</u>	<u>Skew</u>	<u>Kurt</u>
1	315.6	164.9	0.7	-0.8	1	538.8	347.5	0.4	1.02
2	1.8	4.5	2.6	7.0	2	33.2	65.0	2.4	5.7
3	131.6	74.2	-0.1	-0.2	3	784.8	905.1	1.7	2.9
4	47.9	84.8	2.4	6.0	4	396.1	534.5	2.2	4.9
5	8.2	9.3	0.5	-2.1	5	3.4	2.4	-0.4	-2.1
6	0	0	0	0	6	117.4	367.3	2.4	5.9
7	0.6	0.2	0.2	-1.1	7	0.4	0.4	0.6	-1.6
8	0.3	0.3	1.3	0.1	8	0.3	0.2	0.3	-1.3
9	0.2	0.4	2.6	6.9	9	0.8	0.8	0.9	0.3
10	0.3	0.7	2.6	7.0	10	0.1	0.2	1.3	1.3
11	0	0	0	0	11	14.1	14.2	1.4	2.1

Group 1, with the lowest level of organics, was composed predominately of shallow stations. Other stations clustered roughly by geographic area: group 3 contained (mostly) northern stations, group 2 central stations, and group 4 southern stations, although each group contained some stations from other areas. The "B" replicates of G50W, G780, H600E, and H640 all clustered together in group 4 and had extremely high levels of several organics. Therefore, these stations were placed within groups based on "A" replicates.

Mean survival between groups generally decreased with increasing organic level of the flow-through system, but differences between means were slight and could not be statistically distinguished (Table 12.14). No differences between groups were detected for the static system, although the highest and lowest survival occurred in groups 1 and 4, respectively.

12.6 Discussion

12.6.1 Receiving Water Bioassays

12.6.1.1 Control Responses

One of the foremost concerns in establishing any receiving water quality testing program is the selection of a proper "control" water to serve as the "reference standard." In Washington State, the 48-hr oyster embryo assay has served as the receiving water quality standard test and the control water was drawn from Quilcene Bay through a seawater pump system at the State Shellfish Laboratory at Brinnon, Washington. This water proved unsatisfactory on occasion, possibly due to metabolites from phytoplankton blooms in Quilcene Bay. As such, the quality of the "standard" changed with the quality of the water pumped from the Bay, a less than ideal situation for long term comparative testing.

The toxicity studies reported here required that comparative bioassays be conducted to yield data which can be used to compare differences in toxicity both spatially and temporally. This is especially true for the interpretation

of data collected in future post-discharge monitoring programs. What, then, constitutes a satisfactory "control" water for long-term monitoring?

Artificial seawater, while convenient, was dropped from consideration because of the multitude of different formulas available, potential unreliability of supply in future years, the possibility of contamination, and because it simply was not "natural" seawater. West Point seawater has traditionally served as the control water (usually filtered in a variety of ways) for many past studies and was included as a "control" water in this study, but it could not be considered the "reference standard" since it is drawn from the same body of water (Puget Sound) being tested (also, it is very close to the station 1 sampling site).

Therefore, the following requirements for a "reference standard" control water were:

1. It should be "natural" seawater.
2. It should come from a high quality source outside Puget Sound (not close to significant urban/industrial development).
3. Salinity should be in the range of 30 ± 2 ‰ and pH 8.0 ± 0.5 .
4. The water should be collected in a manner that minimizes the presence of phytoplankton metabolites.

Ideally, the reference water should be collected on the day of testing, but requirements in #2 above plus the resulting variability between control samples effectively negates this possibility. Therefore, one-time collection and bulk storage seemed to be the only reasonable alternative.

Two sources of water approximated these requirements:

1. Seawater from bulk underground storage at Kincaid Hall (Zoology) on the U.W. campus. This water is pumped from the Anacortes ferry terminal at high tide, transported by truck in large plastic bags,

and routinely used for marine embryological work without reported difficulties.

2. Water collected from the University's Friday Harbor Laboratory during a high tide in the spring, quickly frozen in clean plastic bottles and kept frozen at 0°F or less until used.

Little information is available on the quality of oyster development and survival in seawater that has been previously frozen. For this reason the Kincaid Hall water was established as the "primary control" water for the receiving water bioassays and the other "control" waters tested with the receiving water samples as backup controls. The primary control responses were then set to zero and all other bioassay responses adjusted relative to this zero point.

Conduct of all sperm bioassays was accomplished without any deviation from the methods outlined by Dinnel et al. (1983). Likewise, oyster embryo bioassays were conducted and analyzed exactly as outlined by ASTM (1980) with only one modification. This modification involved the use of oyster embryo test results when the primary control mortality or abnormality exceeded the proposed ASTM standards.

The "standard" oyster embryo assay (ASTM 1980) relates mortality in the controls to the density of embryos in the beakers at 48 hours vs. the initial density as determined from counts made at the time of fertilized egg inoculation. If the control mortality exceeds 30 percent (or the control abnormality exceeds 10 percent) the test is considered invalid and must be repeated. Obviously, the invalidation of test results means lost data, much of which may be valuable in all but a strictly "legal" or regulatory context. If tests may be easily repeated then only time and money is lost. However, if the tests are temporally related (as in this study), then lost data cannot be

retrieved. During the two year period of bioassay work 45 separate oyster embryo assays were conducted on receiving waters and sewage samples. Of this number 18 percent of the oyster assays exceeded 50 percent primary control mortality and/or abnormality and were automatically discarded. An additional 42 percent had control mortality or abnormality in excess of the ASTM 30/10 percent standard but less than 50 percent mortality. The ASTM standards were established at these levels of control mortality/abnormality because these levels were shown not to compromise the evaluation of the test responses (Cardwell et al. 1977a). It was not, however, established that control responses >30/10 percent would compromise the test results since values >30/10 percent were not observed. Therefore, for the purposes of this study, test results were discarded only when either or both of the primary control responses were >50 percent.

The justification for this modification is not arbitrary. Historical Washington State oyster embryo assays have used control water collected from Quilcene Bay at the time of the assays introducing the possibility of high mortality due solely to the control water quality. In the tests reported here, this factor has been eliminated by the use of two "control" waters carefully selected to be of high and consistent quality through the test season.

If variability in control water mortality is water-related, then the relationship of mortality between the two types of control waters should be a random one. If, on the other hand, the between-test mortality is not water-related but caused by some other extraneous factor, there would exist a relationship between the two control waters used here. The regression of Kincaid Hall and Friday Harbor control water mortality is shown in Figure 12.16. The trend (as described by the regression line with a positive slope

OYSTER EMBRYO MORTALITY RESPONSES

KINCAID VS FRIDAY HARBOR CONTROLS

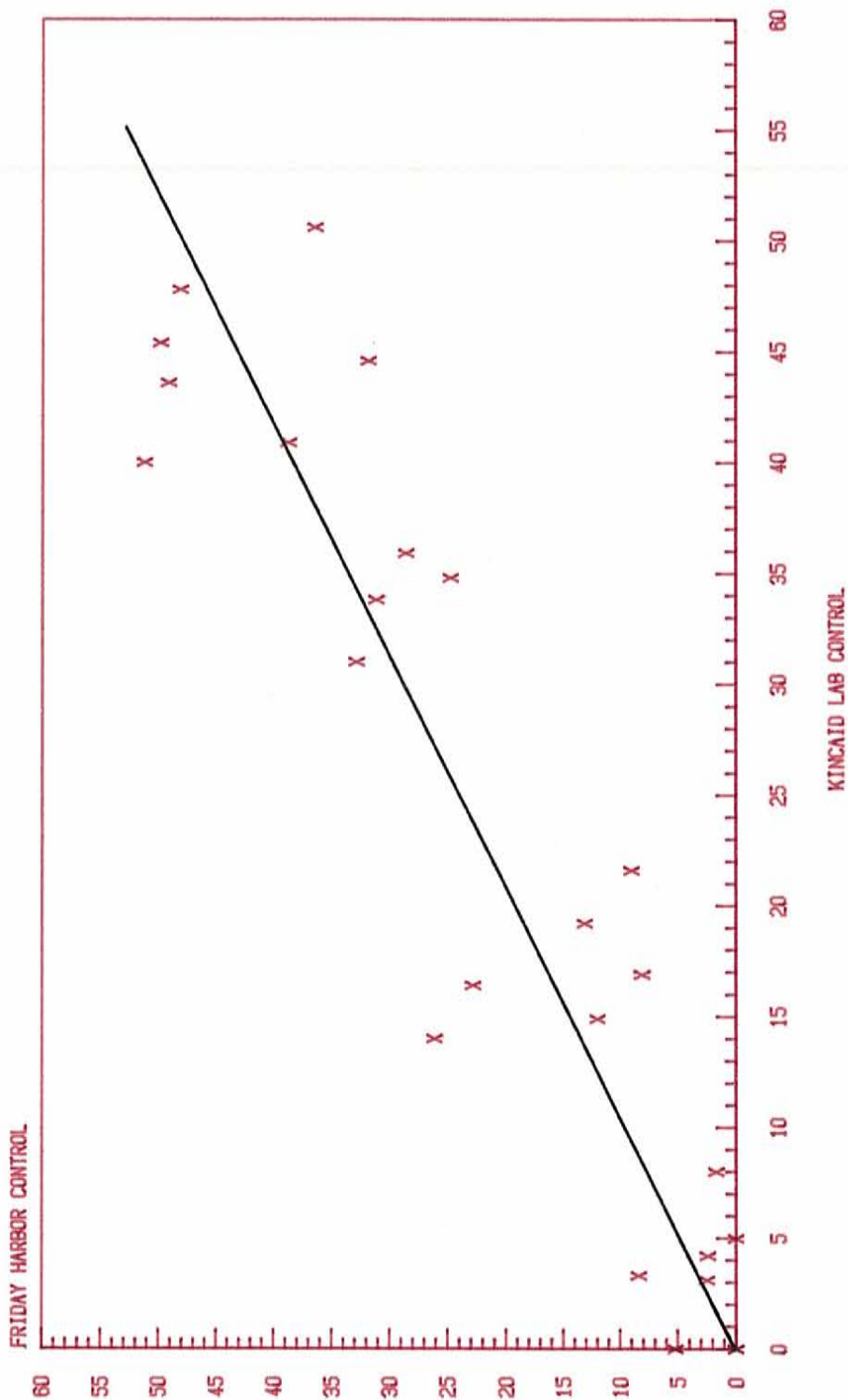


Figure 12.16. Linear regression of the relationship between the percent Kincaid control oyster embryo mortality and percent Friday Harbor (frozen) control oyster embryo mortality. The regression line slope is 0.925, y-intercept is 0.055 and the correlation coefficient is 0.9172 (significant at $p = 0.001$).

of 0.925 and a significant ($p = 0.001$) correlation coefficient of 0.9172) shows that between-test mortality in each type of control water is related, therefore, independent of water quality.

If the control water is eliminated as a source of variability causing fluctuating mortality between tests, then the most probable causes are: inconsistency in test methodology, or gamete quality between different groups of oysters.

Close attention to detail and methodology was given during the conduct of all bioassays. The highest probable source of variability related to the test methodology was the fertilization of the eggs. Over- or under-fertilization could produce a high mortality index in the controls, but this factor was closely controlled by adding only dilute sperm densities ($<10^7/\text{ml}$) to minimize polyspermy (multiple fertilization) and by checking fertilization success by monitoring polar body formation in each batch of fertilized eggs.

Gamete quality is the most probable source of between-test control mortality differences. Gametes within any given adult oyster will be at a peak of ripeness only during a portion of the time in which they contain gametes. If eggs from an underripe or overripe female are used, high embryo mortality will result with gradations of mortality being related to degree of over- or under-ripeness. Therefore, egg (and possibly sperm) quality will be the primary controlling factor of survival in any given test. Thus, if 50 percent of the eggs cannot develop due to being over- or underripe but the remaining half do develop, then the simple dismissal of the undeveloped eggs from consideration should not invalidate the responses of the remaining 50 percent of the eggs in the test samples. This concept is a departure from past views, but there is experimental support for it in the works of Lannan (1980a, b, c) and Cardwell et al. (1977b).

The relationship between the degree of oyster embryo control mortality and abnormality has been poorly defined in past work. The present study found that an increase in control mortality was significantly associated with an increase in the abnormal index but with a fair degree of variability. This is illustrated by the regression of control mortality vs. abnormality (Figure 12.17) for all oyster tests conducted during 1982 and 1983. The calculated linear regression equation is:

$$y = -2.091 + 0.599X; r = 0.659 \text{ (significant at } p = 0.001)$$

Average bioassay control responses for each control water for each year and combined years are summarized in Tables 12.1 through 12.3 above. For the 1982 tests there was no significant difference between any of the average control responses (at the 95% confidence level) with the exception of the average oyster mortality for the West Point water (11.7 percent higher than the average Kincaid primary control responses). These results suggest that the West Point water was impacted by the same factors affecting oyster embryo survival (possibly phytoplankton metabolites) in many of the receiving water surface samples throughout the Sound. The West Point control waters did not, however, produce an elevated average embryo mortality response during the 1983 test season (Table 12.2). This may be related to the amplitude of phytoplankton blooms between the two years. Using station 1 as a guide (the station nearest the West Point Lab seawater intake), Tables 12.1 and 12.2 show that the phytoplankton populations were much stronger during 1982 as compared with 1983, suggesting that phytoplankton metabolites may have adversely affected oyster embryo survival.

For the 1983 tests, the only control response that was significantly different from the Kincaid primary control responses was the average sperm assay response in the Friday Harbor water. While not significant, the average

OYSTER EMBRYO MORTALITY VS. ABNORMALITY

(FOR KINCAID LAB CONTROL WATER)

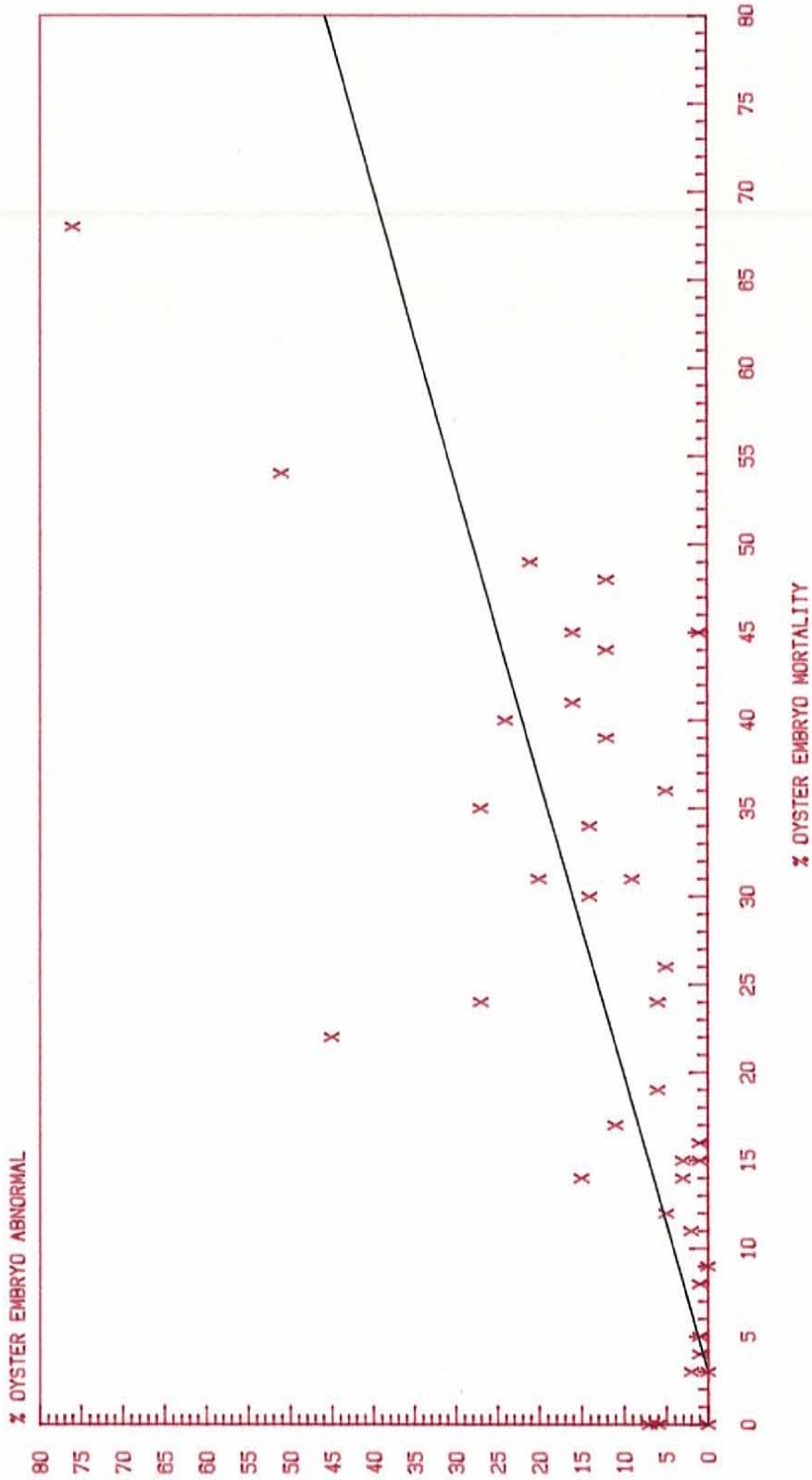


Figure 12.17 . Relationship between oyster embryo mortality and abnormality in Kincaid Lab control water. Linear regression line slope = 0.599, y-intercept = -2.091 and correlation coefficient = 0.659 (significant at $p = 0.001$).

oyster embryo responses for 1983 also point to the possibility of slightly compromised Friday Harbor water quality, a point partially substantiated by the fact that the 1983 batch of Friday Harbor control water had a salinity of only 26.0 ‰ rather than the anticipated 28 to 30 ‰. Whether or not these elevated Friday Harbor control responses were due to the reduced salinity or some other factor(s) is not known.

12.6.1.2 Seahurst Baseline Receiving Water Test Responses

The Seahurst receiving water bioassay work represents the most concentrated amount of effort expended to date to define background water column toxicity in a major basin of Puget Sound. The robustness of the test program is illustrated by the following sampling design parameters:

1. Use of multiple control waters as discussed above;
2. Use of two sensitive gamete-embryo stage marine bioassays to provide three indices of toxicity (i.e., oyster embryo mortality, oyster embryo abnormal development, and sand dollar sperm/egg fertilization impacts);
3. Bioassays of receiving water from 12 stations with multiple depths at each station;
4. Multiple within-year sampling at approximately weekly intervals during a major portion of the biologically active summer/fall period;
5. Repetition of the sampling program over a 2-year period to provide insight into between-year differences in toxicity patterns and;
6. Close coordination of the toxicity testing with the Water Column investigations to provide a wide array of supporting chemical/biological data for each bioassay sample.

The results (Figures 12.8 and 12.9) show that measurable background water

toxicity was found at all stations and depths sampled depending on sample date, location, year, and bioassay type. A high degree of variability in bioassay responses is evident for most stations. Computer assisted reduction and analysis of the sampling data has provided valuable insights into patterns of toxicity by station location, depth, and bioassay type.

