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SURVEY OF BIOLOGICAL EFFECTS OF TOXICANTS UPON PUGET SOUND BIOTA - III. TESTS IN EVERETT HARBOR, SAMISH AND BELLINGHAM BAYS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

U.S. NOAA has supported considerable research in Puget Sound on the existing levels, fates and effects of toxic chemicals. Much of that research was performed by scientists funded through the Marine Ecosystems Analysis (MESA) Puget Sound Project. That Project was incorporated into the Ocean Assessments Division (OAD) in 1982. During both the initial MESA project studies and the continuing OAD studies, an effort has been made to determine whether or not the contaminant mixtures observed in Puget Sound are toxic to biota. The relative toxicity of sediment samples from numerous areas in the vicinity of Bremerton, Seattle and Tacoma showed that those samples that were most toxic were often taken from areas known to be most contaminated. Chemical analyses of sediment samples from many other bays and harbors around the Sound showed that Bellingham Bay and Everett Harbor were relatively highly contaminated. The chemical mixtures in the two areas were somewhat different from those encountered elsewhere. Thus, this study was conducted to determine if sediment samples of these two urban areas were toxic. The Contracting Officer's Technical Representative for this study was Edward R. Long.

EXECUTIVE SUMMARY

The Marine EcoSystems Analysis (MESA) Puget Sound Project has undertaken intensive studies of Puget Sound, with particular emphasis on such highly industrialized areas as Elliott and Commencement Bays and Sinclair Inlet. These studies have involved chemical, biological and oceanographic investigations aimed at determining the concentrations, fates and effects of toxic chemicals in the Puget Sound ecosystem. An integral part of these studies has been toxicity tests with field-collected sediments. The present study was initiated to extend sediment toxicity testing to two previously untested industrialized Puget Sound embayments: Bellingham Bay and Everett Harbor. Relatively high concentrations of toxic chemicals had been reported from these two areas (Malins et al., 1982). The contaminant mixtures discovered there differed somewhat from those observed in other parts of Puget Sound. This study was initiated to determine if sediments from these two areas were toxic or not. Toxicity testing was also conducted south of Bellingham Bay at Samish Bay, chosen as a reference area.

A total of 22 stations were chosen for study: 10 in the Everett Harbor area, 10 in Bellingham Bay, and 2 in Samish Bay. Composite sediment grab samples were collected from each station and tested for acute lethal, sublethal, partial life-cycle, cell reproduction and genotoxic effects. These effects were examined utilizing sensitive test methods applied elsewhere in Puget Sound (Chapman et al., 1982a; in press a, b). An additional station south of Everett Harbor was tested (with negative results) for acute lethal and sublethal effects.

On the basis of acute lethal, sublethal, partial life-cycle, cell reproduction and genotoxic effects testing, Everett Harbor, Bellingham and Samish Bays were less toxic than contaminated areas such as the Duwamish Waterway (Elliott Bay) and the Commencement Bay Waterways. Everett Harbor sediments were more toxic overall than those from Bellingham Bay. Samish Bay sediments only showed toxicity in cell reproduction and genotoxic tests, suggesting very different sediment chemistry in this area.

Partial life-cycle bioassays with oyster larvae (Crassostrea gigas) were conducted by exposing fertilized eggs to settled sediment slurries for 48 h then determining the number of live larvae and any abnormalities. A total of 19 stations demonstrated significant abnormalities or mortalities; the two reference stations showed no significant effects.

Acute lethal bioassays were conducted with the sensitive amphipod Rhepoxynius abronius. Two stations (one each in Bellingham Bay and Everett Harbor) demonstrated significant acute lethal effects; the two reference stations showed no significant effects.

Sublethal effects measurements were conducted with the oligochaete Monopylephorus cuticulatus by exposing the worms to sediment elutriates and measuring respiration rates. Seven stations demonstrated significant respiration rate differences compared to controls; the two reference stations showed no significant effects.

Cell reproduction studies were conducted by exposing rainbow trout gonad (RTG-2) and bluegill fry (BF-2) cells to sediment extracts during logarithmic growth. Eight stations (including one reference station) significantly reduced cell growth in RTG-2 cells, and three stations (including both reference stations) significantly reduced cell growth in BF-2 cells.

Genotoxic tests for chromosomal damage were conducted by exposing RTG-2 cells to sediment extracts and determining mitotic (anaphase aberration) effects. Sediment extracts from eight stations (including one reference station) caused significant chromosomal damage.

Physical and chemical data for tested samples (particle size, total volatile solids, digestible organic carbon, and extractable organic matter) were within the ranges observed for other areas of Puget Sound with the following exceptions. A high clay content was noted in Bellingham Bay sediments and a high percentage of total volatile solids was noted in inner Everett Harbor sediments.

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

One of the intents of the MESA Puget Sound Project is to develop an understanding of the effects of environmental contaminants upon Puget Sound biota. High environmental levels of particular chemicals have been detected in sediments from industrialized embayments of Puget Sound, and a variety of in situ biological effects (e.g. tissue abnormalities in fish and shellfish, changes in biological community structure) occur in areas associated with high levels of various contaminants (Malins et al., 1980, 1982; Dexter et al., 1981; Long, 1982). Direct evidence of toxicity from Puget Sound sediments has recently been provided for three of these industrialized embayments: Elliott Bay, Commencement Bay and Sinclair Inlet (Chapman et al., 1982a, in press a, b).

Contaminant mixtures found in sediments from Bellingham Bay and Everett Harbor were known to differ from those of the previously tested areas. However, the relative toxicity of sediments in these two areas was unknown. Recent indirect evidence of the potential for biological effects among biota captured in the two areas has been collected.

Field studies have recently recorded fin rot and lesions in bottom fish in Bellingham Bay and Everett Harbor (Campana, 1983; Gronlund et al., 1983). The intent of the present study was to determine whether marine sediments from these embayments also exhibited toxic biological effects in direct exposure tests. Accordingly, composited sediment grab samples were obtained from a total of 22 stations (including a non-industrialized reference area, Samish Bay). These samples were tested for possible biological effects using a range of species and test methodologies. Sediment collected from a station in Possession Sound, south of Everett Harbor, was also tested on an opportunistic basis. The results were used to determine the relative toxicity of Everett Harbor and Bellingham Bay samples compared to those from other tested areas of Puget Sound.

1.1 Objectives

The overall objective of this study was to assess the direct toxic effects of marine sediments from previously untested urban embayments. The specific objectives were:

a. To establish 22 stations (10 in Everett Harbor, 10 in Bellingham Bay, and 2 in Samish Bay, a reference area), and obtain composited sediment samples from several Van Veen grabs from each station.

b. To determine the effects of exposure of representative Puget Sound biota to these sediment samples.

c. To relate the degree of observed effects to similar data from other previously-tested areas of Puget Sound.

2.0 METHODS

2.1 Geographic Study Areas

The present study areas were located on the eastern shores of Puget Sound adjacent to Possession Sound and Admiralty Inlet (Fig. 1). Ten stations each from Bellingham Bay and Everett Harbor, plus one station south of Everett Harbor, were selected for testing (Figs. 2 and 3). Stations were sited near potential sources of toxic substances (e.g. industrial locations, sewage discharges). Two stations from Samish Bay were incorporated as non-urban reference samples (Fig. 2).

Precise station location information (e.g. latitude and longitude, water depth, position relative to shore facilities, etc.) is presented in Appendix A.

2.2 Approach

Previous toxicity tests with field-collected sediments from Puget Sound have demonstrated a variety of effects ranging from acute lethal (Swartz et al., 1982), sublethal and genotoxic responses (Chapman et al., 1982a), to life-cycle and reproductive impairment (Chapman et al., in press b). Test methods and species were chosen based on these previous studies to provide the most responsive, sensitive protocols compatible with previous results.

Effects testing included acute lethal (with the amphipod, Rhepoxynius abronius), sublethal (respiration rate of the oligochaete, Monopylephorus cuticulatus), partial life-cycle (with oyster larvae, Crassostrea gigas), cell reproduction and genotoxic effects (with cultured fish cells). Results were used to classify the comparative toxicity of sediment samples, and to identify areas of biological effects in Bellingham Bay and Everett Harbor.

2.3 Sediment Collection

Sediment samples were collected May, 1983 using a 0.1m^2 Van Veen grab modified with top screens and rubber flaps to minimize surface sediment disturbance. Opening of the screens allowed access to the surface of the material in the grab without disturbing the contents. Six to ten grabs were collected at each station.

Sediments were removed from the grab by carefully inserting the barrel of a commercial aluminum cookie press (approximately 5 cm diameter) into the sediment without disturbing the sample. Holes had been bored in the barrel 10 cm above the end of the press to allow the overlying water to escape and to ensure uniform penetration. Between seven and ten subcores were taken from each grab at a station, with the number of subcores dependent on he amount of sediment obtained in the grab.

The subcores from all Van Veen grabs at each station were extruded into a single polyethylene bag and thoroughly homogenized (homogeneous color and texture) to yield approximately 10 L (15 Kg

