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SURVEY OF BIOLOGICAL EFFECTS OF  
TOXICANTS UPON PUGET SOUND BIOTA

I. BROAD-SCALE TOXICITY SURVEY

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## PREFACE

A variety of chemical contaminants have been found in Puget Sound. High concentrations of some chemicals have been geographically linked with the occurrence of biological abnormalities in biota from the Sound. However, no direct evidence had been collected of adverse biologic effects resulting from exposure of Puget Sound biota to contaminants. Thus, the MESA (Marine EcoSystems Analysis) Puget Sound Project supported the study reported here to determine if adverse biologic effects occurred or not as a result of exposure of biota to contaminants found in the Sound. The approach that was selected involved performing a variety of tests with field-collected sediment and water samples from a large number of sites. The sites were selected to reflect the wide range in pollution situations known to exist in the Sound (from highly contaminated to moderately contaminated to pristine). The study was coordinated with other studies supported by the MESA Project and other agencies to assess biologic effects among Puget Sound biota. The Contract Officer's Technical Representative from the MESA Project Office for this study was Edward R. Long.

## EXECUTIVE SUMMARY

Puget Sound is a marine fjord consisting of heavily industrialized embayments (e.g. Seattle harbor) and open, relatively pristine areas. Chemical analyses had shown high levels of various contaminants at different sites, but the significance and potential effect of these substances on the biota was unknown. The present study was therefore undertaken to determine and rank potentially toxic areas of Puget Sound, Washington, based on biological effects testing.

Study results were used to prioritize specific geographic areas of concern. On this basis, the most toxic tested areas of Puget Sound were: near the Denny Way CSO (Elliott Bay); City, Blair and Hylebos Waterways (Commencement Bay). Other tested areas which showed strong biological effects (in descending rank) were: upper Duwamish, Sinclair Inlet, outer Elliott Bay, outer and inner Commencement Bay and East Duwamish Waterway. The control site (Birch Bay) and reference site (Port Madison) were among the least toxic areas but did exhibit some effects. The inference is made that subtle adverse effects previously observed in field surveys occur among Puget Sound fauna associated with the areas shown in the present laboratory study as having the greatest demonstrable biological effects.

A progression of tests ranging from lethal to sensitive sublethal were used to evaluate a total of 97 sediment and 7 bottom water samples from the following areas of Puget Sound: Elliott Bay and the lower Duwamish River, Commencement Bay and its Waterways, Sinclair Inlet, Port Madison (a reference site), and Birch Bay (a control site). Testing methods were chosen that were known to be responsive to toxic chemicals, such as those known to occur in Puget Sound. Lethality bioassays with an oligochaete (*Monopylephorus cuticulatus*), amphipod (*Eogammarus confervicolus*), and fish (threespine stickleback *Gasterosteus aculeatus*) using bottom water samples and slurries of Puget Sound sediments indicated no acute lethality in any areas tested with the exception of amphipod mortalities following exposure to sediment from one station off Denny Way Combined Sewer Overflow (Elliott Bay). The relative sensitivities of the test organisms were documented and confirmed by spiked sediment tests.

Respiration measurements were conducted with *M. cuticulatus* by exposing the worms to the water decanted from centrifuged sediment slurries and directly to the water samples. Standard respiration rates were measured at high dissolved oxygen levels for each sample tested and were compared with control and other test results. A total of 40 sediment and 3 water samples demonstrated significant respiration anomalies.

Genotoxicity tests for chromosomal damage were conducted by exposing Rainbow Trout gonad cells to sediment and water extracts followed by fixation and examination. Different types of mitotic (anaphase aberration) effects were classified, and the lethality of the extracts was also assessed. Inhibition of cell proliferation was documented separately from mitotic effects. A total of 58 sediment and 3 water samples caused significant chromosomal damage.

Physical and chemical data for tested samples (temperature, salinity and extractable organic matter for waters; particle size, total volatile solids, digestible organic carbon, and extractable organic matter for sediments) conformed to results of other studies of Puget Sound and did not provide a clear distinction among samples. Live benthic fauna were noted as part of a cursory visual examination in most of the benthic grab samples, including one station where amphipod mortalities were observed. Consequently, it appears that direct, rapid lethality is not a major factor for the majority of fauna exposed to and living in or near chemically contaminated Puget Sound sites.

However, the results of respiration and genotoxicity testing substantiate previous evidence of sublethal toxicant effects (e.g. liver neoplasia in bottom fish, benthic community changes) in Puget Sound biota from highly contaminated areas. Comparisons of the test results for both respiration and genotoxicity indicated generally very good agreement on broad scale toxicity patterns at different geographic areas. Comparison of these data with chemical data, other studies on mortalities of sensitive amphipod species, and results from the control and reference area, indicate that the approach taken in this study has successfully described various biological responses apparently related to chemical contamination.

## ACKNOWLEDGMENTS

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This report documents the results of a study commissioned by NOAA through the MESA (Marine EcoSystems Analysis) Puget Sound Project to determine the effects of contaminants in Puget Sound sediments and waters on resident biota, using a combination of lethal and sublethal toxicity tests. This study represents the first comprehensive experimental investigation of the possible effects of the suites of chemical contaminants identified by MESA researchers in sediments, biota, water and suspended solids and reviewed by Konasewich et al. (1982) on the basis of their possible significance to Puget Sound.

Previous studies identified concentrations of trace metals much above background levels and many industrial organic compounds in Puget Sound, principally near urban industrialized areas such as Elliott Bay, the lower Duwamish River, Commencement Bay and Sinclair Inlet. However, the full implications of this contamination have been difficult to assess due to limitations in existing analytical methodology for identifying and quantifying all the compounds, and due to limitations in current toxicological methodology. In general, little of the needed information has been available to relate toxicant levels to actual or potential biological impacts. While correlations have been established between sites of high chemical levels and increased incidences of a variety of effects in resident organisms, there was no clear cause-effect relationship(s) between occurrence of concentrations of any chemicals or group of chemicals and observations of adverse biological effects.

This study was initiated to begin bridging the gap between observed biological disorders and chemical contamination by performing a broad-scale biological survey of stations in Puget Sound representing qualitative and quantitative ranges of contamination. A variety of toxicity tests was applied to samples from these stations to develop a spatial delineation of biologically active (e.g. toxic) areas as well as develop an understanding of the types of effects inducible on resident biota.

Previous bioassay studies in Puget Sound include somewhat contradictory in situ studies in Elliott Bay, specifically in the region of the Denny Way Combined Sewer Overflow (CSO). Armstrong et al. (1978, 1980) performed live cage bioassays with the mussel Mytilus edulis and the oyster Crassostrea gigas and noted a decrease in condition index at polluted sites. However, Tomlinson et al. (1980) in a similar study noted no effects and ascribed Armstrong et al.'s (1978, 1980) results to reproductive changes in the bivalves rather than toxicant effects.

In contrast, sediment bioassays from other areas of the Sound have demonstrated adverse effects on tested biota. Shuba et al. (1978) conducted laboratory tests with Duwamish sediments and found that,

although a number of organisms were unaffected, the grass shrimp Palaemonetes suffered significant mortalities compared to controls. Swartz et al. (1979) conducted bioassays with sediment from various areas of Puget Sound including Elliott Bay and the lower Duwamish River and found that, of several species tested, the survival of the clam Macoma inquinata and the amphipod Rhepoxynius abronius (= Paraphoxus epistomus) was affected by some Puget Sound sediments. Work with R. abronius has also shown evidence of spatial differences in sediment toxicity along a Duwamish River - Puget Sound transect (Swartz et al., 1979) and in Commencement Bay (Swartz et al., 1981, 1982). Malins et al. (1980) and McCain (1982) reported increased frequencies of histopathological disorders among demersal fishes and depressed benthos species richness in areas with the highest toxic chemical concentrations.

## 1.1 Objectives

The specific objectives of this study were:

- a) To demonstrate effects, if any, of ambient toxicant levels upon Puget Sound biota.
- b) If effects could be demonstrated, to show as wide a variety of effects as feasible upon as many species as feasible.
- c) If effects could be demonstrated, to show a gradient of effects related to geographic location or chemical concentration, if feasible.
- d) As a secondary objective, to document the important physical-chemical parameters of each sampling site.
- e) To identify biological testing protocols for future assessment of contaminants in the Puget Sound ecosystem.

## 2.0 METHODS

### 2.1 Geographical Study Area

This study was intended to assess the effects of chemically contaminated sediments on biota; hence, testing was primarily done on samples from areas already shown by previous analyses to be most impacted by human pollution, specifically the urbanized embayments and estuaries in central and southern Puget Sound (Figs. 1-7). Specific study stations were selected in Elliott Bay, the lower Duwamish River, Commencement Bay and associated waterways, and Sinclair Inlet. In addition, Port Madison was incorporated as a non-urban reference area, and Birch Bay was selected as a control area outside of central Puget Sound.

Precise station location information (e.g. latitude and longitude, sextant readings from shore facilities, etc.) is presented in Appendix

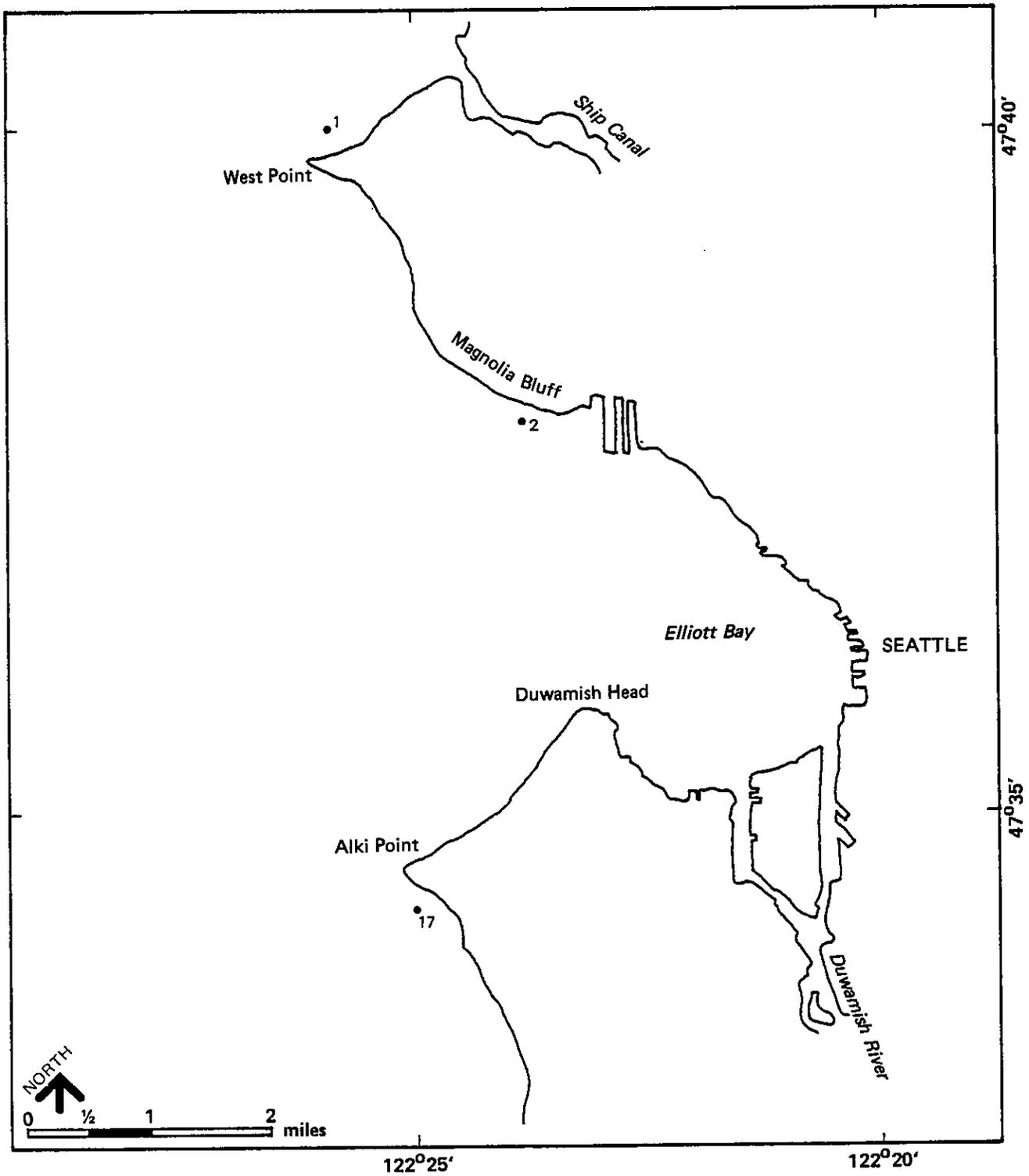


Figure 1. Station Locations at West Point, Alki Point, and Magnolia Bluff.

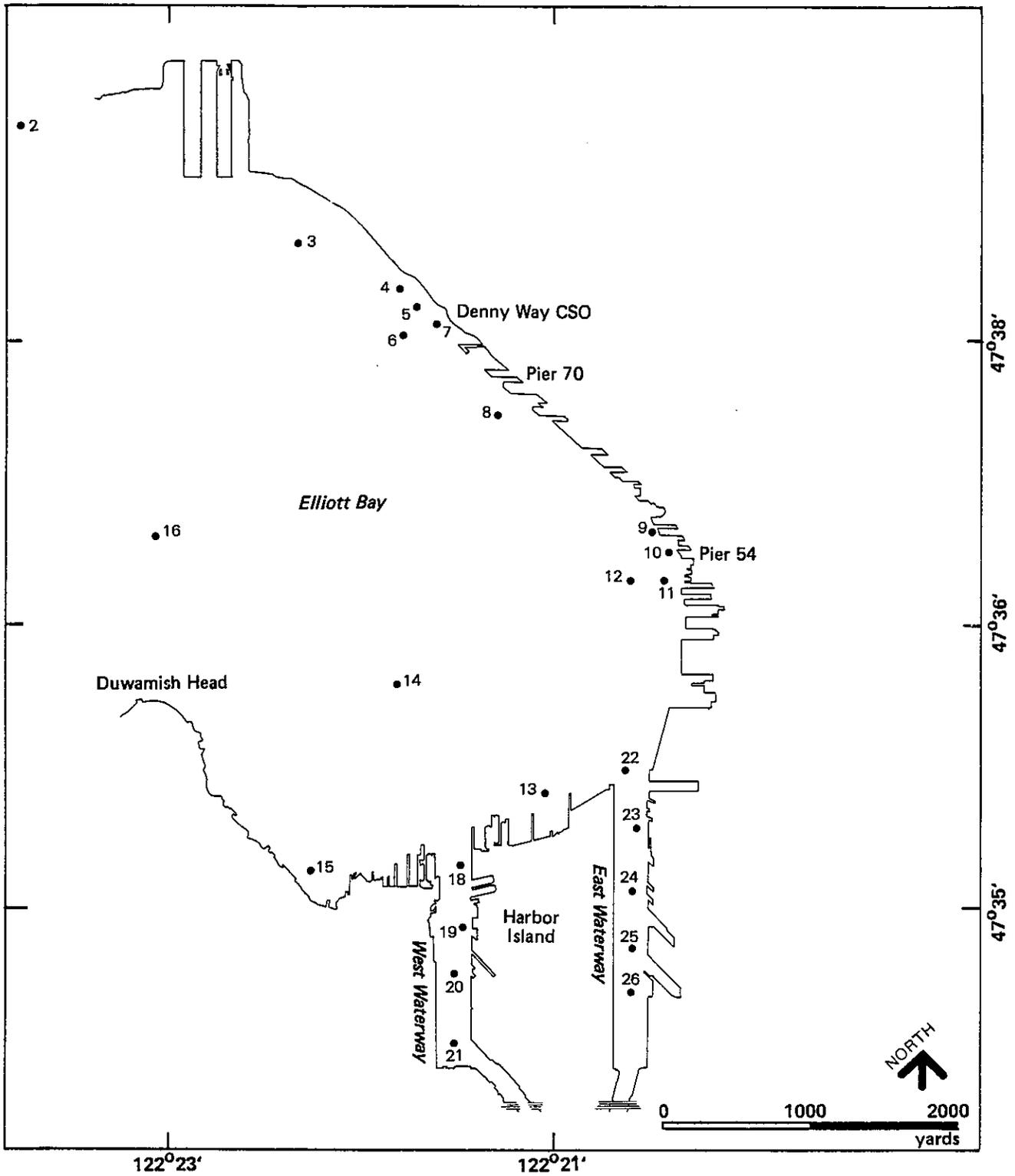


Figure 2. Station Locations in Elliott Bay and the Lower Duwamish River.