In 1982, the oyster embryo mortality index was highest within the Seahurst Bight (stations 4-13) and significantly ($p = 0.05$) greater in surface water samples. Second, there is a trend towards greater oyster embryo abnormality in deeper waters and a greater number of unfertilized sand dollar eggs in both surface and deep waters with a significantly lower sand dollar sperm assay response for the 75-m samples (stations 3, 4, and 7) (Table 12.1 and Appendix Table 12.1).

Analyses of the 1983 bioassay response data showed the same trends and significance responses as the 1982 data except that the overall magnitude of the responses was lower (Table 12.2 and Appendix Table 12.2).

Not surprisingly, an analysis of all 1982 and 1983 data combined shows the same trends and significance responses as noted for 1982 and 1983 data analyzed separately, with the exceptions that the sand dollar eggs unfertilized average response for station 12 and the oyster embryo mortality index for 200 meters depth are significantly lower than their respective population means (Table 12.3 and Appendix Table 12.3).

The physical/chemical data summary presented in Tables 12.1 through 12.3 shows that there is very little to suggest that salinity, pH, total ammonia, or Secchi disk depths are associated with the biological response trends. Average salinity values do increase with depth but only within a range of 1 ‰. Likewise, there is a trend toward lower pH with depth, but again, only within narrow limits (0.2 pH units). Average chlorophyll "a", on the other

hand, shows trends (high at inshore stations and in surface waters) reminiscent of the pattern observed for oyster mortality.

To shed further light on possible relationships between the physical/chemical data and the biological responses, Pearson correlation coefficients (r) were calculated in a pairwise fashion for each of the variables presented in Table 12.3 using combined 1982 plus 1983 data. The strongest meaningful correlation coefficients (Table 12.20) between the various physical/chemical measurements are between pH and chlorophyll "a" (0.6124), salinity and depth (0.5760), pH and depth (-0.3758), Secchi disk depth and chlorophyll "a" (-0.3829), total ammonia and Secchi disk (0.3747), and depth vs. primary productivity (-0.7361). These correlations suggest that pH increases with chlorophyll "a" due to the respiratory/metabolic activity of the phytoplankton during a bloom, while Secchi disk depths decrease (more biomass in the water causing turbidity), and total ammonia decreases (nitrogen being consumed by the phytoplankton). This pattern is inversely related to depth since most phytoplankton must remain in the photic zone for growth. Of the phytoplankton groups measured, primary productivity was most correlated with diatoms (0.8236) which were most responsible for reduction of Secchi disk depths (-0.4687) in the surface waters.

Most of the biological responses are weaker than the physical/chemical associations. The strongest (significant at $p = 0.01$) positive correlations for the oyster embryo mortality index are with chlorophyll "a" (0.3222) and pH (0.1901) while significant negative correlations exist for depth (-0.2610) and salinity (-0.2564). This pattern of correlations reinforces earlier findings by Cardwell et al. (1977 and 1979) that phytoplankton blooms in surface waters adversely affect the survival of oyster embryos.

The oyster embryo abnormality index is significantly ($p = 0.01$)

Table 12.20. Pearson correlation coefficients (r) between the oyster embryo and sand dollar sperm assay results and selected physical/chemical properties from Seahurst water column stations 1-13 for 1982 and 1983 data combined. * = correlation significant at $p > 0.01$.

Parameter	Station	Depth	Oyster Embryo		Sand Dollar		Salinity	pH	Total Ammonia	Chlorophyll "a"	Primary Product.	Diatoms	Dinoflagellates	Other phyto-plankton	Secchi disk depth
			Mortal.	Abnormal	Eggs unfert.	Eggs									
Station	1.000														
Depth	-.3143*	1.000													
Oyster mortality	-.0213	-.2610*	1.000												
Oyster abnormal	-.0380	.0949	.1577*	1.000											
Sand dollar unfertilized	.0186	-.0530	.0225	.2024*	1.000										
Salinity	-.0342	.5760*	-.2564*	.0477	-.0777	1.000									
pH	.2818*	-.3758*	.1901*	.1430*	.1221*	-.4886*	1.000								
Ammonia	-.0705	-.0071	.0393	-.3306*	-.0126	.1383*	-.3341*	1.000							
Chlorophyll	.1238*	-.3735*	.3222*	.0415	.1780*	-.3692*	.6124*	-.1298*	1.000						
Primary productivity	.1925	--	-.0749	.2443	.2127	-.3447*	.6072*	-.1800	.4664*	1.000					
Diatoms	.2126	--	-.2666	.0810	.1156	-.3297*	.3183	-.0924	.0089	.8236*	1.000				
Dinoflagellates	-.0990	--	.1242	-.0041	.0658	-.2926	.0619	.0639	.1043	.1318	.1405	1.000			
Other phyto-plankton	.1826	--	.0930	-.0302	-.0287	-.1923	-.3499*	.0664	-.0122	.2461	.4133*	.3622*	1.000		
Secchi disk depth	.0630	--	-.1488	-.1940*	-.1824*	.6185*	-.4287*	.3747*	-.3829*	-.7361*	-.4687*	-.2562	-.2478	1.000	

correlated with pH (0.1430) and inversely correlated with total ammonia (-0.3306) and Secchi disk depth (-0.1940). Since the correlation with depth (0.0949) is quite low, the cause of these significant correlations may not be related to phytoplankton blooms but to some other unknown factors. Indeed, the highest average percent abnormal values were registered for depths between 75 and 150 m (Table 12.3).

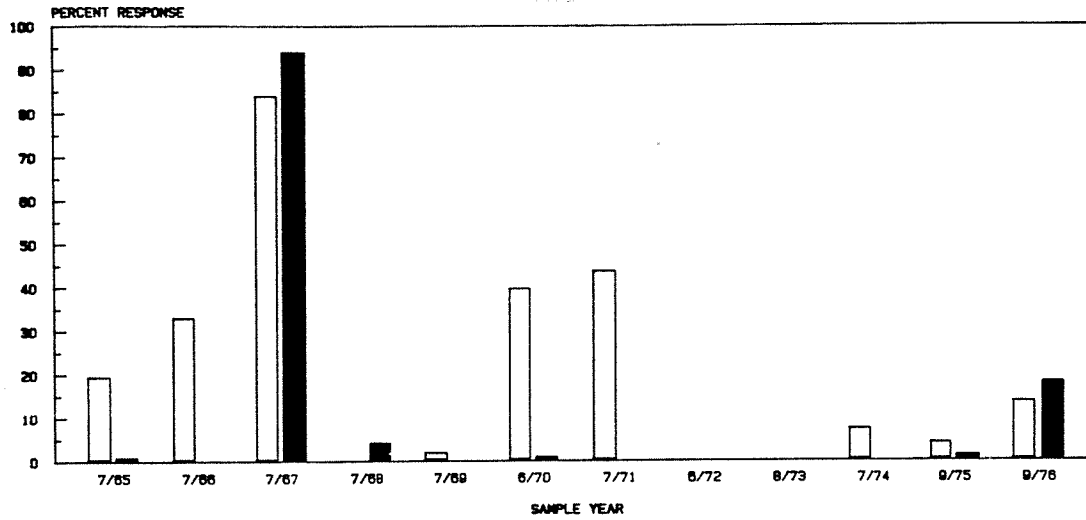
The two highest (significant at $p = 0.01$) correlations for the sand dollar eggs unfertilized index were with chlorophyll "a" (0.1780) and Secchi disk depth (-0.1824) which suggest that the same factor(s) affecting the oyster embryo mortality index in surface waters also affect(s) the successful fertilization of sand dollar eggs but to a lesser degree.

12.6.1.3 Historical Comparisons of Toxicity

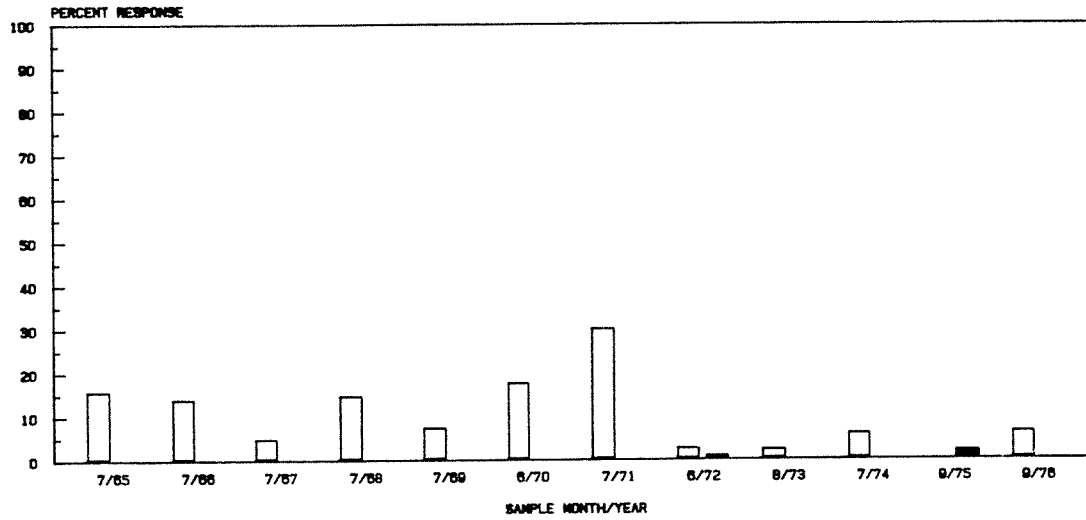
Historically, similar patterns of toxicity have been defined for many areas of Puget Sound using the oyster embryo assay. Cardwell and Woelke (1979) have summarized approximately 12 years of annual water quality monitoring and found embryo mortality most often associated with plankton blooms and embryo abnormality most dramatically affected by pollutant discharges (especially from pulp and paper mills).

State historical data for testing conducted close to some of the Seahurst sampling stations are presented in Figures 12.18 and 12.19. These figures show high variability from year to year with occasional high peaks in both mortality and abnormality, a pattern and magnitude fairly typical of the results presented in this report. Comparisons of mean responses for oyster embryo mortality and abnormality at four selected areas show that there is little difference between the responses measured by the Washington State Shellfish Laboratory for samples collected once per year (1962 to 1976) versus the Seahurst samples which were collected over a two year (1982 and 1983)

102
STATION 04-0112 (DES MOINES) -- 0 METERS



STATION 04-0105 (COLVOS PASS.)- 0 METERS



STATION 04-0115 (WEST POINT) -- 0 METERS

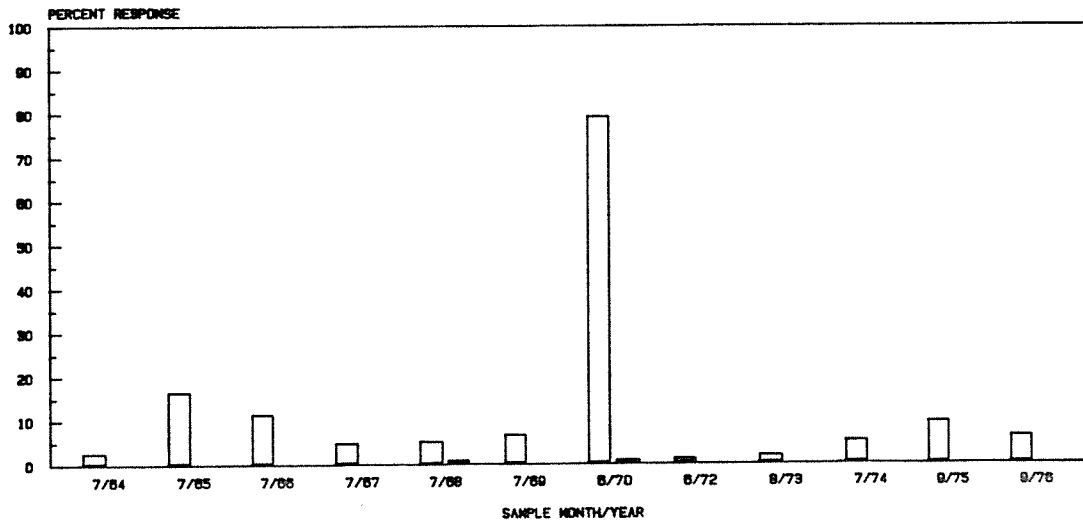
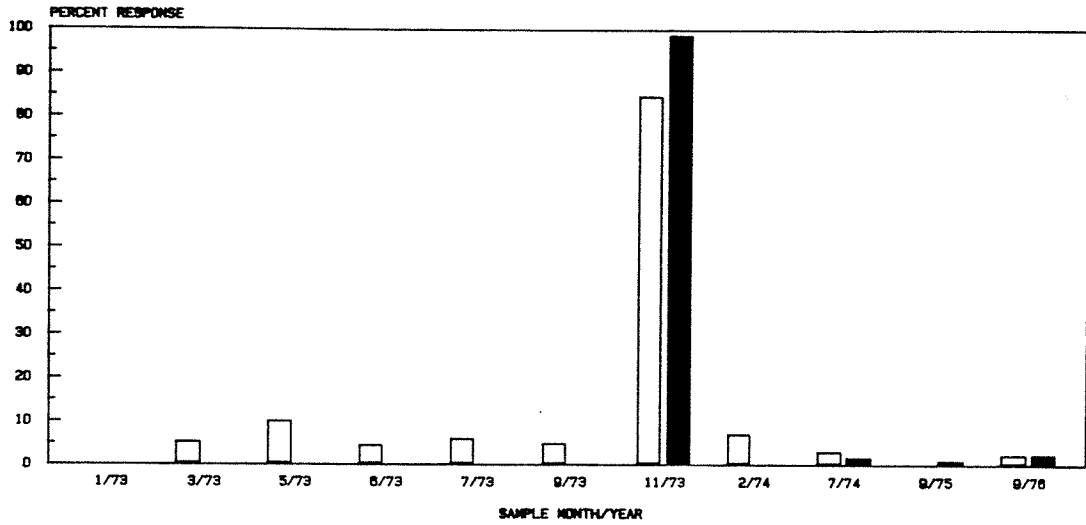
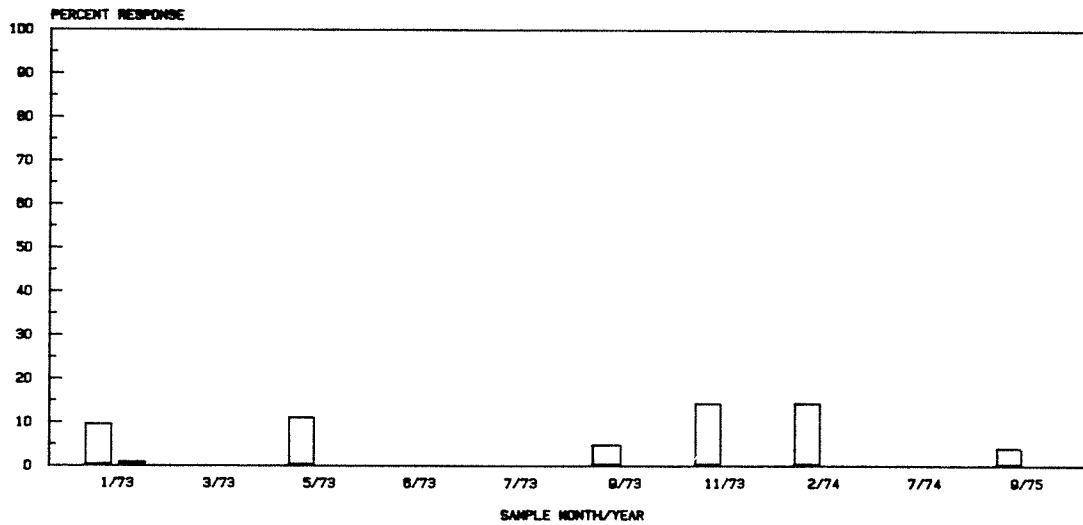


Figure 12.18. Washington State Department of Fisheries' historical data showing 48-hour oyster embryo responses in surface water collected from Des Moines, Colvos Passage and West Point stations, 1964-1976 (adapted from Cardwell and Woelke 1979).
 □ = mortality, ■ = abnormality.

103
STATION 04-0122 (BROWNS PT.) -- 0 METERS



STATION 04-0122 (BROWNS PT.)--9.1 METERS



STATION 04-0122 (BROWNS PT)--18.3 METERS

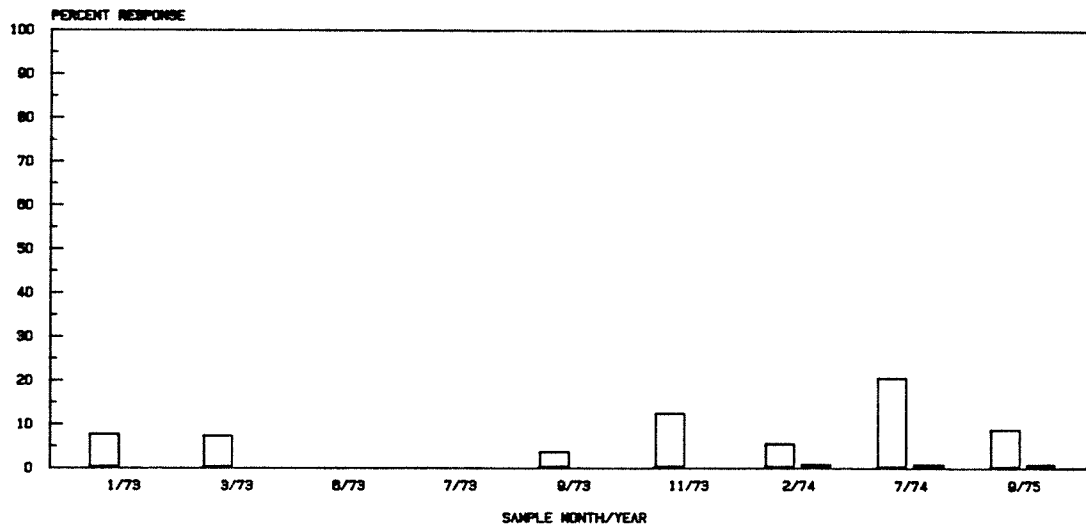


Figure 12.19. Washington State Department of Fisheries' historical data showing 48-hr oyster embryo responses in water collected from the Brown's Point station at three depths, 1973-1976 (adapted from Cardwell and Woelke 1979). = mortality, = abnormality.

period (Table 12.21). These comparisons suggest that the quality of surface waters of Puget Sound within the Central Basin area have not undergone a noticeable decline since the cessation of the state testing program in 1976.

12.6.2 Algal Metabolite Bioassays

Oyster embryos and sand dollar egg fertilization were never adversely affected by concentrations of Renton unchlorinated secondary sewage at or below 2% in seawater (see section 12.7 below), hence, the toxicity observed in the various culture waters was probably due to other factors. The apparent threshold effect for embryo abnormality (Figure 12.10) is evidence that reduced oyster embryo success in surface waters is related to phytoplankton densities. However, conclusive cause-and-effect proof of this relationship was not attained in these experiments since the toxic metabolite(s) or factor(s) were not isolated or measured and remain unknown. Indeed, the type and concentrations of metabolites in any given situation may be species-dependent and variable depending on the culture medium and growth dynamics.