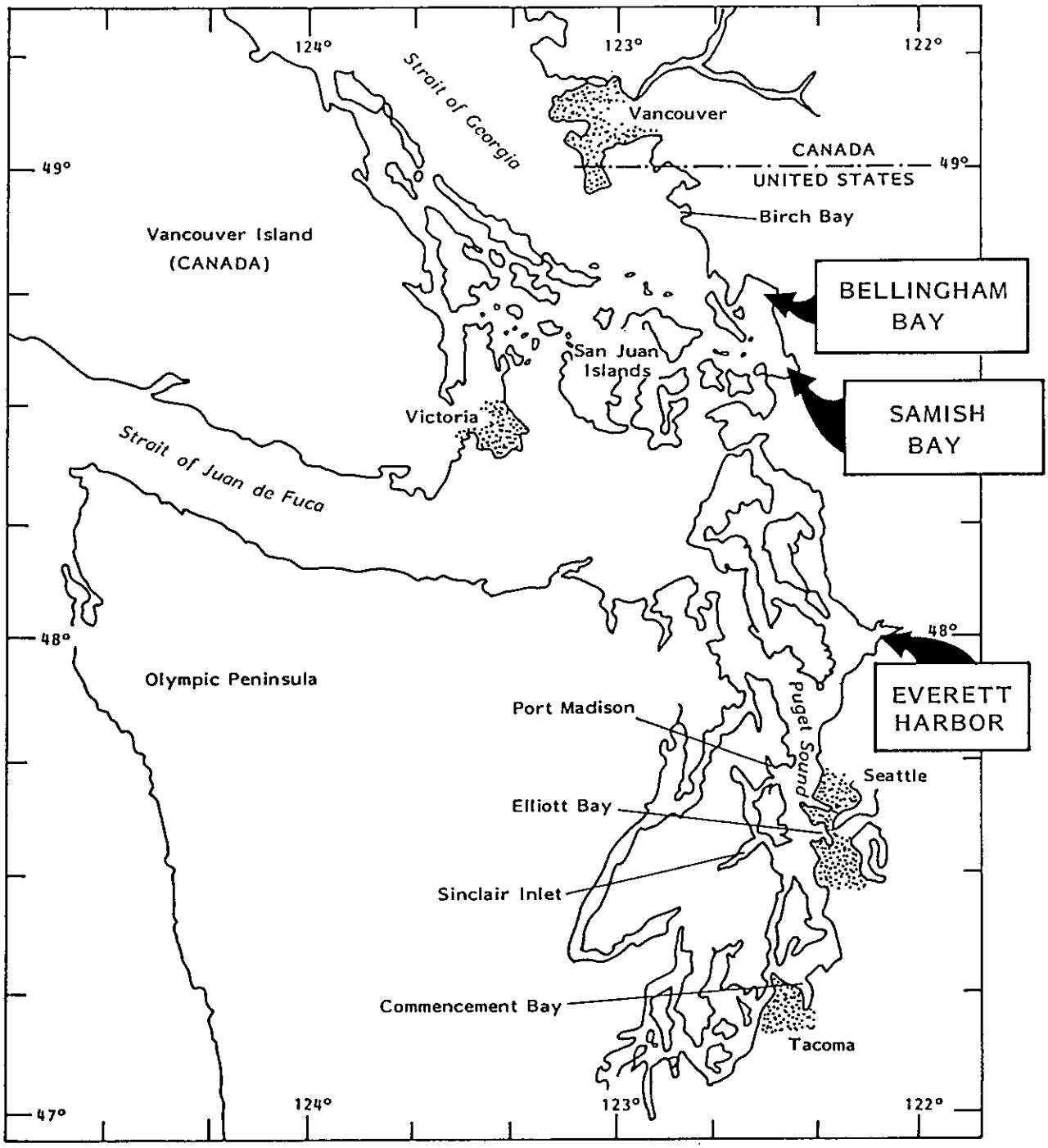

Figure 1 Locations of Study Areas

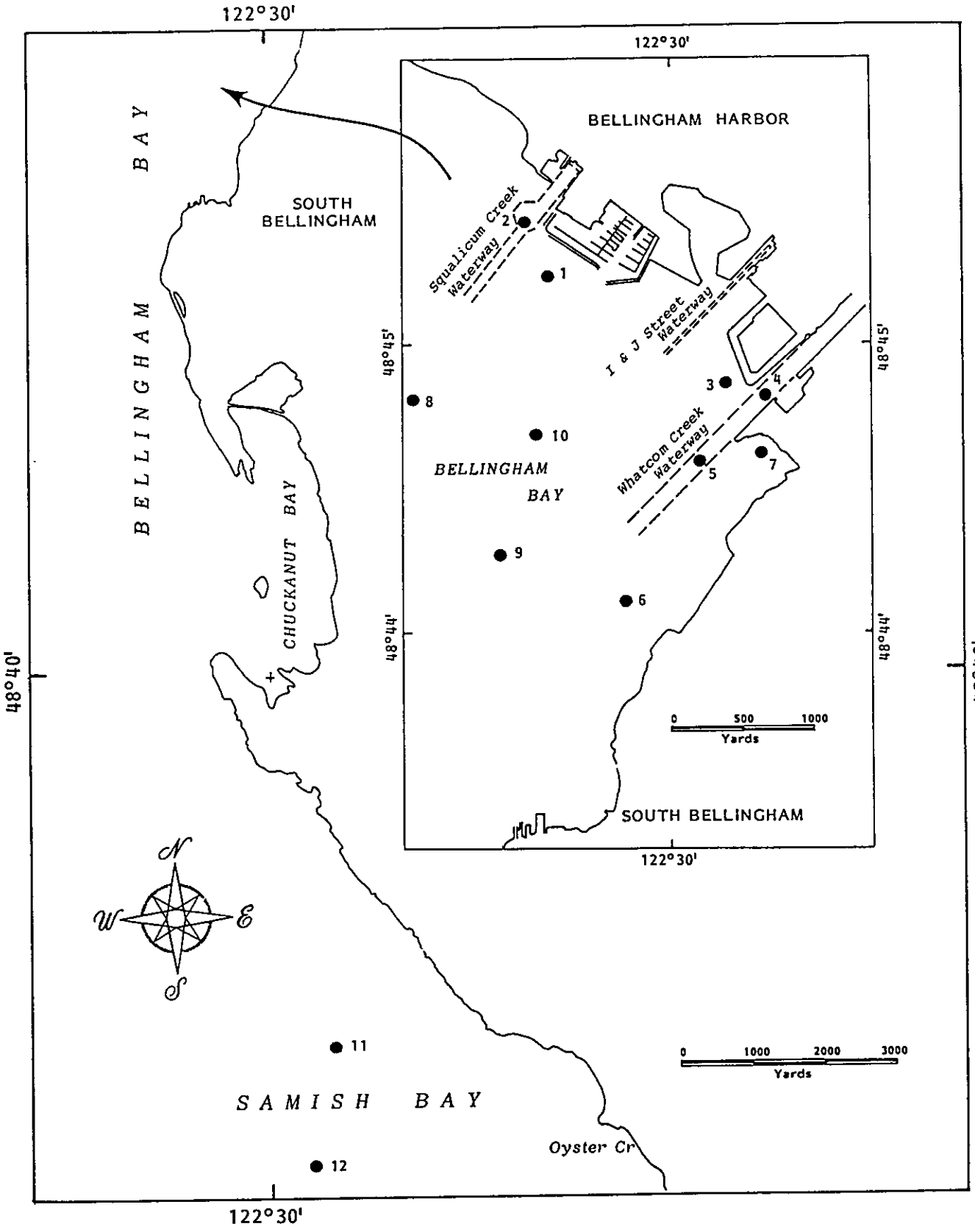

Figure 2 Station Locations in Bellingham and Samish Bays

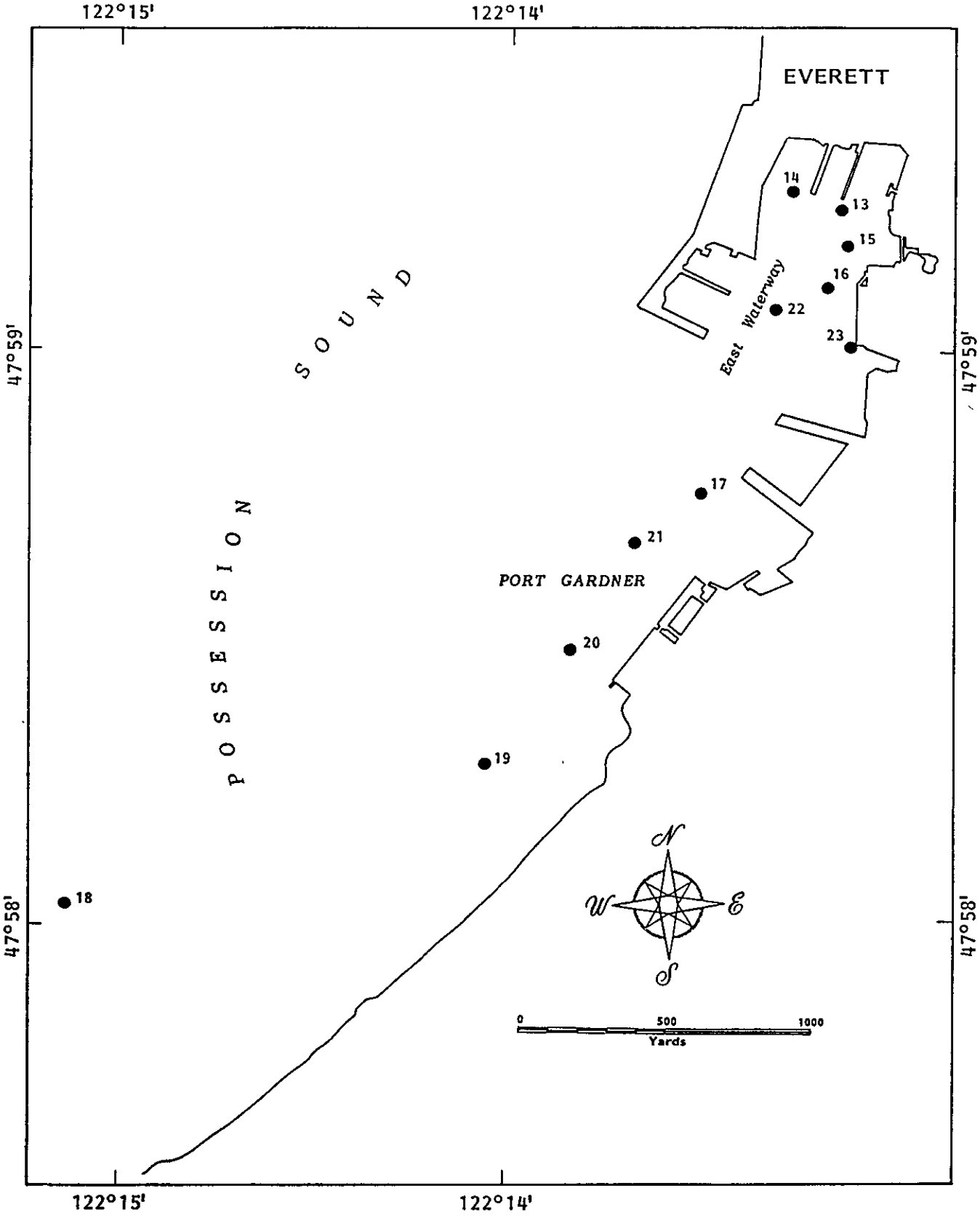

Figure 3 Station Locations in Everett Harbor

wet weight) of sample. Aliquots for possible future chemical analysis were then transferred to solvent-rinsed, 120 mL glass jars with teflon cap liners using a stainless steel spatula. Aliquots for cell reproduction testing were similarly transferred to solvent-rinsed, 475 mL glass jars with teflon cap liners. Aliquots for sediment characterization were placed in whirlpak polyethylene bags. The remainder of the sample was sealed in the polyethylene bag. All sample containers were pre-labelled and the homogenate was transferred immediately to remove all possibility of handling errors.

All samples, except those for sediment characterization, were frozen within 24 h of collection, stored with dry ice during transport, and kept frozen prior to analysis. Subsamples for chemical analysis were delivered to the National Marine Fisheries Service (Montlake Laboratory) for frozen storage. Samples for sediment characterization were stored at 4°C until analysed.

2.4 Sediment Characterization

All analyses were conducted following the basic methodology of Folk (1964) as summarized below. Each sediment sample was homogenized then quartered to yield an approximate 40 g aliquot. Half of this amount was used to determine the percent of water and the percent of carbon; the other half was used for grain size analysis.

The subsample for determinations of water and organic carbon content was placed in a tared beaker and weighed. It was then dried, dessicated, reweighed and the percent of water calculated. The dried sample was split and 10 g transferred to a tared beaker and reweighed. Approximately 40 mL of 30% H_2O_2 was then added to this sample in 10 mL aliquots. The sample was again dried, dessicated, reweighed, and the percent of digestible organic carbon (DOC) in the dried sample was calculated.

An additional subsample of about 10 g of dried sample was placed in a tared crucible and reweighed. This aliquot was then combusted at 550 $\pm$ 10°C overnight, cooled in a dessicator and reweighed for the determination of total volatile solids (TVS). TVS determinations were performed by the U.S. EPA Region 10 Laboratory (Manchester, Washington).

The subsample for grain size analysis was mixed with 10 mL of 30% H_2O_2 in distilled water, allowed to sit for 12 h and wet sieved through a 4 Ø (0.0675 mm) screen. The material that passed through the screen was collected in a 1 L settling cylinder. The residue on the screen was dried and sieved at 1/2 Ø intervals from the largest fraction present to 4 Ø. Any material that passed through the 4 Ø screen was added to the settling cylinder. The remaining gravel and coarse sand fractions were weighed, with notations made of such substances as wood, coal and shell fragments.

A 0.5 g/L solution of dispersing agent (sodium hexametaphosphate) in distilled water was added to the residue in the settling cylinder to make a 1 L volume. Silt and clay fractions were measured by

pipette techniques in 1 Ø intervals from 4 Ø to 12 Ø (Krumbein and Pettijohn, 1938). Laboratory temperature was measured and used in the calculations.

Ten percent of all samples for grain size, DOC and TVS were analysed in duplicate for quality control. Separate subsamples were removed for this determination of analytical reliability following homogenizing and splitting of the entire sample.

2.5 Toxicity Testing

2.5.1 Oyster Larvae Toxicity

Adult Pacific oysters (age 3-4 years) were obtained from Redonda Sea Farms, a commercial oyster farm located in Refuge Cove, British Columbia. Stock oysters were held at ambient seawater temperatures (15°C) in clean seawater.

Prior to testing, the oysters were cleaned of fouling organisms and placed in continuous-flow conditioning trays to permit gonadal maturation. The oysters were thermally conditioned (19±0.5°C) for 10 days, with individual oysters periodically sacrificed to determine the state of gonadal development.

Following maturation, two female oysters were placed together in a shallow Pyrex® spawning dish filled with filtered, UV-treated seawater at 20°C. The spawning dish was then placed in a water bath (30°C) and 1.0 hour later approximately 20 mL of a prepared sperm suspension from a sacrificed male oyster was added. After 0.5 h, the water in the spawning dish was replaced with fresh 20°C treated seawater and the dish placed back into the 20°C water bath. Spawning of one of the females occurred soon afterwards. The other female was removed and 50 mL of a prepared sperm suspension added. After 30 min, the adult oyster was removed from the spawning dish. The fertilized eggs were washed through a Nitex screen (250 µm) to remove excess gonadal tissue, and were then suspended in 4 L of treated seawater at 20°C. Fertilized eggs were examined microscopically for the formation of polar bodies. When polar bodies were observed, density was determined from triplicate counts of the number of embryos in 1 mL samples of a 1:99 dilution of homogeneous embryos suspension.

Sediment bioassays were conducted using the procedure detailed by Chapman and Morgan (1983). Testing was conducted in clean (rinsed with 5% nitric acid) one liter Nalgene polyethylene bottles. Fifteen grams (wet weight) of the appropriate sediment were added to each bottle and the volume brought up to 750 mL with treated seawater to make a final concentration in all test containers of 20 g (wet weight) of sediment per liter seawater. Two controls were prepared and run concurrently. One control contained the same concentration of clean sediment (from off West Beach, Whidbey Island, the collection site for the sensitive amphipod Rhepoxynius abronius). The other control contained clean seawater. All containers were run in duplicate (22 test sediments + 2 controls x 2 = 48 containers).

The sediments were suspended by rotating at 10 rpm for 3 h, following which period the embryos were added and the suspended sediments allowed to settle. No additional agitation was provided.

Within 2 h of fertilization, each container was innoculated with approximately 28,000 developing oyster embryos, to give an approximate concentration of 35/mL. The innoculated cultures were covered with paper towelling and incubated for 48 h at 20±1°C. After 48 h, the overlying water in each container was carefully poured through a 38 µm sieve, without disturbing the settled sediment, thereby retaining and concentrating the surviving oyster larvae (larvae caught in the sediments were invariably dead). The concentrated larvae were washed into a 100 mL graduated cylinder to measure the volume of each sample, transferred to screw-cap glass vials, and preserved with 3% neutral formalin. Preserved samples (equal in volume to that containing 300-400 larvae in controls) were placed in Sedgewick-Rafter cells and examined at 100X magnification. Quality assurance procedures included independent (blind) counts.

Normal and abnormal larvae were enumerated to determine percent survival and percent abnormalities. Percent survival was determined as number of larvae surviving in each test container relative to seawater control survivals. All larvae that failed to transform to the fully shelled, hinged, "D" shaped veliger larval stage were considered abnormal.

Salinity, dissolved oxygen and pH levels were initially adjusted in each container to 25 ppt, 8.0 mg/L and 8.0, respectively. These parameters were measured in each container at the termination of the bioassay.

2.5.2 Amphipod Lethality

The infaunal amphipod Rhepoxynius abronius was collected subtidally from West Beach on Whidbey Island using a bottom trawl. Collections were carried out by personnel from the Environmental Protection Agency. Amphipods were maintained and transported in clean polyethylene containers on ice, and were delivered to the E.V.S. Consultants laboratory within 6 h of collection.

Following their arrival in the laboratory, amphipods were hand sorted from sediments and identifications were confirmed with a Wild M5 dissecting microscope. Damaged and dead individuals were discarded. The remaining amphipods were placed in clean polyethylene containers provided with substrate from the collection site and clean seawater (25 ppt salinity). Test animals were acclimated in a constant environment room adjusted to 10±0.5°C under a 12 h light/dark cycle. Cultures were aerated but not fed during the acclimation phase. Testing was conducted 5 or 6 days after collection.

Acute lethality of whole sediment was determined by the method of Swartz et al. (1982, in press) which involves a 10 d exposure to test sediments. Two hundred grams of each test sediment were placed in

one liter glass jars and covered with 800 mL of clean seawater (25 ppt salinity). Frozen sediment samples were weighed and distributed to the test jars before thawing to prevent losses of interstitial water. Amphipods were randomly and blindly assigned to the test jars at a density of 20 individuals per jar. Five replicate jars (=100 amphipods) were run for each test sediment and for controls. Four control series were run; three contained unfrozen sediment from the collection site; one contained frozen sediment from the collection site. Containers were aerated during testing by means of 1 mL glass pipettes fitted through plastic lids covering individual jars. All testing was conducted at 10±0.5°C under a 12 h light/dark cycle.