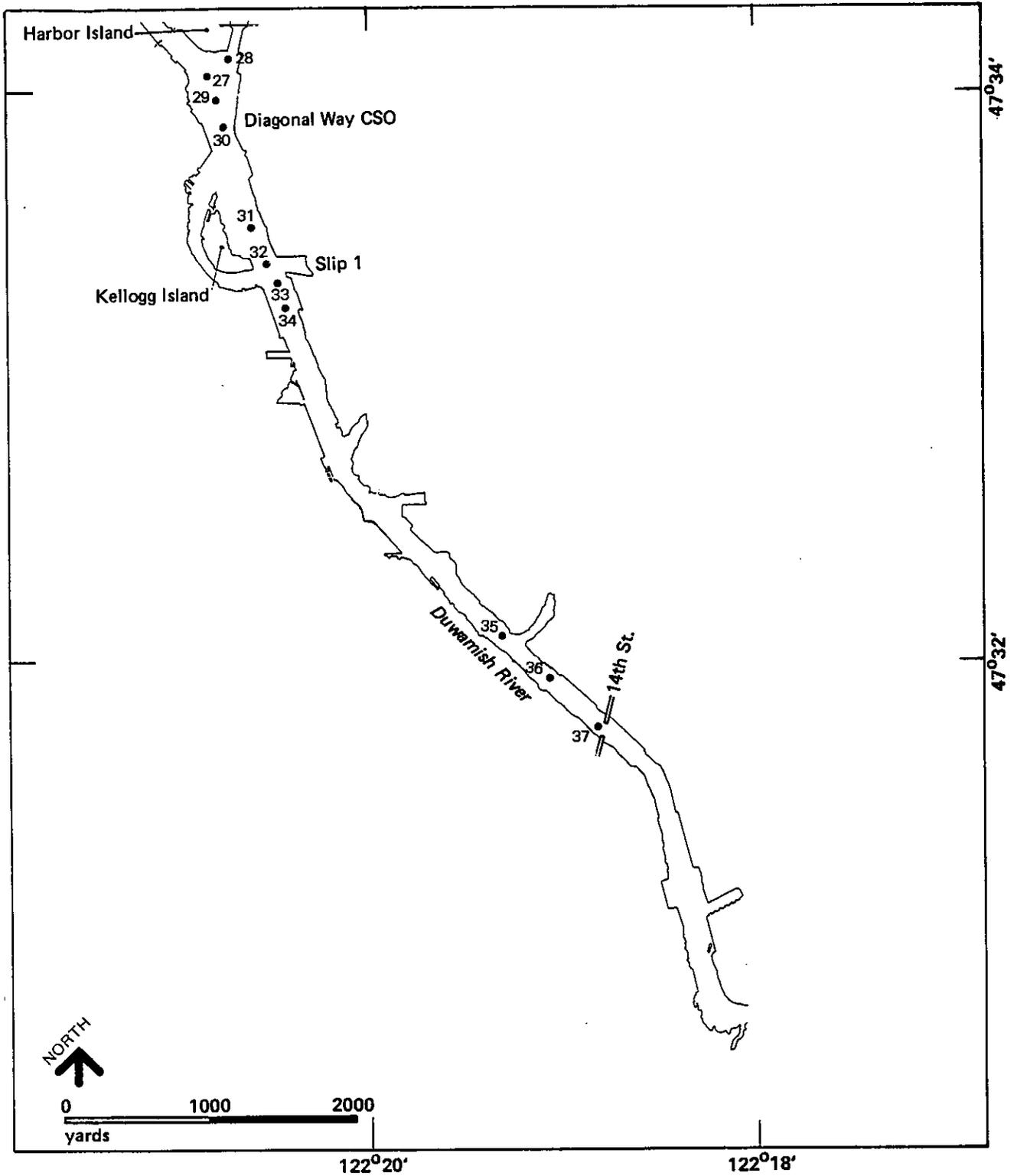


Figure 3. Station Locations in the Upper Duwamish River.

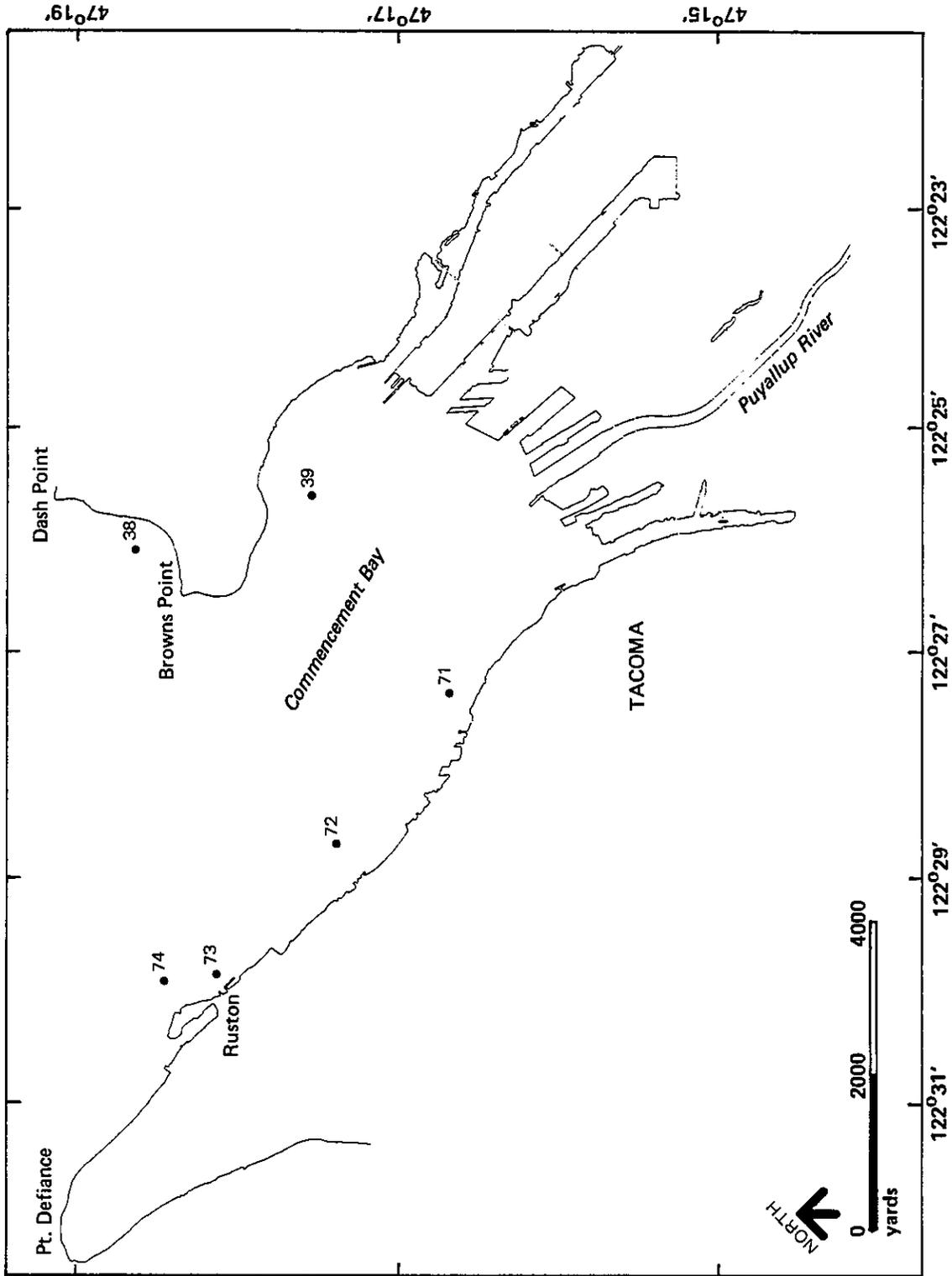


Figure 4. Station Locations in Commencement Bay.

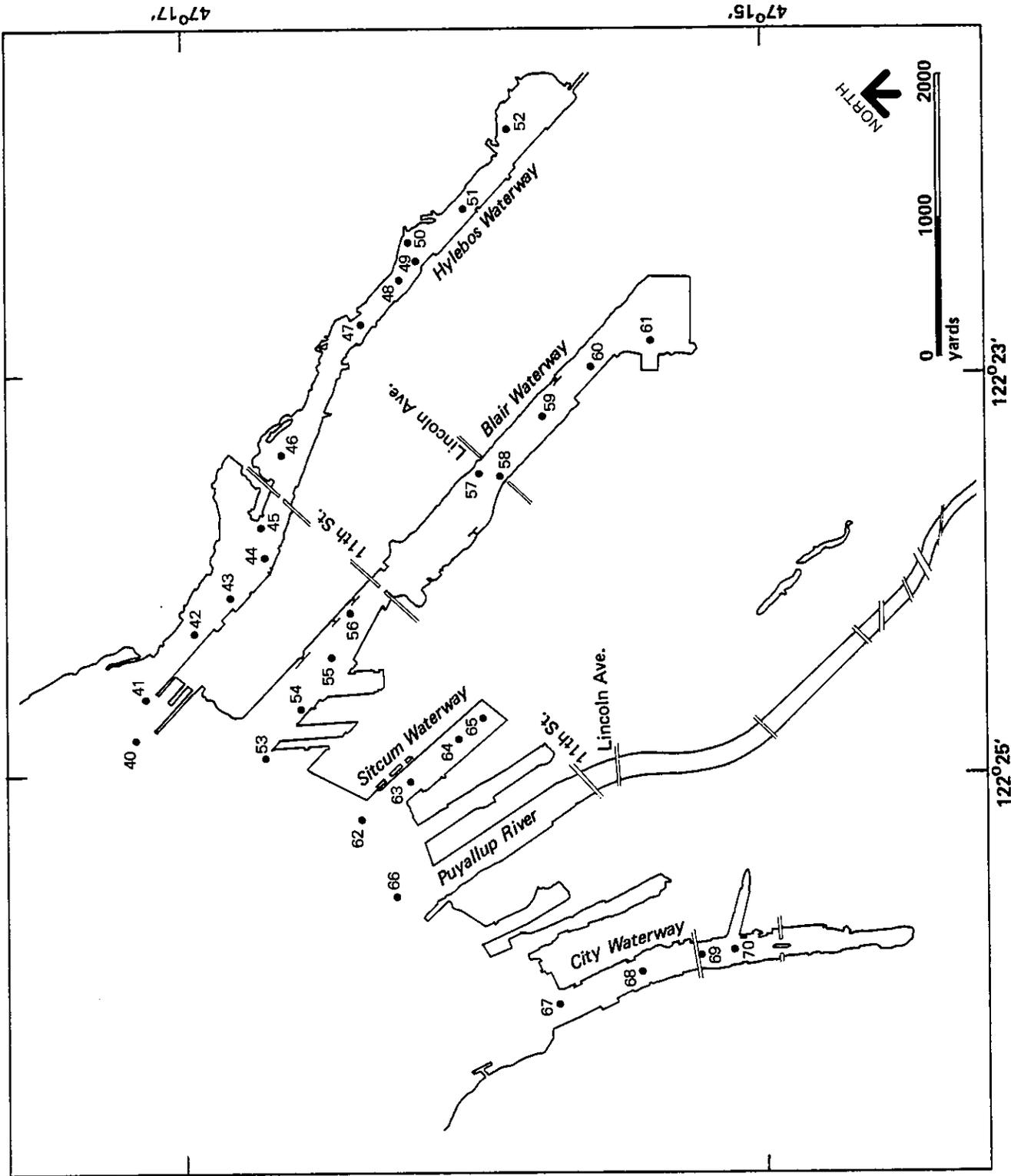


Figure 5. Stations Locations in the Waterways of Commencement Bay.