12.6.3 Renton Sewage Bioassays

12.6.3.1 Direct Water Column Toxicity

Based on the results of the marine bioassays of Renton sewage, discharge of dechlorinated secondary-treated sewage should cause no direct acute toxicity in the water column. All but two bioassay indices reflected acute toxicity levels at >20 percent dechlorinated effluent in seawater (Table 12.9). The summer 1982 sand dollar sperm assay and the winter 1983 sea urchin embryo assay (for abnormality) registered EC50's of 7.13 (effluent only partially dechlorinated for summer 1982 tests) and 17.4 percent effluent, respectively. These values are high enough to provide a substantial margin of safety at an initial minimum effluent dilution of 100:1.

Downgrading of the present effluent treatment could produce measurable

Table 12.21. Comparisons of mean oyster embryo responses for Washington State historical data (calculated from Cardwell and Woelke 1979) with Seahurst data for 1982 and 1983 combined. State historical data was generally collected once per year from 1962 to 1976. All calculations are based on surface water data only (historical data based on Cardwell and Woelke 1979).

Sample Location	Mean Oyster Embryo Responses. (95% confidence limits)	
	Mortality	Abnormality
<u>West Point</u>		
Historical (Sta. #04-0115)	10.8 (-1.3 to 22.9)	0.4 (- 0.1 to 0.8)
Seahurst (Sta. #1)	14.4 (6.3 to 22.6)	-0.3 (- 1.2 to 0.6)
<u>Colvos Pass</u>		
Historical (Sta. #04-0105)	8.1 (3.1 to 13.0)	0.4 (0.0 to 0.8)
Seahurst (Sta. #2)	10.4 (2.5 to 18.3)	-0.1 (- 1.7 to 1.5)
<u>Brown's Point</u>		
Historical (Sta. #04-0122)	11.6 (-3.8 to -27.0)	8.6 (-10.1 to 27.3)
Seahurst (Sta. #3)	9.8 (0.1 to 19.5)	0.1 (- 2.8 to 3.0)
<u>East Passage</u>		
Historical (Sta. #04-0112)	17.7 (4.0 to 31.5)	8.0 (- 5.9 to 22.0)
Seahurst (Sta. #8)	16.4 (4.2 to 28.5)	-2.7 (-16.3 to 10.9)

toxic effects in marine waters. Deletion of the dechlorination step and the subsequent discharge of chlorinated effluent could add substantially to the expected acute toxicity depending on the magnitude of residual chlorine remaining in the effluent at the point of discharge. The EC50 values associated with the chlorinated secondary sewage range from 0.43 to >20 percent sewage (Table 12.9). Assuming an initial dilution of 100:1, these data predict that acute toxicity might be detected in the near-field discharge area at certain times using a sensitive sperm cell assay. Primary treatment would increase the effluent toxicity to marginal levels of safety since EC50 values ranged as low as 2.9 percent effluent.

The acute toxicity of primary sewage approaches that of the chlorinated secondary but qualitative differences are important. The oxidative component of chlorine is very toxic to sensitive marine life but decays rapidly in marine waters due to chemical reactions with naturally occurring organics (Goldman et al. 1979) and bromide (Wong and Davidson 1977). Hence, any toxic effects should be limited to an area close to the discharge. The acute toxicity of primary sewage, however, is probably due to many toxicants and their combined effects, which have not been removed by the secondary treatment process. While not as acutely toxic as chlorine, these toxicants may be more conservative in marine waters and result in sublethal effects at greater distances from the discharge, particularly if toxicant-laden solids settle to the bottom. The discharge of chlorinated primary effluent could affect both near and far-field toxicity.

Variations other than level of treatment can affect the measures of toxicity generated for a given sewage plant, such as the difference in sensitivity of each of the various bioassay organisms/life stages (Table 12.9). For the summer 1982 tests, the sand dollar sperm assay was clearly a

more sensitive indicator of toxicity than the oyster embryo assay. For the winter 1983 tests, crab zoea and the sea urchin embryo mortality indices were least sensitive while the sea urchin sperm and embryo abnormal indices proved most sensitive. The sperm assays are probably most sensitive to sewage (especially chlorinated sewage) because the assays are conducted within a short time after the addition of sewage to seawater while embryo/larval stages require exposure periods of 48 to 96 hours over which time toxic components may be volatilized, adsorbed onto particulates, or chemically degraded. Likewise, the sensitivity of embryonic stages may change through time as the egg divides and develops into a complex embryo. Kobayashi (1980) has shown that the later stages of sea urchin embryos are typically more sensitive than the dividing zygote and blastula stages. Maximum embryo sensitivity may occur at a point where the toxicant has decreased in concentration and may have changed chemical form (Figure 12.20). The later stages of embryos may be more sensitive to toxicants but the sperm assay is a more sensitive test due to the protocol and time frames involved. Sperm assays are faster, easier, and cheaper to conduct; they can be used to test more replicates/treatments at a time and can be conducted year round using closely related sand dollars (summer) and sea urchins (winter). For these reasons, we recommend that sperm bioassays be the primary tool for routine post-discharge monitoring of acute, sewage-related toxicity in the water column.

There is changing toxicity between seasons and between years (Table 12.9). Using the sperm assays as a guide there appears to be a reduction in toxicity for the winter 1983 and 1984 tests as compared to those of summer 1982 tests, using the primary sewage level as the basis of comparison. This pattern of toxicity correlates with the physical/chemical data measured by Renton treatment plant laboratory personnel and summarized by sewage type in

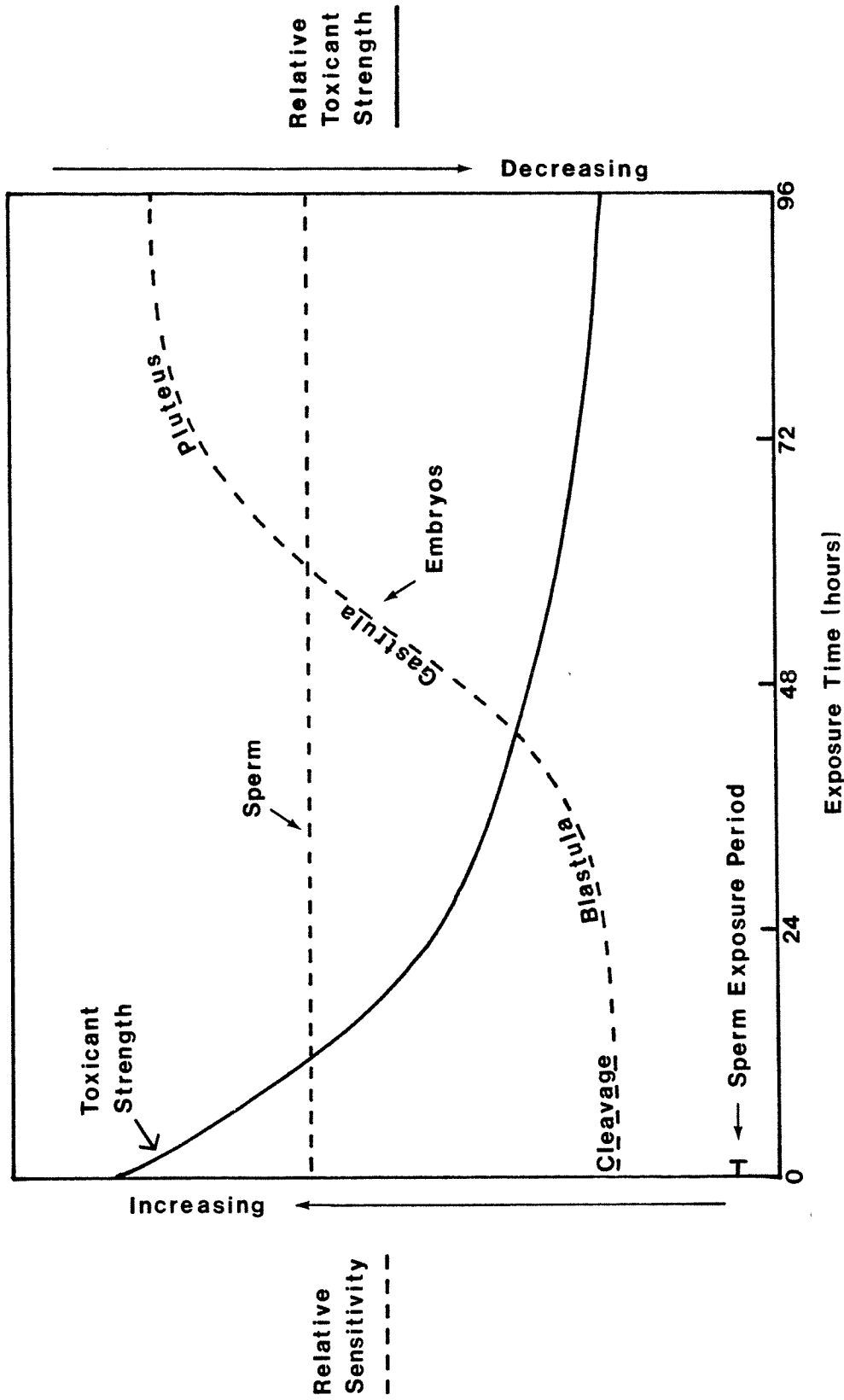


Figure 12.20. Hypothetical relationship between 60-min sperm cell and 96-hr embryo bioassay sensitivities to toxicants which degrade or are lost from the test system with time. From Dinneil (1984).

Tables 12.22 (influent), 12.23 (primary) and 12.24 (final dechlorinated effluent). The Renton treatment plant flow of primary sewage was approximately 21 percent less during the summer and contained higher levels of ammonia, suspended and volatile solids, and registered higher COD's and BOD's than during winter 1983 (Table 12.23). The lower summer flow served to concentrate the toxicants relative to the winter period. Higher values for all metals in the final secondary summer 1983 effluent (Table 12.24) also serve to illustrate this point. The winter 1983 sperm assay EC50 of 0.43 percent for chlorinated sewage is the exception to the summer-winter pattern but may have been due to higher residual chlorine levels during the winter period as measured in the sewage samples (Tables 12.10). The calculated average residual chlorine values associated with summer 1982 and winter 1983 sperm assay EC50's were 0.017 and 0.009 mg/liter, respectively. The effective chlorine concentrations may have been even lower due to the rapid oxidation of chlorine in seawater.

There is a substantial difference in toxicity between summer 1982 and summer 1983 tests as shown by sand dollar sperm assays for both years (Table 12.9). While flows through the plant were higher during summer 1983, the ammonia, solids, COD, BOD, and metals measurements were not substantially different between years (Tables 12.22 through 12.24). Reasons for between-year toxicity fluctuations are unknown but may be associated with unmeasured toxicant inputs to the Renton plant and/or insufficient test replication.

12.6.3.2 Indirect Water Column Toxicity

As noted above, significant direct toxicity of the effluent to marine organisms is improbable due to the high level of treatment of Renton treatment plant effluent and the expected dilution. However, the potential for indirect toxicity must be considered.

Table 12.22. Average physical/chemical characteristics of Renton treatment plant Influent Sewage. Values are averages of METRO's 24-hr composite sampling data corresponding to the days which sewage bioassay grab samples were collected (10 days each season).

Parameter	Sewage Sample Period			
	Summer 1982	Winter 1983	Summer 1983	Winter 1984
Flow (MGD)	42.9	54.2	44.4	52.2
pH	7.1	7.4	7.4	7.5
NH ₃ -N (mg/l)	15.6	12.2	17.9	15.1
Suspended solids (mg/l)	277.7	210.9	255.0	199.8
Volatile suspended solids (mg/l)	225.6	180.3	211.7	164.3
Total COD (mg/l)	507.9	360.7	558.5	426.2
Five-day BOD (mg/l)	235.4	171.9	267.9	221.0

Table 12.23. Average physical/chemical characteristics of Renton treatment plant Primary Effluent. Values are averages of METRO's 24-hr composite sampling data corresponding to the days which sewage bioassay grab samples were collected (10 days each season).

Parameter	Sewage Sample Period			
	Summer 1982	Winter 1983	Summer 1983	Winter 1984
Flow (MGD)	44.8	56.7	46.6	54.4
NH ₃ -N (mg/l)	16.4	13.4	16.4	14.9
Suspended solids (mg/l)	69.3	72.3	67.5	71.8
Volatile suspended solids (mg/l)	57.7	55.8	57.0	56.5
Total COD (mg/l)	267.3	214.1	262.4	252.4
Five-day BOD (mg/l)	148.7	114.4	147.1	134.4

Table 12.24. Average physical/chemical characteristics of Renton treatment plant Final Secondary (Dechlorinated) Effluent. Values are averages of METRO's 24-hr composite sampling data corresponding to the days which sewage bioassay grab samples were collected (10 days each season).

Parameter	Sewage Sample Period			
	Summer 1982	Winter 1983	Summer 1983	Winter 1984
Flow (MGD)	38.0	51.1	45.4	52.9
Turbidity (J.T.U.) ¹	4.5	4.2	3.9	2.9
pH	7.2	6.9	6.7	7.0
NH ₃ -N (mg/l)	14.0	11.7	15.4	13.3
Suspended solids (mg/l)	7.7	7.0	4.8	5.9
Volatile suspended solids (mg/l)	6.2	6.2	4.5	5.0
Total COD (mg/l)	50.5	39.4	42.6	43.2
Five-day BOD (mg/l)	11.5	5.2	6.0	5.1
Chlorination set point (mg/l)	2.60	2.46	1.60	1.30
Residual chlorine (dechlorinated) (mg/l)	0.20	<0.002	<0.001	<0.001
<u>Metals (µg/l)²</u>				
Mercury	<0.40	0.28	<0.32	<0.22
Cadmium	<4.9	<4.0	<4.1	<4.0
Copper	30.0	16.0	26.0	<24.0
Lead	<24.0	<20.0	<23.0	<23.0
Zinc	40.0	27.9	41.3	24.0
Nickel	<28.0	<26.0	29.0	<30.0
Total Chromium	<20.0	<20.0	<20.0	<31.0

¹Turbidity was determined by averaging the values from effluent collection chambers 1 and 2.

²When daily metals values were recorded as less than (<) values, the detection limit was used as the value for that day in calculating the 10-day averages. Since this method overestimates the average value, the averages were therefore noted as < values.

Presently, there is one possible scenario which appears plausible, although a large degree of speculation must be incorporated into the discussion at this point. In brief, background toxicity has been observed in ambient water samples and is most highly associated with surface and inshore stations. These data suggest that a portion of this toxicity is associated with toxic phytoplankton metabolites. If the discharged effluent serves to stimulate greater than "normal" phytoplankton blooms, "background toxicity" in Puget Sound could gradually increase with possible adverse effects on the sensitive larval stages of a variety of marine animals. There are many bits and pieces of experimental data, causal observations, and experiences with eutrophication associated with other marine outfalls, some of which support this scenario, and some of which do not. Outlined below are some of the major considerations involved both for and against the potential for stimulated blooms and increased "background toxicity:"

1. The nutrient found to be most limiting to marine phytoplankton growth is nitrogen (Welch 1980; Thomas et al. 1974). Nitrogen compounds are relatively high in sewage effluents and may provide forms of nitrogen (nitrate and urea) preferentially used by some forms of algae (Mahoney and McLaughlin 1977).
2. While nitrogen has been shown to stimulate algal growth in laboratory experiments, nitrogen seems to be rarely limiting in estuaries (as contrasted to open ocean waters) and only small amounts (~10 percent) are actually used for primary production (Garside et al. 1976; Ingram 1979).
3. Sewage may contain other factors which serve to stimulate certain types of algae. These include natural organic (humic) substances, and chelators which affect the biological availability of trace

metals (Saunders et al. 1982).

4. Physical factors controlling water column stability and light availability probably exert the biggest control over phytoplankton bloom dynamics. These factors include winds, convective heating or cooling, tidal currents, turbidity, cloud cover, storm activity and water depth. Some algal species, however, are heterotrophs which do not require residence in the photic zone for growth.
5. In recent years paralytic shellfish poisoning (PSP) caused by toxic species of dinoflagellates has spread to previously unaffected areas throughout the world, and the outbreaks appear to be increasing in intensity. This trend has become apparent locally in recent years with the spread of PSP into the central Puget Sound basin (Saunders et al. 1982). The relationship between PSP increases and eutrophication processes are presently unknown but cannot be discounted at this time.
6. Oyster embryo bioassays conducted by the State of Washington have repeatedly associated high water toxicity (as related to larval mortality) with blooms of certain phytoplanktonic organisms (dinoflagellates), especially in areas with high water column stability (Cardwell et al. 1977b and 1979). Confirmatory laboratory bioassays of water in which the dinoflagellates Ceratium fusus and Gymnodinium splendens were grown imparted high toxicity to oyster embryos (Cardwell 1979). Additional evidence suggests that bloom metabolites are also toxic to other species of bivalve larvae and may extend to adult mortalities as well. Cardwell et al. (1979) conclude that "with continued urbanization of the innermost southern Puget Sound region, it is conceivable that receiving water toxicity

problems due simply to blooms of toxic dinoflagellates and perhaps other phytoplankters could be exacerbated."

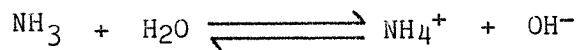
It should be noted that all of the comments outlined above are discussed in a generic sense and not directed at any one point discharge. If eutrophication within Puget Sound does become a future concern, all anthropogenic sources (both point and non-point) must be addressed.

Relative to the placement of a specific outfall, several factors seem important for minimizing the potential for stimulating phytoplankton growth:

1. Location of the outfall as close to the main channel as possible to maximize effluent dispersion and minimize effluent concentration in areas of relatively high water column stability.
2. Maximize the potential for the effluent dispersal in an ocean-bound direction.
3. The deeper the discharge the greater the dilution of the effluent before it gets into the photic zone.

12.6.4 Ammonia Bioassays

Ammonia toxicity is primarily dependent on chemical state. The following equilibrium:



is characteristic of ammonia in aqueous solutions (NAS 1972). This equilibrium is dependent primarily on pH, temperature, and alkalinity or salinity (Emerson et al. 1975). Unionized ammonia (NH_3) has been shown to be the toxic component of ammonia solutions, as ionized ammonia (NH_4^+) apparently is unable to pass tissue barriers (Milne et al. 1958).

Buckley (1978) conducted studies of ammonia toxicity to coho salmon

(Oncorhynchus kisutch) in Duwamish River water and found that the 96-hr LC50's were associated with 0.45 mg/liter unionized ammonia. Based on equilibrium models, the concentration of NH_3 in marine waters (pH 7.8-8.2, temperature 8-12°C, salinity 28-30 ‰) would approximate 1 to 4 percent of the total ammonia (Emerson et al. 1975). Using a representative value of 2 percent for the unionized ammonia fraction in seawater, the EC50's for the oyster embryo abnormality index and the sperm assay would be approximately 0.23 to 0.70 mg/liter NH_3 . These values indicate that the bioassay marine life stages exhibit a similar degree of sensitivity to ammonia as coho salmon (0.45 mg/liter; Buckley 1978). The average concentration of 13.7 mg/liter total NH_3 in Renton effluent (EPA 1981) would be diluted to less than 0.005 mg/liter unionized ammonia, after an initial dilution of 100:1, reflecting a safety factor of roughly 50X for acute ammonia toxicity.