Test jars were checked daily and numbers of amphipods on the surface recorded. Amphipods trapped at the air/water interface were gently pushed below the interface, after which they would typically swim to the bottom of the test vessel and burrow in the sediment.

After the 10 d exposure period, the sediments were sieved (0.5 mm) and the number of surviving and dead amphipods counted. Missing individuals were assumed to have died and decomposed (Swartz et al., 1982).

Amphipod avoidance response was also determined, from counts of numbers of amphipods that had emerged from the sediments at the time each mortality check was made. Data were pooled at the end of the 10 d exposure period to calculate means and standard deviations. These results were compared with amphipod survival in sediments.

Any significant differences in survival between test sediments was determined by analysis of variance. Differences in mean survival between test and control sediments was determined by Dunnets procedure (Steel and Torrie, 1960). One-tailed Dunnet t tables were used to determine if mean survival in each test series was significantly less than control values.

2.5.3 Oligochaete Respiration Rate

Monopylephorus cuticulatus were collected intertidally from beach sediments near Port Alice, B.C. and transported to E.V.S. Consultants laboratories in clean coolers with ice. Worms were hand sorted in the laboratory and placed in aquarium cultures consisting of natural substrate and 25 ppt salinity seawater. Cultures were maintained at 10±0.5°C under a 12 h light/dark cycle. Aeration was continuous and culture water was replaced weekly. Cultures were fed a mixture of ground Enteromorpha and commercial Tetramin ad libidum; feeding was discontinued 24 h prior to testing.

Respiratory responses of the oligochaetes were measured during exposure to sediment elutriates prepared as follows. Ten grams of test sediment were placed in a clean 500 mL volumetric flask containing 500 mL of filtered (0.45 µm) seawater (25 ppt salinity) and the flask was then shaken vigorously for one minute. One

hundred mL of test solution was decanted and centrifuged for 10 min at 1000 rpm to settle particulates. The prepared elutriate test solutions were then decanted and tested.

Oxygen consumption was determined potentiometrically using a Radiometer PHM73 Blood Gas Analyzer, with 10 mL disposable syringes as respiration chambers. The analyzer was connected to a Haake Constant Temperature Circulator (10±0.5°C). Worms (10-20 depending on biomass) were cleaned of adhering matter and placed in syringes containing test elutriate or water. The syringes were carefully capped to exclude all air bubbles, and incubated upside-down in a darkened water bath (10±0.5°C) for 1 h prior to testing. Duplicate syringes plus a blank syringe consisting of test solution without worms were tested for each sample (=3 syringes).

Syringes were removed from the water bath at 0.6-1.5 h intervals, inverted twice to disrupt possible oxygen gradients and aliquots were injected directly into the Radiometer analyser chamber. An initial 400 µL aliquot was injected to flush the chamber, the electrode was allowed to equilibrate, and then three consecutive 200 µL measurements were taken on each sample. The Radiometer electrode was calibrated prior to the first measurement and subsequently recalibrated after each set of readings. Syringes were returned to the water bath between measurements.

Between three and five consecutive series of measurements were taken for each syringe over 3-5 h, following which the worms were removed, placed on pre-weighed aluminum weigh boats and dried overnight at 80°C. Readings in mm Hg were converted to percent saturation using the oxygen solubility tables of Truesdale and Downing (1954). Respiration rates in µL O_2 were calculated directly from the change in percent saturation over time divided by the dry weight of worms tested following adjustment for microbial respiration in the blanks and for any oxygen probe calibration drift.

Mean respiration rates (n=3-5 values per syringe x 2) were compared statistically with control values determined using the same procedure but with worms in clean dilutent water. A t-test was used to compare means.

2.5.4 Cell Reproduction and Genotoxicity

2.5.4.1 Preparation of Sediment Extracts

Sediment samples were frozen and stored until just prior to extraction. Each sample was then thawed, rehomogenized by careful but thorough stirring, and an aliquot (approximately 20 g wet weight) transferred to a clean tared beaker. This aliquot was dried to constant weight (80°C), dessicated and reweighed to determine the percent water. A second aliquot (approximately 150 g wet weight) was transferred to a tared, solvent-cleaned, 315 mL stainless steel centrifuge bottle with a teflon-lined screw cap, and weighed. The sample was then serially extracted with pesticide-grade solvents using the procedure of Malins et al. (1980) as previously used by Chapman et al. (1982a, in press b), and summarized below.

Methanol (50 mL) was added to each centrifuge bottle, which was tightly capped and shaken vigorously for 2 min followed by centrifugation at 2000 rpm for 5 min. The clear solvent was decanted into a 1 L separatory funnel. The procedure was repeated twice more and the methanol extracts were combined in the separatory funnel, which was then closed and covered with aluminum foil.

One hundred mL of a dichloromethane/methanol (2:1 v/v) solution were added to the centrifuge bottle, the cap closed tightly and the bottle was shaken vigorously for two minutes to ensure complete mixing. The bottle was then placed in a shaker table overnight (approximately 18 h), following which the sediment was settled by centrifugation at 2000 rpm for 5 min and the solvent decanted into the separatory funnel with the methanol. A second 100 mL aliquot of the dichloromethane/methanol (2:1) was added, the bottle shaken vigorously and placed on the shaker table for 6 h. The sediments were again settled with centrifugation and the solvents decanted.

The remaining sample was shaken vigorously for 2 min with approximately 30 mL of dichloromethane; it was centrifuged, and the solvent decanted into the separatory funnel. Another 100 mL of dichloromethane was added to the bottle, the cap secured, the bottle shaken vigorously, and placed on the shaker table overnight. The sediments were again settled with centrifugation and the solvent was decanted into the separatory funnel. A final 30 mL rinse of dichloromethane was added and the bottle shaken vigorously, followed by centrifugation and decanting. The sediment was then discarded.

Approximately 500 mL of cleaned, distilled water were added to the combined solvents in the separatory funnel. The funnel was carefully swirled and inverted (with frequent venting) for 2 min. The liquid phases were allowed to separate and the dichloromethane (lower) layer was drained into a 500 mL separatory funnel. The aqueous layer was re-extracted twice with 20 mL of dichloromethane and the remainder discarded. The dichloromethane fractions were combined in the 50 mL funnel and transferred, with rinsing, back to the 1 L funnel and re-extracted with another 500 mL of distilled water. The dichloromethane was drained into the 500 mL funnel and the aqueous layer was extracted once more with 20 mL of dichloromethane. The latter solvent was added to the 500 mL funnel and the aqueous layer was discarded.

The dichloromethane was drained from the 500 mL separatory funnel through approximately 20 g of combusted and washed anhydrous sodium sulfate that was held in a 30 mL glass conical centrifuge tube with the tip cut off. The effluent from this mini-column was discharged into a 500 mL Kuderna-Danish flask with a 15 mL receiver. When empty, the 500 mL separatory funnel was rinsed with 20 mL of dichloromethane which was drained through the sodium sulfate column into the flask. The column was washed a final time with 10 mL of dichloromethane which was also drained into the flask.

Boiling chips were added to the Kuderna-Danish Flask and a 3-ball Snyder column was placed on top. The solvent volume was reduced to about 5 mL on a hot water bath. When cooled, the sides of the flask were rinsed into the receiver with dichloromethane. The receiver was removed and the contents were quantitatively transferred to a tared conical centrifuge tube with a ground glass stopper. The sample was taken almost to dryness on the hot water bath, followed by storage in a dessicator wrapped in aluminum foil with the stopper open slightly, until a constant weight was achieved upon reweighing the tube. This weight was the amount of extractable organic material.

After weighing, the tube was closed and wrapped fully in aluminum foil ready for cell reproduction testing. Extracts were treated with 1 mL of spectrophotometric grade DMSO for 24 h with frequent stirring on a vortex mixer. The DMSO was then removed to a glass vial and used as "stock" solution. Since not all extracted material was dissolved in the DMSO during testing, the centrifuge tubes were dried and reweighed to determine the exact amount used in testing (fraction soluble). Both stock and extract solutions were stored in the dark under nitrogen until applied to the cell cultures.

2.5.4.2 Cell Reproduction

Cells from two species of fish were used for these studies: rainbow trout gonad cells (cell line RTG-2) and bluegill fry cells (cell line BF-2). The RTG-2 cells possess an active mixed function oxygenase (MFO) system which processes and activates many toxins that require metabolic activation. The BF-2 cells possess little or no MFO activity, hence are relatively unaffected by toxins requiring metabolic activation. Both cell types are susceptible to direct acting toxins. Both cell types were cultured in Leibovitz L-15 medium supplemented with 10% fetal bovine serum, antibiotics and buffered (pH 7.1 to 7.3) with sodium phosphate. Cells from mass culture were seeded into Corning 2 cm^2 multiwell plates and incubated at 10°C (RTG-2 cells) and 25°C (BF-2 cells) for 96 h in the presence of the sediment extracts.

Sediment extracts were weighed and re-extracted with DMSO for 24 h, after which the centrifuge tubes which had contained the extracts were reweighed to determine the exact amount of organic extract present in the DMSO solvent. On the basis of these data and previous experience with toxicity testing of Puget Sound sediment extracts (Chapman et al., 1982a, in press b), six dilutions of each extract were prepared (50, 25, 10, 5, 2 and 1 µg/mL). Dilutions ranged from non-toxic (1 µg/mL) to 100% toxic (50 µg/mL). Cells were exposed to exactly comparable amounts of organic extract in contrast to previous testing in which the absolute values of exposure concentrations were calculated from organic concentrations for each sample. Each concentration of each extract was tested against both cell types in triplicate and the mean of the three values used for comparison against control cultures.

Control cultures consisted of six replicates of untreated cells, and two replicates of solvent (0.5% DMSO) treated cells. In addition, to test the activity of the MFO enzyme systems and the sensitivity of the two types of cell, positive controls consisting of three replicates of six concentrations of benzo(a)pyrene (B(a)P) were run for each cell type.

After a 96 h exposure period, both test cultures and controls were rinsed once with phosphate buffered saline (pH 7.1) to remove any unattached dead cells. The remaining live cells were removed for counting by the addition of 0.01% EDTA/Trypsin. The total number of cells in each replicate was determined using a Coulter electronic particle counter (Model ZB1) and the mean for each concentration of extract was determined.

Mean number of cells for each treatment was compared to the mean control values (96 h controls = 100%) and any concentration which reduced the final cell count by 20% (equivalent to approximately 2 standard deviations of the mean of the controls and thus representing 95% confidence limits) or more, was considered inhibitory. When cell numbers at the end of 96 h were below the starting cell number (seeding cell density), that concentration of extract was considered to be cytotoxic (causing cell death) rather than inhibitory to cell reproduction.

2.5.4.3 Genotoxicity

Based on the results of the cell reproduction testing, two extract concentrations were chosen for genotoxicity (= anaphase aberration) testing: the highest concentration which did not inhibit mitosis in RTG-2 cells and a second concentration one dilution lower. RTG-2 cells from stock cultures were then plated onto cover slips in Leighton tubes with 1 mL of culture medium. These cultures were incubated overnight and the appropriate sample extract was added to the culture medium the following morning. Two cultures per dilution of each extract were then incubated for 48 h at 10°C, following which the cover slips were removed from the culture medium, rinsed, fixed in methanol:acetic acid (3:1) for 1 h, air dried and stained with 3% Giesma stain in Sorensen's buffer (pH 6.8). A minimum of 100 anaphases per slide were microscopically examined under 470x power for each anaphase concentration and the percents of normal and aberrent anaphases were recorded. Controls were run with each series of extracts tested and consisted of a set of untreated cells and a set treated with 0.5% DMSO. In addition, to test the response of these cells to a concentration of known mutagen previously shown to be capable of producing high levels of aberrations, a set of three cultures was treated with 0.25 µg/mL B(a)P.

Cells were scored as abnormal if they contained any of the previously described chromosomal lesions reported for this test (Nichols et al., 1972; 1977; Kocan et al., 1982; Chapman et al., 1982a).

3.0 RESULTS

3.1 Sediment Characteristics

The observed values for grain size, percent water, percent digestible organic carbon (DOC), percent total volatile solids (TVS), and percent extractable organic matter are summarized in Table 1 for each sediment sample. Detailed sediment grain-size data are included in Appendix B. For visualization of differences in the sediment characteristics among the stations, the sediment texture (expressed as the percent of the sample in the sand and gravel class; the coarser fraction), and the percents of the organic fractions have been plotted as bar diagrams at the charted station locations in Figs. 4 and 5.

Living organisms were noted in all samples with the exception of Station 15, in inner Everett Harbor (Appendix A). Although no attempt was made as part of the present study to conduct either qualitative or quantitative assessments of the benthic fauna, such data will be available in the future from the U.S. EPA for Bellingham Bay and Samish Bay stations (E. Long, NOAA, pers. comm.).

3.1.1 Grain Size

Bellingham Bay sediments consisted predominantly of grey-green mud with comparatively low quantities of sand and organic matter (Fig. 4). Organically enriched, anoxic sediments were observed at Stations 3, 4 and 7 in inner Bellingham Bay.

Everett Harbor sediments were generally coarser than those from Bellingham Bay, but many contained considerably more organic matter (Fig. 5). Sediments from the inner harbor consisted of black, anoxic muds with large quantities of wood debris. Most of the sediment fraction in the gravel size class noted for Everett Harbor samples in Table 1 consisted of bark and other wood debris. Samples from the outer harbor were coarser and gray-green in color. Station 21 was the only station in the outer harbor with an H_2S odor.

The two sediment samples from the reference area, Samish Bay (Fig. 4), were both gray-green in color. However, Station 11 comprised a low organic matter sample while Station 12 comprised slightly anoxic mud of intermediate organic enrichment.