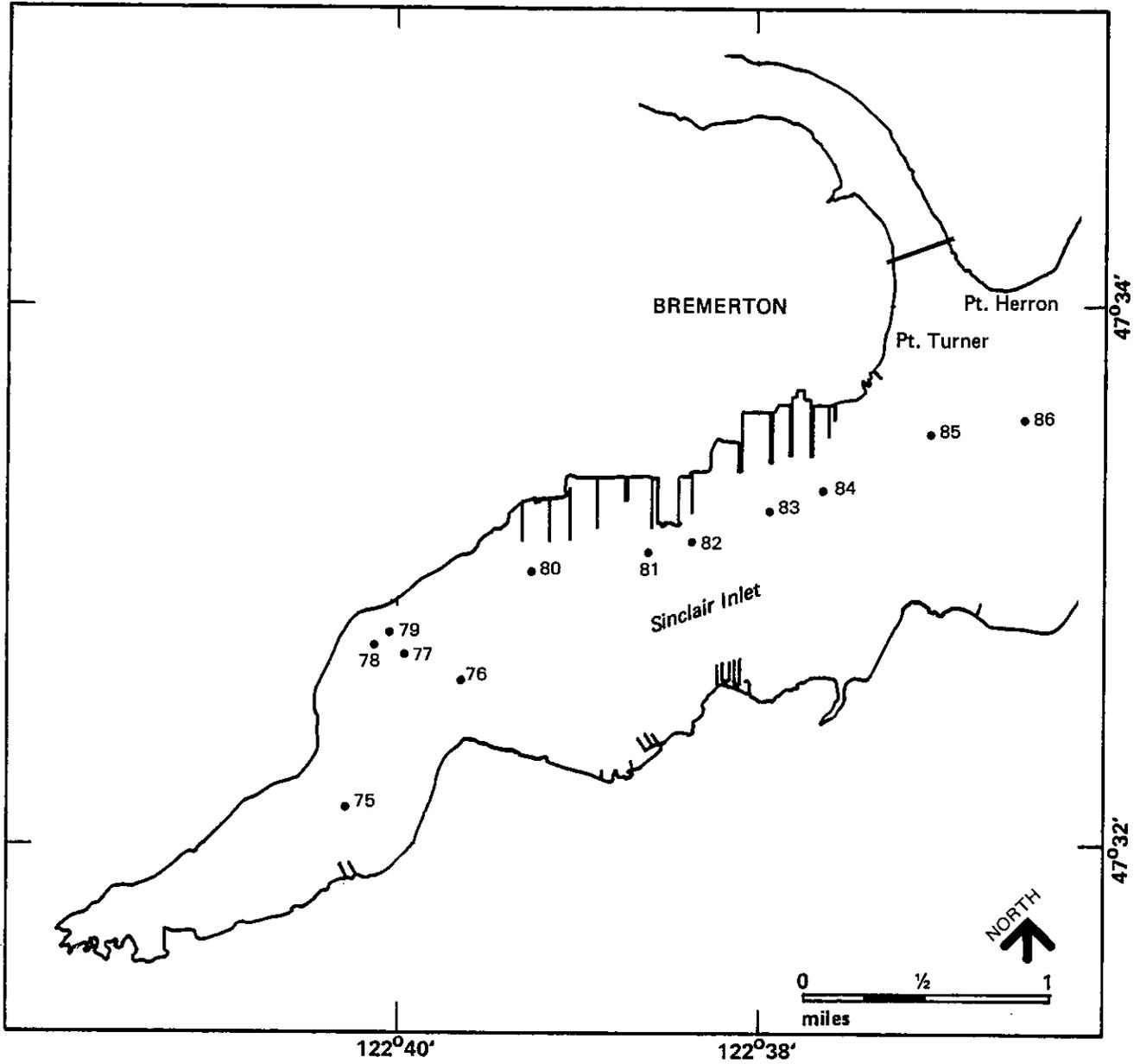


Figure 6. Station Locations in Sinclair Inlet.

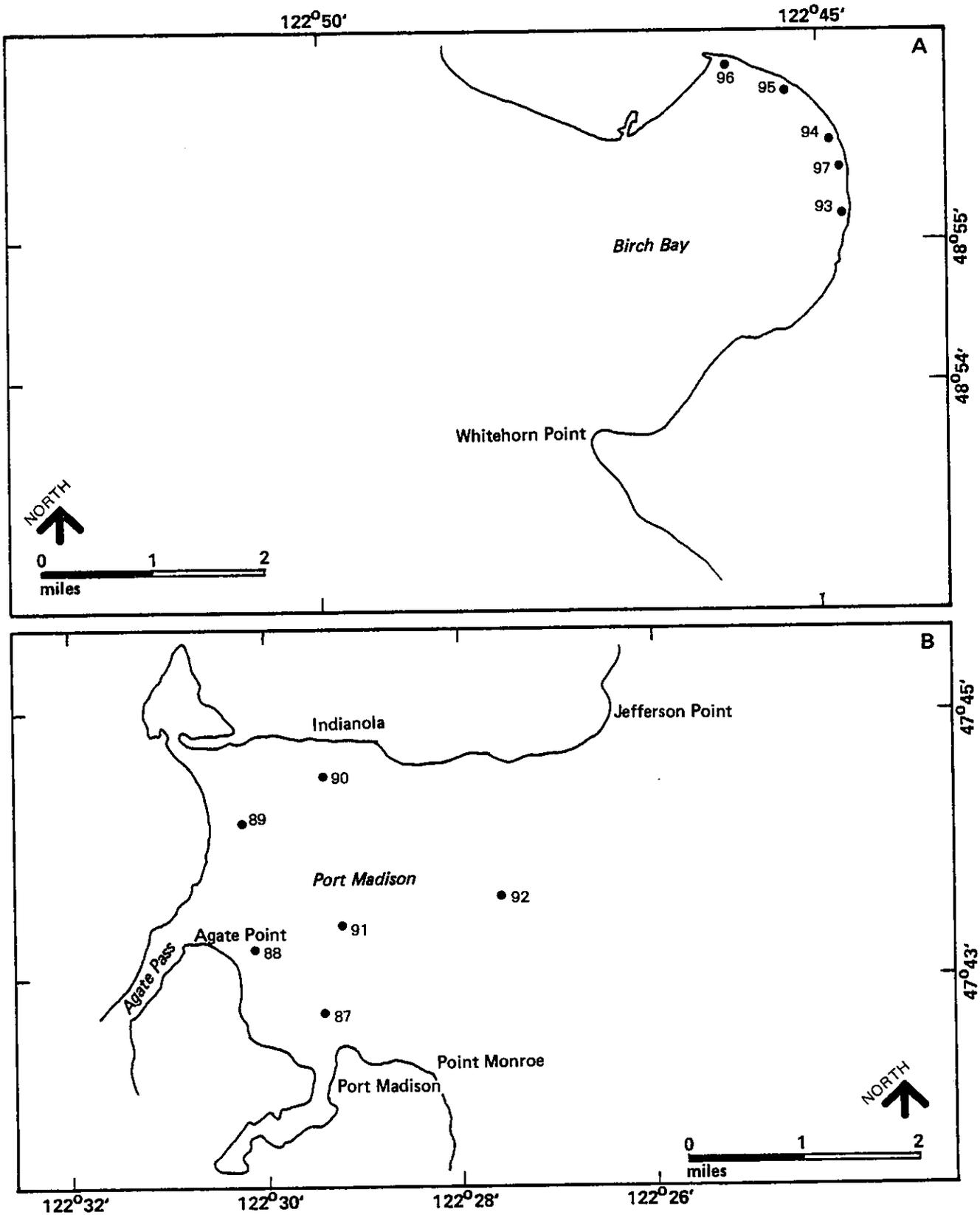


Figure 7. Station Locations in (A.) Birch Bay and (B.) Port Madison.

A. The majority of stations were located coincident with stations occupied in previous studies (e.g. Malins et al., 1980; Riley et al., 1980, 1981) to make use of available physical and chemical data. Though contaminant distributions were known to be highly patchy and chemical data from previous sampling would not necessarily represent conditions in our samples, we used data from the previous chemical surveys as a general guide for station location selection. Stations were also sited between previous station locations to improve the spatial resolution of selected areas. Additional stations were sited near potential sources of toxic substances not previously sampled (e.g. near storm drains, combined sewer overflows, and some industrial locations). Selection of station locations was weighted toward areas known to be highly or moderately contaminated, thus the data are not necessarily representative of the predominant conditions, overall, in Puget Sound.

## 2.2 Approach

Previous experience and the results of toxicity testing carried out by various Puget Sound researchers (Armstrong et al., 1978, 1980; Tomlinson et al., 1980; Shuba et al., 1978; Swartz et al., 1979, 1981, 1982) have shown that in highly contaminated areas the sediments are more toxic than the overlying water. Thus, the methodology employed in this study concentrated on testing sediments to assess "worst case" toxic effects. Consequently, a total of 97 sediment samples was tested in comparison to 7 water samples.

Testing involved three levels of assessment varying from mortality to sublethal effects: acute lethal bioassays, respiration rate tests and chromosomal (anaphase) aberration tests. Results were used to classify the comparative toxicity of sediment and water samples and to identify biologically active areas in Puget Sound. The basic approach involved use of tests known to be responsive to toxic substances such as those known to occur in the Sound. A multiplicity of tests was used to assure collection of as broad a picture as possible of effects.

An experimental approach was used to evaluate sediment and water toxicities in relation to three specific effects criteria:

- a) acute lethality using three different organisms (threespine stickleback fish, Gasterosteus aculeatus; amphipod crustacean, Eogammarus confervicolus; and oligochaete worm, Monopylephorus cuticulatus);
- b) respiratory response using the oligochaete M. cuticulatus; and
- c) induction of chromosomal damage to rainbow trout (Salmo gairdnerii) cells.

Species tested were chosen based on the following criteria (as recommended by APHA, 1980; Shuba et al., 1981; Buikema et al., 1982):

- a) representative importance in the trophic relationships of the Puget Sound ecosystem;
- b) relative sensitivity and consistency of response as test organisms;
- c) availability (in terms of numbers and seasonal occurrence); and
- d) ease of transport, acclimation, culturing and testing relative to budgetary constraints.

### 2.3 Relative Sensitivity of Tests and Organisms

Species of different genera show different susceptibilities to toxicants. Warner (1967) notes that: "no single bioassay, regardless of its sensitivity or sophistication, can provide more than a fragment of information on the consequences of releasing a toxicant into the environment". He gives the example of herbicides, which are often innocuous to marine fauna, but may have profound effects on the flora.

Thus, a multiparametric approach was taken in the present study, using a diversity of bioassays and test organisms, and following the EPA/COE Implementation Manual regarding selection of test species. The three species chosen for LT50 testing are representative Puget Sound biota, occupying distinct ecological niches and representing different feeding modes. M. cuticulatus is an infaunal, bacterial-feeding benthic invertebrate, while E. confervicolus is a representative epibenthic detritivore/carnivore known to feed on M. cuticulatus. Moreover, this amphipod species is an important prey item for juvenile salmonids. The stickleback, G. aculeatus, is a representative pelagic carnivore which feeds predominantly on zooplankton.

The three test species are available and can be collected with minimal difficulty year-round. In addition, all can be maintained and cultured with relative ease, and are highly amenable to laboratory toxicological testing. An account of their relative sensitivities, and of the sensitivity of the respiration and anaphase aberration tests is presented in the following sections.

#### 2.3.1 Eogammarus confervicolus

Tables 1 and 2 provide data from the literature and from preliminary testing conducted in the present study regarding the sensitivity of E. confervicolus as a test organism. Bioassay results with this species versus sodium pentachlorophenate (see Section 2.7.3) are compared to literature values in Table 1. E. confervicolus is among the more sensitive species and 96 h LC50 values are comparable with those for the sensitive larvae of Crangon crangon and Palaemonetes pugio.

Table I

## Toxicity of Sodium Pentachloropheno/Phenates to Marine Biota

<u>Test Organism</u>	<u>Test Conditions</u>	<u>Concentration (ppm)</u>	<u>Results</u>	<u>Reference</u>
<u>Crangon septemspinosa</u> (shrimp)	static, seawater lab study @ 10°C	3.3	66 h LT50	McLeese et al. (1979)
"mussel, anemones, barnacles"	static, seawater lab study	1.0	Killed all organisms in 3 days (i.e. 72 h LT100)	Turner et al. (1948)
<u>Palaemonetes pugio</u> (grass shrimp)	static, seawater lab study	2.63	96 h LC50- intermoult	Conklin & Rao (1978)
		2.74	96 h LC50- early pre-moult	
		0.44	96 h LC50 - moult	
<u>Crangon crangon</u> (sand shrimp)	static, seawater lab study @ 15°C	1.79	96 h LC50 - adults	van Dijk et al. (1977)
		0.11	96 h LC50 - larvae	
<u>Palaemonetes varians</u> (brown shrimp)	static, lab study @ 15°C	5.09	96 h LC50 - adult	van Dijk et al. (1977)
		0.36	96 h LC50 - larvae	
<u>Palaemonetes pugio</u> (grass shrimp)	static, seawater lab study @ 25°C	0.649	96 h LC50 - larvae	Borthwick & Schimmel (1978)
<u>Palaemonetes pugio</u> (grass shrimp)	constant flow, seawater lab study	0.515	96 h LC50	Schimmel et al. (1978)
<u>Eogammarus confervicolus</u> (amphipod)	static, seawater lab study @ 10°C	0.41	96 h LC50	present study

Table 2

Comparative Sensitivity of Eogammarus confervicolus to a Variety of Toxicants

<u>Toxicant</u>	<u>Species</u>	<u>Result</u>	<u>Reference</u>
Bleached kraft mill effluent	stickleback/rainbow trout	96 h LC50 range 12-35%	E.V.S. Consultants Ltd. (1977b)
Kaolin (suspended)	<u>Eogammarus confervicolus</u>	96 h LC50 range 21-24%	Levings et al. (1976)
	"fish"	200 h LC50 < 55 g/l	Peddicord et al. (1976)
Puget Sound sediment sample #4	<u>Eogammarus confervicolus</u>	200 h LC50 = 55 g/l	present study
	<u>Callinassa californiensis</u>	60% mortality at 10 d	
		no mortality at 10 d	

Table 2 shows that E. confervicolus is comparably sensitive to fish, and in terms of Puget Sound sediment, is more sensitive than the ghost shrimp, Callinassa californiensis. Sediment bioassays were performed on both species using procedures described in Section 2.7.1 and sediment from Station 4 in Elliott Bay. After 10 d, mortalities of 60% had occurred among replicates of E. confervicolus, while C. californiensis suffered no mortalities or evidence of stress.

The above results and comparisons indicate that E. confervicolus are relatively sensitive bioassay organisms, although a recent inter-laboratory comparison conducted by E.V.S. Consultants with R. Swartz of the U.S. EPA (unpub. data, 1982) indicated that the infaunal amphipod species Rhepoxynius abronius is more sensitive. However, E. confervicolus is both an important food source for such commercially important species as salmonids, and an important component of Puget Sound benthos (e.g. Meyer and Vogel, 1978). Thus, this species was an appropriate test organism based on both its relative sensitivity and its importance in the Puget Sound food chain.

### 2.3.2 Monopylephorus cuticulatus

In contrast to E. confervicolus, the infaunal oligochaete M. cuticulatus is relatively tolerant to a variety of toxicants in 96 h LC50 tests (Chapman et al., 1982a,b) and was chosen partly for this reason in order to provide a counterpoint to the more sensitive amphipod and fish species. In addition, the use of this species in both LC50 and respiratory stress testing provided continuity of information; because this species is tolerant, there is little "noise" in its response to contaminated sediments and water. Respiratory tests (Chapman et al., 1982c; Brinkhurst et al., 1983) have indicated that this species has a wide range of response to toxicants while showing insignificant variation in respiration rate under baseline conditions.

In addition, there is a great deal of background tolerance/effects data available for this species, including histopathological studies (Thompson et al., 1982). M. cuticulatus is an intertidal species which feeds mainly on bacteria while providing an important food source for crustaceans, fish and birds. It is important in the bioturbation of sediments and subsequent recycling of nutrients (and toxicants) to the water column and is particularly common in the receiving waters near pulp mills with marine discharges. Its presence or absence has been suggested as an indication of the extent of effluent effects (Coates and Ellis, 1980). Based on this information, M. cuticulatus was selected as an acute lethal test organism that could also be used in respiratory stress testing.

### 2.3.3 Gasterosterus aculeatus

Threespine stickleback is a wide-ranging, circumpolar species found in both fresh and salt water. They are present year round in Puget Sound and are an important food item for game fish as well as

providing a food source for fur seal, American mergansers and other fish-eating birds.