12.6.5 Baseline Sediment Bioassays

Amphipods have been increasingly used in a variety of toxicant studies of 96-hr to several months duration. Laboratory bioassays have involved exposures via water to single compounds such as phenol, cadmium, copper, chromium, mercury, nickel, lead, and zinc (Oksama and Kristoffersson 1979; Rehwoldt et al. 1973; Spehar et al. 1978) and several compounds in concert such as pharmaceutical wastes, petroleum hydrocarbons, and bleached pulp kraft mill effluent and heavy metals (Lee and Arnold 1983; Lee and Nicol 1978; Lee et al. 1977; Levings et al. 1975; Moulder 1980). Laboratory studies have also investigated amphipod response and uptake, depuration, and biotransformation in sediment exposures of anthracene, benz(a)pyrene, cadmium, fluoride, and various compound mixtures (Landrum and Scavia 1983; Connell and Airey 1979; Reichart et al. in press). In the field, distribution and/or diversity of naturally occurring populations of amphipods were used to map pollution levels

in lakes and embayments (Bellan-Santini 1980; Wagemann et al. 1978; Zauke 1977). All of these studies show that when amphipods are exposed under controlled conditions to single compounds or contrived mixtures of compounds, responses can be readily interpreted in terms of toxicant effects.

Amphipods have also been used in bioassays with naturally occurring sediments known or suspected to be contaminated with a wide variety of chemicals (Chapman et al. 1982a; Ott et al. in preparation; Pierson et al. 1982; Swartz et al. 1979, 1982, 1984). These test results were often associated with a high degree of variability and, in the past, were rarely equated to the actual toxicant burdens in the test sediments. Data from bioassays using different methodologies and amphipod species resulted in poor agreement between laboratories on the degree of toxicity of sediment (Swartz et al. 1982; Pierson et al. 1982). Much of the confusion in this body of literature is a result of incomplete understanding of combined effects on amphipod responses of multiple toxicants, natural organics and sediment grain size under different bioassay test regimes.

In this study, the marine amphipod Rhepoxynius abronius was exposed to 27 sediments from central Puget Sound using three bioassay systems over a two year period. Data was also collected on sediments for grain size, total volatile solids (TVS), percent carbon, biological oxygen demand (BOD), 15 heavy metals, and 65 organic compounds. The pattern of survival of amphipods in Seahurst sediments correlates with grain size, heavy metal sediment burden, and, to a lesser extent, organic chemical sediment load when a static system was employed. With a flow-through system, reduced survival consistently occurred in sediments from transects A, B, C, and H and there was a trend for data to correlate with grain size and sediment toxicant load, but this was not as pronounced as with the static system. Amphipod survival was generally

lower with a static system, especially in finer-grained substrates. All correlations with survival, although statistically significant, were very low and no single chemical compound or sediment parameter could account for the observed survival pattern.

Swartz et al. (1984) reported similar findings for correlations between R. abronius survival and 15 parameters (Eh, percent solids, total volatile solids, total organic carbon, ammonia nitrogen, free sulfides, hydrocarbon and total oil/grease, Ag, Cd, Cu, Cr, Ni, Pb, and Zn). Swartz et al. concluded that "different combinations of stresses involving multiple or unmeasured factors were probably responsible for observed (toxic) effects."

In a sediment bioassay, test organism response depends partially upon the fraction of toxicant which is bioavailable. For many trace metals and heavier weight organics in a marine environment, degree of bioavailability is largely a function of sediment grain size and redox (pH, Eh) conditions (Cross and Sunda 1978). Finer-grained substrates tend to be more contaminated, as the toxicant burden is a function of surface area (grain size), but binding affinity also increases with surface area, due to increased TVS (Comiskey et al. 1983). For heavy metals, bioavailability is equated with concentration of free (dissolved) ion and studies with polychaetes and bivalves have shown that response was more affected in metal-spiked sands than in similarly-spiked silts (Pesch 1979; Phelps et al. 1983). Other metals, chlorides (salinity), and ligands (different microflora/fauna) can all act as chelating agents to reduce metal bioavailability and, hence, toxicity (Cross and Sunda 1978; Lumoa and Jenne 1978; Leckie and James 1974). Interaction between organics and metals can also influence toxicant bioavailability (Crececius et al. 1980). Redox conditions influence sediment adsorption rates of both organics and metals. Under increasingly anoxic conditions, Mn and Fe are solubilized while

Cd, Cu, Ni, Pb, and Zn are adsorbed (Elderfield et al. 1981; Riley 1971). Redox potential affects both adsorption and decomposition rates of organic compounds (Gambrell et al. 1984).

Interaction between redox potential and grain size can account for the observed differences in survival between the static and flow-through systems. Differences in redox regime for sediments in situ versus those in a bioassay are most likely to increase with sampling depth. Thus, in a bioassay situation with oxidizing conditions, finer-grained sediments from a reducing environment are more likely to leach toxicants into the seawater. Finer-grained sediments may also have a higher toxicant load (i.e., more to leach). Leaching would be maximized with aeration (static system) which would accentuate oxidizing conditions and could also change types and amounts of ligands and chelates present in the sediment (Seelye et al. 1982). In a static system, compounds released into seawater would remain and be available to animals. Enhanced accumulation of metals and organics occurred in fish when exposed to aerated versus non-aerated sediments (Seelye et al. 1982). Similar differences in toxicant accumulation are suggested to occur in amphipods exposed to different bioassay systems and are supported by the stronger correlations between toxicant levels and survival in the static system.

Amphipod survival in the Seahurst sediments may have been influenced by grain size alone, besides interactions between grain size, toxicant, and redox conditions. Grain size in Seahurst sediments ranged from gravel to fine clay. Many infaunal amphipods demonstrate a preference for a certain grain size range (Fenchel et al. 1975; Meadows 1964) and grain size is also a major factor influencing distribution of small invertebrates (Wieser 1959). The preferred habitat of R. abronius is a fine-grained sand (2.2-3.2 mean phi) and

a threshold response in survival was observed between 20-63 um (medium to coarse silt) in sediments devoid of organics (TVS) and toxicants (Ott in preparation). However, only moderately reduced survival occurred in fine-grained sediments with an intact bacteria organic matrix. In addition, this same study found a sublethal effect (slightly reduced reburial) occurred in coarse-grained sediments (2-mm sands) (Ott, in preparation). An upper grain size tolerance limit for R. abronius has also been suggested by Swartz et al. (1982). Reduced survival was observed in both coarse- and fine-grained Seahurst sediments with a static system. However, this response cannot be easily partitioned from toxicant effects for these sediments. It is suggested that although R. abronius may tolerate finer or coarser sediments, unsuitable sediments may impart a sublethal stress, which, when in combination with other stresses (such as toxicant), may become lethal.

Variability in responses for a given station over time was observed in 13 of the 26 Seahurst stations (station G780 was only tested once). Although distribution and bioavailability of both metals and organics fluctuate seasonally (Elderfield et al. 1981; Lee and Swartz 1980; Tsai et al. 1979; Wagemann et al. 1978; Zauke 1977), fluctuations in survival were not consistent with season, or grain size. Variability generally increased with increasing sediment toxicity. Thus, in sediments from transects A, B, C, and H, 8 out of 13 stations were significantly different between seasons compared to 5 out of 13 for the other stations. In the "toxic" control (Duw SH), survival was not significantly different between seasons, but the same sediment was retested in 3 out of 4 seasons (i.e., same sediment chemistry in 3 out of 4 seasons). Although no consistent differences were noted between fall and spring, in 10 out of 13 of the more toxic stations (A, B, C, and H), survival was higher during spring 1984 with a flow-through system. However,

survival was low in these same sediments with a static system. These observations suggest that the within-stations variability in survival is probably not due either to increased amphipod tolerance or to a general improvement in sediment quality of the central Sound in spring 1984, but instead may be due to differences in bioavailability of toxicants during different sampling periods or with different bioassay methods.

The relatively high overall survival of amphipods in the Seahurst area sediments may be due to the isolation of south central Puget Sound from significant industrial or municipal discharges. Survival in most Seahurst sediments was well above that in the Duwamish South Harbor (Duw SH) "toxic" control. Organic chemical levels in those stations with survival below that of Duw SH (notably in A and C transects) were an average of an order of magnitude lower than those in Duw SH. However, since these stations also had a smaller average grain size than Duw SH, it is suggested that survival reflected an interaction of stresses between grain size and toxicant. The reduced survival in the northern transects may be associated with proximity of these sites to Elliott or Commencement Bays (via recirculation from Colvos Passage). Recent work by NOAA-PMEL researchers (Curl 1982) suggest that the East Passage may be a depositional site for sediments (and sediment bound toxicants) being discharged into and around these Bays. Sediments which caused reduced amphipod survival also were found to impact infaunal trophic indices (ITI). However, the ITI index is typically not used to monitor chemical contamination (versus organic enrichment). Instead, the lower-than-normal indices in the northern stations may reflect an area of increased deposition which encompasses the stations in A, B, and C transects (Figure 6.21, Volume V, Section 6). Higher-than-normal TVS and organic carbon values were associated with this area of increased deposition (Figures 6.23, 6.25,

Volume V, Section 6). Typically, as sediment carbon content increases, ITI values decrease. Thus, ITI values were most likely reflecting changes in conventional chemicals (TVS, BOD, C). However, since pollutants most readily associate with finer-grained particles (Comiskey et al. 1983), areas with increased deposition, TVS, and carbon content are more likely to have increased levels of priority pollutants and, thus, to impact amphipod survival..

Because Duwamish South Harbor sediments (obviously contaminated with at least petroleum products) were only moderately toxic to amphipods in a 10-day bioassay, the question is raised whether or not the amphipod test is sensitive enough to define the type and magnitude of toxicity expected to result from the discharge of secondary sewage effluent into marine waters. Additional types of sediment bioassays should be conducted in conjunction with amphipod tests to help refine estimates of toxicity.

Nonetheless, it is clear that amphipod bioassays of sediments from the Seahurst area have provided valuable baseline data. A range of survival from high to moderate was observed for Seahurst area sediments. This range of survival also occurred among fine-grained sediments. Clearly, amphipods were stressed in some of the baseline sediments by more than grain size alone and this stress was translated into reduced survival in a 10-day test.

Therefore, any additional chemical contamination of Seahurst sediments from sewage effluents should cause an even greater reduction in amphipod survival, especially for sediments from stations which have already effected reduced survival. However, the degree of contamination which will effect a significant reduction of amphipod survival is presently unknown.

12.7 Summary and Conclusions

The toxicological portion of the Seahurst Baseline studies focused on three areas of concern: 1) definition of predischage levels of water column toxicity within the south central Puget Sound basin, 2) determination of the toxicity of various treatment stages of Renton sewage to sensitive life stages of marine organisms and 3) determination of baseline levels of sediment toxicity using a 10-day amphipod bioassay.

Baseline levels of water column toxicity were measured at eleven stations within the Seahurst study area and at one station (West Point) outside the Seahurst basin. Water samples were collected several times per month during summer and fall of 1982 and 1983 at two or three depths per station. Toxicity determinations were based on the results of two standard bioassay tests: 1) 48-hr oyster embryo survival and normal development and 2) success of sand dollar egg fertilization following 60-min sperm exposures to the test waters. The major findings of the water column bioassays were:

1. Measurable background water toxicity was found at all stations and depths sampled. A high degree of variability in bioassay responses was evident for most stations. The degree of variability was dependent on sample date, location, year and bioassay type.
2. Generally, the oyster embryo mortality index was highest in surface waters and within the Seahurst area of East Passage. The oyster embryo abnormality index was slightly higher in deeper waters while sand dollar egg fertilization was slightly inhibited in both surface and deep (below 150 m) waters.
3. Significant ($p \leq 0.01$) positive correlation coefficients (r) were found between the oyster embryo mortality index versus chlorophyll "a" and pH while significant negative correlations existed for depth

and salinity. This pattern of correlations reinforces earlier findings by Cardwell et al. (1977b and 1979) that phytoplankton blooms in surface waters adversely affect the survival of oyster embryos. Significant ($p < 0.01$) negative correlations were also found for sand dollar egg fertilization success versus chlorophyll "a" and Secchi disk depths. This suggests that the same factors affecting the oyster embryo mortality index in surface waters also affect(s) the successful fertilization of sand dollar eggs but to a lesser degree.

4. Comparisons of the 1982-1983 Seahurst oyster embryo data with data generated by the Washington State Fisheries Laboratory from 1962 to 1976 show that there are no substantial changes in oyster embryo responses either in the magnitude of the responses or degree of variability for four similar stations.

Bioassays of five stages of Renton sewage were conducted using a variety of gamete, embryo and larval stages of common marine animals. Each bioassay organism was tested ten times each to account for daily changes in sewage toxicity. Sand dollar and sea urchin sperm (egg fertilization) assays were repeated a second year to provide for between-year comparisons in toxicity. The sewage bioassay results showed the following:

1. Generally, the relative toxicity of five stages of Renton sewage was: chlorinated secondary > influent > primary > dechlorinated secondary > unchlorinated secondary.
2. The relative sensitivity of the various bioassay indices was: sand dollar and sea urchin sperm (egg fertilization) > oyster and sea urchin embryo abnormality > oyster and sea urchin embryo mortality > crab zoea mortality.

3. Primary treatment afforded only a slight reduction in acute toxicity over that observed for the influent sewage. Secondary treatment effected a very marked reduction in acute toxicity over that observed for primary-treated sewage. Unchlorinated and chlorinated-dechlorinated secondary sewage were essentially equal in toxicity (assuming complete dechlorination).
4. Chlorinated secondary sewage was more acutely toxic than influent or primary sewage. However, the rapidity of chlorine decay in seawater may "dilute out" the toxic effects of chlorine a short distance from the discharge point. Primary (or influent) sewage was less toxic than chlorinated secondary sewage. However, the toxicity associated with these stages is probably due to the interactive toxicity of a wide variety of pollutants (i.e., metals, xenobiotic organics, petroleum hydrocarbons, pesticides, detergents, etc.) which are probably more conservative than chlorine in the receiving waters.
5. The discharge of either unchlorinated or dechlorinated secondary sewage to Puget Sound should not cause any discernable acute toxicity in the water column to sensitive life stages of marine organisms assuming an initial dilution of 100:1 or greater. The discharge of either chlorinated secondary or primary (especially chlorinated primary) sewage could cause acute toxicity on some occasions at initial dilutions less than 200:1.

Ten-day amphipod bioassays of sediments from 27 Seahurst area stations were conducted twice per year for a two year period. The amphipod/sediment bioassay results suggest the following:

1. Reduced amphipod survival occurs in sediments from the northern Seahurst transects A, B, C, and H. Increased numbers of

sublethally-stressed amphipods (as measured by the post-exposure reburial tests) occurred in sediments from transects A, H, and I. Results were correlated with grain size and toxicant load. Survival in static bioassays tended to be lower than in flow-through bioassays, which is probably more a function of seawater rather than sediment contamination.

2. Seahurst sediment bioassay results are generally consistent over time, showing no seasonal trends in survival (with the possible exception of run 1, spring 1983). Within station variability in survival was not consistently different between seasons and is possibly associated with differences in bioavailability of toxicants during different sampling periods.
3. Bioassays should include at least three controls including a native sand, a fine-grained uncontaminated sediment to gauge response due to grain size, and contaminated or "toxic" control to gauge response due to possible shifts in animal sensitivity over time.
4. Amphipod bioassays are recommended as one tool to monitor sediment toxicity over time. Results suggest that future addition of toxicants to Seahurst area sediments may result in lower amphipod survival. Ideally, sediment should be collected and tested at least twice to minimize response due to patchy distribution or variable bioavailability of chemicals.
5. Additional types of sediment bioassays should be conducted to help refine estimates of sediment toxicity. These may include, but not be limited to, solid phase tests with additional species, behavioral assays, chronic and life cycle assays and elutriate assays with gamete or embryo stages.

12. 8 LITERATURE CITED

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12.9 APPENDIX

Appendix Table 12.1

BREAKDOWN (SPSS) OF THE 1982 CONTROL AND TEST RESPONSES FOR EACH OF THE RECEIVING WATER BIOASSAY INDICES.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE AVERAGE PERCENT OYSTER EMBRYO MORTALITY
BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN MORTALITY	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	16)
STATION (KIN. CARRY-ALONG)	91.	-3.28 TO 6.64	26.9000	1.6813	9.0786	82.4203	16)
STATION (WEST POINT)	92.	3.70 TO 19.68	187.0000	11.6875	14.5931	212.9572	16)
STATION (FRIDAY HARBOR)	93.	-7.72 TO 7.42	-2.1000	-.1500	12.7384	162.2658	14)

TOTAL CASES = 65
MISSING CASES = 3 OR 4.6 PCT.

***** ANOVA TABLE *****

SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS 1507.5670	(3)	502.5223
WITHIN GROUPS 6540.1169	(58)	112.7606
TOTAL 8047.6839	(61)	

F = 4.4565 SIG. = .0069 ETA SQRD = .1873

Appendix Table 12.1 (continued)

BREAKDOWN (SPSS) OF THE 1982 CONTROL RESPONSES FOR THE OYSTER EMBRYO ABNORMAL INDEX TOGETHER WITH THE 95% CONFIDENCE LIMITS FOR THE MEAN ABNORMALITY.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE AVERAGE PERCENT OYSTER EMBRYO ABNORMAL
BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	16)
STATION (KIN. CARRY-ALONG)	91.	-7.23 TO 2.86	-34.9000	-2.1813	9.2313	85.2163	(16)
STATION (WEST POINT)	92.	-11.39 TO 8.50	-23.1000	-1.4437	18.1579	329.7106	(16)
STATION (FRIDAY HARBOR)	93.	-21.02 TO 4.16	-118.0000	-8.4286	21.1734	448.3145	(14)

TOTAL CASES = 65
MISSING CASES = 3 OR 4.6 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	604.4348	(3)	201.4783
WITHIN GROUPS	12051.9923	(58)	207.7930
TOTAL	12656.4271	(61)	

F = .9696 SIG. = .4133 ETA SQD = .0478

Appendix Table 12.1 (continued)

BREAKDOWN (SPSS) OF THE 1982 CONTROL RESPONSES FOR THE SAND DOLLAR SPERM ASSAY INDEX TOGETHER WITH THE 95% CONFIDENCE LIMITS FOR THE MEAN PERCENT EGGS UNFERTILIZED.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED

BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN UNFERTILIZED	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	(17)
STATION (KIN. CARRY-ALONG)	91.	-0.60 TO 2.32	14.7000	.8647	2.7737	7.6937	(17)
STATION (WEST POINT)	92.	-0.76 TO 0.45	-2.8000	-.1647	1.1407	1.3012	(17)
STATION (FRIDAY HARBOR)	93.	-2.79 TO 0.85	-13.6000	-.9714	3.0663	9.4022	(14)

TOTAL CASES = 65

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	26.3393	(3)	8.7798
WITHIN GROUPS	266.1462	(61)	4.3631
TOTAL	292.4855	(64)	

F = 2.0123 SIG. = .1216 ETA SQRD = .0901

Appendix Table 12.1 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1982 SEAHURST BASELINE OYSTER EMBRYO MORTALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN MORTALITY.