3.1.2 Organic Matter

Three measures were made of organic matter associated with the sediments. Total volatile solids (TVS) was the most rigorous technique, in which all combustible organic matter in the samples was destroyed. In comparison, digestible organic matter (DOC: oxidized by H_2O_2) represented a fraction of the TVS which is susceptible to less rigorous degradation. In particular, DOC measurements should exclude much of the larger woodchips and coal fragments known to be present in many of the sediments from Puget Sound (Pavlou et al., 1978). The solvent extractable organic matter

TABLE 1 Sediment Physical and Chemical Parameters

Station Number	Gravel %	Sand[a] %	Silt[a] %	Clay[a] %	Water %	DOC[b] %	TVS[b] %	Extractables[b] %	Soluble[c] %
Bellingham Bay									
01	0.00	1.72	53.58	44.70	43.28	1.42	5.9	0.0693	84
02	0.00	2.31	68.93	28.76	49.08	2.25	8.7	0.0603	98
03*[d]	3.48	18.08	44.30	34.14	61.21	3.32	14.8	0.1746	66
04*	0.64	5.29	66.80	27.28	58.35	1.03	11.9	0.1965	76
05	0.00	2.98	49.83	47.19	55.33	1.22	8.1	0.1113	83
06	0.00	2.18	62.94	34.88	58.39	1.54	8.7	0.1493	82
07*	0.14	7.17	48.37	44.38	60.19	1.50	9.8	0.1875	72
08	1.26	23.13	43.56	32.04	45.56	1.02	5.6	0.0944	78
09	0.00	5.46	49.11	45.43	63.11	1.35	7.6	0.1393	85
10	1.06	23.46	44.48	31.00	47.04	1.29	6.4	0.1065	85
Samish Bay									
11	0.28	78.40	11.08	10.25	31.62	0.38	3.0	0.0334	68
12*	0.00	8.76	59.76	31.48	64.32	1.32	7.9	0.0789	67

Table 1 (Cont'd)

Station Number	Gravel %	Sand[a] %	Silt[a] %	Clay[a] %	Water %	DOC[b] %	TVS[b] %	Extractables[b] %	Soluble[c] %
Everett Harbor									
13*	0.23	28.35	50.17	21.25	62.91	5.72	18.6	0.4257	78
14*	2.51	9.04	54.77	33.67	74.59	7.20	18.9	0.4158	65
15*-[d]	11.01	32.30	45.36	11.33	56.86	7.06	18.7	0.4021	43
16*	0.80	34.00	47.38	17.82	66.22	7.69	23.1	0.3691	42
17	1.30	38.57	40.58	19.54	51.82	3.43	11.4	0.1894	53
18	3.06	60.18	24.15	12.61	36.01	1.36	4.2	0.0658	58
19	0.04	81.63	12.72	5.61	27.15	0.78	2.6	0.0464	77
20	0.07	40.31	42.97	16.64	47.62	1.23	7.9	0.1114	78
21*	1.77	40.90	37.12	20.20	51.61	1.26	9.9	0.1737	49
22*	8.55	16.60	42.95	31.90	64.54	2.45	17.3	0.3503	38
23*	3.13	42.15	37.24	17.48	66.19	6.34	25.6	0.3148	43

a. Phi size ranges: sand, -2 to +4; silt, >4 to 8; clay, >8.
b. Percent of dry weight of sediments.
c. Material which dissolved in DMSO during genotoxicity testing. Expressed as percent of extractable organic matter (extractables).
d. A minus (-) sign after the station number indicates that no living organisms were observed in the sediments during sampling. A star (*) indicates that a bad odor, e.g. H_2S, was noted in the sample during collection.

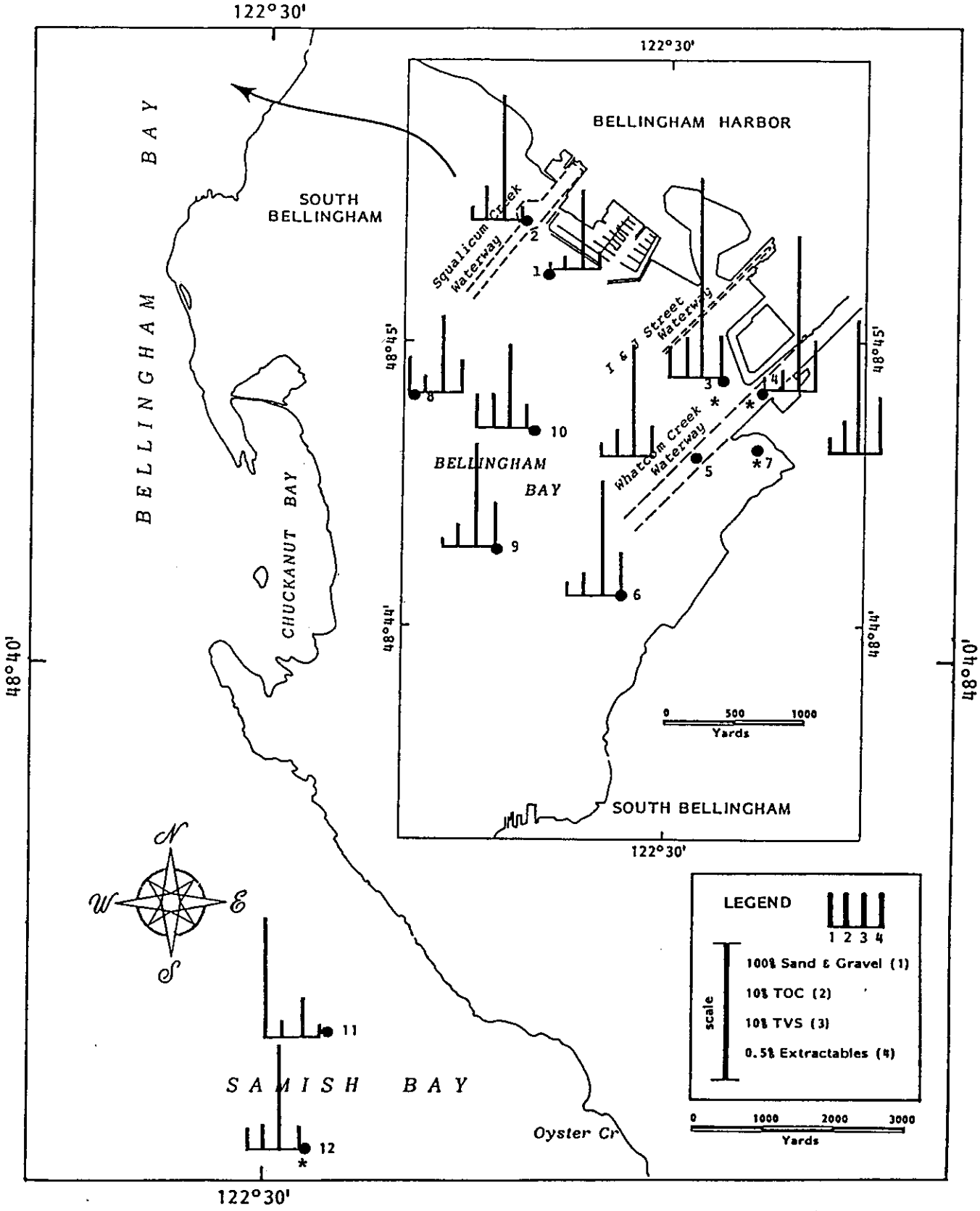

Figure 4 Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in Bellingham and Samish Bays

A star (*) indicates that malodorous sediments were noted during collection.

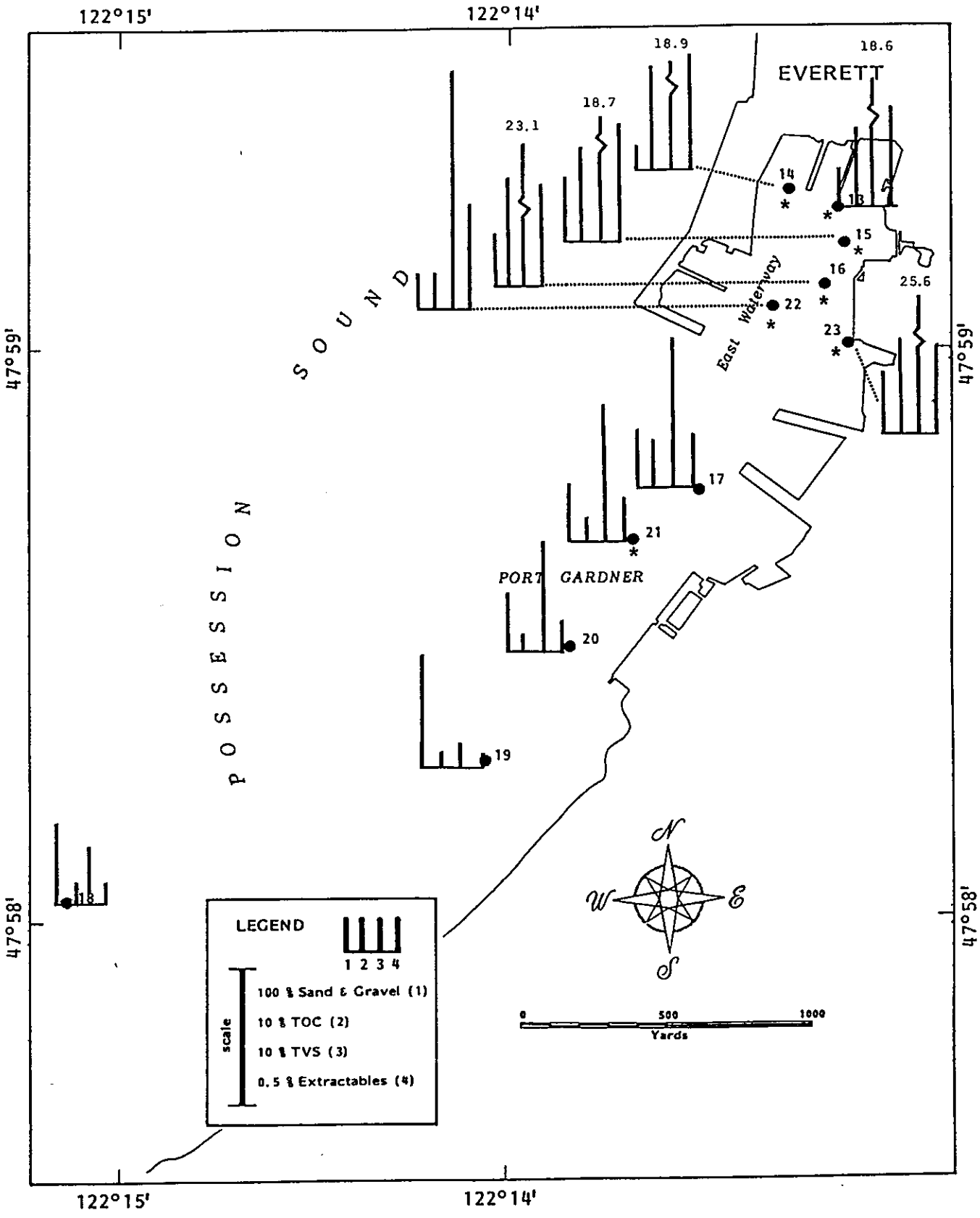

Figure 5 Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in Everett Harbor

A star (*) indicates that malodorous sediments were noted during collection.

was also monitored while preparing extracts for the anaphase aberration testing. This fraction should include the low molecular weight, nonpolar to medium polar compounds, and would include the toxic organic compounds measured in other studies (e.g. Malins et al., 1980, 1982). All three measures indicated generally lower levels of organic matter in Bellingham Bay compared to Everett Harbor (Table 1). This difference may partly reflect a sampling bias, with more samples from Everett Harbor being taken in the organically enriched inner harbor area.

Correlations between the percents of DOC and TVS and the sediment grain size did not indicate strong spatial trends although slight trends were observed for Everett Harbor samples (Figs. 6 and 7). Both organic parameters decreased with increasing percent coarse material in the sediments (as determined by eye). No such relationship was apparent for the Bellingham Bay samples. Too few samples were collected in Samish Bay to confirm a relationship. These correlations, particularly for the Everett samples, are complicated by the fact that the coarse fractions themselves contain large quantities of organic matter (wood debris) which tends to skew the DOC and TVS measurements toward higher values at larger percents of sand and gravel. In comparison with the above, the solvent extractable organic matter showed a very strong dependence on the percent DOC and percent TVS (Fig. 7).

3.2 Toxicity Testing

3.2.1 Oyster Larvae Toxicity

The results of the oyster larvae bioassays are summarized in Table 2. In addition to larval numbers, bioassay response was expressed in terms of mean percent abnormal larvae, and mean percent relative survival (compared to controls). Salinity, pH and dissolved oxygen values determined at the termination of the bioassay are provided in Appendix B. These parameters remained at acceptable levels in all test cultures including those eliciting adverse larval response: salinity range 25.0-25.2 ppt; pH range 7.4-7.9; dissolved oxygen range 6.4-6.9 mg/L in controls, 4.6-6.0 mg/L in test sediments.

The Pacific oyster embryos used in the bioassays were of excellent quality, as indicated by the low percentage of abnormal larvae in the control seawater (2.2%) and control sediment (1.6%) cultures. These values are well below the 3% abnormality rate suggested by Woelke (1972) as acceptable for oyster larvae bioassay controls.

Sediment samples gave responses ranging from highly toxic to non-toxic. The percentage of abnormal larvae exhibited by oyster embryos exposed to sediments from Stations 1, 5, 8, 13, 14 and 23 exceeded the single sample marine quality criterion of 20% larval abnormality, proposed by Woelke (1972). The following additional stations exceeded Woelke's (1972) proposed multiple sample quality criterion of 5% larval abnormality: Stations 2, 3, 4, 6, 7, 9, 15, 16, 17, 20, 21 and 22. Stations 10, 11, 12 and 19 all had less than 5% larval abnormalities.