There is extensive literature on the use of stickleback in bioassays (e.g. Katz, 1961; Stewart et al., 1967; Hazel et al., 1971; E.V.S. Consultants Ltd., 1977a). In addition, stickleback have been widely used in behavioral and distributional studies and in experiments to determine the effects of environmental parameters on fin, vertebral and body plate development (Leim and Scott, 1966).

Comparative tests indicate that stickleback are relatively sensitive to a variety of toxicants. Katz (1961) found them to be more tolerant than rainbow trout in 14 out of 21 96-h LC50 tests with insecticides, and similarly tolerant as coho and chinook salmon. Clarke (1974) recorded a 96 h LC50 of 0.8 mg/L for G. aculeatus exposed to nickel, compared to a value of 46.2 mg/L for the mummichog, Fundulus heteroclitus, both in saltwater. Threespine stickleback were 3-5 times more sensitive than the demersal shrimp Crangon communis when exposed to dredge spoil leachate or pentachlorophenate (E.V.S. Consultants Ltd., 1977a), and comparably sensitive to herring and rainbow trout exposed to pentachlorophenate and bleached Kraft mill effluent (E.V.S. Consultants Ltd., 1977b). They have been classed among the most sensitive fish species for acute saltwater bioassays (E.V.S. Consultants Ltd., 1977b). Davis and Hoos (1976) found them to be similarly sensitive to NaPCP as rainbow trout. Thus, based on relative sensitivity and importance in the Puget Sound ecosystem and food chain, G. aculeatus was selected as a test species.

#### 2.3.4 Sublethal Respiration Rate Tests

Respiration is a physiological process that is responsive to sublethal toxicant effects. Brinkhurst et al. (1983) have shown the value of using respiration to detect sublethal stress, and have shown that significant changes (elevation or depression) in respiration rate of M. cuticulatus can be caused by exposure to sublethal levels of metals and organic toxicants.

Respiration rate tests respond to the presence of metals, as well as to all organic compounds that affect respiratory enzyme function (e.g. phenols, chlorophenols, pesticides, PCBs) but are probably not sensitive to known carcinogens such as PAHs. The mechanism(s) by which respiration is affected have not been determined precisely, but appear to reflect various adverse physiological effects. For instance, depression of respiration rate may be due to a build-up of oxaloacetate leading to a reduction of tissue oxygen consumption (cf. Cantelmo et al., 1978). Elevated respiration may be due to interference in normal osmoregulatory processes, making osmotic adjustment energetically more costly (cf. Laughlin and Neff, 1979).

#### 2.3.5 Anaphase Aberration Tests

The anaphase aberration test is a relatively simple procedure which allows one to visualize the segregation of chromosomes prior to

telophase. To perform the test in vitro, one simply cultures a desired cell type on glass microscope slides, exposes the growing cells to the test compound, and then fixes and stains them. Examination of the cells is done with a conventional light microscope at 200-430 X magnification. The test requires only that one examine anaphase cells (rather than the chromosomes which comprise them) for the presence of such defects as lagging/attached fragments, bridges or multi-polar bodies. The anaphase aberration test is highly sensitive to a wide variety of organic mutagens (Kocan et al., 1982) and has been recommended by the Ad Hoc Committee of the Environmental Mutagen Society and the Institute for Medical Research as an integral part of any mutagenicity testing.

## 2.4 Field Collections

### 2.4.1 Sediment

Sediment samples were collected using a 0.1 m<sup>2</sup> 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.

Sediments were removed from the grab by means of a scoop (6 cm depth, 15 cm width) using a polyethylene bag stretched over an aluminum frame. This scoop was drawn through the surface of soft sediments. In the case of very consolidated sediments, the upper 6 cm were transferred to the bag with a stainless steel spatula. Between 3 to 5 kg (wet weight) of sediment was collected, and immediately homogenized by careful and thorough kneading. Aliquots for possible future chemical analyses were then transferred to solvent-rinsed, 120 ml glass jars with teflon cap liners using a stainless steel spatula and frozen. Aliquots for anaphase aberration testing were similarly transferred to solvent-rinsed, 475 ml glass jars with teflon cap liners. A final aliquot for grain size and total organic carbon analysis was collected in Whirl-Pak bags. The remainder of the sample was sealed in the collection bag and retained refrigerated for toxicity testing.

Sampling in Birch Bay, which was conducted intertidally, deviated slightly from this procedure. Sediments to a depth of 6 cm were scooped directly from the beach just above the water-line at low tide on a calm day. Once sufficient sediment was collected in the bag, each sample was kneaded and aliquoted as described for subtidal samples.

Samples were stored on ice following collection and during transport. Samples for chemical analysis were delivered to the National Marine Fisheries Service (Montlake Laboratory) for disposition within 24 h of collection; samples for anaphase aberration testing were frozen; and, samples for toxicity testing were transported on ice to EVS Consultants laboratories within 24 h of collection. Samples for grain size, digestible organic carbon and total volatile solids were stored refrigerated (at 4°C) prior to analysis.

## 2.4.2 Water

Water samples were collected from seven selected stations (12, 29, 43, 65, 70, 82, 96) using a 25 L stainless steel sampler triggered via a rotating ball valve. The sampler was deployed empty and closed. The ball was rigged with weights on cables so that it opened approximately 30 cm above the sediment-water interface allowing the sampler to fill. Lifting the sampler from the bottom closed the ball valve allowing retrieval of a discrete sample. Two 25 L samples were collected consecutively from each site.

Upon retrieval, the sampler was immediately discharged into clean 25 L polyethylene carboys. While the carboys were being filled, a laboratory thermometer was inserted to measure water temperature. A 100 ml aliquot was retained in a clean plastic container for the determination of salinity. An additional aliquot (3.5 L) was poured into a solvent-cleaned brown glass jug with a teflon cap liner for anaphase aberration testing. Immediately after filling, 100 ml of pesticide grade hexane was added to the jug for preservation and to initiate extraction necessary for testing.

Water samples from Birch Bay were collected approximately 7 m offshore, by wading into 0.75 m of water. Two closed 25 L polyethylene carboys were held 15-20 cm above the bottom, opened and filled. Care was taken to avoid disturbance of the bottom material. Water temperature was measured by holding the thermometer tip at the sampling depth.

Samples were stored on ice following collection and during transport. The carboys containing water for toxicity testing were transported to E.V.S. Consultants laboratories within 24 h of collection. Samples for anaphase aberration testing and salinity determination were stored refrigerated (at 4°C) prior to analysis.

## 2.4.3 Test Organisms

Both *E. confervicolus* and *M. cuticulatus* were collected intertidally from the mouth of Union Bay Creek, Patricia Bay, B.C., an area free from any significant pollutant inputs. Whole sediment samples were removed with a trowel and placed in polyethylene buckets. Seawater was added and the resulting slurry gently agitated, then sieved (0.25 mm). Large shells, rocks and other debris were removed, and the residue kept in seawater in clean polyethylene containers with ice for transportation to E.V.S. Consultants laboratories.

Stickleback (*G. aculeatus*) were collected at night from a clean area of Burrard Inlet, Vancouver, B.C. using dipnets and nightlights. They were transported to E.V.S. Consultants laboratories in clean 65 L polyethylene containers provided with lids and a constant supply of air from compressed air cylinders.

All organisms were returned to the laboratory as soon as possible following collection. In the case of invertebrate species, this time period was less than 24 h; in the case of stickleback, it was less than 1 h.

## 2.5 Physical/Chemical Characterization

### 2.5.1 Water Temperature and Salinity

The temperatures of the water samples were measured immediately after collection as described above. Salinities were determined in the laboratory with an inductive salinometer.

### 2.5.2 Sediment Grain Size and Organic Analyses

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 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<sub>2</sub>O<sub>2</sub> 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.

The remaining 10 g of dried sample was placed in a tared crucible and reweighed. This aliquot was then combusted at 500 ± 100°C overnight, cooled in a dessicator and reweighed for the determination of total volatile solids (TVS).

The subsample for grain size analysis was mixed with 10 ml of 30% H<sub>2</sub>O<sub>2</sub> in distilled water, allowed to sit for 12 h and then 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.

An approximately 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.

## 2.6 Holding/Acclimation of Test Organisms

### 2.6.1 Benthic Invertebrates

Worms and amphipods were hand sorted following their arrival in the laboratory. Confirmatory identifications of species were conducted using a Wild M5 dissecting microscope. Damaged or dead individuals were removed prior to placing the worms and amphipods in separate aquaria provided with natural substrate (from the collection area) and clean seawater (25 ppt salinity). Cultures were kept in a constant environment room adjusted to  $10 \pm 0.5^\circ\text{C}$  under a 12 h light/dark cycle. Cultures were aerated and fed mixtures of ground Enteromorpha and commercial Tetramin ad libidum; feeding was discontinued 24 h prior to testing.

Amphipod culture aquaria were additionally provided with breeding chambers made of fine netting into which copulatory pairs were placed. Adults were periodically removed from these chambers and brood pouch juveniles retained and reared within.

### 2.6.2 Fish

Stickleback were maintained prior to testing in a flow-through acclimation pool (5 m diameter) in clean ambient Burrard Inlet seawater (salinity range 23.5-28 ppt; temperature range 7-11°C). Stocks were fed daily with frozen euphausiids ad libidum. Mortalities in the acclimation chambers were less than 0.1% per day. Test fish were transferred to laboratory holding tanks at  $10 \pm 0.5^\circ\text{C}$  in 25 ppt salinity water 24 h before testing, and feeding was discontinued at this time.

## 2.7 Toxicity Testing

### 2.7.1 Acute Lethal Bioassays

The acute lethality of sediment and overlying water samples was determined by conducting 96 h LT50 (time to 50% mortality) tests on amphipods, worms and fish. These tests are among the most useful means of initially estimating toxicity (Cairns et al., 1978; Buikema et al., 1982) and represent a sensitive short-term protocol for comparing relative toxicities of sediments.

The methodology employed was modified from APHA Standard Methods (1980) and the EPA/COE Technical Committee on Criteria for Dredged and Fill Material (1978) to allow for evaluation of "composite" toxicity of solid, particulate and elutriate sediment phases. Sediments from each sampling station were homogenized and

mixed with diluent water (25 ppt salinity) to yield a 20,000 mg/L (=ppm) weight/volume sediment/water slurry. This concentration of clean sediment was not lethal to the test organisms over a 10 d period, and represents a maximum that can be kept in suspension by air agitation and which will produce mortalities with contaminated sediments (e.g. sediments contaminated with copper - Chapman et al., 1979; sediments contaminated with creosote - E.V.S. Consultants Ltd., unpublished data). The slurry was added to the test chambers and stirred vigorously for 15-20 min to suspend particulates and liberate the water soluble fraction in the liquid phase. The sediments were then allowed to settle prior to the introduction of test species. This procedure assured that sediment mixing was representative for each sample and minimized irregularities from partially stirred sediments. Bottom water samples were also stirred for 15-20 min.

A temperature of 10°C was considered to be representative of shallow Puget Sound water below the thermocline and was used for the present work. Salinities in the Sound range up to 33 ppt, with most indigenous species capable of tolerating salinities from 15 to 33 ppt. A salinity of 25 ppt was used in the present study. Water samples were adjusted with artificial R.I.L.A. sea salt and distilled water as necessary and kept at  $10 \pm 0.5^\circ\text{C}$  in controlled environment rooms under 12 h light/dark regimes. Sediment slurry samples were similarly treated. Both types of samples were aerated as necessary to raise dissolved oxygen concentrations to saturation prior to testing.

Organism loading density (g of organism/L of slurry) is a major variable that was controlled to minimize artifacts. Loading densities were maintained below 0.5 g/L to maximize the sensitivity of acute lethal toxic responses, provide sufficient test material, and allow interspecies comparisons. Test organisms were not fed during the test period.

Sediment and water samples were tested within 72 h of collection; testing with each sample was initiated for each of the 3 species within a 1 h period. Three separate collections of sediment and water were made in Puget Sound and were received at the Vancouver bioassay laboratories within 24 h. Testing began the next day, with 1/2 of the total samples tested that day and the remainder the following day (e.g. 72 h after collection). Controls consisting of clean seawater and sediment from the Union Bay Creek collection area were run concurrently with each series of samples.

Test initiation involved pouring samples for testing (either bottom water or sediment slurries) into test containers immediately following mixing. Randomly sorted healthy individuals of the appropriate test species were then introduced.

Mortality checks were conducted at 10, 20, 40, 80, 160, and 320 min intervals for the first 6 h of each test. Subsequent checks were made at 24 h intervals. Testing was terminated at 96 h providing no mortalities and/or sublethal effects were noted. Otherwise, testing

was continued with 24 h checks for 10 d, an exposure time considered by Shuba et al. (1981) to be sufficient for bioassay studies based on a literature review.

#### 2.7.1.1 Eogammarus confervicolus

Tests with E. confervicolus have been shown to be responsive to chlorophenols, pulp mill effluents and other contaminants (Section 2.3.1).

Amphipods were tested in inert plastic containers consisting of 20 separate chambers. Individuals were tested separately due to the possibility of cannibalism. A total of 20 amphipods was tested for each sample, consisting of 2 equal replicates of 10 individuals. Test containers were fitted with a covering mesh screen to prevent escape or communication between chambers.

#### 2.7.1.2 Monopylephorus cuticulatus

Tests with M. cuticulatus have been shown to be responsive to such contaminants as mercury, cadmium, chlorophenols, and black liquor (Chapman et al., 1982a).

Worms were tested in polyethylene petri dishes. Ten similar sized worms were randomly placed in each container without separation and duplicate containers were run per sample.

#### 2.7.1.3 Gasterosterus aculeatus

Tests with G. aculeatus have been shown to be responsive to such contaminants as insecticides, heavy metals and chlorophenols (Section 2.3.3).

Stickleback were tested in 20 L rectangular glass aquaria (one aquarium per sample) with initial and final pH and D.O. being recorded. A total of 20 fish (mean weight 0.38 g, mean length 3.4 cm) were introduced into each aquaria by dipnet, and the aquaria were covered with glass lids.

#### 2.7.2 Sublethal Respiration Rate Tests

Respiration rates have been documented to change (increase or decrease) as a response to contaminant exposure (Chapman et al., 1982c; Chapman and Brinkhurst, 1983; Brinkhurst et al., 1983).

Respiratory responses were measured for the oligochaete M. cuticulatus exposed to water samples and sediment slurries treated and prepared as previously described for LT50's. Test solutions were prepared, then centrifuged at 1,000 rpm for 10 min and the resulting elutriate was decanted and tested. Centrifugation minimized bacterial respiration in tests and removed particulates. The use of a control syringe (test elutriate without worms) provided a correction value for changes in oxygen level not due to worm respiration.

Testing was performed potentiometrically using a Radiometer PHM 73 Blood Gas Analyzer, with 10 ml disposable syringes as respiration chambers. The analyzer was connected to a Haake Constant Temperature Circulator ( $10 \pm 0.5^\circ\text{C}$ ). 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 ( $10 \pm 0.5^\circ\text{C}$ ) for 1 h prior to testing. Duplicate syringes plus a control syringe (consisting of test solution without worms) were tested for each sample (=3 syringes).