D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO MORTALITY	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION	10.09 TO 15.34	2860.5000	12.7133	20.1136	404.5562	(225)
STATION 1	4.20 TO 15.02	201.8000	9.6095	11.6093	134.7749	(21)
STATION 2	5.24 TO 14.48	207.1000	9.8619	9.9182	98.3715	(21)
STATION 3	2.18 TO 11.29	141.5000	6.7381	9.7871	95.7875	(21)
STATION 4	6.87 TO 20.70	372.1000	13.7815	17.1871	295.3962	(27)
STATION 5	8.29 TO 23.10	423.8000	15.6963	18.3860	338.0465	(27)
STATION 6	6.52 TO 25.31	429.7000	15.9148	23.3742	546.3521	(27)
STATION 7	-0.99 TO 19.94	255.9000	9.4778	25.9788	674.8964	(27)
STATION 8	2.04 TO 30.16	289.8000	16.1000	27.5942	761.4388	(18)
STATION 9	5.47 TO 32.02	337.4000	18.7444	26.0692	679.6026	(18)
STATION 10	-0.18 TO 22.56	201.4000	11.1889	22.3159	497.9975	(18)

TOTAL CASES = 243
MISSING CASES = 18 OR 7.4 PCT.

S U M O F S Q U A R E S D E G R E E S O F F R E E D O M M E A N S Q U A R E

BETWEEN GROUPS	2856.2895	(9)	317.3655
WITHIN GROUPS	87764.3105	(215)	408.2061
TOTAL	90620.6000	(224)	

S I G. = .6374 E T A S Q R D = .0315

Appendix Table 12.1 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1982 SEAHURST BASELINE OYSTER EMBRYO ABNORMALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN ABNORMALITY.

DESCRIPTION OF SUBPOPULATIONS									
CRITERION VARIABLE BROKEN DOWN BY STATION	AVERAGE OYSTER EMBRYO ABNORMAL	95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL	SUM	MEAN	STD DEV	VARIANCE	N		
FOR ENTIRE POPULATION	-5.57 TO 0.86		-529.5000	-2.3533	24.5710	603.7316	(225)		
STATION 1	-2.24 TO 4.87		27.6000	1.3143	7.6632	58.7243	(21)		
STATION 2	-1.86 TO 0.93		-9.7000	-.4619	2.9777	8.8665	(21)		
STATION 3	-0.57 TO 3.51		30.8000	1.4667	4.3717	19.1113	(21)		
STATION 4	-11.58 TO 12.26		9.2000	.3407	29.6308	877.9864	(27)		
STATION 5	-13.30 TO -0.86		-191.1000	-7.0778	15.4530	238.7956	(27)		
STATION 6	-12.58 TO 10.94		-22.2000	-.8222	29.2377	854.8441	(27)		
STATION 7	-17.57 TO 9.32		-111.3000	-4.1222	32.6301	1064.7218	(27)		
STATION 8	-21.33 TO 9.52		-106.3000	-5.9056	30.2513	915.1441	(18)		
STATION 9	-23.00 TO 12.26		-96.7000	-5.3722	34.5764	1195.5245	(18)		
STATION 10	-19.57 TO 12.62		-59.8000	-3.3222	31.3020	979.8183	(18)		

TOTAL CASES = 243
MISSING CASES = 18 OR 7.4 PCT.

ANOVA TABLE			
SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	
BETWEEN GROUPS	(9)	224.2796	
WITHIN GROUPS	(215)	619.6156	
TOTAL	(224)		
F = .3620	SIG. = .9519	ETA SQD = .0149	

Appendix Table 12.1 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1982 SEAHURST BASELINE SAND DOLLAR SPERM ASSAY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED.

DESCRIPTION OF SUBPOPULATIONS									
CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED	95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED		SUM	MEAN	STD DEV	VARIANCE	N	
VARIABLE		3.52	TO 7.79	1374.0000	5.6543	17.0277	289.9427	(243)
FOR ENTIRE POPULATION									
STATION 1		-1.07	TO 14.36	139.6000	6.6476	16.5966	275.4476	(21)
STATION 2		-2.56	TO 11.46	93.5000	4.4524	15.0456	226.3706	(21)
STATION 3		0.07	TO 4.40	46.9000	2.2333	4.6669	21.7803	(21)
STATION 4		0.05	TO 8.21	123.9000	4.1300	10.7762	116.1263	(30)
STATION 5		-1.26	TO 6.75	82.3000	2.7433	10.5784	111.9019	(30)
STATION 6		-3.07	TO 8.49	81.4000	2.7133	15.2578	232.8012	(30)
STATION 7		-1.61	TO 9.05	111.7000	3.7233	14.0606	197.7005	(30)
STATION 8		3.42	TO 36.22	396.4000	19.8200	34.2552	1173.4164	(20)
STATION 9		-2.41	TO 18.53	161.2000	8.0600	21.8772	478.6109	(20)
STATION 10		-0.13	TO 13.84	137.1000	6.8550	14.6149	213.5942	(20)

TOTAL CASES = 243

ANOVA TABLE									
	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE						
BETWEEN GROUPS	5149.9970	(9)	572.2219						
WITHIN GROUPS	65016.1459	(233)	279.0393						
TOTAL	70166.1430	(242)							
F = 2.0507	SIG. = .0349	ETA SQRD = .0734							

Appendix Table 12.1 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1982 SEAHURST BASELINE OYSTER EMBRYO MORTALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN MORTALITY.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO MORTALITY DEPTH IN METERS	95% CONFIDENCE LIMITS OF THE MEAN MORTALITY	SUM	MEAN	STD DEV	VARIANCE	N
DEPTH	0	16.45 TO 25.67	1769.0000	21.0595	21.0733	444.0858	(84)
DEPTH	10	-12.98 TO 28.94	71.8000	7.9778	25.7195	661.4944	(9)
DEPTH	25	-11.01 TO 29.48	83.1000	9.2333	24.8379	616.9200	(9)
DEPTH	50	3.93 TO 13.82	381.5000	8.8721	15.8476	251.1454	(43)
DEPTH	75	-3.78 TO 15.74	149.5000	5.9800	23.1526	536.0425	(25)
DEPTH	100	3.34 TO 12.27	249.7000	7.8031	12.2162	149.2345	(32)
DEPTH	150	-2.71 TO 18.31	124.8000	7.8000	19.0925	364.5227	(16)
DEPTH	200	-4.32 TO 13.20	31.1000	4.4429	8.7584	76.7095	(7)

TOTAL CASES = 308
MISSING CASES = 21 OR 6.8 PCT.

* * * * * A N O V A T A B L E * * * * *

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	13768.0980	(8)	1721.0123
WITHIN GROUPS	89101.6151	(278)	320.5094
TOTAL	.1029E+06	(286)	

F = 5.3696 SIG. = .0000 ETA SQRD = .1338

Appendix Table 12.1 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1982 SEAHURST BASELINE OYSTER EMBRYO ABNORMALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN ABNORMALITY.

D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO ABNORMAL DEPTH IN METERS	SUM	MEAN	STD DEV	VARIANCE	N
DEPTH 0	-11.47 TO 0.81	-447.9000	-5.3321	28.0271	785.5198	(84)
DEPTH 10	-24.31 TO 10.69	-61.3000	-6.8111	21.4683	460.8861	(9)
DEPTH 25	-32.39 TO 19.26	-59.1000	-6.5667	31.6723	1003.1350	(9)
DEPTH 50	-11.17 TO 2.76	-181.0000	-4.2093	22.3547	499.7342	(43)
DEPTH 75	-6.70 TO 15.26	106.9000	4.2760	26.0723	679.7652	(25)
DEPTH 100	-3.54 TO 9.85	101.0000	3.1562	18.2884	334.4645	(32)
DEPTH 150	-11.39 TO 12.64	10.0000	.6250	21.8250	476.3327	(16)
DEPTH 200	-1.39 TO 1.94	1.9000	.2714	1.6660	2.7757	(7)

TOTAL CASES = 308
MISSING CASES = 21 OR 6.8 PCT.

S U M O F S Q U A R E S D E G R E E S O F F R E E D O M M E A N S Q U A R E

BETWEEN GROUPS	3503.7741	(8)	437.9718
WITHIN GROUPS	.1444E+06	(278)	519.4244
TOTAL	.1479E+06	(286)	

S I G . = .5653 E T A S Q R D = .0237

Appendix Table 12.1 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1982 SEAHURST BASELINE SAND DOLLAR SPERM ASSAY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED.

D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED
BROKEN DOWN BY DEPTH IN METERS

95% CONFIDENCE LIMITS
OF THE MEAN EGGS UNFERTILIZED

VARIABLE	MEAN	STD DEV	VARIANCE	N
DEPTH 0	9.3176	23.6173	557.7786	91)
DEPTH 10	3.1800	6.0196	36.2351	10)
DEPTH 25	3.3300	5.9343	35.2157	10)
DEPTH 50	2.7511	11.1663	124.6860	47)
DEPTH 75	.3370	1.3978	1.9540	27)
DEPTH 100	3.0706	10.8302	117.2924	34)
DEPTH 150	6.7471	12.4215	154.2926	17)
DEPTH 200	14.7857	25.3982	645.0681	7)

TOTAL CASES = 308

S U M O F S Q U A R E S D E G R E E S O F F R E E D O M M E A N S Q U A R E

BETWEEN GROUPS	4981.6843	(8)	622.7105
WITHIN GROUPS	67131.7163	(299)	224.5208
TOTAL	72113.4006	(307)	

S I G . = .0057 E T A S Q R D = .0691

Appendix Table 12.2

BREAKDOWN (SPSS) OF THE 1983 CONTROL AND TEST RESPONSES FOR EACH OF THE RECEIVING WATER BIOASSAY INDICES.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE AVERAGE PERCENT OYSTER EMBRYO MORTALITY

BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN MORTALITY	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	13)
STATION (KIN. CARRY-ALONG)	91.	-9.90 TO 3.15	-43.9000	-3.3769	10.4540	109.2869	(13)
STATION (WEST POINT)	92.	-9.93 TO 10.10	1.2000	.0857	16.8162	282.7859	(14)
STATION (FRIDAY HARBOR)	93.	-7.93 TO 15.08	42.9000	3.5750	17.5147	306.7639	(12)

TOTAL CASES = 81
MISSING CASES = 29 OR 35.8 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	301.7165	(3)	100.5722
WITHIN GROUPS	8362.0627	(48)	174.2096
TOTAL	8663.7792	(51)	

F = .5773 SIG. = .6327 ETA SQRD = .0348

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) OF THE 1983 CONTROL RESPONSES FOR THE OYSTER EMBRYO ABNORMAL INDEX TOGETHER WITH THE 95% CONFIDENCE LIMITS FOR THE MEAN ABNORMALITY.

----- DESCRIPTION OF SUBPOPULATIONS -----

CRITERION VARIABLE AVERAGE PERCENT OYSTER EMBRYO ABNORMAL
BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	13)
STATION (KIN. CARRY-ALONG)	91.	-0.11 TO 2.14	13.2000	1.0154	1.8078	3.2681	(13)
STATION (WEST POINT)	92.	-2.67 TO 1.88	-5.5000	-.3929	3.8337	14.6976	(14)
STATION (FRIDAY HARBOR)	93.	-2.14 TO 9.58	44.6000	3.7167	8.9189	79.5470	(12)

TOTAL CASES = 81
MISSING CASES = 29 OR 35.8 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	128.7254	(3)	42.9085
WITHIN GROUPS	1105.3029	(48)	23.0271
TOTAL	1234.0283	(51)	

F = 1.8634 SIG. = .1484 ETA SQD = .1043

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) OF THE 1983 CONTROL RESPONSES FOR THE SAND DOLLAR SPERM ASSAY INDEX TOGETHER WITH THE 95% CONFIDENCE LIMITS FOR THE MEAN PERCENT EGGS UNFERTILIZED.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED
BROKEN DOWN BY STATION

VARIABLE	CODE	.95% CONFIDENCE LIMITS OF THE MEAN UNFERTILIZED		SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	0	(18)
STATION (KIN. CARRY-ALONG)	91.	-0.95 TO	0.69	-2.4000	-.1333	1.6066	2.5812	(18)
STATION (WEST POINT)	92.	-1.54 TO	1.68	1.3000	.0684	3.2563	10.6034	(19)
STATION (FRIDAY HARBOR)	93.	0.20 TO	6.67	61.9000	3.4389	6.3487	40.3060	(18)

TOTAL CASES = 81
MISSING CASES = 8 OR 9.9 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	162.6373	(3)	54.2124
WITHIN GROUPS	919.9438	(69)	13.3325
TOTAL	1082.5811	(72)	

F = 4.0662 SIG. = .0102 ETA SQD = .1502

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1983 SEAHURST BASELINE OYSTER EMBRYO MORTALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN MORTALITY.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO MORTALITY	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION	1.77 TO 6.87	945.7000	4.3183	19.2487	370.5115	(219)
STATION 1	-4.59 TO 17.55	58.3000	6.4778	13.5706	184.1619	(9)
STATION 2	0.05 TO 11.86	53.6000	5.9556	7.2397	52.4128	(9)
STATION 3	-10.61 TO 11.34	3.3000	.3667	13.4601	181.1750	(9)
STATION 4	-6.37 TO 9.88	57.8000	1.7515	22.5557	508.7595	(33)
STATION 5	0.52 TO 14.04	138.4000	7.2842	13.6881	187.3636	(19)
STATION 6	-3.79 TO 10.10	104.1000	3.1545	19.2779	371.6388	(33)
STATION 7	-2.61 TO 11.48	146.5000	4.4394	19.5877	383.6768	(33)
STATION 8	0.41 TO 16.00	114.9000	8.2071	13.0262	169.6807	(14)
STATION 9	-2.10 TO 26.06	167.7000	11.9786	23.5129	552.8572	(14)
STATION 10	-2.93 TO 19.88	186.5000	8.4773	25.2026	635.1733	(22)
STATION 12	-16.69 TO 9.89	-40.8000	-3.4000	20.0159	400.6364	(12)
STATION 13	-14.55 TO 7.11	-44.6000	-3.7167	16.3156	266.1997	(12)

TOTAL CASES = 309
MISSING CASES = 90 OR 29.1 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	3539.7327	(11)	321.7939
WITHIN GROUPS	77231.7742	(207)	373.1004
TOTAL	80771.5069	(218)	

F = .8625 SIG. = .5780 ETA SQRD = .0438

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1983 SEAHURST BASELINE OYSTER EMBRYO ABNORMALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN ABNORMALITY.

DESCRIPTION OF SUBPOPULATIONS												
CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO ABNORMAL	95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL		SUM	MEAN	STD DEV	VARIANCE					
VARIABLE		-0.44	TO 2.89									
FOR ENTIRE POPULATION				268.7000	1.2269	12.5187	156.7188	(219)			
STATION 1		-0.70	TO 0.17	-2.4000	-.2667	.5500	.3025	(9)			
STATION 2		-0.76	TO 1.45	3.1000	.3444	1.3593	1.8478	(9)			
STATION 3		-0.49	TO 0.98	2.2000	.2444	.9015	.8128	(9)			
STATION 4		-3.76	TO 9.23	90.4000	2.7394	18.0384	325.3831	(33)			
STATION 5		-2.43	TO 1.59	-7.9000	-.4158	4.0833	16.6736	(19)			
STATION 6		-3.06	TO 14.69	191.9000	5.8152	24.6733	608.7695	(33)			
STATION 7		-2.47	TO 0.34	-35.2000	-1.0667	3.9106	15.2929	(33)			
STATION 8		-2.59	TO 1.26	-9.3000	-.6643	3.1977	10.2255	(14)			
STATION 9		-1.78	TO 2.50	5.0000	.3571	3.5606	12.6780	(14)			
STATION 10		-2.04	TO 5.75	40.8000	1.8545	8.6154	74.2255	(22)			
STATION 12		-4.10	TO 2.90	-7.2000	-.6000	5.2783	27.8600	(12)			
STATION 13		-2.51	TO 2.06	-2.7000	-.2250	3.4396	11.8311	(12)			

TOTAL CASES = 309
MISSING CASES = 90 OR 29.1 PCT.

ANOV A T A B L E												
		SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE								
BETWEEN GROUPS	(11)	1165.5233		105.9567								
WITHIN GROUPS	(207)	32999.1677		159.4163								
TOTAL	(218)	34164.6911										
F =	.6647	SIG. = .7709	ETA SQRD = .0341									

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1983 SEAHURST BASELINE SAND DOLLAR SPERM ASSAY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED.

DESCRIPTION OF SUBPOPULATIONS												
CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED	95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED		SUM	MEAN	STD DEV	VARIANCE	N				
VARIABLE	0.78 TO	3.44	594.9000	2.1096	11.3729	129.3439	(282)				
STATION 1	-1.16 TO	3.21	18.5000	1.0278	4.2718	18.2480	(18)				
STATION 2	-0.71 TO	0.89	1.6000	.0889	1.5718	2.4705	(18)				
STATION 3	-5.90 TO	13.51	68.5000	3.8056	19.0447	362.6994	(18)				
STATION 4	-2.05 TO	12.03	194.6000	4.9897	21.4796	461.3730	(39)				
STATION 5	-0.12 TO	4.25	43.3000	2.0619	4.6937	22.0305	(21)				
STATION 6	-0.50 TO	2.86	45.9000	1.1769	5.1258	26.2739	(39)				
STATION 7	0.00 TO	1.38	26.9000	.6897	2.1072	4.4404	(39)				
STATION 8	-0.30 TO	5.23	34.5000	2.4643	4.6095	21.2471	(14)				
STATION 9	-1.38 TO	5.48	28.7000	2.0500	5.7240	32.7642	(14)				
STATION 10	-3.38 TO	11.92	111.0000	4.2692	18.5971	345.8526	(26)				
STATION 12	-1.66 TO	1.53	-1.2000	-0.0667	3.1158	9.7082	(18)				
STATION 13	-1.12 TO	3.63	22.6000	1.2556	4.6664	21.7756	(18)				

TOTAL CASES = 309
MISSING CASES = 27 OR 8.7 PCT.

ANOVA TABLE												
SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE										
BETWEEN GROUPS	803.9033	(11)	73.0821									
WITHIN GROUPS	35541.7209	(270)	131.6360									
TOTAL	36345.6241	(281)										
F = .5552	SIG. = .8639	ETA SQRD = .0221										

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1983 SFAHURST BASELINE OYSTER EMBRYO MORTALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN MORTALITY.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO MORTALITY		SUM	MEAN	STD DEV	VARIANCE	N
VARIABLE	95% CONFIDENCE LIMITS OF THE MEAN MORTALITY						
DEPTH 0	7.38	TO 16.87	1027.1000	12.5256	23.2563	540.8558	(82)
DEPTH 10	-8.34	TO 10.98	14.5000	1.3182	13.7771	189.8096	(11)
DEPTH 25	-6.67	TO 12.16	19.2000	2.7429	9.4407	89.1262	(7)
DEPTH 40	-38.02	TO 24.47	-27.1000	-6.7750	17.0099	289.3358	(4)
DEPTH 50	-3.16	TO 6.24	60.1000	1.5410	14.2762	203.8088	(39)
DEPTH 60	-30.83	TO 11.18	-39.3000	-9.8250	11.4351	130.7625	(4)
DEPTH 75	-7.91	TO 5.17	-34.2000	-1.3680	15.5074	240.4781	(25)
DEPTH 100	-2.88	TO 5.32	31.7000	1.2192	9.9382	98.7672	(26)
DEPTH 150	-14.48	TO 10.40	-28.6000	-2.0429	20.7830	431.9349	(14)
DEPTH 200	-24.90	TO 2.70	-77.7000	-11.1000	13.8250	191.1300	(7)

TOTAL CASES = 390
MISSING CASES = 119 OR 30.5 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	11303.9277	(10)	1130.3928
WITHIN GROUPS	78913.5674	(260)	303.5137
TOTAL	90217.4951	(270)	

F = 3.7244 SIG. = .0001 ETA SQRD = .1253

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1983 SEAHURST BASELINE OYSTER EMBRYO ABNORMALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL.