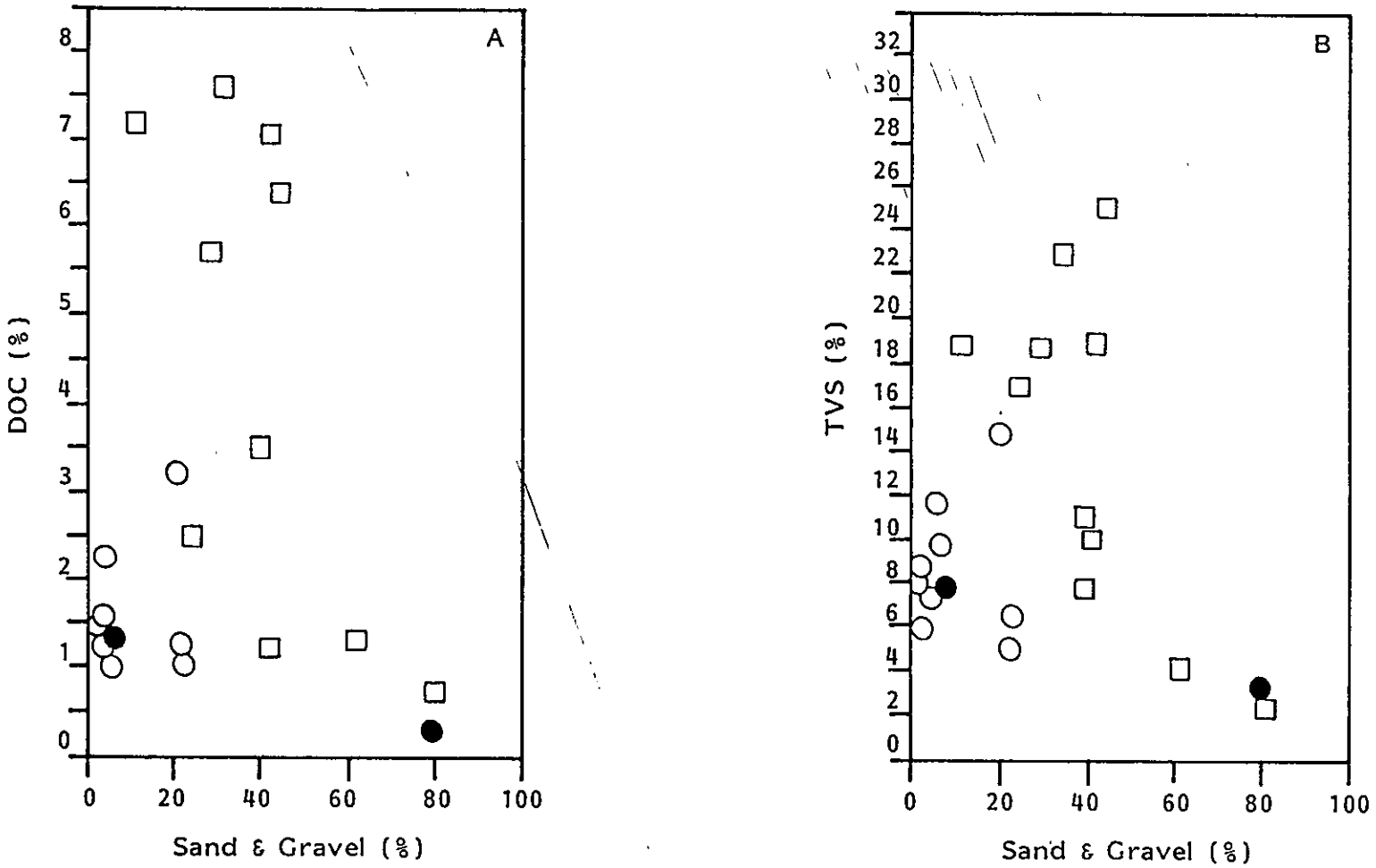

Figure 6 Plots of the Concentrations of (A) DOC and (B) TVS Versus the Sediment Grain Size for Samples from Bellingham (○) and Samish (●) Bays, and Everett Harbor (□)

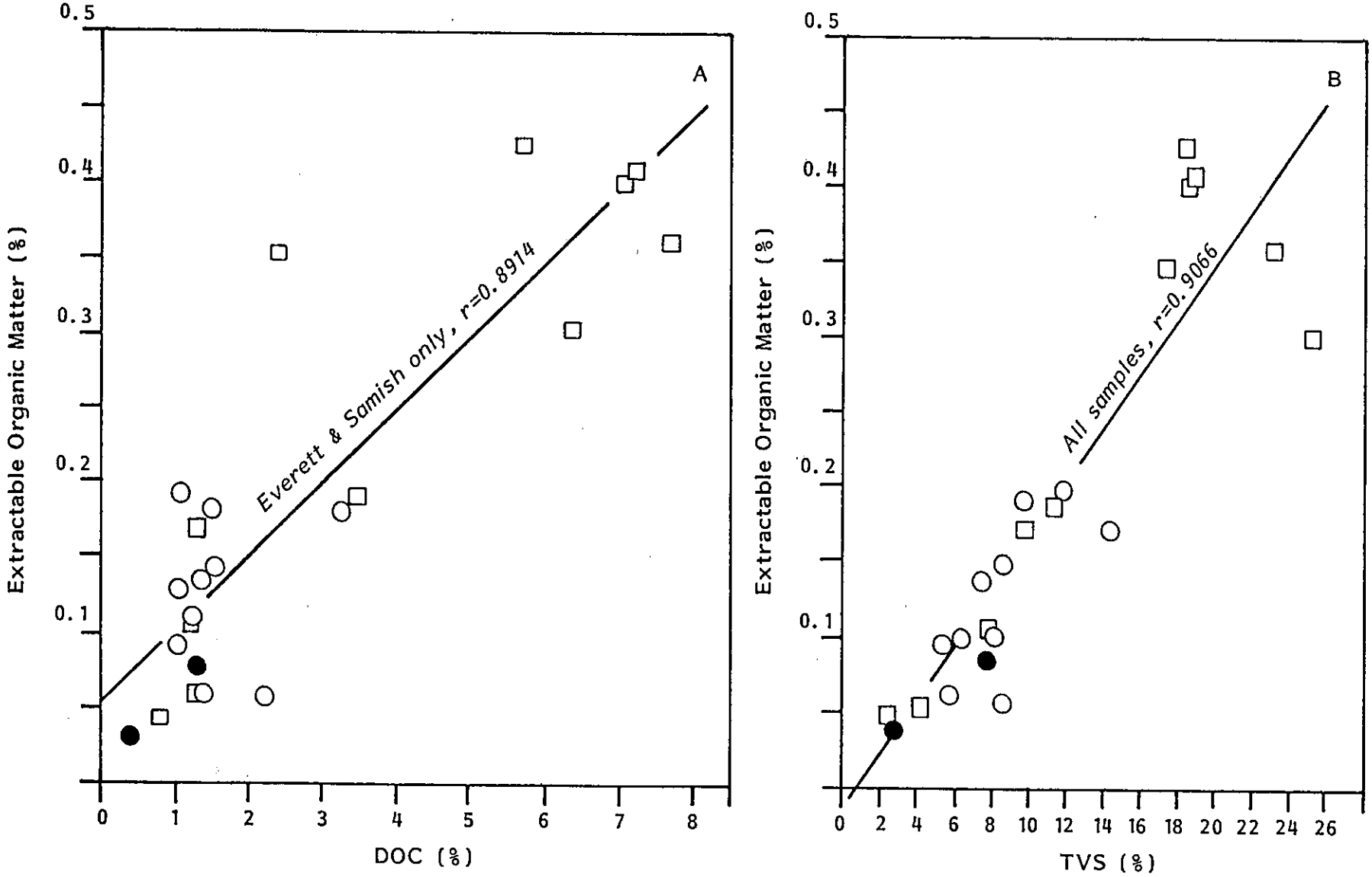

Figure 7 Plots of the Concentrations of Extractable Organic Matter in the Sediments of Bellingham (○) and Samish (●) Bays, and Everett Harbor (□) Versus the Concentrations of (A) DOC and (B) TVS.

Lines represent linear correlation analysis.

TABLE 2 Oyster Larvae Bioassay Data

Station	Replicate	Total Larvae	Normal Larvae Total	Normal Larvae %	Abnormal Larvae Total	Abnormal Larvae %	Mean Values: Number of Larvae	Mean Values: Percent Abnormal	Mean Values: % Relative Survival[a]	Relative Toxicity[b]
1	A	66	48	72.7	18	27.3	110	25.1	26	3
	B	153	116	75.8	37	24.2				
2	A	140	128	91.4	12	8.6	166	11.8	40	2
	B	191	164	85.9	27	14.1				
3	A	181	165	91.2	16	8.8	250	6.0	60	1
	B	318	304	95.6	14	4.4				
4	A	100	89	89.0	11	11.0	67	11.3	16	2
	B	33	29	87.9	4	12.1				
5	A	84	64	76.2	20	23.8	65	26.2	16	3
	B	46	32	69.6	14	30.4				
6	A	192	176	91.7	16	8.3	232	6.5	56	1
	B	272	258	94.9	14	5.1				
7	A	47	39	83.0	8	17.0	32	18.8	8	3
	B	17	13	76.5	4	23.5				
8	A	90	59	65.6	31	34.4	106	24.6	25	3
	B	121	100	82.6	21	17.4				
9	A	181	168	92.8	13	7.2	184	6.3	44	1
	B	186	176	94.6	10	5.4				
10	A	69	64	92.8	5	7.2	136	4.4	33	1
	B	203	196	96.6	7	3.4				
11	A	266	257	96.6	9	3.4	245	2.7	59	0
	B	224	220	98.2	4	1.8				

Table 2 (Cont'd)

Station	Replicate	Total Larvae	Normal Larvae Total	Normal Larvae %	Abnormal Larvae Total	Abnormal Larvae %	Mean Values: Number of Larvae	Mean Values: Percent Abnormal	Mean Values: % Relative Survival[a]	Relative Toxicity[b]
12	A	400	395	98.8	5	1.2	307	1.8	74	0
	B	214	208	97.2	6	2.8				
13	A	19	8	42.1	11	57.9	32	46.7	8	3
	B	44	27	61.4	17	38.6				
14	A	55	48	87.3	7	12.7	96	31.7	23	3
	B	136	92	67.6	44	32.4				
15	A	119	104	87.4	15	12.6	124	12.5	30	2
	B	128	112	87.5	16	12.5				
16	A	66	56	84.8	10	15.2	105	15.8	25	2
	B	143	120	83.9	23	16.1				
17	A	184	163	88.6	21	11.4	189	10.1	45	2
	B	193	176	91.2	17	8.8				
18				not tested						
19	A	319	312	97.8	7	2.2	311	2.1	75	0
	B	302	296	98.0	6	2.0				
20	A	123	104	84.6	19	15.4	147	12.9	35	2
	B	171	152	88.9	19	11.1				
21	A	248	232	93.5	16	6.5	236	8.1	57	1
	B	223	201	90.2	22	9.8				

Table 2 (Cont'd)

Station	Replicate	Total Larvae	Normal Larvae Total	Normal Larvae %	Abnormal Larvae Total	Abnormal Larvae %	Mean Values: Number of Larvae	Mean Values: Percent Abnormal	Mean Values: % Relative Survival[a]	Relative Toxicity[b]
22	A	179	163	91.1	16	8.9	119	11.3	29	2
	B	59	48	81.4	11	18.6				
23	A	56	35	62.5	21	37.5	57	26.5	14	3
	B	57	48	84.2	9	15.8				
Sediment Control	A	408	402	98.5	6	1.5	382	1.6	92	-
	B	356	350	98.3	6	1.7				
Seawater Control	A	449	438	97.6	11	2.4	415	2.2	100	-
	B	381	374	98.2	7	1.8				
Combined Sediment and Seawater Controls							396 (n=4; S.D.=42; $\bar{x}$±2S.D.=312-480)			

a. In terms of mean seawater control survivals which, following standard (Cummins, 1973, 1974; APHA, 1980) procedures, are assigned a survival value of 100%.

b. Relative toxicity of sediment extracts was scored on the following scale:

>20% abnormalities = 3 - high toxicity
10-19% abnormalities = 2 - intermediate toxicity
4-9% abnormalities = 1 - low toxicity
<3% abnormalities = 0 - no toxicity

The survival rate data generally agreed with those for abnormalities. For the seven stations with greater than 20% larval abnormalities, mean relative survival was low, with a range of 8-26%. Of the 11 stations with between 20% and 5% mean larval abnormalities, four stations (Stations 3, 6, 9 and 21) had mean survivals between 44 and 57% which, coupled with a low 6.0-8.1% rate of larval abnormalities, indicates low toxicity at these stations. The four stations with less than 5% abnormalities had generally good survival rates (59-75%) with the exception of Station 10 which had a mean survival rate of 33% indicating high acute lethality at this station.

Based on the combined data for the oyster larvae bioassay, the following stations showed high toxicity: 1, 5, 7, 8, 13, 14 and 23. Stations 2, 4, 15, 16, 17, 20 and 22 showed intermediate toxicity. Stations 3, 6, 9, 10 and 21 showed low toxicity. Stations 11, 12 and 19 were non-toxic.

3.2.2 Amphipod Lethality

The results of the amphipod bioassay tests are summarized in Table 3. Detailed data tables including daily observations of amphipods on the surface are provided in Appendix D.

Analysis of variance indicated no significant difference in mean survival between the controls, however there were differences between the test sediments. Pooled control data compared with test sediment data indicated that the survivals in two test sediments (Station 3, Bellingham Bay and Station 14, Everett Harbor) were significantly lower ($P<0.05$) than controls. All other test series were not significantly different than controls.

Data for avoidance (amphipods emerging from the sediments, Table 3), did not correspond well with the survival data. The four test sediments with the greatest avoidance response (Stations 2, 11, 13 and 19) had similar survival levels to controls. Significantly lower survival was noted in sediments from Stations 3 and 14, however amphipods displayed a marginal avoidance response to these sediments. The least avoidance response was exhibited by amphipods in control sediments and sediments from Stations 5 and 9.

3.2.3 Oligochaete Respiration Rate

Respiration rate data sheets are shown in Appendix E and the results are summarized in Table 4.

All testing was carried out at oxygen saturation levels greater than 60% (96-156 mm Hg). Mean respiration rates (in μL O_2/mg dry wt/h) ranged from 0.19 to 0.32 in test solutions; control respiration rate was 0.24 ± 0.4. Significant respiratory anomalies compared to control values were detected in three of ten sediments from Bellingham Harbor, four of ten sediments from Everett Harbor, and none of the sediments from Samish Bay.