Syringes were removed from the water bath at 1-2 h intervals, inverted to disrupt possible oxygen gradients and aliquots were injected directly into the Radiometer analyzer chamber. An initial 400  $\mu\text{L}$  aliquot was injected to flush the chamber, the electrode was allowed to equilibrate, and then three consecutive 200  $\mu\text{L}$  measurements were taken on each sample. The Radiometer electrode was calibrated prior to the first measurement, was checked hourly and recalibrated as necessary following changes in barometric pressure. Syringes were returned to the water bath between measurements.

Between three and five consecutive series of measurements were taken for each syringe over 4-8 h, following which the worms were removed, placed on pre-weighed aluminum weigh boats and dried overnight at  $80^\circ\text{C}$ . Readings in mm Hg were converted to percent saturation using the oxygen solubility tables of Truesdale and Downing (1954). Respiration rates in  $\mu\text{L O}_2$  were calculated directly from the change in percent saturation over time divided by the dry weight of worms tested following any adjustment for controls.

Mean respiration rates ( $n = 3-5$  values per syringe  $\times 2$ ) were compared statistically with control values determined using the same procedure but with worms in clean diluent water. Four separate control measurements were made at approximately monthly intervals throughout the study. The objective was to determine if mean respiration was significantly different than controls, and a one-tailed student t-test was used to compare means.

### 2.7.3 Sediment Spike

In order to determine the sensitivity of the LT50 and respiration tests and of the test species, a sediment spike test was conducted using a reference toxicant. Such use of reference toxicants is commonly recommended (Buikema et al., 1982). Sodium pentachlorophenate (NaPCP), which was chosen for use in this study, is frequently used for this purpose (e.g. Alderdice, 1963; Davis and Hoos, 1976; E.V.S. Consultants Ltd. - numerous bioassay programs).

The 96 h LC50 values for NaPCP, in the absence of sediment for the three test species under a defined set of bioassay conditions (25 ppt, pH 7.0,  $10^\circ\text{C}$ ), were as follows (in mg/L): stickleback, 0.06; oligochaetes, 0.55; amphipods, 0.41. Sediments tend to modify the toxic effects of contaminants (Chapman et al., 1982a), thus we selected a concentration for sediment testing (3.4 mg NaPCP/L of

toxic effects of contaminants (Chapman et al., 1982a), thus we selected a concentration for sediment testing (3.4 mg NaPCP/L of slurry) which was an order of magnitude higher than the mean 96 h LC50 value for all three species without sediment.

The possible interactions of sediment and toxicant (i.e. whether the toxicant interacts with the sediment, is adsorbed by the sediment, or reacts and changes in toxic character) are dependent upon surface area and organic content. However, a stabilized and defined condition was obtained by preparing large batches of test sediments as slurries, and then washing the slurry for 48 h in a 3.4 mg/L NaPCP solution with continuous slow stirring. The sediment was then allowed to settle, the overlying water decanted and the wet sediment treated in the same way as the field collected sediment, which it resembled.

A new sediment slurry was made up from the spiked samples and both LT50 and respiration effects testing was conducted as previously detailed.

Two sediments were spiked: a sandy control sediment from Birch Bay (Station 95) and a muddy sediment from Commencement Bay (Station 50). At the end of testing, the water and sediment in the stickleback bioassay aquaria were sampled, and then analyzed for chlorophenols using the methodology of Renberg (1974), involving solvent extraction, anionic ion exchange, and gas chromatography.

#### 2.7.4 Anaphase Abberation Tests

##### 2.7.4.1 Preparation of Sediment Extracts

Sediment samples were stored frozen until just prior to extraction. Each sample was then thawed, rehomogenized by careful but thorough stirring, and an aliquot (approximately 150 g wet weight) 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 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 shaken vigorously for two minutes to insure 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, 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 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 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 and 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 quantitatively transferred to a tared conical centrifuge tube with a ground glass stopper. The sample was then taken almost to dryness on the hot water bath, followed by storage in a dessicator wrapped in aluminum foil and 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 anaphase aberration testing. Since not all extracted material was dissolved during testing, the tubes were reweighed upon

return to estimate the amount actually used in testing (fraction soluble).

#### 2.7.4.2 Preparation of Water Extracts

Water samples preserved with hexane were stored in the dark prior to analysis. The hexane was effective in retarding microbial growth and it accumulated the majority of the organic residues prior to the initiation of the formal extraction procedure, which is summarized below.

A teflon-coated magnetic stirring bar was added to the original sample jug. The jug was placed on a magnetic stirrer and a strong vortex, sufficient to draw solvent to the bottom of the jug, was maintained for 20 min. Stirring was then stopped and the liquid phases allowed to separate (20 min). The hexane layer was drawn off by vacuum through a teflon tube into a 1 L separatory funnel. This stirring-extraction procedure was repeated twice more with additional 100 ml portions of hexane.

The quantity of water extracted was determined by emptying the contents of the jug, after the final hexane layer was removed, into a suitable graduated cylinder. Any water brought over into the separatory funnel was combined during this step. The volume was determined with a minimum precision of  $\pm 20$  ml (less than 1%) and the water discarded.

The combined hexane extracts were eluted through a drying column of anhydrous sodium sulfate into a 1 L Kuderna-Danish flask with two 25 ml rinses of the separatory funnel. The flask was fitted with a three-ball Snyder column. The extract volume was reduced to approximately 5 ml on a hot water bath. The cooled extract was transferred quantitatively to a tared conical centrifuge tube with a ground glass stopper and taken almost to dryness on the hot water bath. The final drying and weighings were completed as with the sediment samples.

#### 2.7.4.3 Testing

Extracts were treated with 1 ml 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. Based on the original dry weights, the samples were diluted to a concentration of 20 mg/ml ( $5 \mu\text{L} = 100 \mu\text{g}$ ). Both stock and 20 mg/ml solutions were stored in the dark under  $\text{N}_2$  until applied to the cell cultures.

Rainbow trout (*Salmo gairdnerii*, Richardson) gonad cells (RTG - 2) were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), glutamine (100 mM), non-essential amino acids (1 mM), sodium bicarbonate (8.9 mM), penicillin (15 units/ml) and streptomycin (15  $\mu\text{g}/\text{ml}$ ). A pH of 7.0 to 7.3 was maintained by cultivation in 5%  $\text{CO}_2$ .

A total of  $5 \times 10^4$  cells were plated onto  $2 \text{ cm}^2$  wells of a Costar dish (24 wells/dish) and the unknown extract added to a final concentration of 100, 50, 10 and  $1 \mu\text{g/ml}$  of culture medium (0.5% DMSO total for all concentrations). These cultures were then allowed to grow for 72 h, examined microscopically and scored as to cell death, mitotic inhibition or no effect. Based on this visual score, two concentrations were chosen for the anaphase aberration test: the highest concentration which did not inhibit mitosis and a second concentration one dilution lower.

Once the working dilutions of the extracts were known, cells from stock cultures were plated onto acid cleaned glass slides in 1 ml of culture medium. The slides were then incubated overnight in square petri plates and the appropriate sample extract was added to the culture medium the following morning. Two slides per dilution of each extract were then incubated for 48 h at  $18^\circ\text{C}$ , following which the slides 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 470 X power for each anaphase concentration and the percent of normal and aberrant anaphases were recorded. Controls were run with each series of stations tested and consisted of: 1) a set of untreated cells; 2) a set treated with 0.5% spectrophotometric grade DMSO; and 3) a set treated with  $0.25 \mu\text{g/ml}$  benzo(a)pyrene. From these controls, determinations were made of the spontaneous background level of anaphase aberrations, the effect, if any, of the solvent used in exposures, and whether the system responded to a concentration of known mutagen previously shown to be capable of producing high levels of aberrations in these cells.

Throughout the experiments the various types of aberration observed were classified on the basis of previously described abnormalities (Nichols et al., 1972, 1977; Kocan et al., 1982).

### 3.0 RESULTS

#### 3.1 Physical/Chemical Sample Characterization

##### 3.1.1 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 3 for each sediment sample. More detailed sediment grain-size data are presented in Appendix B. For visualization of the relative differences in sediment characteristics, the sediment texture, expressed as the percent of the sample in the silt and clay class (the finer fraction), and the percents of the organic fractions have been plotted as bar diagrams at the charted sample locations in Figs. 8-14.

Table 3

## Sediment Physical and Chemical Parameters

<u>Station Number</u>	<u>Sand<sup>a</sup> %</u>	<u>Silt<sup>a</sup> %</u>	<u>Clay<sup>a</sup> %</u>	<u>Water %</u>	<u>DOC<sup>b</sup> %</u>	<u>TVS<sup>b</sup> %</u>	<u>Extractables<sup>b</sup> %</u>	<u>Soluble<sup>c</sup> %</u>
<u>Elliott Bay</u>								
1	91.3	5.4	3.3	28.9	1.3	2.2	0.048	46
2	97.4	0.9	1.7	27.2	1.4	2.2	0.025	80
3+ <sup>d</sup>	77.4	15.9	6.7	26.4	1.1	2.4	0.114	47
4+* <sup>d</sup>	8.2	85.7	6.1	41.9	3.2	9.5	0.419	28
5+*	49.0	45.4	5.6	32.3	5.3	3.5	0.224	39
6+*	8.1	67.0	24.9	50.7	11.9	8.9	0.596	81
7 *	5.0	88.7	6.3	47.1	5.2	7.4	0.640	75
8+	60.7	33.4	5.9	37.9	2.2	5.3	0.500	67
9+	34.0	55.5	10.5	46.5	1.5	8.1	0.499	58
10+	55.3	32.3	12.4	43.8	7.8	8.6	0.722	46
11+	34.5	53.7	11.8	56.1	16.0	12.1	1.395	72
12	30.0	61.5	8.5	40.6	5.9	6.4	0.291	25
13	45.7	49.7	4.6	33.5	1.4	9.0	0.061	21
14	64.9	22.9	12.2	40.6	5.1	18.4	0.169	27
15+	56.8	35.2	7.9	31.4	5.1	4.5	0.091	33
16	58.1	26.7	15.2	35.2	1.2	10.0	0.087	50
17+	94.7	1.9	3.4	26.9	1.4	0.9	0.035	68
<u>Duwamish River</u>								
18	49.9	38.2	11.9	42.7	6.1	10.2	0.207	9
19	49.4	30.0	20.6	44.0	4.1	9.1	0.284	40
20+	48.1	46.4	5.5	46.7	3.6	5.4	0.423	23
21+	31.7	54.2	14.1	45.6	3.2	6.5	0.680	65
22	56.6	37.3	6.1	42.7	2.9	5.3	0.163	43
23	25.9	66.2	7.9	45.8	3.6	5.2	0.367	60
24	44.8	49.7	5.5	43.7	3.1	4.9	0.545	24
25	12.8	77.8	7.4	45.7	3.4	5.9	0.501	28
26	15.2	74.1	10.7	47.9	3.9	7.9	0.649	80
27	30.9	51.4	17.7	49.2	2.0	3.9	0.492	27
28	4.7	88.6	6.7	50.8	0.9	8.7	0.622	22
29+	96.2	1.9	1.9	21.9	0.2	2.5	0.033	32
30	22.8	60.1	17.1	45.0	2.7	5.0	0.537	37
31+	21.7	58.9	19.4	45.6	0.8	11.2	0.404	44
32+	12.7	76.9	10.4	49.4	8.5	8.9	0.353	14
33+	14.3	68.1	17.6	45.8	5.6	8.3	0.371	32
34+	10.5	65.1	24.4	46.1	4.1	6.2	0.318	23
35	14.7	67.7	17.6	46.4	4.6	5.9	0.175	27
36	14.4	67.5	18.1	48.0	5.7	7.6	0.471	70
37+	39.8	46.2	14.0	47.3	5.1	6.9	0.098 (1/2) <sup>e</sup>	54

Table 3 (Contd)

<u>Station Number</u>	<u>Sand<sup>a</sup> %</u>	<u>Silt<sup>a</sup> %</u>	<u>Clay<sup>a</sup> %</u>	<u>Water %</u>	<u>DOC<sup>b</sup> %</u>	<u>TVS<sup>b</sup> %</u>	<u>Extractables<sup>b</sup> %</u>	<u>Soluble<sup>c</sup> %</u>
<u>Commencement Bay</u>								
38	96.8	1.5	1.7	7.2	0.2	2.3	0.017	14
39+	10.1	82.0	7.9	44.5	4.0	7.2	0.158	55
40	13.2	67.3	19.5	37.4	2.8	3.2	0.159	40
41	18.3	64.2	17.5	87.1	5.0	7.3	1.221	33
42+	24.2	64.7	11.1	46.5	3.9	6.5	0.353	25
43	33.8	52.8	13.4	43.8	3.4	15.6	0.298	6
44+	25.1	52.5	22.4	47.0	5.3	8.2	0.347	35
45 *	29.5	58.0	12.5	53.7	4.6	6.3	0.472 (1/3)	28
46+	40.0	52.0	8.0	47.5	6.2	7.1	0.231	--
47+	40.3	48.3	11.4	47.3	8.1	8.3	0.441	21
48+	13.2	64.7	22.1	56.7	5.0	5.5	0.623	48
49 *	10.2	69.0	20.8	59.9	6.6	6.8	0.763	25
50 *	7.4	75.8	16.8	60.5	5.3	5.7	0.518	49
51 *	18.8	49.9	31.3	74.4	13.8	12.9	1.226 (1/4)	25
52+	11.8	64.3	23.9	60.9	6.6	6.8	0.932	20
53	82.1	13.3	4.6	26.9	1.0	5.7	0.034	29
54+	37.1	59.6	3.3	40.7	2.1	2.9	0.147	44
55	66.5	29.1	4.4	32.1	2.8	3.4	0.089	40
56	29.2	60.7	10.1	38.0	6.2	6.2	0.177	47
57+	23.7	69.8	6.5	44.7	2.1	5.4	0.160	13
58+	37.7	55.7	6.6	41.6	5.6	6.2	0.142	40
59	8.5	83.5	8.0	48.4	5.6	6.7	0.290	62
60+	41.6	53.1	5.3	39.6	2.4	4.1	0.192	60
61	13.9	71.1	15.0	47.0	2.8	6.1	0.247	45
62	17.7	66.6	15.7	35.7	2.4	5.4	0.227	62
63+	15.8	78.4	5.8	40.7	5.1	6.8	0.278	38
64	28.4	64.2	7.4	38.5	6.0	23.7	0.241	54
65	3.4	88.8	7.8	44.5	6.0	7.1	0.313	65
66	10.1	76.0	13.9	31.1	3.3	4.0	0.078	57
67	71.8	23.1	5.1	33.2	4.2	4.9	0.162	49
68+	10.3	70.6	19.1	53.7	6.6	7.3	0.724	58
69 *	52.0	36.6	11.4	39.8	5.8	8.1	0.430	43
70 *	13.9	69.2	16.9	59.7	8.8	9.2	1.413	50
71+	31.7	48.8	19.5	46.5	3.2	8.1	0.167	53
72+	85.1	9.0	5.9	27.1	3.7	3.9	0.049	42
73+	89.3	4.8	5.9	27.6	3.7	3.7	0.029	53
74+	97.2	2.2	0.6	19.9	1.6	0.6	0.018	10