D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT OYSTER EMBRYO ABNORMAL DEPTH IN METERS	95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL		SUM	MEAN	STD DEV	VARIANCE	N
DEPTH	0	-1.23	TO 4.35	127.7000	1.5573	12.5892	158.4889	(82)
DEPTH	10	-2.51	TO 1.51	-5.5000	-.5000	2.8425	8.0800	(11)
DEPTH	25	-2.19	TO 1.19	-3.5000	-.5000	1.6803	2.8233	(7)
DEPTH	40	-4.99	TO 5.44	.9000	.2250	2.8430	8.0825	(4)
DEPTH	50	-2.09	TO 0.06	-39.6000	-1.0154	3.2693	10.6882	(39)
DEPTH	60	-9.23	TO 9.48	.5000	.1250	5.0947	25.9558	(4)
DEPTH	75	-2.27	TO 0.86	-17.6000	-.7040	3.7351	13.9512	(25)
DEPTH	100	-4.03	TO 12.24	106.7000	4.1038	19.7447	389.8540	(26)
DEPTH	150	-8.69	TO 23.59	104.3000	7.4500	26.9224	724.8135	(14)
DEPTH	200	-4.19	TO 2.71	-5.2000	-.7429	3.4640	11.9995	(7)

TOTAL CASES = 390
MISSING CASES = 119 OR 30.5 PCT.

T A B L E

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	1147.3889	(10)	114.7389
WITHIN GROUPS	34253.3860	(260)	131.7438
TOTAL	35400.7749	(270)	

F = .8709 SIG. = .5610 ETA SQD = .0324

Appendix Table 12.2 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1983 SFAHURST BASELINE SAND DOLLAR EGGS UNFERTILIZED RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED.

D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED
BROKEN DOWN BY DEPTH IN METERS

VARIABLE	95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED	SUM	MEAN	STD DEV	VARIANCE	N
DEPTH 0	0.93 TO 7.05	411.3000	3.9932	15.5416	241.5404	(103)
DEPTH 10	-0.95 TO 0.79	-1.0000	-.0769	1.4037	1.9703	(13)
DEPTH 25	-2.43 TO 5.69	11.4000	1.6286	4.0742	16.5990	(7)
DEPTH 40	-5.97 TO 10.33	13.1000	2.1833	7.0881	50.2417	(6)
DEPTH 50	-0.18 TO 1.43	28.6000	.6217	2.6995	7.2871	(46)
DEPTH 60	-3.07 TO 3.41	1.0000	.1667	2.8147	7.9227	(6)
DEPTH 75	-1.10 TO 0.37	-11.8000	-.3688	2.0104	4.0416	(32)
DEPTH 100	-0.56 TO 2.56	37.9000	.9974	4.7106	22.1895	(38)
DEPTH 150	-6.07 TO 15.74	91.9000	4.8368	22.0239	485.0513	(19)
DEPTH 200	-2.63 TO 4.72	12.5000	1.0417	5.5351	30.6372	(12)

TOTAL CASES = 390
MISSING CASES = 35 OR 9.0 PCT.

T A B L E * * * * *

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	1046.8089	(10)	104.6809
WITHIN GROUPS	36475.9155	(344)	106.0346
TOTAL	37522.7244	(354)	

F = .9872 SIG. = .4541 ETA SQRD = .0279

Appendix Table 12.3

BREAKDOWN (SPSS) OF THE 1982 AND 1983 (COMBINED) CONTROL AND TEST RESPONSES FOR EACH OF THE RECEIVING WATER BIOASSAY INDICES.

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----- D E S C R I P T I O N   O F   S U B P O P U L A T I O N S -----
CRITERION VARIABLE   AVERAGE PERCENT OYSTER EMBRYO MORTALITY
BROKEN DOWN BY     STATION
-----
VARIABLE           CODE      95%   CONFIDENCE LIMITS      SUM      MEAN      STD DEV      VARIANCE      N
                   90.      0      OF THE MEAN MORTALITY
STATION (KINCAID LAB)  90.      0      0      0      0      0      0      0      ( 29)
STATION (KIN. CARRY-ALONG) 91.     -4.41 TO 3.24     -17.0000     -1.5862     9.8765     97.5448     ( 29)
STATION (WEST POINT)  92.      0.02 TO 12.52     188.2000     6.2733     16.4795     271.5724     ( 30)
STATION (FRIDAY HARBOR) 93.     -4.58 TO 7.72     40.8000     1.5692     14.9312     222.9406     ( 26)

```

TOTAL CASES = 146
MISSING CASES = 32 OR 21.9 PCT.

```

***** ANOVA TABLE *****
SUM OF SQUARES      DEGREES OF FREEDOM      MEAN SQUARE
*****
BETWEEN GROUPS      860.3859      ( 3)      286.7953
WITHIN GROUPS      16180.3685     ( 110)     147.0943
TOTAL              17040.7544     ( 113)
*****
F = 1.9497      SIG. = .1258      ETA SQD = .0505
*****

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Appendix Table 12.3 (continued).

BREAKDOWN (SPSS) OF THE 1982 + 1983 COMBINED CONTROL RESPONSES FOR THE OYSTER ABNORMAL INDEX TOGETHER WITH THE 95% CONFIDENCE LIMITS FOR THE MEAN ABNORMALITY

----- DESCRIPTION OF SUBPOPULATIONS -----
 CRITERION VARIABLE AVERAGE PERCENT OYSTER EMBRYO ABNORMAL
 BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	29)
STATION (KIN. CARRY-ALONG)	91.	-3.47 TO 1.97	-21.7000	-.7483	7.0477	49.6697	(29)
STATION (WEST POINT)	92.	-6.00 TO 4.09	-28.6000	-.9533	13.3196	177.4129	(30)
STATION (FRIDAY HARBOR)	93.	-10.02 TO 4.37	-73.4000	-2.8231	17.5000	306.2490	(26)

TOTAL CASES = 146
 MISSING CASES = 32 OR 21.9 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	116.4914	(3)	38.8305
WITHIN GROUPS	14191.9532	(110)	129.0178
TOTAL	14308.4446	(113)	

F = .3010 SIG. = .8246 ETA SQD = .0081

Appendix Table 12.3 (continued).

BREAKDOWN (SPSS) OF THE 1982 + 1983 COMBINED CONTROL RESPONSES FOR THE SAND DOLLAR SPERM ASSAY INDEX TOGETHER WITH THE 95% CONFIDENCE LIMITS FOR THE MEAN PERCENT EGGS UNFERTILIZED.

D E S C R I P T I O N O F S U B P O P U L A T I O N S

CRITERION VARIABLE AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED
BROKEN DOWN BY STATION

VARIABLE	CODE	95% CONFIDENCE LIMITS OF THE MEAN UNFERTILIZED	SUM	MEAN	STD DEV	VARIANCE	N
STATION (KINCAID LAB)	90.	0	0	0	0	0	35)
STATION (KIN. CARRY-ALONG)	91.	-0.44 TO 1.14	12.3000	.3514	2.2732	5.1673	(35)
STATION (WEST POINT)	92.	-0.89 TO 0.81	-1.5000	-.0417	2.4621	6.0619	(36)
STATION (FRIDAY HARBOR)	93.	-0.53 TO 3.55	48.3000	1.5094	5.5666	30.9873	(32)

TOTAL CASES = 146
MISSING CASES = 8 OR 5.5 PCT.

***** ANOVA TABLE *****

SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS 51.9777	(3)	17.3259
WITHIN GROUPS 1348.4621	(134)	10.0632
TOTAL 1400.4398	(137)	

F = 1.7217 SIG. = .1655 ETA SQD = .0371

Appendix Table 12.3 (continued).

BREAKDOWN (SPSS) BY STATION OF THE 1982 + 1983 COMBINED SEAHURST BASELINE SAND DOLLAR EGGS UNFERTILIZED RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED.

DESCRIPTION OF SUBPOPULATIONS												
CRITERION VARIABLE BROKEN DOWN BY STATION	AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED	95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED		SUM	MEAN	STD DEV	VARIANCE					
VARIABLE	FOR ENTIRE POPULATION	2.52	TO 4.99	1968.9000	3.7503	14.3665	206.3967					
STATION 1		-0.11	TO 8.22	158.1000	4.0538	12.6961	161.1915					
STATION 2		-1.22	TO 6.10	95.1000	2.4385	11.1850	125.1040					
STATION 3		-1.37	TO 7.29	115.4000	2.9590	13.2043	174.3541					
STATION 4		0.36	TO 8.87	318.5000	4.6159	17.5367	307.5349					
STATION 5		0.03	TO 4.89	125.6000	2.4627	8.5924	73.8300					
STATION 6		-0.75	TO 4.44	127.3000	1.8449	10.7030	114.5540					
STATION 7		-0.27	TO 4.29	138.6000	2.0087	9.4387	89.0896					
STATION 8		2.91	TO 22.44	430.9000	12.6735	27.5526	759.1444					
STATION 9		-0.52	TO 11.69	189.9000	5.5853	17.2478	297.4849					
STATION 10		0.34	TO 10.45	248.1000	5.3935	16.8524	284.0042					
STATION 12		-1.66	TO 1.53	-1.2000	-0.0667	3.1158	9.7082					
STATION 13		-1.12	TO 3.63	22.6000	1.2556	4.6664	21.7756					

TOTAL CASES = 552
MISSING CASES = 27 OR 4.9 PCT.

ANOVA TABLE												
		SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE								
BETWEEN GROUPS		4011.3504	(11)	364.6682								
WITHIN GROUPS		.1041E+06	(513)	203.0029								
TOTAL		.1082E+06	(524)									
F = 1.7964		SIG. = .0518	ETA SQD = .0371									

Appendix Table 12.3 (continued)