TABLE 3 Amphipod Bioassay Data

Station	Survival[a]		Avoidance[b]	
	Mean	S.D.	Mean	S.D.
1	18.6	0.5	1.3	2.9
2	18.6	1.7	3.8	5.1
3	17.2	1.3	1.1	2.1
4	18.4	0.9	2.1	3.3
5	19.2	0.8	0.4	1.3
6	17.4	2.2	2.8	4.9
7	18.0	1.6	0.6	1.6
8	18.8	1.3	2.0	3.0
9	19.2	1.3	0.4	1.5
10	18.4	1.5	1.4	3.2
11	19.0	1.2	3.3	2.5
12	19.2	1.3	0.7	1.6
13	19.4	0.5	3.3	3.0
14	15.0	2.0	1.6	2.3
15	18.4	1.5	1.5	2.2
16	18.6	1.1	1.3	1.9
17	18.6	0.9	1.9	2.5
18	19.0	0.7	1.2	2.0
19	19.6	0.5	3.1	2.6
20	19.2	0.8	1.4	2.4
21	19.2	0.8	1.6	2.3
22	19.4	0.9	0.7	1.3
23	19.4	0.5	2.4	3.0
C1[c]	19.2	1.3	0.3	0.6
C2[c]	19.4	0.9	0.4	0.7
C3[c]	19.0	0.7	0.3	0.6
FC[d]	18.6	0.5	0.1	0.4

a. 5 replicates; seeded with 20 amphipods per replicate
b. number of amphipods on the surface per jar per day
c. controls with fresh sediment
d. control with frozen sediment

TABLE 4 Oligochaete Respiration Rate Data

Station	Mean Respiration ($\mu L O_2$/mg dry wt/h)	S.D.	n	Level of Significance[a]
1	0.32	0.10	7	**
2	0.23	0.05	7	n.s.
3	0.19	0.03	7	*
4	0.20	0.04	7	n.s.
5	0.28	0.06	7	n.s.
6	0.19	0.02	7	*
7	0.25	0.05	7	n.s.
8	0.26	0.04	7	n.s.
9	0.26	0.06	7	n.s.
10	0.22	0.04	7	n.s.
11	0.22	0.02	6	n.s.
12	0.24	0.05	7	n.s.
13	0.22	0.03	7	n.s.
14	0.31	0.05	7	**
15	0.29	0.07	6	*
16	0.21	0.03	6	n.s.
17	0.28	0.04	6	*
18	0.27	0.05	7	n.s.
19	0.19	0.03	7	*
20	0.20	0.03	6	n.s.
21	0.24	0.06	6	n.s.
22	0.20	0.03	6	n.s.
23	0.21	0.05	6	n.s.
Control	0.24	0.04	29	

a. Level of significance of difference compared to control value:
n.s. = not significant; * = $0.05>P>0.01$; ** = $0.01>P>0.001$

Of the significantly different respiration rates recorded, approximately equal numbers were depressed (3/7) as those elevated (4/7). No clear spatial trends were noted.

3.2.4 Cell Reproduction and Genotoxicity

3.2.4.1 Cell Reproduction

Complete cell reproduction test data are provided in Appendix F. Both cell types showed an approximately 100% increase in numbers over 96 h. Neither the DMSO solvent nor the extraction blank affected cell proliferation. B(a)P had little effect on the BF-2 cultures, but did reduce proliferation of RTG-2 cells (Fig. F-1, Appendix F). This result indicates the importance of an active MFO system in metabolizing otherwise inert compounds such as B(a)P to form toxic products.

Table 5 summarizes the data obtained from cell cultures exposed to sediment extracts. In all cases extract additions of 50 µg/mL were toxic. Because of this, three basic responses were observed: positive, negative and questionable. Extracts which reduced cell proliferation by more than two standard deviations from the mean of the controls ($P<0.05$) were considered positive (toxic) if the response occurred at a concentration of 10 µg/mL or less, and negative (non-toxic) if only the 50 µg/mL concentration elicited a response. Those cultures which were significantly different from the controls at 25 µg/mL were considered to be questionable as no clear dose response was observed and the next highest concentrations were toxic from all sites. The universal toxic response at 50 µg/mL organic content was due to a disruption of cell function as a result of the large quantities of organics which were dissolved in the membranes, and did not necessarily reflect the presence of exogenously introduced toxic substances in the extract.

The three types of response seen for each cell type (i.e. positive, negative and questionable) are compared in Appendix F (Figs. F-2, F-3). Similar response patterns were noted for both cell types. An obvious dose response resulted from exposure to toxic extracts (positive) with no response or a stimulatory response observed with exposure to non-toxic extracts (negative). Those extracts classified as questionable showed a significant drop in cell number only at 25 µg/mL. Effects observed at 25 µg/mL may be related to membrane disruption by organics, hence significant responses observed at 25 µg/mL are regarded as questionable.

As observed in previous studies (Chapman et al., in press a, b), the greatest number of positive responses was observed in the RTG-2 cultures, for which eight extracts were positive (Stations 1, 12, 13, 14, 17, 19, 20, 21). The BF-2 cultures produced three positive responses (Stations 3, 11, 12). Among the 22 stations tested, four were negative for both cell lines, and one was positive for both cell lines (Station 12).

TABLE 5 Fish Cell Reproduction Data

Station	Lowest Organic Concentration Giving Significant Response[a]		Relative Toxicity[b]	
	RTG-2	BF-2	RTG-2	BF-2
1	10	50	+	n.s.
2*	25	25	(+)	(+)
3	25	10	(+)	+
4*	50	50	n.s.	n.s.
5	25	50	(+)	n.s.
6*	50	50	n.s.	n.s.
7*	50	50	n.s.	n.s.
8	50	25	n.s.	(+)
9	25	50	(+)	n.s.
10	25	50	(+)	n.s.
11	25	10	(+)	+
12*	5	10	+	+
13	10	50	+	n.s.
14	10	50	+	n.s.
15*	50	50	n.s.	n.s.
16	25	50	(+)	n.s.
17	10	50	+	n.s.
18	not tested			
19	10	25	+	(+)
20	2	25	+	(+)
21	10	25	+	(+)
22	25	50	(+)	n.s.
23	25	50	(+)	n.s.

a. values in µg sediment extract/mL culture medium
b. n.s. = negative; significant response ($P<0.05$) only at 50 µg/mL
(+) = questionable; significant response ($P<0.05$) only at 25 µg/mL
\+ = positive; significant response ($P<0.05$) at 10 µg/mL or less

* = same rating for both RTG-2 and BF-2 cells

Station 20 was positive at 2 µg/mL for RTG-2 cells, and Stations 12 and 20 were positive at 5 µg/mL also for RTG-2 cells. All other positive responses were recorded at 10 µg/mL concentrations.

3.2.4.2 Genotoxicity

The results of the genotoxicity (anaphase aberration) testing are summarized in Table 6. Control cultures exhibited an abnormality frequency of 10.8% with no treatment and 10.0% when treated with DMSO. These abnormality frequencies are within the range of previous studies (Chapman et al., 1982a) in which control cultures had between 8 and 15% aberrant anaphases.

A total of 8 of the 22 tested stations induced significant increases in the number of anaphase aberrations present in the RTG-2 cells when compared to the controls ($P<0.01$): Stations 3, 6, 8, 9, 10, 11, 15 and 22. Unlike previous studies (Chapman et al., 1982a), there were no intermediate responses (i.e. $P<0.05$) in this test; the extracts either had no effect or were highly genotoxic.

4.0 DISCUSSION

4.1 Sediment Characteristics

The percents of clay, DOC, TVS and extractable organic matter observed in the present study are compared with other areas of Puget Sound in Table 7. Inner Everett Harbor sediments showed high levels of organic enrichment, probably due to the inputs of wood and other organic debris associated with the local pulp and paper mills. Large quantities of low density organic material and fine-grained sediments exist in inner Everett Harbor, which is consistent with the area being poorly flushed. In contrast, outer Everett Harbor sediments were coarser and had lower organic content, more typical of Puget Sound sediments from well flushed areas.

Bellingham Bay sediments differed from most other areas sampled in Puget Sound. These samples contained greater percentages of clay than observed in other areas (Table 7). However, this area was not particularly rich in organic matter, except in the inner harbor, near the pulp and paper mill. These data indicate that much of Bellingham Bay is strongly influenced by the deposition of fine-grained, inorganic "glacial flour" from the Nooksack River, even though Bellingham Bay is relatively shallow and open to wind and wave action.

The two sediment samples from Samish Bay were very different (Table 1, Fig. 4) suggesting a high level of heterogeneity in this area. Measured parameters were, however, all well within the "normal" range for Puget Sound sediments (Table 7) and hence Samish Bay could be a useful reference embayment if further studies indicate that it is chemically uncontaminated.

The measured parameters in the individual sediment samples collected for the present study from Everett Harbor, Bellingham and Samish Bays, were almost all within the ranges observed for other

TABLE 6 Fish Cell Anaphase Aberration Data

Station	Number of Abnormalities Per 100 Cells	P< a
1	11	n.s.
2	6	n.s.
3	17	0.01
4	12	n.s.
5	13	n.s.
6	21	0.01
7	10	n.s.
8	26	0.01
9	16	0.05
10	48	0.01
11	18	0.01
12	6	n.s.
13	7	n.s.
14	8	n.s.
15	23	0.01
16	2	n.s.
17	9	n.s.
18	Not Tested	
19	10	n.s.
20	8	n.s
21	11	n.s.
22	18	0.01
23	7	n.s.

Controls:

5 cultures; 100 anaphase cells/culture = 500 cells
Mean = 10.8% abnormalities
S.D. = 1.92

a. Significance is determined based on the confidence limits (C.L.) for abnormalities in 500 anaphase cells from control cultures: upper 95% C.L. = 15 abnormal cells/100; upper 99% C.L. = 17 abnormal cells/100.

TABLE 7 Comparison of the Physical and Chemical Characteristics of the Sediments from Everett Harbor, Bellingham and Samish Bays, with those from Other Areas of Puget Sound

Area	% Clay	% DOC	% TVS	% Extractables
Elliott Bay[a]				
Elliott Bay (n=17)	8.7±5.6[b]	4.5±4.2	7.0±4.4	0.35±0.36
Duwamish River (n=20)	12.7±6.2	3.7±2.0	6.8±2.2	0.39±0.18
Commencement Bay[a]				
Outer Bay (n=10)	9.5±7.1	2.6±1.3	4.4±2.2	0.09±0.08
Waterways (n=27)	12.7±7.1	5.4±2.4	7.0±2.6	0.46±0.36
Sinclair Inlet[a] (n=12)	16.1±5.0	5.3±2.6	10.7±4.6	0.30±0.10
Port Madison[a] (n=6)	9.0±9.5	1.1±0.7	4.3±3.1	0.05±0.04
Birch Bay[a] (n=5)	0.6±0.2	3.5±2.2	3.9±2.5	0.01±0.01
Bellingham Bay (n=10)	53.2±9.6	1.6±0.7	8.8±2.9	0.13±0.05
Samish Bay (n=2)	20.9	0.85	5.45	0.06
Everett Harbor				
Inner Harbor (n=6)	22.2±8.8	6.1±1.9	20.4±3.2	0.38±0.04
Outer Reach (n=5)	14.9±6.0	1.6±1.0	7.2±3.7	0.12±0.06

a. data from Chapman et al. (1982a)
b. values presented as means ± one standard deviation

areas of Puget Sound. The only exceptions were the high clay content in Bellingham Bay sediments and the high percent TVS in inner Everett Harbor sediments.

4.2 Oyster Larvae Toxicity Tests

Partial life-cycle tests with oyster larvae measure both survival after a 48 h exposure of developing embryos to sediments, and the induction of abnormal development. Significant mortalities and abnormalities compared to controls are indicative of chemical toxicity effects (Cardwell et al., 1979).

The data collected during this study indicated that seven of the 22 stations tested were highly toxic to oyster larvae; seven stations were of intermediate toxicity, five stations were of low toxicity, and three were non-toxic. Chemical toxicity, as defined by the development of significant larval abnormalities, was indicated for those stations showing a positive response with the following possible exception. Station 10 had a low level of abnormalities (4.4%, within the defined range for no toxicity), but a survival rate of only 33%. Neither of the two Samish Bay reference stations showed evidence of significant toxicity effects on oyster larvae.

The highest level of oyster larvae abnormalities was 46.7% (Station 13, Everett Harbor). Much higher levels of oyster larvae abnormalities (up to 94%) have been documented for chemically contaminated sites in the Duwamish River and Commencement Bay Waterways (Chapman and Morgan, 1983; Chapman et al., in press a, b). Based on this comparison, the bottom sediments collected from Everett Harbor and Bellingham Bay were substantially less toxic than those collected from Elliott and Commencement Bays.

A comparison of relative toxicity by area also indicated that sediment toxicity, as measured by the oyster larvae bioassay, was less in Bellingham Bay and Everett Harbor than in areas such as the Duwamish River. Chapman et al. (in press b) found that 100% of stations in the Duwamish (4/4) tested by the oyster larvae bioassay induced levels of abnormalities of 10% or greater. In the present study, percentages of stations with this level of abnormalities were 60% (6/10) in Bellingham Bay, and 80% (8/10) in Everett Harbor.

4.3 Amphipod Lethality Tests

The use of Rhepoxynius abronius to determine the acute lethality of field-collected sediments has been documented by Swartz et al. (1982, in press), Chapman et al. (1982a, b), and Chapman and Fink (in prep.). This amphipod species is a sensitive indicator of contaminated areas both by its absence in natural populations from such areas (Swartz et al., 1982; Comiskey et al., in press), and by its response to contaminated sediments in laboratory studies (Swartz et al., in press).

In the present study, sediments from only one station in inner Bellingham Bay (Station 3) and one station in inner Everett Harbor (Station 14) caused significant acute lethality to R. abronius.

Amphipod mortality at these stations was 14 and 25% respectively. Significant mortality was not noted at the two Samish Bay reference stations.