Table 3 (Contd)

<u>Station Number</u>	<u>Sand<sup>a</sup> %</u>	<u>Silt<sup>a</sup> %</u>	<u>Clay<sup>a</sup> %</u>	<u>Water %</u>	<u>DOC<sup>b</sup> %</u>	<u>TVS<sup>b</sup> %</u>	<u>Extractables<sup>b</sup> %</u>	<u>Soluble<sup>c</sup> %</u>
<u>Sinclair Inlet</u>								
75+	16.3	66.0	17.7	63.4	3.7	17.8	0.473	23
76+	4.0	76.3	19.7	67.3	4.6	11.5	0.420	25
77+	5.3	75.7	19.0	64.7	9.1	8.8	0.318	34
78	2.0	79.5	18.5	64.9	1.5	10.0	0.138	32
79	1.6	81.4	17.0	64.4	6.4	20.0	0.272	41
80	1.5	80.1	18.4	63.7	5.2	7.3	0.308	40
81	6.0	79.6	14.4	64.1	6.0	6.7	0.329	57
82	7.1	74.5	18.4	64.4	7.6	8.2	0.327	31
83	11.1	70.2	18.7	58.4	2.2	10.0	0.294	52
84	16.1	64.1	19.8	24.5	7.7	7.0	0.146	49
85	52.4	40.3	7.3	48.7	4.7	4.1	0.089	52
86+	89.3	5.9	4.8	28.7	1.9	1.9	0.036	60
<u>Port Madison</u>								
87+	78.6	16.2	5.2	26.5	1.2	8.0	0.032	43
88	94.2	3.8	2.0	24.6	1.1	5.6	0.017	58
89	49.9	43.1	7.0	30.3	0.2	2.2	0.050	73
90	85.5	11.3	3.2	29.7	0.9	1.3	0.035	44
91+	69.4	20.6	9.1	28.7	1.0	1.2	0.064	57
92	19.0	53.3	27.7	52.4	2.4	7.2	0.103 (1/4)	77
<u>Birch Bay</u>								
93	99.5	0.2	0.3	10.2	5.4	5.7	0.007	89
94	99.2	0.1	0.7	20.3	1.4	1.8	0.008	55
95	98.2	1.1	0.7	16.9	4.9	5.4	0.015	44
96	98.6	0.7	0.7	20.1	0.8	1.3	0.024	100
97	99.4	0.2	0.4	13.4	4.9	5.1	0.012	47

- a. Phi size ranges: sand, -2 to +4; silt, >4 to 8; clay, >8.
- b. Percent of dry weight sediments.
- c. Expressed as percent of extractable organic matter (extractables).
- d. A plus (+) notation after the station number indicates that live organisms were observed in the sediments during sampling. A star (\*) indicates that a bad odor, e.g., H<sub>2</sub>S, was noted in the sample during collection.
- e. Fractions in parentheses indicate approximate amount of extract lost during sample preparation.

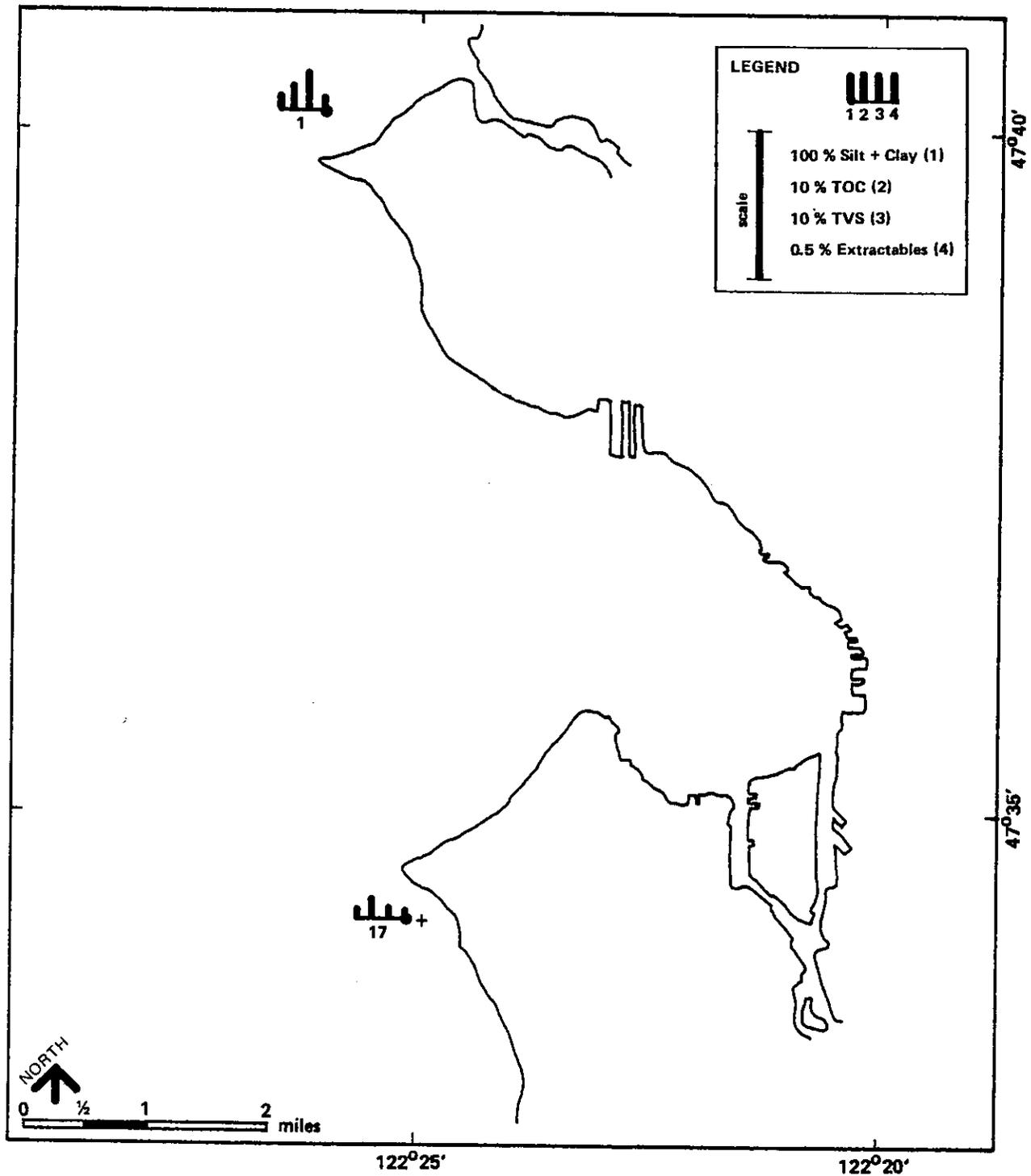


Figure 8. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments at West Point and Alki Point. A plus sign (+) indicates that living organisms were observed at the time of collection.

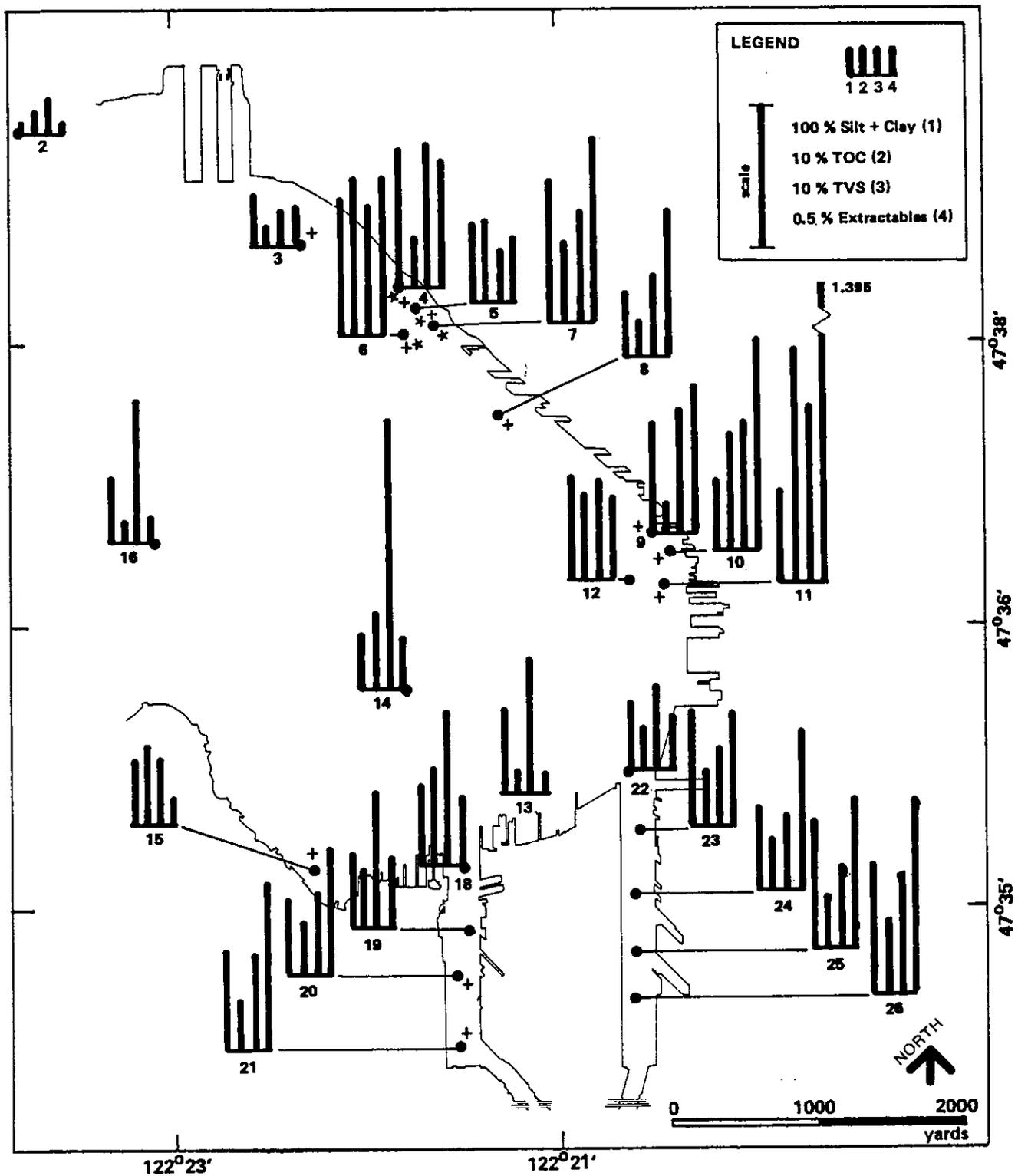


Figure 9. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in Elliott Bay and the Lower Duwamish River. A plus sign (+) indicates that living organisms were observed at the time of collection. A star (\*) indicates that malodorous sediments were noted during collection.

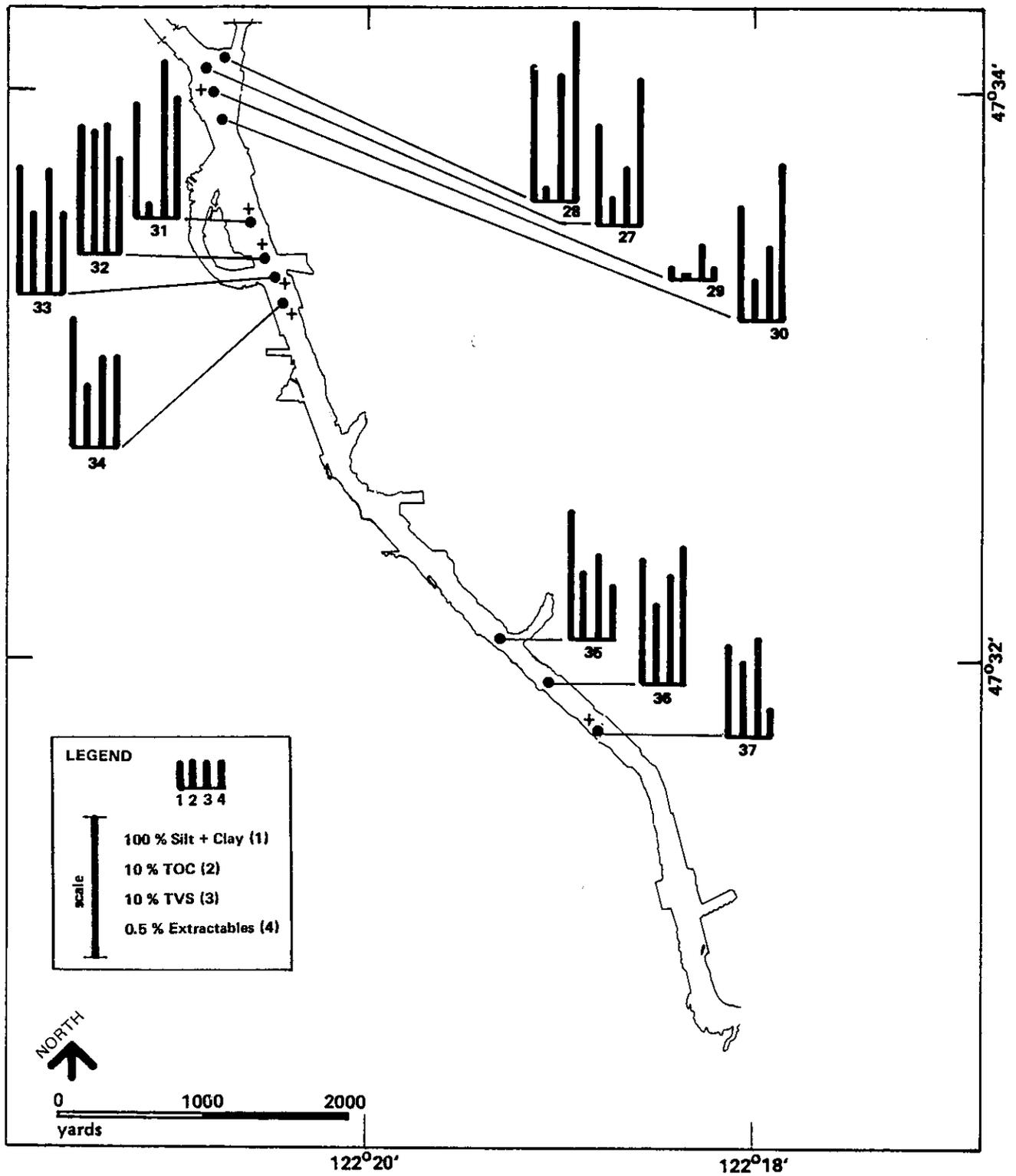


Figure 10. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in the Upper Duwamish River. A plus sign (+) indicates that living organisms were observed at the time of collection.