BREAKDOWN (SPSS) BY DEPTH OF THE 1982 + 1983 COMBINED SEAHURST BASELINE OYSTER EMBRYO MORTALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN MORTALITY.

```

----- D E S C R I P T I O N   O F   S U B P O P U L A T I O N S -----
CRITERION VARIABLE   AVERAGE PERCENT OYSTER EMBRYO MORTALITY
BROKEN DOWN BY     DEPTH IN METERS
-----
VARIABLE             95% CONFIDENCE LIMITS
                   OF THE MEAN MORTALITY
                   SUM             MEAN             STD DEV             VARIANCE             N
DEPTH 0             13.41 TO 20.28             2796.1000             16.8440             22.5214             ( 166)
DEPTH 10            -5.13 TO 13.76             86.3000              4.3150             19.7479             ( 20)
DEPTH 25            -4.21 TO 16.99             102.3000             6.3937             19.3838             ( 16)
DEPTH 40            -38.02 TO 24.47             -27.1000             -6.7750             17.0099             ( 4)
DEPTH 50             1.96 TO 8.81              441.6000             5.3854             15.4728             ( 82)
DEPTH 60            -30.83 TO 11.18           -39.3000             -9.8250             11.4351             ( 4)
DEPTH 75            -3.40 TO 8.01             115.3000             2.3060             19.8522             ( 50)
DEPTH 100           1.77 TO 7.93             281.4000             4.8517             11.6357             ( 58)
DEPTH 150           -4.45 TO 10.86           96.2000              3.2067             20.1771             ( 30)
DEPTH 200          -11.50 TO 4.84           -46.6000             -3.3286             13.7353             ( 14)

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TOTAL CASES = 698
MISSING CASES = 140 OR 20.1 PCT.

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***** ANOVA TABLE *****
SUM OF SQUARES   DEGREES OF FREEDOM   MEAN SQUARE
BETWEEN GROUPS   24625.4434   ( 10)   2462.5443
WITHIN GROUPS    .1757E+06   ( 547)   321.2369
TOTAL            .2003E+06   ( 557)
F = 7.6658   SIG. = .0000   ETA SQD = .1229

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Appendix Table 12.3 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1982 + 1983 COMBINED SEAHURST BASELINE OYSTER EMBRYO ABNORMALITY RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL.

CRITERION VARIABLE		Average percent oyster abnormal		BROKEN DOWN BY DEPTH IN METERS		95% CONFIDENCE LIMITS OF THE MEAN ABNORMAL		SUM	MEAN	STD DEV	VARIANCE	N
VARIABLE												
DEPTH	0	-5.28	TD	1.42		-320.2000		-1.9289	22.0200	484.8802	(166)
DEPTH	10	-10.27	TD	3.61		-66.8000		-3.3400	14.4460	208.6867	(20)
DEPTH	25	-16.76	TD	8.94		-62.6000		-3.9125	23.3623	545.7958	(16)
DEPTH	40	-4.99	TD	5.44		.9000		.2250	2.8430	8.0825	(4)
DEPTH	50	-6.30	TD	0.92		-220.6000		-2.6902	16.3313	266.7113	(82)
DEPTH	60	-9.23	TD	9.48		.5000		.1250	5.0947	25.9558	(4)
DEPTH	75	-3.56	TD	7.13		89.3000		1.7860	18.6039	346.1061	(50)
DEPTH	100	-1.40	TD	8.56		207.7000		3.5810	18.7914	353.1163	(58)
DEPTH	150	-5.34	TD	12.96		114.3000		3.8100	24.1514	583.2885	(30)
DEPTH	200	-1.83	TD	1.36		-3.3000		-.2357	2.6639	7.0963	(14)

TOTAL CASES = 698
MISSING CASES = 140 OR 20.1 PCT.

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	2888.2163	(10)	288.8216
WITHIN GROUPS	.1823E+06	(547)	333.2100
TOTAL	.1852E+06	(557)	

F = .8668 SIG. = .5644 ETA SQRD = .0156

Appendix Table 12.3 (continued).

BREAKDOWN (SPSS) BY DEPTH OF THE 1982 + 1983 COMBINED SEAHURST BASELINE SAND DOLLAR EGGS UNFERTILIZED RESPONSES TOGETHER WITH THE 95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED.

----- D E S C R I P T I O N O F S U B P O P U L A T I O N S -----

CRITERION VARIABLE BROKEN DOWN BY	AVERAGE PERCENT SAND DOLLAR EGGS UNFERTILIZED DEPTH IN METERS	95% CONFIDENCE LIMITS OF THE MEAN EGGS UNFERTILIZED				SUM	MEAN	STD DEV	VARIANCE	N
DEPTH 0	3.69 TO 9.29	1259.2000	6.4907	19.8709	394.8542	(194)				
DEPTH 10	-0.56 TO 3.24	30.8000	1.3391	4.3155	18.6234	(29)				
DEPTH 25	-0.11 TO 5.36	44.7000	2.6294	5.1748	26.7785	(17)				
DEPTH 40	-5.97 TO 10.33	13.1000	2.1833	7.0881	50.2417	(6)				
DEPTH 50	0.01 TO 3.39	157.9000	1.6978	8.1886	67.0530	(93)				
DEPTH 60	-3.07 TO 3.41	1.0000	.1667	2.8147	7.9227	(6)				
DEPTH 75	-0.51 TO 0.41	-2.7000	-.0458	1.7782	3.1618	(59)				
DEPTH 100	0.04 TO 3.91	142.3000	1.9764	8.1955	67.1661	(72)				
DEPTH 150	-0.41 TO 11.89	206.6000	5.7389	17.9144	320.9242	(36)				
DEPTH 200	-2.16 TO 14.37	116.0000	6.1053	16.7374	280.1416	(19)				

TOTAL CASES = 698
MISSING CASES = 35 OR 5.0 PCT.

***** ANOVA TABLE *****

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE
BETWEEN GROUPS	4626.0002	(10)	462.6000
WITHIN GROUPS	.1061E+06	(652)	162.7795
TOTAL	.1108E+06	(662)	

F = 2.8419 SIG. = .0018 ETA SQD = .0418

Appendix Table 12.4. Calculated EC50's and 95% fiducial limits for the Renton sewage bioassays.

Test	EC50 (% sewage in seawater)	95% fiducial limits
<u>Summer 1982</u>		
Sand Dollar Sperm:		
Influent	1.88	1.70-2.13
Primary	2.90	2.60-3.28
Secondary	17.62	14.72-21.35
Chlorinated Sec.	1.02	0.83-1.26
Dechlorinated Sec.	7.13	6.42-7.97
Freshwater	>20	---
Oyster Embryo Mortality:		
Influent	5.68	4.34-7.80
Primary	7.31	5.67-9.78
Secondary	>20	---
Chlorinated Sec.	2.26	2.48-4.58
Dechlorinated Sec.	>20	---
Freshwater	>20	---
Oyster Embryo Abnormal:		
Influent	4.21	3.30-5.63
Primary	5.90	5.41-6.46
Secondary	>20	---
Chlorinated Sec.	2.26	2.11-2.43
Dechlorinated Sec.	>20	---
Freshwater	>20	---
<u>Winter 1983</u>		
Green Urchin Sperm:		
Primary	10.54	9.89-11.26
Secondary	>20	---
Chlorinated Sec.	0.43	0.41-0.46
Dechlorinated Sec.	>20	---
Freshwater	>20	---
Green Urchin Embryo Mortality:		
Primary	>20	---
Secondary	>20	---
Chlorinated Sec.	>20	---
Dechlorinated Sec.	>20	---
Freshwater	>20	---

Appendix Table 12.4 (continued).

Test	EC50 (% sewage in seawater)	95% fiducial limits
<u>Green Urchin Embryo Abnormal:</u>		
Primary	15.85	14.97-16.80
Secondary	18.08	17.19-19.01
Chlorinated	8.67	6.57-11.92
Dechlorinated Sec.	17.40	---
Freshwater	>20	---
<u>Dungeness Crab Zoea Mortality:</u>		
Primary	>20	---
Secondary	>20	---
Chlorinated Sec.	>20	---
Dechlorinated Sec.	>20	---
Freshwater	>20	---
<u>Summer 1983</u>		
<u>Sand Dollar Sperm:</u>		
Influent	12.95	9.71-17.26
Primary	10.71	8.45-13.57
Secondary	>20	---
Chlorinated Sec.	2.26	2.06-2.48
Dechlorinated Sec.	>20	---
<u>Winter 1984</u>		
<u>Green Urchin Sperm:</u>		
Influent	10.54	9.86-11.28
Primary	12.63	11.73-13.59
Secondary	>20	---
Chlorinated Sec.	0.85	0.41-1.78
Dechlorinated Sec.	>20	---

Appendix Table 12.5. Total ammonia, pH, and total residual chlorine measured in the bioassay sewage samples during summer, 1982. Dashes mean not measured.

Sample Date	pH			Total Ammonia (mg/l)			Residual chlorine (mg/l)			
	Inf	Pri	Sec	Inf	Pri	Sec	Cl	DeCl	DeCl	
21 July	6.7	7.0	7.2	7.0	-	-	-	-	1.68	0.05
23 July	6.9	7.0	7.2	7.1	-	-	-	-	1.62	0.11
30 July	6.8	6.8	7.2	7.0	-	-	-	-	1.73	0.08
4 Aug	6.8	7.1	7.4	7.3	24.3	15.2	14.8	14.8	1.44	0.07
6 Aug	7.2	7.3	7.4	7.3	19.9	12.7	14.8	10.0	1.59	0.13
13 Aug	7.0	7.2	7.1	7.2	12.3	6.8	7.2	6.7	1.82	0.00
3 Sept	6.8	6.9	7.1	7.0	23.8	13.8	14.0	13.9	1.67	0.02
15 Sept	6.9	7.1	7.3	7.4	15.4	10.0	10.2	10.1	1.76	0.06
20 Sept	6.6	6.8	7.2	7.3	17.1	12.8	13.3	13.5	1.63	0.07
28 Sept	6.7	6.7	7.1	7.0	16.2	10.2	10.3	10.7	1.61	0.00
Average:	6.84	6.99	7.22	7.10	18.4	11.6	12.1	11.4	1.655	0.059
Standard Deviation:	0.17	0.19	0.11	0.16	4.5	2.8	2.9	2.8	0.105	0.043
Range:	6.7-7.2	6.7-7.3	7.1-7.4	6.9-7.4	12.3-23.8	6.8-15.2	7.2-14.8	6.7-14.8	1.44-1.82	0-0.13

Appendix Table 12.6 Total ammonia, pH, and total residual chlorine measured in the bioassay sewage samples during winter, 1983. Dashes mean not measured.

Sample Date	pH			Total Ammonia (mg/l)			Residual chlorine (mg/l)		
	Pri	Sec	DeCl	Pri	Sec	DeCl	CI	DeCl	DeCl
7 Feb	7.23	7.11	7.14	-	-	-	2.07	-	0.00
9 Feb	7.06	7.01	6.99	13.7	12.2	12.0	1.97	12.5	0.00
10 Feb	7.16	7.08	7.05	12.8	10.2	10.5	1.84	11.0	0.00
11 Feb	6.59	6.97	7.02	12.3	11.2	11.4	1.96	11.8	0.00
12 Feb	7.10	7.10	7.08	14.3	10.2	9.9	1.94	10.3	0.00
14 Feb	6.99	7.13	7.11	12.2	11.7	11.6	-	12.2	-
15 Feb	7.11	7.02	7.01	12.8	10.7	10.7	1.99	11.2	0.00
16 Feb	7.09	7.02	6.99	12.0	10.1	10.1	2.44	10.5	0.00
17 Feb	7.05	6.98	7.00	11.7	10.4	10.0	1.90	11.2	0.00
18 Feb	6.70	6.99	6.98	10.4	9.4	9.3	1.90	9.7	0.00
Average:	7.01	7.04	7.04	12.5	10.7	10.6	2.001	11.2	0.000
Standard Deviation:	0.20	0.06	0.06	1.1	0.9	0.9	1.18	0.9	0.0
Range:	6.59-7.23	6.97-7.13	6.98-7.4	10.4-14.3	9.4-12.2	9.3-12.0	1.84-2.44	9.7-12.5	-

Appendix Table 12.7. Total ammonia, pH, and total residual chlorine measured in the bioassay sewage samples during summer, 1983. Dashes mean not measured.

Sample Date	pH				Total Ammonia (mg/l)				Residual chlorine (mg/l)			
	Inf	Pri	Sec	Cl	DeCl	Inf	Pri	Sec	Cl	DeCl	Cl	DeCl
12 Sept	6.72	6.80	6.84	6.89	7.02	27.0	24.6	22.3	22.2	-		
13 Sept	6.95	6.87	7.00	6.95	7.12	19.0	17.0	17.1	18.1	19.7		
15 Sept	6.81	6.70	6.89	6.98	7.02	19.6	18.9	18.8	18.6	18.8		
19 Sept	6.85	7.00	7.05	6.88	7.15	25.1	24.9	23.5	21.2	18.0		
20 Sept	7.0	6.81	6.81	6.79	6.79	22.0	20.0	17.9	18.2	19.1		
22 Sept	6.86	6.82	6.93	6.90	6.92	24.7	21.3	20.1	18.5	17.0		
23 Sept	6.64	6.59	6.88	6.82	6.88	24.3	20.2	19.6	18.1	18.3		
26 Sept	6.65	6.78	6.90	6.86	6.88	24.5	24.7	23.6	22.8	22.6		
27 Sept	6.82	6.82	6.89	6.84	6.82	25.0	22.2	23.1	21.3	20.9		
29 Sept	6.90	6.80	6.80	6.85	6.80	21.1	23.7	19.0	18.5	-		
Average:	6.82	6.79	6.90	6.88	6.94	23.2	21.8	20.5	19.8	19.3		
Standard Deviation:	0.12	0.10	0.08	0.06	0.13	2.6	2.7	2.4	1.9	1.8		
Range:	6.64-7.00	6.59-7.00	6.80-7.05	6.79-6.98	6.79-7.15	19.0-27.0	17.0-24.9	17.1-23.6	18.1-22.8	17.0-22.6		

Broken buret tip
No measurements

Appendix Table 12.8. Total ammonia, pH, and total residual chlorine measured in the bioassay sewage samples during winter, 1984. Dashes mean not measured.

Sample Date	pH				Total Ammonia (mg/liter)				Residual chlorine (mg/l)		
	Inf	Pri	Sec	DeCl	Inf	Pri	Sec	Cl	DeCl	Cl	DeCl
21 Feb	6.9	7.2	6.7	6.7	-	-	-	-	-	-	-
22 Feb	6.7	6.6	6.7	6.6	-	-	-	-	-	-	-
23 Feb	6.6	6.6	6.7	6.7	13.5	9.7	8.7	8.5	8.6	-	-
7 March	7.0	7.0	7.2	7.0	16.7	13.0	11.3	10.9	10.8	0.90	0.00
8 March	6.8	6.7	6.8	6.8	17.2	13.1	10.3	10.4	10.4	1.06	0.00
9 March	6.8	6.4	6.9	6.8	15.7	13.5	10.7	10.2	10.3	0.83	0.00
12 March	6.8	6.9	7.0	6.8	16.3	12.8	12.6	12.3	12.0	0.97	0.00
13 March	7.0	7.2	7.0	6.9	16.2	11.7	9.6	9.9	9.1	1.04	0.00
14 March	7.0	7.2	7.1	7.0	16.8	13.8	11.4	11.4	11.3	0.89	0.04
15 March	6.8	7.0	6.8	6.8	15.0	11.8	8.8	8.8	8.7	0.88	0.00
Average:	6.84	6.88	6.89	6.84	15.9	12.4	10.4	10.3	10.2	0.94	<0.01
Standard Deviation:	0.13	0.29	0.18	0.14	1.2	1.3	1.4	1.3	1.2	0.09	0.01
Range:	6.6-7.0	6.4-7.2	6.7-7.2	6.6-7.0	13.5-17.2	9.7-13.8	8.7-12.6	8.5-12.3	8.6-12.0	0.83-1.06	0.00-0.04

Appendix Table 12.9. Summer, 1982 sewage bioassay control water salinity, pH, and temperature. Dashes mean not measured.

Date	West Pt. Control Seawater			Kincaid Control Seawater	
	Salinity ‰	pH	Temp. °C	Salinity ‰	pH
21 July	28.99	7.4	12.4	29.71	7.3
23 July	28.18	8.0	12.3	29.71	7.9
30 July	29.14	7.6	13.6	29.71	8.6
4 Aug	29.35	-	13.0	29.71	7.8
6 Aug	29.37	7.6	12.3	29.71	7.6
13 Aug	29.45	7.7	12.4	29.71	7.5
3 Sept	29.77	7.4	12.4	29.71	8.7
15 Sept	29.86	7.5	12.8	29.71	7.3
20 Sept	29.97	7.6	12.3	29.71	8.3
28 Sept	29.80	-	12.4	29.71	7.4
Average:	29.49	7.62	12.59	29.71	7.84
Standard Deviation:	0.34	0.18	0.43	0	0.53
Range:	28.99-29.97	7.4-8.0	12.3-13.6	0	7.3-8.7

Appendix Table 12.10. Winter, 1983 sewage bioassay control water salinity, pH, and temperature.

Date	West Pt. Control Seawater			Kincaid Control Seawater	
	Salinity ‰	pH	Temp. °C	Salinity ‰	pH
7 Feb	28.48	8.00	8.7	29.71	7.92
9 Feb	28.71	7.98	8.9	29.71	8.12
10 Feb	28.85	7.97	8.8	29.71	7.98
11 Feb	28.86	7.90	9.1	29.71	7.91
12 Feb	28.85	7.94	8.9	29.71	8.11
14 Feb	28.88	7.91	9.0	29.71	7.90
15 Feb	28.91	7.95	9.0	29.71	7.98
16 Feb	28.96	7.98	8.8	29.71	8.04
17 Feb	28.96	7.98	8.9	29.71	7.93
18 Feb	28.81	7.96	8.9	29.71	8.22
Average:	28.83	7.96	8.9	29.71	8.01
Standard Deviation:	0.14	0.03	0.1	0	0.11
Range:	28.48-28.96	7.90-8.00	8.7-9.1	0	7.90-8.22

Appendix Table 12.11. Summer, 1983 sewage bioassay control water salinity, pH, and temperature.

Date	West Pt. Control Seawater			Kincaid Control Seawater	
	Salinity ‰	pH	Temp. °C	Salinity ‰	pH
12 Sept	29.74	8.15	13.4	29.59	8.31
13 Sept	29.73	8.25	13.4	29.59	8.22
15 Sept	29.78	8.00	13.3	29.59	8.11
19 Sept	28.82	8.03	13.7	29.59	8.02
20 Sept	29.52	8.03	13.5	29.59	8.05
22 Sept	29.36	8.05	13.6	29.59	8.00
23 Sept	29.46	8.11	13.8	29.59	7.98
26 Sept	29.67	8.09	13.2	29.59	9.12
27 Sept	29.79	8.08	13.3	29.59	8.03
29 Sept	29.84	8.06	13.0	29.59	8.23
Average:	29.57	8.08	13.4	29.59	8.21
Standard Deviation:	0.31	0.07	0.2	0	0.34
Range:	28.82-29.84	8.00-8.25	13.0-13.8	0	7.98-9.12

Appendix Table 12.12. Winter, 1984 sewage bioassay control water salinity, pH, and temperature.

Date	West Pt. Control Water			Kincaid Control Water	
	Salinity ‰	pH	Temp. °C	Salinity ‰	pH
21 Feb	29.3	8.0	9.0	29.59	7.8
22 Feb	29.2	7.9	8.9	29.59	7.8
23 Feb	29.1	7.9	8.8	29.59	7.7
7 March	28.1	8.0	9.1	29.59	7.8
8 March	28.5	7.9	9.3	29.59	8.0
9 March	28.5	8.0	9.5	29.59	9.2
12 March	29.6	7.9	9.1	29.59	7.8
13 March	28.7	7.8	9.0	29.59	7.8
14 March	28.7	7.9	9.1	29.59	7.6
15 March	29.1	7.8	9.0	29.59	7.4
Average:	28.88	7.91	9.08	29.59	7.89
Standard Deviation:	0.45	0.07	0.20	0.00	0.47
Range:	28.1-29.6	7.8-8.0	8.8-9.5	0	7.4-9.2

Appendix Table 12.13 Sediment size classes used in grain size analyses.

Class name*	Metric units	Phi (ϕ) units
Gravel	(millimeters)	
Very coarse	64-32	-5
Coarse	32-16	-4
Medium	16-8	-3
Fine	8-4	-2
Very fine	4-2	-1
Sand	(microns)	
Very coarse	2000-1000	0
Coarse	1000-500	1
Medium	500-250	2
Fine	250-125	3
Very fine	125-62	4
Silt		
Coarse	62-31	5
Medium	31-16	6
Fine	16-8	7
Very fine	8-4	8
Clay		
Coarse	4-2	9
Medium	2-1	10
Fine	1-0.5	11
Very fine	0.5-0.24	12

*Lang, E.W., et al. 1947. Report of the sub-committee on sediment terminology. Trans. Am. Geophys. Union 28: 936-938.

Appendix Table 12:14, Runs 1-3. Responses of the marine amphipod, Rhepoxynius abronius, to Seahurst baseline sediments from 1982 to 1984. Season is fall (F) or spring (SP). Run numbers are for comparisons to control survival. Mean % survival + S.D. given under each group; asterisk means significantly different from control with Dunnett's test ($p = 0.05$) for respective run. Numbers in () are the number of amphipods out of the sediment (recorded only for SP 84). Bioassay temperature from 9°C to 14°C; salinity from 28 to 30 ‰/‰.

Station	Season- year (Run No.)	Replicate		Alive		Moribund		Season- year (Run No.)		Replicate		Alive		Moribund		Grand $\bar{X} \pm \text{S.D.}$ (N)		
		No.	No.	No.	%	No.	%	No.	No.	No.	No.	No.	%	No.	No.			
N50W	F83 (1)	1	20	100		0		1	20	100		0		1	20	100	92.9 + 7.8 (12)	
		2	20	100		0		2	20	100		0		2	20	100		
		3	19	95		0		3	19	95		0		3	19	95		
		4	16	80		0		4	16	80		0		4	16	80		
	SP84 (1)				90.0 ± 9.1					90.0 ± 9.1							92.9 + 7.8 (12)	
		1	20	100		0	(0)	1	20	100		0	(0)	1	20	100		
		2	18	90		0	(0)	2	18	90		0	(0)	2	18	90		
		3	20	100		0	(0)	3	20	100		0	(0)	3	20	100		
	A400E	F83 (1)	1	18	90		0		1	18	90		0		1	18	90	84.1 ± 8.4 (16)
			2	16	80		0		2	16	80		0		2	16	80	
			3	18	90		0		3	18	90		0		3	18	90	
			4	18	90		0		4	18	90		0		4	18	90	
		SP84 (1)				77.5 ± 8.6					77.5 ± 8.6							84.1 ± 8.4 (16)
			1	16	80		0	(3)	1	16	80		0	(3)	1	16	80	
			2	18	90		0	(2)	2	18	90		0	(2)	2	18	90	
			3	16	80		0	(0)	3	16	80		0	(0)	3	16	80	