Data on amphipod avoidance (leaving the sediments) were obtained for all stations. The lowest avoidance response occurred in control sediments, however a variable response was observed in test sediments, and trends in avoidance data did not correspond to those for lethality. The significance of these data are presently unknown.

Previous R. abronius acute lethality studies in Commencement Bay recorded 100% mortality in some areas of the Waterways (Swartz et al., 1982). Previous tests in Elliott Bay and the Duwamish River recorded mortality levels as high as 70% (Chapman et al., 1982b; Chapman and Fink, in prep.). Based on this comparison, the bottom sediments collected from Everett Harbor and Bellingham Bay were substantially less toxic than those collected from Elliott and Commencement Bays.

A comparison of relative toxicity by area also indicates that sediment toxicity, as measured by the amphipod acute lethal bioassay, is less in Bellingham Bay and Everett Harbor than in areas such as Commencement Bay Waterways. Swartz et al. (1982) found that 37% (10/27) of stations in the Blair Waterway, and 75% (50/65) of stations in the Hylebos Waterway tested with this bioassay had less than 75% survival. In the present study, the two stations with significantly lower survival than controls had survival levels of 75 and 86%.

4.4 Oligochaete Respiration Rate Tests

Oligochaete respiration as a measure of sediment toxicity has been documented by Chapman et al. (1982a, b). Significant depression or elevation of respiration rate compared to baseline indicates sublethal effects (Brinkhurst et al., 1983).

The data collected during this study indicate that sediments from seven of the 22 stations tested induced sublethal stress in oligochaete worms. The highest level of difference noted between test and control respiration rates was $P<0.01$. Significant respiration effects were not noted at the two Samish Bay reference stations.

Previous sublethal respiration rate tests in other areas of Puget Sound have recorded significantly higher differences between test sediments and controls ($P<0.001$). These higher response levels were recorded for Elliott and Commencement Bays (particularly in the Duwamish River and the Waterways) and in Sinclair Inlet (Chapman et al., 1982a, b; Chapman and Fink, in prep.). Based on oligochaete respiration rate tests, the bottom sediments collected from Everett Harbor and Bellingham Bay were significantly less toxic than those collected from Elliott and Commencement Bays.

A comparison of relative toxicity by area also indicates that sediment toxicity, as measured by the sublethal oligochaete respiration test, is lower in Bellingham Bay and Everett Harbor than

in other areas of Puget Sound. Chapman et al. (1982a) found the following percentages of stations with significant sublethal (respiratory) effects: Duwamish Waterway, 40% (8/20); Hylebos Waterway, 58% (7/12); Blair Waterway, 55% (5/9); Sinclair Inlet waterfront, 83% (5/6). In the present study, 30% (3/10) of stations in Bellingham and 40% (4/10) of stations in Everett showed significant sublethal (respiration rate) effects.

4.5 Cell Reproduction and Genotoxicity Tests

Tests of cell reproduction and genotoxicity (anaphase aberrations) with cultured fish cells assess cytotoxic and genotoxic responses at the cellular level. Cell reproduction tests measured lethal (cytotoxic) and sublethal growth and reproduction effects of organic sediment extracts on two fish cell lines with very different MFO activity. Differences between the two cell lines detected sediments containing direct acting toxins requiring no metabolic conversion (affecting RTG-2 and BF-2 cells) and indirect or pro-toxins requiring conversion to a toxic form (affecting only RTG-2 cells). Anaphase aberrations observed in tests with RTG-2 cells were the result of exposure of actively dividing cells to genotoxic agents.

4.5.1 Cell Reproduction

The data obtained in this study indicated that extracts of sediments from nine of the 22 stations tested significantly impaired reproduction in cultured fish cells. However, at only one station (Station 12) were both BF-2 and RTG-2 cells significantly affected. Comparisons of the various stations were made on the basis of equal concentrations of total organics, and not on precise knowledge of organic compounds present in the extracts. As a result, each of these stations may be chemically very different from one another. The wide differences observed in effective concentration (2 µg/mL to 25 µg/mL) indicate that some stations contain higher concentrations of toxic or inhibitory compounds than do others. These results indicate that cell reproduction tests detected the presence of toxic compounds. The response (reduced cell proliferation) is restricted to organic compounds but could have resulted from a wide range of man-made or natural substances. Laboratory tests (Kocan, unpub. data) have shown that metals (i.e. Cd, Hg, Zn, Cu) are only toxic at levels 10 to 100X those considered toxic in marine ecosystems, while most natural or man-made organic compounds known to be cytotoxic or inhibitory, produce a response in this test system.

Most positive responses were in the RTG-2 cell line, indicating that the majority of toxicants detected require metabolic activation to their toxic forms (i.e. comprise pro-toxins). The only station exhibiting positive response with both cell lines (Station 12) may have contained a single direct acting toxin. It is highly improbable that two distinct toxic substances were present in the sediment from this station, and that each produced an identical effect on each cell type.

The results indicate that the majority of toxic compounds in Everett Harbor and Bellingham Bay require metabolic conversion to become active. This observation suggests that those species with the most active MFO systems and which are extensively exposed to these sediments may be the ones most likely to be adversely affected.

Significant cell reproduction effects were observed in both Samish Bay reference stations, which may reflect "natural" toxicity resulting from the presence of mutagens in marine plants (Ames, 1983). Because defined concentrations of specific organics were not determined in previous cell reproduction testing in Elliott and Commencement Bays (Chapman et al., in press b), it is not possible to directly compare the level of toxic response obtained in Everett Harbor, Bellingham and Samish Bays, with responses from other areas of Puget Sound, except on a relative basis.

Relative comparisons of cell reproduction effects by area indicate that Everett Harbor, Bellingham and Samish Bays have lower levels of toxicants requiring metabolic activation to elicit cell reproduction responses than the Duwamish River. Chapman et al. (in press b) found that 75% of stations (3/4) in the Duwamish River tested for cell reproduction effects on RTG-2 cells showed responses at 10 µg/mL extract concentrations or less. In the present study, the following comparative data were recorded: Bellingham Bay, 10% (1/10); Samish Bay, 50% (1/2); Everett Harbor, 60% (6/10). Similar comparisons for direct-acting toxins using cell reproduction effects on BF-2 cells could not be made due to data incompatibility.

4.5.2 Genotoxicity

The data obtained in this study indicate that extracts of sediments from eight of the 22 tested stations induced significant genotoxic responses in RTG-2 cells. Consistent with previous studies (Chapman et al., 1982a), there was no correlation between inhibition of cell proliferation and genotoxicity, indicating that two distinct forms of toxic response were being measured.

All seven stations in Everett Harbor and Bellingham Bay showing significant genotoxicity also showed toxicity to oyster larvae. Although more stations than these showed oyster larvae toxicity, correspondence between the two tests may reflect the fact that young, rapidly growing organisms are most susceptible to the effects of mutagenic/carcinogenic substances because of the large proportion of mitotically active cells in their body.

A positive genotoxicity response was obtained for one station in Samish Bay, the reference area. Because there are no available data yet on the specific organic substances present in each of the tested sediment samples, it is not possible to assess why this response was observed. The physical/chemical data obtained in this study did not indicate differences between Samish Bay sediments and other areas. However, genotoxic substances may be present in areas thought to be pristine due to the decomposition of plant species containing genotoxic substances which evolved as a means of protecting plants from parasites and predators (Ames, 1983; Colwell, 1983). Although

these substances are not present in large quantities, massive amounts of vegetation can be deposited by tides and currents in small areas of the sea bottom where they decompose leaving a residue of these chemicals that accumulate over long periods of time. Prediction of such "natural" genotoxicity would include determination of which species of plants contain such mutagenic compounds, and their distribution in study areas.

Because concentrations of specific organics were not used in previous genotoxicity testing in Elliott and Commencement Bays (Chapman et al., 1982a), it is not possible to directly compare the level of toxic response obtained in Everett Harbor, Bellingham and Samish Bays, with responses from other areas of Puget Sound, except on a relative basis.

Relative comparisons of genotoxicity (anaphase aberration) effects by area indicate that Everett Harbor, Bellingham and Samish Bays show a lower level of sediment toxicity than do other areas of Puget Sound. Chapman et al. (1982a) found the following percentages of stations with significant anaphase aberration effects: Duwamish Waterway, 60% (12/20); Hylebos Waterway, 58% (7/12); Blair Waterway, 77% (7/9); Sinclair Inlet waterfront, 67% (4/6). In the present study, the following comparative data were recorded: Everett Harbor, 20% (2/10); Samish Bay, 50% (5/10); Bellingham Bay, 50% (5/10).

4.6 Combined Test Results

Summary bar diagrams of biological effects at each station tested are presented in Figs. 8 and 9. Two of the tested stations exhibited positive responses for all levels of biological effects testing (lethal, sublethal, partial life-cycle, cell reproduction and genotoxic): Station 3 in inner Bellingham Bay, and Station 14 in inner Everett Harbor. The remaining 20 stations subjected to all biological effects test methods showed some level of response to at least one of the five different tests employed (cell reproduction using two different cell lines is considered a single test). The two Samish Bay reference stations did not exhibit significant lethal, sublethal or partial life-cycle effects, but did exhibit significant cell reproduction and genotoxic responses. No other stations of those tested responded solely to the cellular bioassays.

Where direct comparisons were possible, test results indicated that sediments from Everett Harbor, Bellingham and Samish Bays were significantly less toxic than sediments from such highly industrialized areas of Puget Sound as parts of Sinclair Inlet, Elliott and Commencement Bays. Such direct comparisons were possible for amphipod acute lethality, respiration effects, and oyster larvae testing. Only relative comparisons were possible for cell reproduction and genotoxicity testing.

Relative comparisons of the percentage of stations with toxic responses in particular areas support the results of direct comparisons. Relative comparisons using lethal, sublethal, partial life-cycle, cell reproduction and genotoxic results indicated that

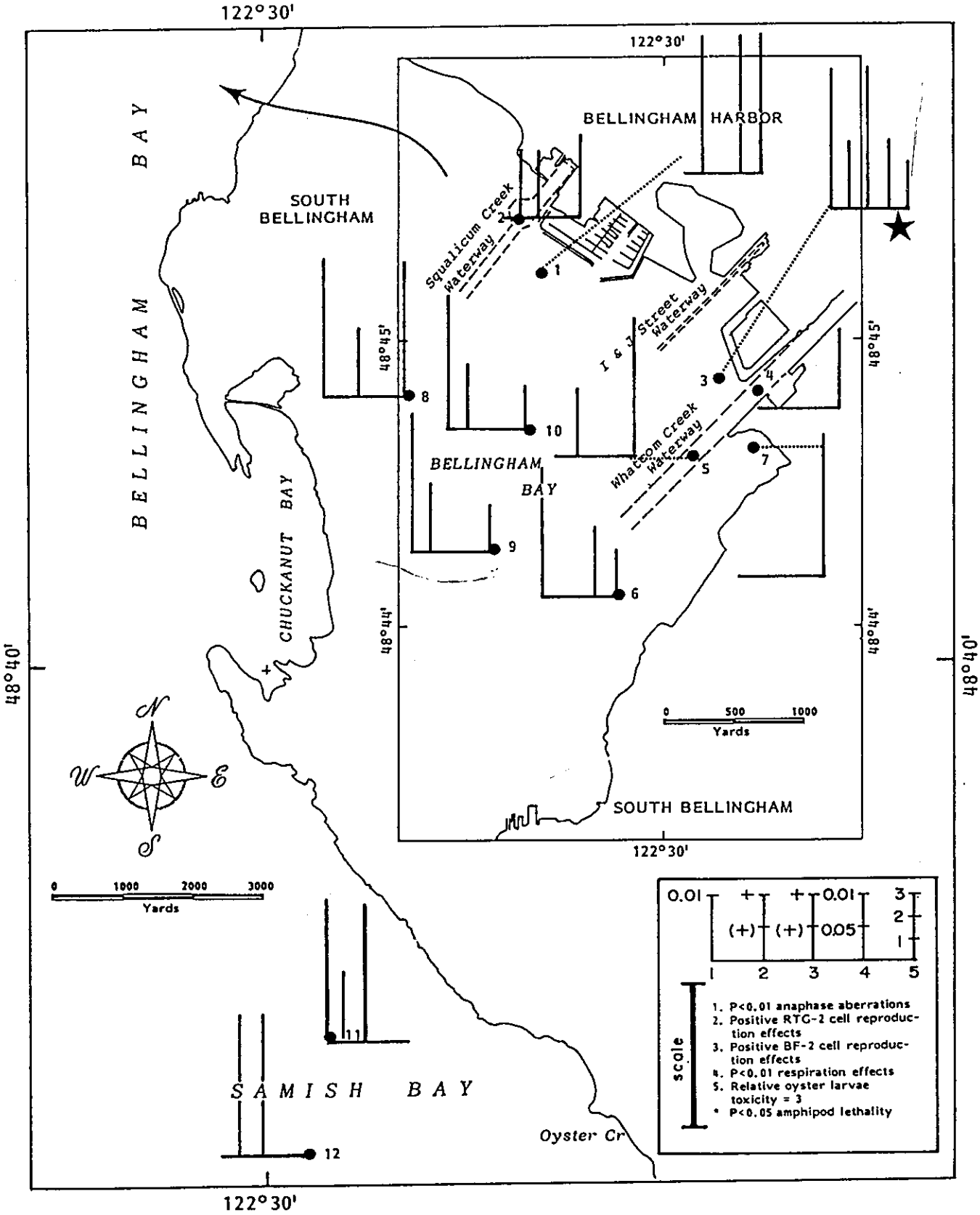

Figure 8 Bar Diagrams of Biological Effects Data for Bellingham and Samish Bays

Refer to the text for explanations of symbols used in the figure legend. Fig. 9 follows the same pattern.