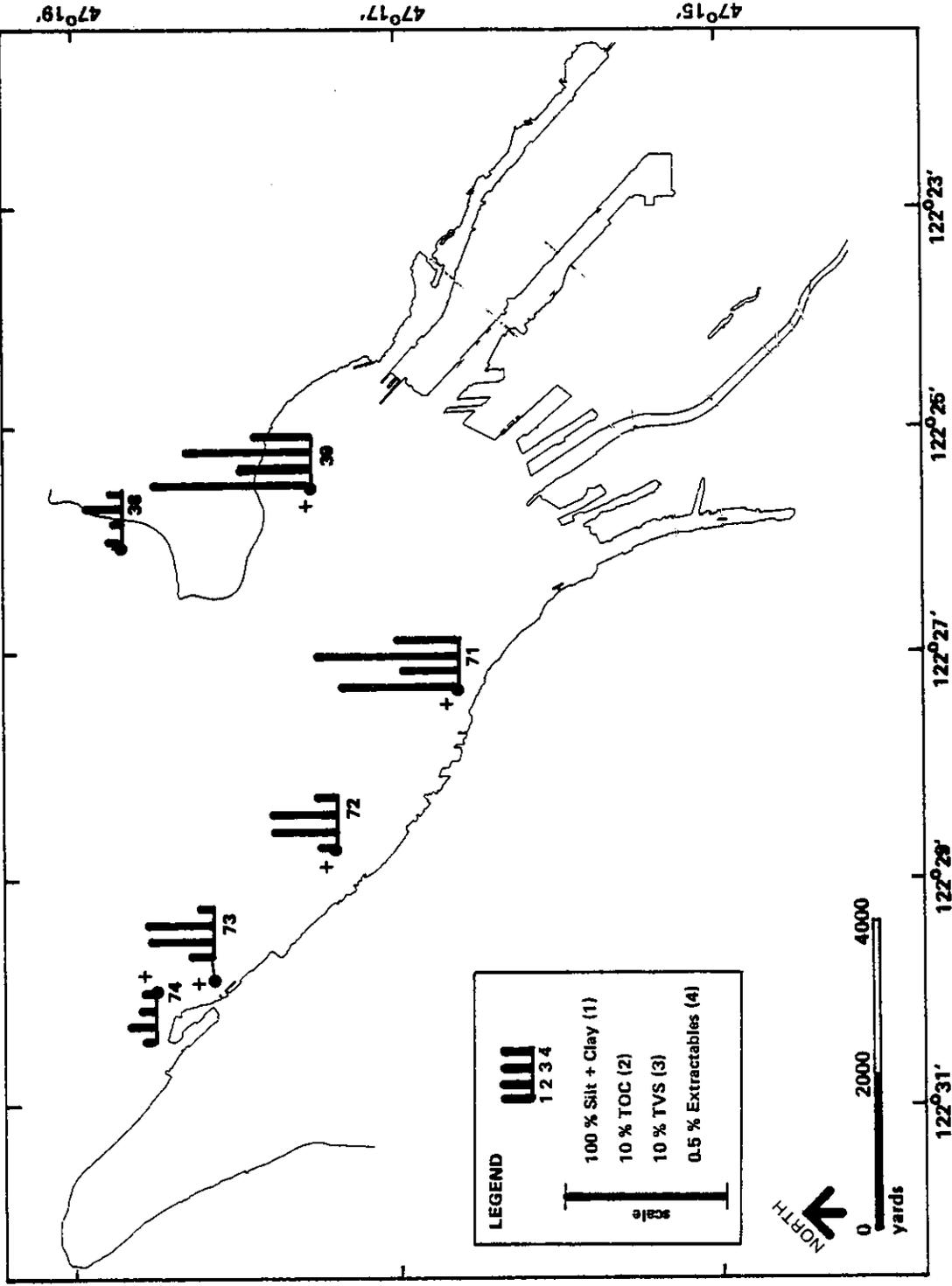


Figure 11. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in Commencement Bay. A plus sign (+) indicates that living organisms were observed at the time of collection.

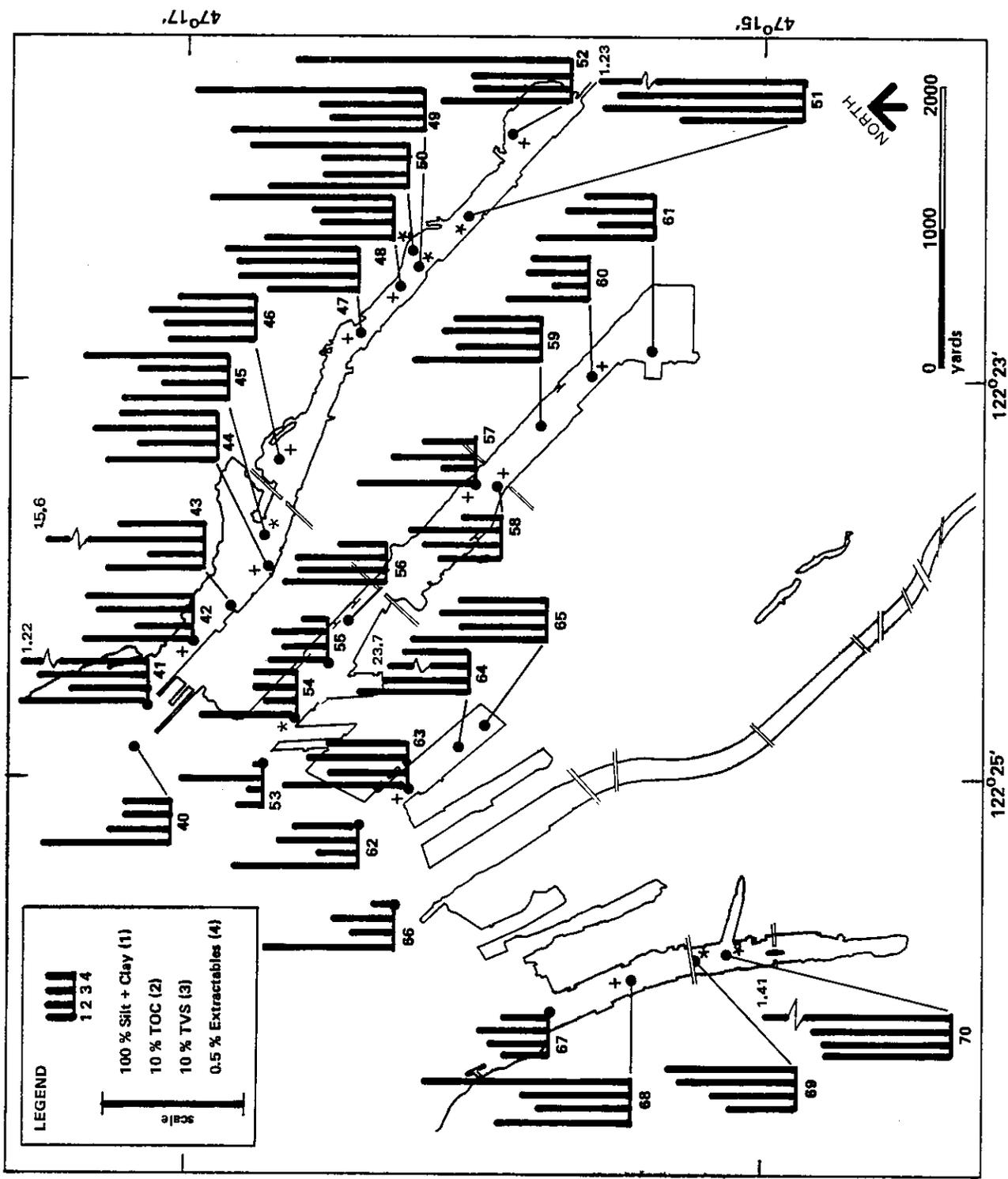


Figure 12. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in the Waterways of Commencement Bay. A plus sign (+) indicates that living organisms were observed at the time of collection. A star (\*) indicates that malodorous sediments were noted during collection.

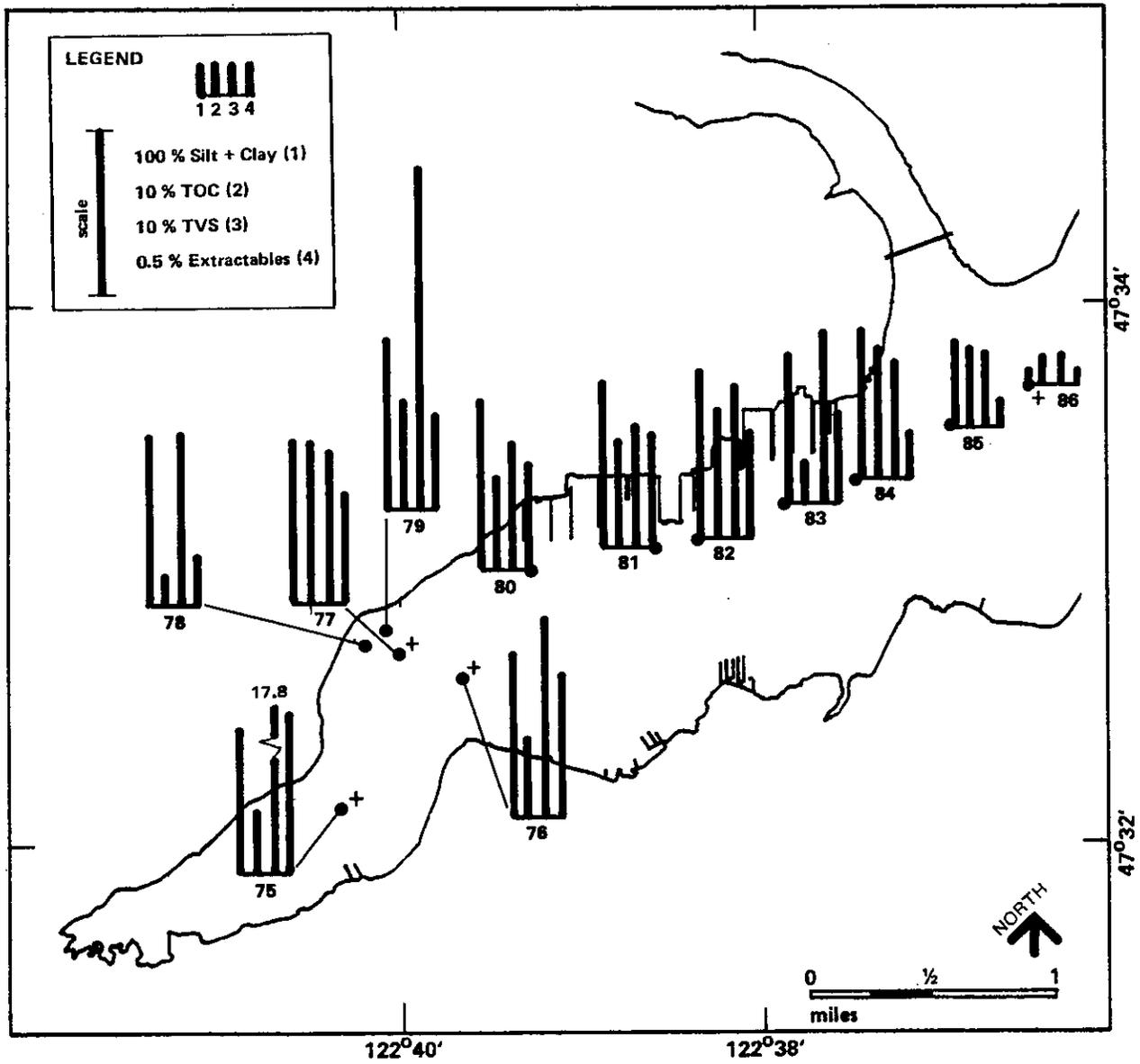


Figure 13. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments in Sinclair Inlet. A plus sign (+) indicates that living organisms were observed at the time of collection.

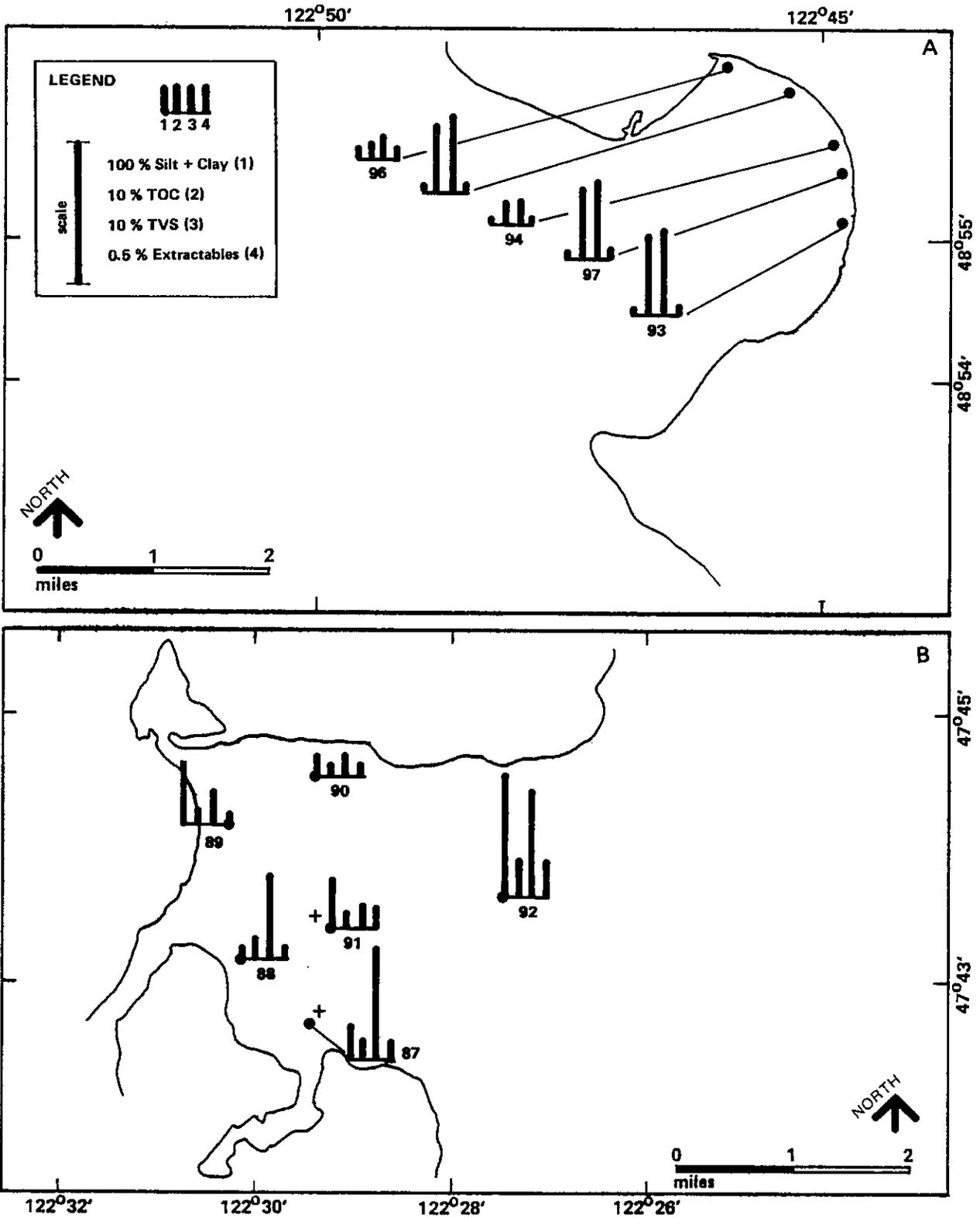


Figure 14. Bar Diagrams of Selected Physical and Chemical Characteristics of the Sediments at (A) Birch Bay and (B) Port Madison.