SP83 (1)					83.8 ± 4.8					83.8 ± 4.8							84.1 ± 8.4 (16)	
		1	17	85		0	(2)	1	17	85		0	(2)	1	17	85		
		2	16	80		0		2	16	80		0		2	16	80		
		4	17	85		0		4	17	85		0		4	17	85		
A600E		F83 (1)	1	12	60		1		1	12	60		1		1	12	60	72.5 ± 19.6 (12)
			2	12	60		0		2	12	60		0		2	12	60	
			3	7	35		0		3	7	35		0		3	7	35	
			4	12	60		1		4	12	60		1		4	12	60	
	SP84 (1)				53.7 ± 12.5					53.7 ± 12.5							72.5 ± 19.6 (12)	
		1	12	60		1		1	12	60		1		1	12	60		
		2	12	60		0		2	12	60		0		2	12	60		
		3	7	35		0		3	7	35		0		3	7	35		
	SP83 (1)				53.7 ± 12.5					53.7 ± 12.5							72.5 ± 19.6 (12)	
		1	12	60		1		1	12	60		1		1	12	60		
		2	12	60		0		2	12	60		0		2	12	60		
		3	7	35		0		3	7	35		0		3	7	35		
	SP84 (1)				53.7 ± 12.5					53.7 ± 12.5							72.5 ± 19.6 (12)	
		1	12	60		1		1	12	60		1		1	12	60		
		2	12	60		0		2	12	60		0		2	12	60		
		3	7	35		0		3	7	35		0		3	7	35		
SP83 (1)				53.7 ± 12.5					53.7 ± 12.5							72.5 ± 19.6 (12)		
	1	12	60		1		1	12	60		1		1	12	60			
	2	12	60		0		2	12	60		0		2	12	60			
	3	7	35		0		3	7	35		0		3	7	35			
SP84 (1)				53.7 ± 12.5					53.7 ± 12.5							72.5 ± 19.6 (12)		
	1	12	60		1		1	12	60		1		1	12	60			
	2	12	60		0		2	12	60		0		2	12	60			
	3	7	35		0		3	7	35		0		3	7	35			

Appendix Table 12.14 (continued)

Station	Season-year (Run No.)		Replicate No.		Alive		Moribund		Season-year (Run No.)		Replicate No.		Alive		Moribund		Grand $\bar{X}\% \pm \text{S.D.} / (N)$		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	$\bar{X}\%$	S.D.	
OT-2E	SP83 (2)		1	19	95	0		1	18	90	1	1	18	90	1	0			
			2	19	95	0		2	20	100	0	0	20	100	0	0			
			3	18	90	0		3	20	100	0	0	20	100	0	0			
			4	18	90	0		4	20	100	0	0	20	100	0	0			
			92.5	± 2.8		97.5	± 5.0												
G50W	F82		1	20	100	0		1	15	75	0		15	75	0				
			2	20	100	0		2	19	95	0		19	95	0				
			3	20	100	0		3	17	85	0		17	85	0				
			4	20	100	0		4	15	75	0		15	75	0				
			100	± 0		82.5	± 9.5												
SP83 (2)		1	20	100	0		1	18	90	1	1	18	90	1	2				
		2	16	80	0		2	20	100	0		20	100	0	1				
		3	16	80	0		3	20	100	0		20	100	0	1				
		4	19	95	0		4	20	100	0		20	100	0	1				
			88.7	± 10.3		97.5	± 5.0												
G780	F82		1	19	95	0													
			2	19	95	0													
			3	17	85	0													
			4	17	85	1													
			90.0	± 0.3															
			88.7	± 10.3		90.0	± 0.3												
H75W	F82		1	18	90	0		1	19	95	0		19	95	0				
			2	17	85	0		2	20	100	0		20	100	0				
			3	17	85	0		3	19	95	0		19	95	0				
			4	15	75	0		4	19	95	0		19	95	0				
			*83.7	± 6.2		96.2	± 2.5												
SP83 (2)		1	17	85	0		1	19	95	0		19	95	0	0				
		2	18	90	0		2	19	95	0		19	95	0	0				
		3	18	90	0		3	20	100	0		20	100	0	0				
		4	18	90	0		4	18	90	0		18	90	0	0				
			88.7	± 2.3		95.0	± 4.1												
			91.0	± 6.4		91.0	± 6.4												

Appendix Table 12.14 (continued).

Station	Season- year (Run No.)		Replicate		Alive		Moribund		Season- year (Run No.)		Replicate		Alive		Moribund		Grand $\bar{X}\% \pm \text{S.D.}$ (N)																											
	No.	No.	No.	No.	No.	%	No.	%	No.	%	No.	No.	No.	%	No.	%																												
H400E	SP83 (2)	1	15	75	0					F83 (2)	1	19	95	0			85.4 \pm 12.5 (12)																											
																		2	17	85	1	16	80	1	(2)																			
																		3	13	65	0	16	80	0	(1)																			
																		4	16	80	0	15	75	0	(3)																			
	SP84 (2)	1	76.2 \pm 8.3			SP84 (2)	*86.2 \pm 20.3					1	16	80	1	(2)																												
																			2	17	85	1	16	80	0	(1)																		
																			3	13	65	0	15	75	0	(3)																		
																			4	16	80	0	14	70	0	(2)																		
																													*76.3 \pm 4.8															
																			SP83 (2)	1	81.2 \pm 13.1			F83 (2)	19	95	0			1	19	95	0			91.7 \pm 10.7 (12)								
																																					2	20	100	0	20	100	0	(1)
																																					3	20	100	0	20	100	0	(4)
	4	20	100	0	20	100	0	(3)																																				
											98.2 \pm 2.5																																	
	SP84 (2)	1				SP84 (2)	20	100	0			1	20	100	0	(1)																												
																																					2	18	90	0	18	90	0	(4)
3																																					19	95	0	19	95	0	(3)	
4																			19	95	0	19	95	0	(2)																			
																											95.0 \pm 4.1																	
F82																			1	20	100	0	F83 (2)	17	85	0			1	17	85	0			86.6 \pm 8.3 (16)									
																																				2	17	85	0	16	80	0	(1)	
																																				3	17	85	0	16	80	1	(0)	
	4	16	80	0	17	85	0	(3)																																				
											*82.3 \pm 2.8																																	
	SP83 (2)	1	17	85	1	SP84 (2)	19	95	0			1	19	95	0	(1)																												
																																				2	19	95	0	17	85	0	(0)	
																																				3	19	95	1	14	70	0	(3)	
4																			16	80	0	20	100	0	(0)																			
																											87.5 \pm 13.2																	

Appendix Table 12.14 (continued)

Station	Season- year (Run No.)		Replicate		Alive		Moribund		Season- year (Run No.)		Replicate		Alive		Moribund		Grand $\bar{X}\% \pm \text{S.D.}$ (N)	
	No.	No.	No.	No.	%	No.	No.	%	No.	No.	No.	No.	%	No.	No.	%	No.	No.
I690	F82		1	20	100	0				F83 (2)	1	19	95	0				88.2 ± 8.9 (16)
			2	19	95	0					2	15	75	0				
			3	16	80	0					3	16	80	1				
			4	16	80	0					4	19	95	0				
	SP83 (1)		1	88.7 ± 10.3		1				SP84 (2)	1	* 85.0 ± 8.1						
			2	19	95	1					2	17	85	0	(0)			
			3	17	85	1					3	17	85	0	(1)			
			4	15	75	1					4	20	100	0	(0)			
	J50E			1	17	85	1			F83 (2)	1	19	95	0				92.1 ± 8.4 (12)
				2	17	85	1					2	17	85	0			
				3	15	75	1					3	19	95	0			
				4	17	85	1					4	18	90	0			
		SP83 (2)		1	86.2 ± 10.3					SP84 (2)	1	* 91.2 ± 4.7						
				2	19	95	0					2	20	100	0	(0)		
				3	18	90	0					3	19	95	0	(0)		
				4	14	70	0					4	20	100	0	(0)		
J75E				1	18	90	0			F83 (2)	1	20	100	0				95.9 ± 5.6 (12)
				2	18	90	0					2	20	100	0			
				3	18	90	0					3	20	100	0			
				4	17	85	0					4	20	100	0			
		SP83 (2)		1	88.7 ± 2.5					SP84 (2)	1	100 ± 0						
				2	18	90	0					2	20	100	0	(2)		
				3	18	90	0					3	19	95	0	(0)		
				4	17	85	0					4	20	100	0	(0)		
			1	88.7 ± 2.5						1	98.8 ± 2.5							
			2	18	90	0					2	20	100	0	(0)			
			3	18	90	0					3	20	100	0	(0)			
			4	17	85	0					4	20	100	0	(0)			

Appendix Table 12.14 (continued).

Station	Season-year		Replicate		Alive		Moribund		Season-year		Replicate		Alive		Moribund		Grand $\bar{X}\% \pm \text{S.D.}$ (N)
	(Run No.)	(Run No.)	No.	%	No.	%	No.	%	(Run No.)	(Run No.)	No.	%	No.	%	No.	%	
Control (West Beach) F82			1		20	100	0		F83	1	20	100	0				95.0 \pm 8.5 (28)
			2		20	100	0		(1)	2	20	100	0				
			3		19	95	0			3	19	95	0				Without SP83 Run 1
			4		18	90	0			4	18	90	0				97.5 \pm 3.9 (24)
SP83 (1)			1		96.2 \pm 4.7						96.2 \pm 4.7						
			2		18	90	0		F83	1	20	100	0				
			3		17	85	0		(2)	2	20	100	0				
			4		17	85	0			3	20	100	0				
SP83 (2)			1		12	60	0			4	19	95	0				
			2		80.0 \pm 13.5						98.7 \pm 2.3						
			3		20	100	0		SP84	1	20	100	0	(0)			
			4		18	90	0		(1)	2	20	100	1	(0)			
Control (Bowman Bay)			1		20	100	0			3	18	90	0	(0)			
			2		20	100	0			4	20	100	1	(0)			
			3		19	95	0				97.5 \pm 5.0						
			4		96.2 \pm 4.7				SP84	1	19	95	0	(1)			
Control (Bowman Bay)			1		20	100	0		(2)	2	20	100	0	(0)			
			2		18	90	0			3	19	95	0	(0)			
			3		20	100	0			4	20	100	0	(0)			
			4		19	95	0				97.5 \pm 2.9						

Appendix Table 12.14 (continued).

Station	Season-year (Run No.)		Replicate No.		Alive		Moribund		Season-year (Run No.)		Replicate No.		Alive		Moribund		Grand $\bar{X} \pm \text{S.D.} (N)$	
	No.	No.	No.	%	No.	%	No.	%	No.	year	(Run No.)	No.	No.	No.	%	No.	%	$\bar{X} \pm \text{S.D.} (N)$
Duwamish South Harbor	F82		1	15	75	0				F83		1	14	70	0			76.1 ± 11.7 (28)
			2	15	75	0					(1)	2	9	45	0			
			3	16	80	0						3	17	85	0			
			4	13	65	0						4	14	70	0			
	SP83 (1)		1	*73.7 ± 6.2									*67.4 ± 16.5					
			2	16	80	0				F83		1	16	80	0			
			3	14	70	1				(2)	2	17	85	0				
			4	14	70	1					3	17	85	0				
	SP83 (2)		1	14	70	3						4	19	95	0			
			2	77.5 ± 9.5									*86.2 ± 6.2					
			3	17	85	0					SP84		1	17	85	0	(5)	
			4	14	70	2				(1)	2	17	85	0	(4)			
	SP84 (2)		1	15	75	0						3	17	85	0	(9)		
			2	15	75	0						4	16	80	0	(8)		
			3	15	75	0						*83.8 ± 2.5						
			4	15	75	0					SP84		1	15	50	0	(0)	
			*76.2 ± 6.2					(2)	2	16	80	0	(1)					
										3	17	85	0	(2)				
										4	11	55	0	(3)				
											*67.5 ± 17.6							

Appendix Table 12.15. Responses of the marine amphipod, *Rhepoxynius abronius*, in a static system to Seahurst baseline sediments collected in spring 1984 Run 1. * indicates mean significantly different from control with Dunnett's test ($p = 0.05$). Temperature = 9.3°C , salinity = $28.3 \text{ }^{\circ}/\text{oo}$.

Station	Replicate number	Amphipod survival			Surviving amphipods not reburying	No. of amphipods out of sediment
		Number	%	$\bar{X}\% \pm \text{S.D.}$		
<u>Static Tests</u>						
<u>Controls</u>						
Bowman Bay	1	20	100		0	0
	2	20	100	100.0 ± 0.0	0	0
	3	20	100		0	0
	4	20	100		0	0
Duwamish	1	14	70		0	7
	2	16	80	$73.8 \pm 11.1^*$	0	5
	3	17	85		0	3
	4	12	60		0	8
<u>Seahurst</u>						
A400 E	1	17	85		0	1
	2	17	85	$88.8 \pm 4.8^*$	0	3
	3	18	90		0	0
	4	19	95		0	1
A600 E	1	18	90		1	0
	2	13	65	$78.8 \pm 10.3^*$	0	0
	3	16	80		0	0
	4	16	80		0	0
B75 W	1	19	95		0	0
	2	20	100	97.5 ± 2.9	0	0
	3	19	95		0	0
	4	20	100		0	0
C400 E	1	16	80		0	0
	2	18	90	$85.0 \pm 5.8^*$	0	0
	3	18	90		0	1
	4	16	80		0	1
C600 E	1	14	70		0	0
	2	15	75	$70.0 \pm 7.1^*$	0	0
	3	15	75		0	1
	4	12	60		0	3
OT-1	1	18	90		0	2
	2	16	80	$76.3 \pm 11.1^*$	0	1
	3	14	70		0	0
	4	13	65		0	1
OT-2	1	13	65		0	0
	2	14	70	$68.8 \pm 8.5^*$	0	0
	3	16	80		0	1
	4	12	60		0	2

Appendix Table 12.15. Responses of the marine amphipod, Rhepoxynius abronius, in a static system to Seahurst baseline sediments collected in spring 1984, Run 2. * indicates mean significantly different from control with Dunnett's test ($p = 0.05$). Temperature = 9.2°C, salinity = 28.5 ‰.

Station	Replicate number	Amphipod survival			Surviving amphipods not reburying	No. of amphipods out of sediment
		Number	%	$\bar{X}\% \pm \text{S.D.}$		
<u>Static Controls</u>						
Bowman Bay	1	19	95		0	1
	2	20	100	98.8 \pm 2.5	0	0
	3	20	100		0	0
	4	20	100		0	0
Duwamish	1	16	80		0	3
	2	12	60	67.5 \pm 9.6*	0	4
	3	12	60		0	4
	4	14	70		0	2
<u>Seahurst</u>						
H75 W	1	19	95		0	1
	2	19	95	86.3 \pm 10.3*	0	1
	3	16	80		0	4
	4	15	75		0	4
H400 E	1	17	85		0	0
	2	17	85	91.3 \pm 7.5	0	1
	3	19	95		0	0
	4	20	100		0	0
H600 E	1	19	95		0	0
	2	17	85	83.8 \pm 8.5*	0	1
	3	16	80		0	0
	4	15	75		0	1
H640	1	18	90		0	0
	2	16	80	88.8 \pm 6.3*	0	0
	3	19	95		0	0
	4	18	90		0	0
I690	1	18	90		0	0
	2	17	85	92.5 \pm 6.5	0	0
	3	19	95		0	0
	4	20	100		0	6
J600 E	1	16	80		0	1
	2	18	90	90.0 \pm 8.2	0	0
	3	18	90		0	1
	4	20	100		0	1

Appendix Table 12.16. Grain size composition of Seahurst Sediments and controls used in amphipod - sediment bioassays.

	Percentage Composition				Moment Measures				Dry/wet
	Gravel	Sand	Silt	Clay	X phi	Std. dev.	Skewness	Kurtosis	
N50 W	1.00	92.71	1.14	5.14	2.20	2.06	2.72	11.26	.674
A400 E	1.08	56.34	15.14	27.44	4.71	3.73	0.33	1.65	.480
A600 E	0.00	6.88	40.57	52.56	8.08	2.21	-0.83	3.32	.334
B75 W	26.98	68.43	0.88	3.71	0.72	2.46	1.87	8.18	.788
B200 E	35.82	46.83	6.17	11.17	1.56	3.83	1.04	3.16	.850
C75 E	0.03	97.14	0.66	2.18	2.15	1.45	3.94	23.38	.797
C200 E	1.83	93.09	1.57	3.51	2.26	1.86	2.55	12.66	.802
C400 E	0.59	73.90	12.25	13.26	3.83	2.88	1.20	3.43	.534
C600 E	0.00	11.65	39.61	47.84	7.86	2.43	-0.74	2.77	.341
C640	0.02	4.52	45.46	50.00	8.15	2.08	-0.68	3.12	.325
OT-1E	0.00	6.59	44.81	48.60	7.83	2.43	-0.67	2.54	.309
OT-2E	0.34	6.23	40.49	52.94	7.95	2.46	-0.87	3.35	.334
G50 W	0.31	97.16	0.72	1.80	2.32	1.36	3.80	24.53	.736
G780	0.02	7.17	33.12	59.69	8.31	2.28	-1.11	3.88	.369
H75 W	0.04	94.21	1.63	4.13	2.98	1.68	3.38	15.25	--
H400 E	2.47	49.01	15.76	32.76	5.13	3.84	0.10	1.56	.677
H600 E	0.00	5.27	38.56	56.17	8.32	2.10	-0.97	3.83	.325
H640	0.00	7.86	39.11	53.02	8.09	2.27	-0.97	3.62	.310
I690	0.00	15.14	33.04	51.82	7.64	2.92	-1.00	3.03	.336
J50 E	0.00	97.63	0.38	1.99	2.08	1.40	4.35	26.84	.761
J75 E	0.03	97.66	0.62	1.69	2.14	1.30	4.09	26.84	.769
J400 E	0.64	85.06	4.11	10.19	2.97	2.75	1.73	5.27	--
J600 E	0.38	7.25	34.45	57.92	8.25	2.30	-1.18	4.32	.315
K400 E	0.06	44.25	27.78	27.91	5.54	3.37	0.11	1.60	.367
L-alt 50E	0.29	87.97	5.29	6.45	2.30	2.47	2.05	6.82	.728
L-alt 200E	2.55	34.60	33.09	29.76	5.73	3.46	-0.22	1.98	.533
L-alt 400E	0.00	23.79	35.24	40.97	6.79	2.99	-0.35	1.98	.428
Controls:									
West Beach	0.00	98.71	0.46	0.83	2.23	0.47	0.57	6.99	.790
Bowman Bay	0.00	97.21	1.73	1.06	3.22	0.47	0.57	8.43	.761
Duw SH	1.12	33.96	46.29	18.63	5.45	2.91	0.29	2.60	--

Appendix Table 12.17. Concentrations of trace metals in the Seahurst sediment samples. Values are in ppm except Cd and Hg which are in ppb. Trace metal concentrations are per dry weight of sediment.

Station designation Code	Ag (ppm)	As (ppm)	B (ppm)	Ba (ppm)	Be (ppm)	Cd (ppb)	Co (ppm)	Cr (ppm)	Cu (ppm)	Hg (ppb)	Mn (ppm)	Ni (ppm)	Pb (ppm)	V (ppm)	Zn (ppm)	Dry/wet	
L ALT 50 E	1.0	5.6	16.5	17.9	2.9	249	8.1	23.4	20.6	68	194	15.1	8.1	28.8	31.6	0.728	
L ALT 200 E	2.1	6.7	13.7	48.8	5.1	315	15.2	41.3	54.4	225	251	26.3	20.1	58.2	63.8	0.533	
L ALT 400 E	2.6	--	15.0	67.8	5.6	306	17.1	51.4	65.4	304	392	30.4	27.1	65.4	81.8	0.428	
A-600 E	3.6	12.4	35.9	92.8	7.2	377	21.8	74.8	68.8	329	572	47.9	44.6	77.8	123.0	0.334	
A-400 E	2.1	6.7	22.9	54.2	5.4	275	18.9	50.0	37.5	104	421	37.5	15.4	62.5	70.8	0.480	
B-75 W	2.7	11.5	2.3	31.7	2.5	227	10.4	20.3	10.4	25	2170	19.0	9.8	30.4	35.5	0.788	
B-200 E	1.1	6.8	12.9	25.9	2.7	126	9.8	29.4	12.9	23	414	25.9	7.5	30.6	36.5	0.850	
C-75 E	0.7	3.6	--	22.6	2.0	245	6.4	28.8	3.9	1	155	18.8	1.1	25.1	20.0	0.797	
C-200 E	0.8	5.7	10.9	28.7	2.9	69	9.7	28.7	9.1	25	539	28.9	5.7	29.9	31.2	0.802	
C-400 E	1.4	14.1	10.9	41.2	3.9	161	14.8	43.1	26.2	187	444	33.7	17.2	43.1	56.2	0.534	
C-600 E	2.1	32.2	12.0	79.2	6.5	261	18.8	64.5	61.6	411	610	41.1	44.9	67.4	106.0	0.341	
C-640	2.1	24.3	2.7	89.2	6.5	462	19.1	67.7	67.7	338	695	43.1	39.6	70.8	105.0	0.325	
OT-1	2.3	--	22.0	93.8	6.8	388	20.1	71.2	64.7	356	612	45.3	51.1	74.4	113.0	0.309	
OT-2	2.1	26.2	29.9	89.8	6.6	299	20.1	71.8	62.9	329	578	43.7	42.5	80.8	117.0	0.334	
G-780	2.3	23.1	14.6	78.6	6.0	252	17.6	62.3	62.3	217	615	37.9	38.7	65.0	100.0	0.369	
G-50W	0.7	6.1	3.5	24.4	2.2	58	6.8	34.0	9.5	14	148	28.5	2.6	25.8	27.2	0.736	
H-400 E	2.0	19.8	21.7	53.1	6.4	166	20.3	64.2	42.0	177	524	37.6	28.1	59.7	90.7	0.452	
H-600 E	2.8	22.1	28.3	92.3	7.1	215	20.3	83.1	55.4	246	800	43.1	41.8	80.0	117.0	0.325	
H-640	2.6	27.5	27.7	80.6	7.4	235	20.3	83.9	51.6	258	868	45.2	42.6	77.4	122.0	0.310	
I-690	2.4	21.1	25.3	74.4	6.5	360	18.8	74.4	41.7	119	964	38.7	37.5	71.4	104.0	0.336	
J-600 E	3.2	34.0	15.9	102.0	6.7	--	27.8	102.0	76.2	--	1104	54.0	--	79.4	130.0	0.315	
J-75 E	1.0	4.3	7.2	16.9	2.3	51	6.5	27.3	3.8	13	202	10.6	6.6	28.6	24.7	0.769	
J-50 E	0.9	2.5	7.1	15.8	2.0	91	5.8	26.3	3.2	13	184	8.7	6.3	23.6	26.3	0.761	
K-400 E	2.7	16.5	22.9	59.9	6.3	166	20.2	73.6	38.1	218	496	35.4	31.0	70.8	103.0	0.367	
N-50 W	1.1	4.2	10.4	14.1	2.2	68	7.6	34.1	5.6	15	266	22.2	4.8	26.7	34.1	0.674	
Mean	1.9	14.7	16.3	55.8	4.9	226	15.1	52.0	38.2	167	569	32.7	23.9	54.1	74.8	0.520	
S.D.	0.8	3.8	9.1	30.0	2.0	113	6.2	22.7	25.3	135	420	12.1	16.8	21.8	38.8	0.201	
n	25	23	24	25	25	24	24	24	25	24	25	25	24	25	25	25	