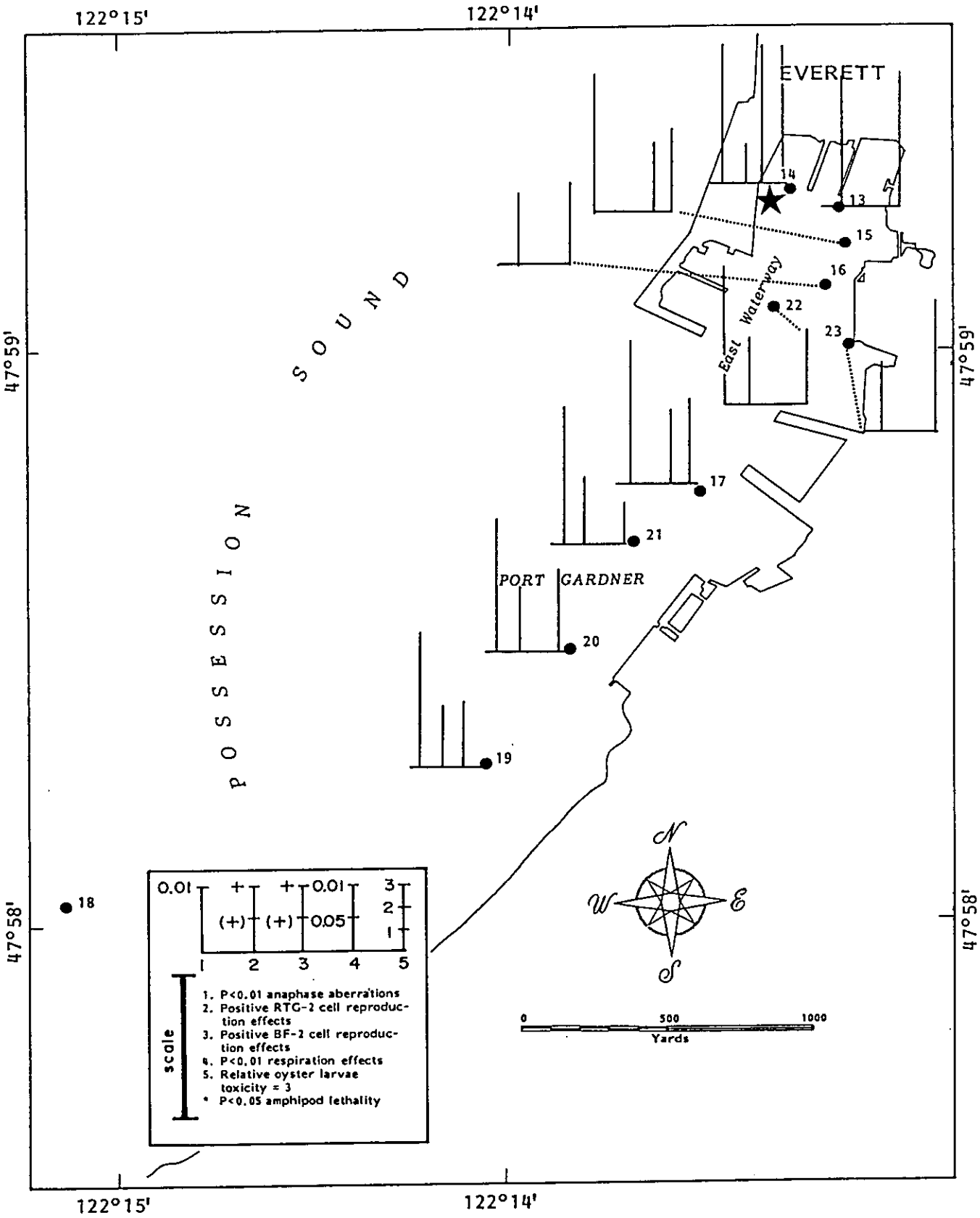

Figure 9 Bar Diagrams of Biological Effects Data for Everett Harbor

Note: Amphipod lethality and respiration effects testing were conducted at Station 18 with negative results.

sediments from Everett Harbor, Bellingham and Samish Bays were less toxic than sediments from highly industrialized areas of Puget Sound such as Sinclair Inlet, Elliott and Commencement Bays.

Comparisons between the present sediment bioassay data for Everett Harbor and Bellingham Bay indicate that Everett Harbor sediments are more toxic overall than Bellingham Bay sediments. A higher percentage of stations showing toxic responses were observed in Everett Harbor for oyster larvae (80% versus 60%), oligochaete respiration (40% versus 30%), and cell reproduction tests (60% versus 10%). A single significant acute lethal amphipod response was observed in each area. Only genotoxic effects were higher in Bellingham Bay (50%) than Everett Harbor (20%).

Samish Bay sediments showed no toxic responses based on lethal, sublethal and life-cycle tests. However, relative cell reproduction effects were intermediate for Samish Bay (1 out of 2 stations) relative to data for Bellingham Bay (1/10) and Everett Harbor (6/10), while relative genotoxic effects were greater than those in Everett Harbor (50% versus 20%) and equal to those in Bellingham Bay (50%). However, the sample size (2 stations) is too small to conclude that there is a significant toxicity problem in Samish Bay.

4.6.1 Relationship to Chemical Data

Presently available chemical data within broad chemical classes and over wide spatial areas are summarized in Table 8. Generally four major classes of toxic compounds have been monitored in Puget Sound: metals, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs) and a large group of other chlorinated organic compounds (ClHs).

To date, the observed levels of all classes of toxic compounds detected in the sediments of Everett Harbor and Bellingham Bay have been substantially lower than those detected in areas such as the Duwamish Waterway (Elliott Bay) and Commencement Bay Waterways, which are generally recognized as being severely contaminated. This observation corresponds with the results of bioassay testing in the present study. No determinations of sediment toxicants have yet been made in Samish Bay.

The response of cellular effects tests to Samish Bay samples may reflect the presence of substantially different chemical groups in Samish Bay compared to Everett Harbor and Bellingham Bay. However, verification of this possibility must await chemical analysis of sediment samples from these areas, which are presently being undertaken by the U.S. EPA Region X Laboratories (Manchester).

4.6.2 Toxic Areas

The utility of a progression of sediment bioassay tests in determining comparative toxicity has been demonstrated by Chapman et al. (1982a, in press a). The use of these tests as part of a comprehensive assessment of marine pollution has been described by Chapman and Long (1983).

TABLE 8 Summary of Contaminant Distribution Data Related to the Study Area

Site (Station No.)	Possible Pollutant Inputs	Primary Pollutants Identified[a]	References and Comments
Bellingham Bay			
Whatcom Creek Waterway Area (3-5, 7)	pulp and paper mill effluent; residual contamination from historical mercury cell chlor-alkali plant	Hg(historical)	Malins et al., 1982 Bothner, 1973
Outer Bellingham Bay (1, 2, 6, 8-10)	secondary effluent from pulp and paper mill (esp. Stn. 9); limited municipal inputs	Hg(historical)	Malins et al., 1982 Bothner, 1973
Samish Bay (11, 12)	reference area; no known local inputs; removed from major municipal and industrial areas	not studied	no data available
Everett Harbor			
Inner Harbor (13-16, 22, 23)	pulp and paper mill effluent; some CSO and light industry inputs	some degree of increase above background for most trace metals, esp. Cu, Pb, Zn; moderate levels of PCBs and PAHs; high concentrations of unresolved organic compounds	U.S. EPA, 1982 Malins et al., 1982 WDOE, 1982 Gronlund et al., 1983
Outer Harbor (17-21)	limited municipal sewage inputs (esp. near Stn. 19); advection from inner harbor	only slight elevations above Puget Sound baseline concentrations for all measured compounds	U.S. EPA, 1982 Malins et al., 1982

a. PAHs = polynuclear aromatic hydrocarbons; PCBs = polychlorinated biphenyls

Acute toxicity tests with the sensitive amphipod Rhepoxynius abronius indicate that acute lethal toxicity is not a general feature of the areas tested, in contrast to contaminated areas in Elliott and Commencement Bays. Observations of the presence of benthic infauna in grab samples support this conclusion. The composition of the benthic community at tested stations is presently being determined by taxonomic analysis of benthic grab samples. These analyses are being conducted by Western Washington University (Bellingham) on behalf of the U.S. EPA, but are not presently available. Based on observations made during sample collection (Appendix A), it seems probable that conditions in many areas of Everett and Bellingham Bay approach anoxia due to organic decomposition, which would affect benthic communities even in the absence of chemical contamination.

Possible chemical contaminant effects were reflected in the lethal, sublethal, partial life-cycle, cell reproduction and genotoxic responses. Previous sediment lethality testing with the amphipod R. abronius at two stations in Everett Harbor (Chapman and Fink, in prep.) also recorded significant toxicity, with slightly lower mean survivals (65%) than the lowest mean survival recorded in the present study (75%). The U.S. EPA recently conducted a broad survey of sediment toxicity in Everett Harbor using the R. abronius bioassay, and recorded survival values as low as 15% in unreplicated tests (J. Cummins, EPA, pers. comm.). The lower survival values (=higher sediment lethality) recorded by the EPA may be due to their testing of fresh sediment compared to testing of frozen sediment in the present study. Reduction of toxicity with freezing is possible based on recent work by the EPA Manchester Laboratories (J. Cummins, EPA, pers. comm.).

No previous toxicity testing has been conducted with bottom sediments in Bellingham or Samish Bays. Campana (1983) found a high incidence of skin tumors in starry flounder (Platichthys stellatus) from Bellingham Bay, but ascribed this to non-anthropogenic sources.

Histopathological abnormalities have been detected in resident bottom fish collected from Everett Harbor by Gronlund et al. (1983). English sole (Parophrys vetulus) collected from their Station 1 (in the vicinity of our Station 17) showed a higher incidence of liver lesions than levels previously recorded for the Duwamish Waterway. High levels of liver lesions were also noted in rock sole collected from this area. Lower incidences of liver lesions in English sole were documented from their Station 2 (in the vicinity of our Station 20). Very low incidences of liver lesions in English sole were documented at stations outside the area of present biological effects testing. No histopathological studies were conducted with fish from inner Everett Harbor (near our Stations 13-16). These liver lesions were considered characteristic of urban areas containing high concentrations of toxic chemicals in sediment (Gronlund et al., 1983).

Significant biological effects including cellular toxicity due to activation of toxins were observed in the present study in areas where Gronlund et al. (1983) documented high incidences of histopathological abnormalities in bottom fish. This correspondence is not, however, conclusive evidence that sediment contaminants are responsible for fish histopathological abnormalities. Cellular and sub-cellular tests are generally more sensitive than whole animal tests, and can exhibit toxic effects when the whole, integrated organism is not affected.

Based on present biological effects testing, Everett Harbor sediments were more toxic than Bellingham Bay sediments. Highest sediment toxicity in Everett was found in the inner Harbor; in Bellingham, highest toxicity was found in the inner Bay. Positive cell reproduction and genotoxic responses in Samish Bay, the reference area, in the absence of other positive responses, suggest a different chemistry for this area than that of the industrialized embayments of Everett Harbor and Bellingham Bay.

5.0 CONCLUSIONS

1. Partial life-cycle tests involving oyster larvae indicated that sediments from Everett Harbor and Bellingham Bay caused toxic effects (mortality and/or abnormalities).

2. Acute lethal responses were recorded at one station from inner Everett Harbor and one station from inner Bellingham Bay.

3. Sublethal tests using oligochaete respiration indicated that sediments from Everett Harbor and Bellingham Bay caused sublethal toxic effects.

4. Cell reproduction studies indicated that sediment extracts from Everett Harbor, Bellingham and Samish Bays affected fish cell proliferation in vitro. Cell lines with and without an active mixed function oxydase (MFO) enzyme system responded positively to Samish Bay sediments indicating the presence of a material that is genotoxic without enzymatic modification.

5. Genotoxicity testing indicated that sediment extracts from all tested areas (Everett Harbor, Bellingham and Samish Bays) induced genetic damage in cultured fish cells.

6. Direct comparison of the results of the lethal, sublethal and partial life-cycle effects testing indicated that sediments from Everett Harbor, Bellingham and Samish Bays were significantly less toxic than contaminated areas of Sinclair Inlet, Elliott and Commencement Bays.

7. Relative comparisons of the proportion of stations in different areas showing sediment toxicity indicated that sediments from Everett Harbor, Bellingham and Samish Bays were less toxic than contaminated areas of Sinclair Inlet, Elliott and Commencement Bays. Everett Harbor sediments were more toxic than those from

Bellingham Bay and Samish Bay sediments were the least toxic overall. These comparisons involved lethal, sublethal, partial life-cycle, cell reproduction and genotoxic tests.

8. There was good correspondence between the results of all bioassay tests and the general low levels of chemical contaminants recorded in other studies of these areas.

9. In contrast to other tested areas, only cell reproduction and genotoxic effects were recorded in Samish Bay. These results suggest that very different, possibly "natural" chemical toxicity is present in Samish Bay compared to Everett Harbor and Bellingham Bay.

10. The results of this study provide additional direct evidence linking specific geographic locations with specific levels of biological effects. Completion of chemical analyses of sediments and benthic infaunal taxonomic analyses for these areas will provide additional data related to biological effects and chemical contamination.

6.0 RECOMMENDATIONS

1. Fish histopathology data for Everett Harbor and environs indicate a very high frequency of liver lesions in English sole. Similar data are not available for Bellingham and Samish Bays. Sediment bioassay and chemistry data indicate that these three areas are all less contaminated/toxic than other areas of Puget Sound where lower incidences of bottomfish liver abnormalities have been observed. It is recommended that bottomfish histopathology data be obtained for Bellingham and Samish Bays to determine whether levels of liver lesions in these areas are comparable to Everett Harbor.

2. Data on benthic community structure and levels of sediment contaminants in Everett Harbor sediments will shortly be available. Statistical analyses should be undertaken to determine the extent and significance of correlations among chemical and biological data sets.

3. Cell reproduction and genotoxicity test results indicate high levels of toxicity in Samish Bay. These results may be due to "natural" toxicity, however this needs to be determined both to verify the test methodology and to determine whether there is cause for concern in Samish Bay.

4. The sources of contamination related to specific subareas should be determined. This information is essential to allow mitigation of possible adverse environmental effects in the affected areas.

5. All five different tests used in the present study (amphipod acute lethality, oyster larvae partial life-cycle toxicity, oligochaete sublethal respiratory response, cell reproduction and genotoxicity) provided useful information regarding different aspects of sediment toxicity. These tests are all recommended for future use in Puget Sound.

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