Generally, black, fine-grained, organic-rich sediments were found in the industrialized areas: along the Seattle waterfront, in the lower Duwamish River, in the waterways of Commencement Bay. Most other areas had green, brown and gray sediments of varying tone. Only a few of these samples had an apparent odor, usually from H<sub>2</sub>S; Stations 4, 5, 6, and 7, near the Denny Way CSO in Elliott Bay; Station 45 in the lower Hylebos Waterway; Stations 49, 50, and 51, in the upper Hylebos; and, Stations 69 and 70 in the upper City Waterway. All of these samples contained high levels of organic matter; Station 70 contained the greatest concentration of extractable organic matter noted in this study.

At the same time, living organisms, particularly polychaetes, and other worms and small bivalves, were observed in many of the sediment samples. No special effort was made during the cruises to look for organisms; only readily obvious biota were recorded. As a result, many of the stations undoubtedly had viable organisms which were not observed. No regional subarea, with the exception of the East Waterway of the Duwamish River, was devoid of organisms. However, no organisms were noted at any of the stations in Commencement Bay waterways which also had odors associated with the sediments.

#### 3.1.1.1 Grain Size

No clear spatial trends among the samples were apparent from the grain size data. In general, samples from shallower stations and from areas exposed to stronger currents and waves had coarser sediments. Examples of these stations would be all of the Birch Bay stations (93-97) as well as Browns Point (Station 38). The samples which exhibited the finest sediments were collected from deeper areas and from the inlets and waterways protected from strong currents and waves. Most of the stations from Sinclair Inlet are examples of this trend (Stations 75-84).

A few areas appeared to be affected by local influences. Stations 73 and 74, near Point Defiance in Commencement Bay, consisted largely of coarse, glassy, brown/black sediments, presumably slag from a nearby smelter. Stations 4, 6, and 7, near the Denny Way CSO in Elliott Bay, were relatively fine-grained in comparison with the other stations in the area at comparable depths. In addition, the sediments from Stations 4 and 7 were strongly dominated by silt-sized particles. These characteristics may reflect the influence of CSO discharge on the local sediments. Similar characteristics, i.e., fine sediments dominated by silts, were also observed at Station 28, south of Harbor Island in the Duwamish River, and at Station 65 at the south end of Sitcum Waterway. Station 28 is located near the Diagonal Way CSO while local sources to the Sitcum Waterway were not identified.

### 3.1.1.2 Organic Matter

Three measures were made of organic matter associated with the sediments. Total volatile solids (TVS) was the most rigorous technique, destroying all combustible organic matter in the samples. In comparison, digestible organic matter (DOC; oxidized by  $H_2O_2$ ) represented a fraction of the TVS which is more 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 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).

Few clear spatial trends were apparent in any of the organic matter parameters (Figs. 15-18). TVS and DOC generally increased in the finer sediment fractions. Extractable organic matter correlated better with TVS in Sinclair Inlet than with DOC, but correlated better with DOC in the other embayments. Extractable organic matter correlated poorly with either TVS or DOC in the Duwamish River, probably reflecting high spatial variability in that system.

Regional comparisons based on combinations of stations from local areas are presented in Table 4. For all three parameters measured, higher values were observed in industrial areas than those of less developed areas. However, the differences were not large for either DOC or TVS. Much larger differences were noted in the levels of extractable organic matter, with high values measured near known pollutant sources. The greatest concentrations of extractable organic matter, both in terms of average levels and for individual stations, were observed in the Hylebos and City Waterways of Commencement Bay and near Pier 54 in Elliott Bay. However, in all cases, the sample size was too small to warrant statistical analysis.

### 3.1.2 Water Characteristics

Table 5 presents the results of physical and organic analyses of the seven water samples. The samples collected in Elliott Bay (Station 12) and the Sitcum (Station 65) and City (Station 70) Waterways had high salinities typical of autumn, when salinities are greatest due to low freshwater runoff (Dexter et al., 1981). Salinities in the Duwamish River (Station 29) and Hylebos Waterway (Station 43) exhibited lower salinities than the other Elliott or Commencement Bay stations. The Duwamish River value can be ascribed to riverine inputs and mixing in the estuary. The Hylebos station may have been affected by freshwater from the Puyallup River, input from Hylebos Creek, or local freshwater inputs from industrial effluent or street runoff.

Samples from Sinclair Inlet (Station 82) and Birch Bay (Station 96) were obtained later in the year than other samples (see Station Logs,

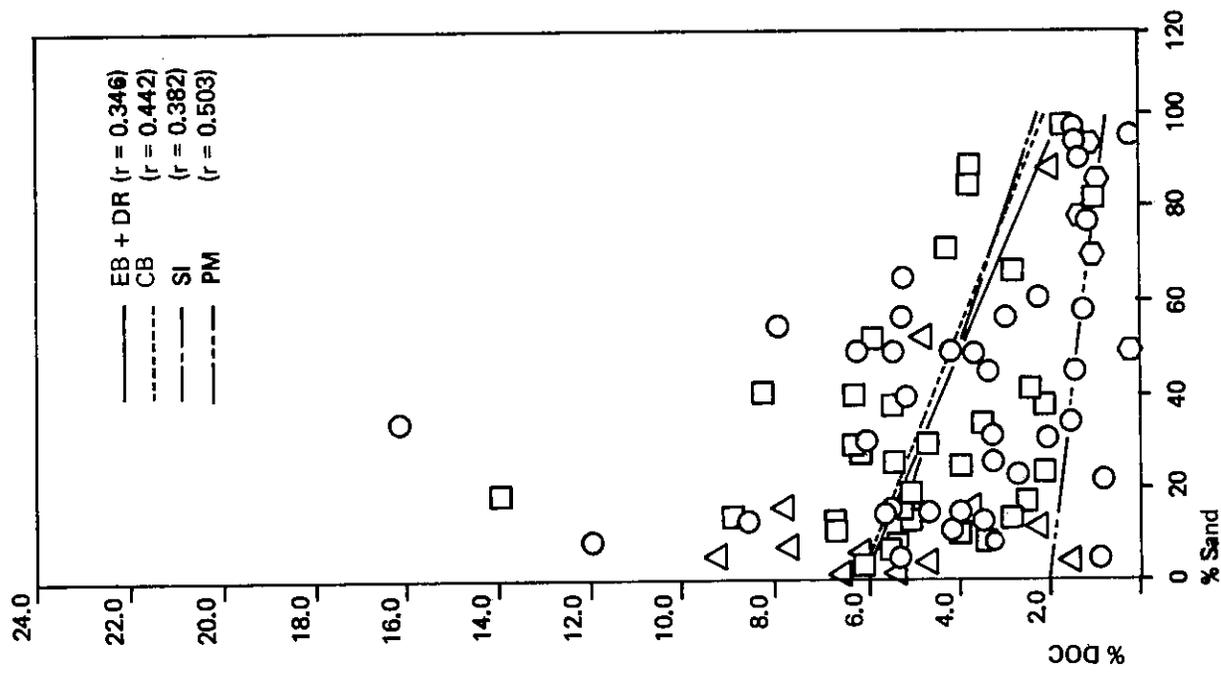
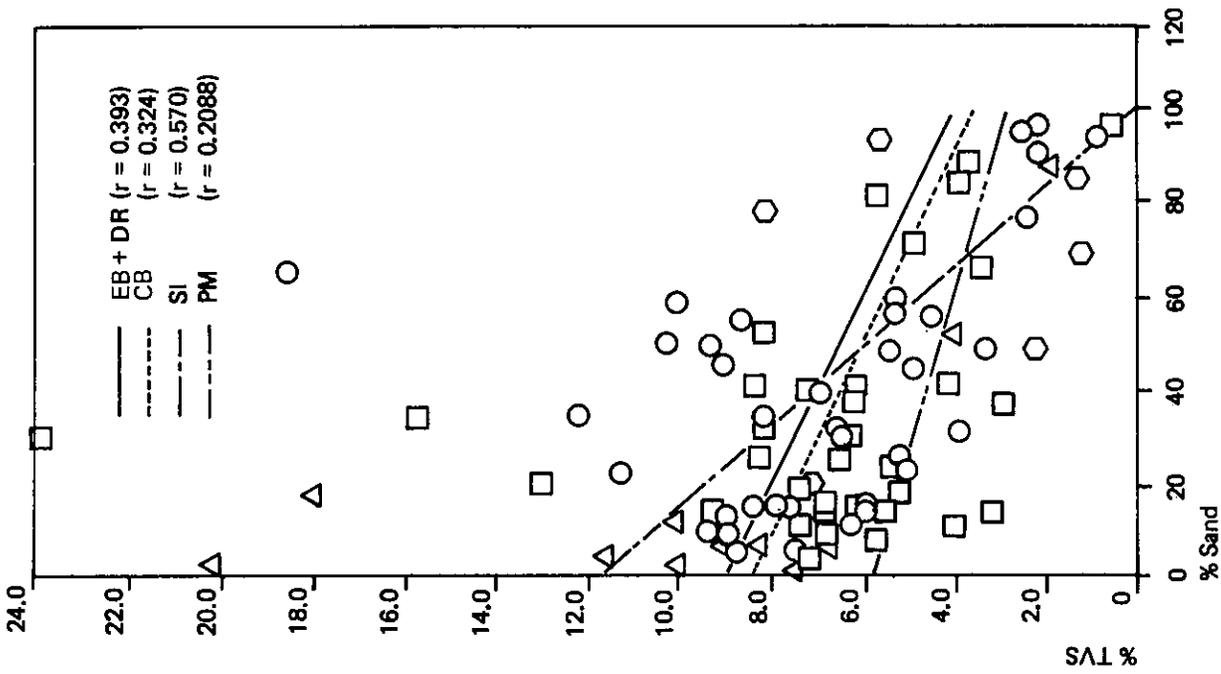


Figure 15. Plots of the Concentrations of (A.) TVS and (B.) DOC versus the Sediment Grain-Size for Samples from Elliott Bay and the Duwamish River (O), Commencement Bay (□), Sinclair Inlet (Δ) and Port Madison (O). Lines represent linear correlation analysis.

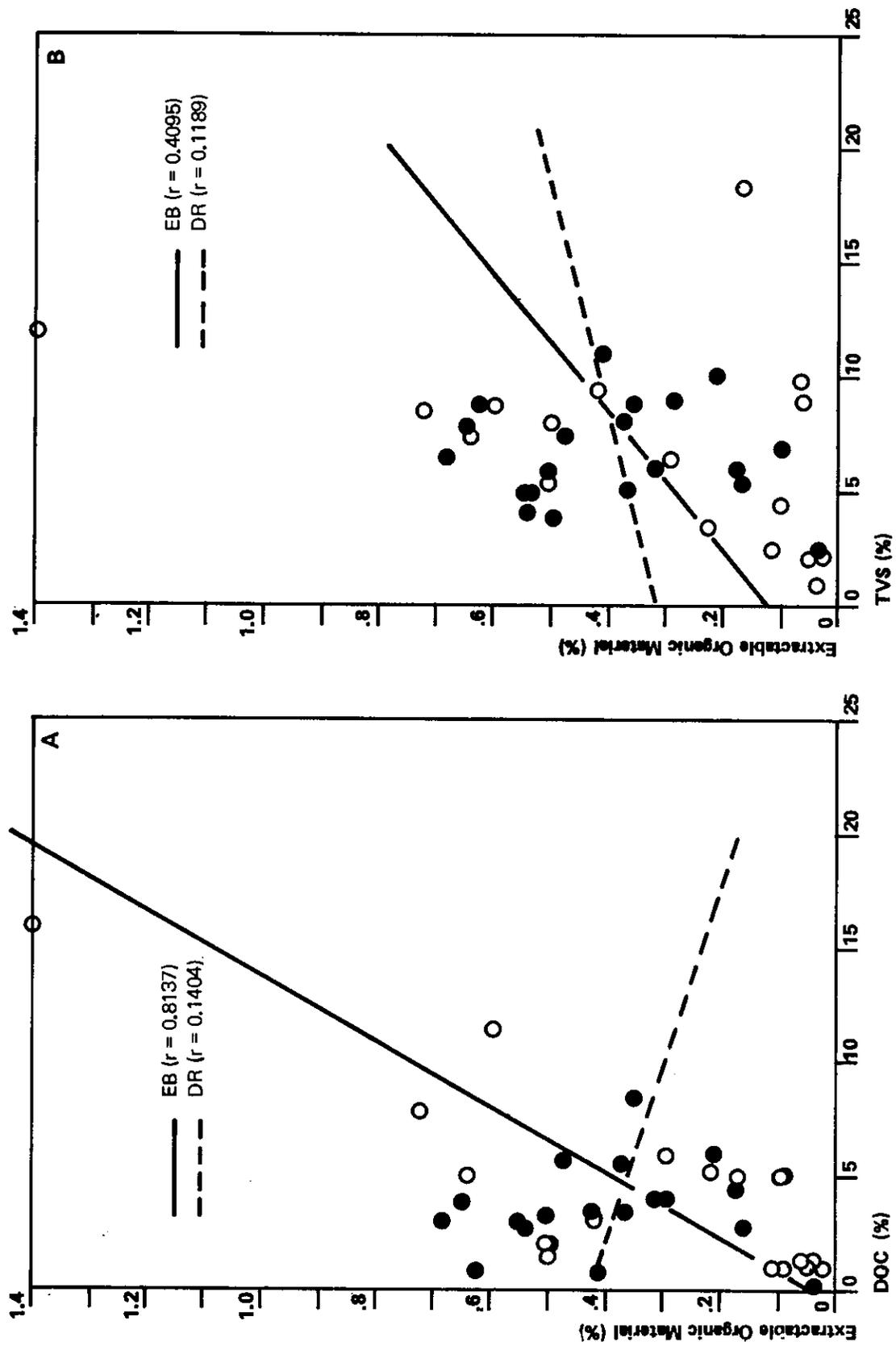


Figure 16. Plots of the Concentrations of Extractable Organic Matter in the Sediments of Elliott Bay (O) and the Duwamish River (●) versus the Concentrations of (A.) DOC and (B.) TVS. Lines represent linear correlation analysis.

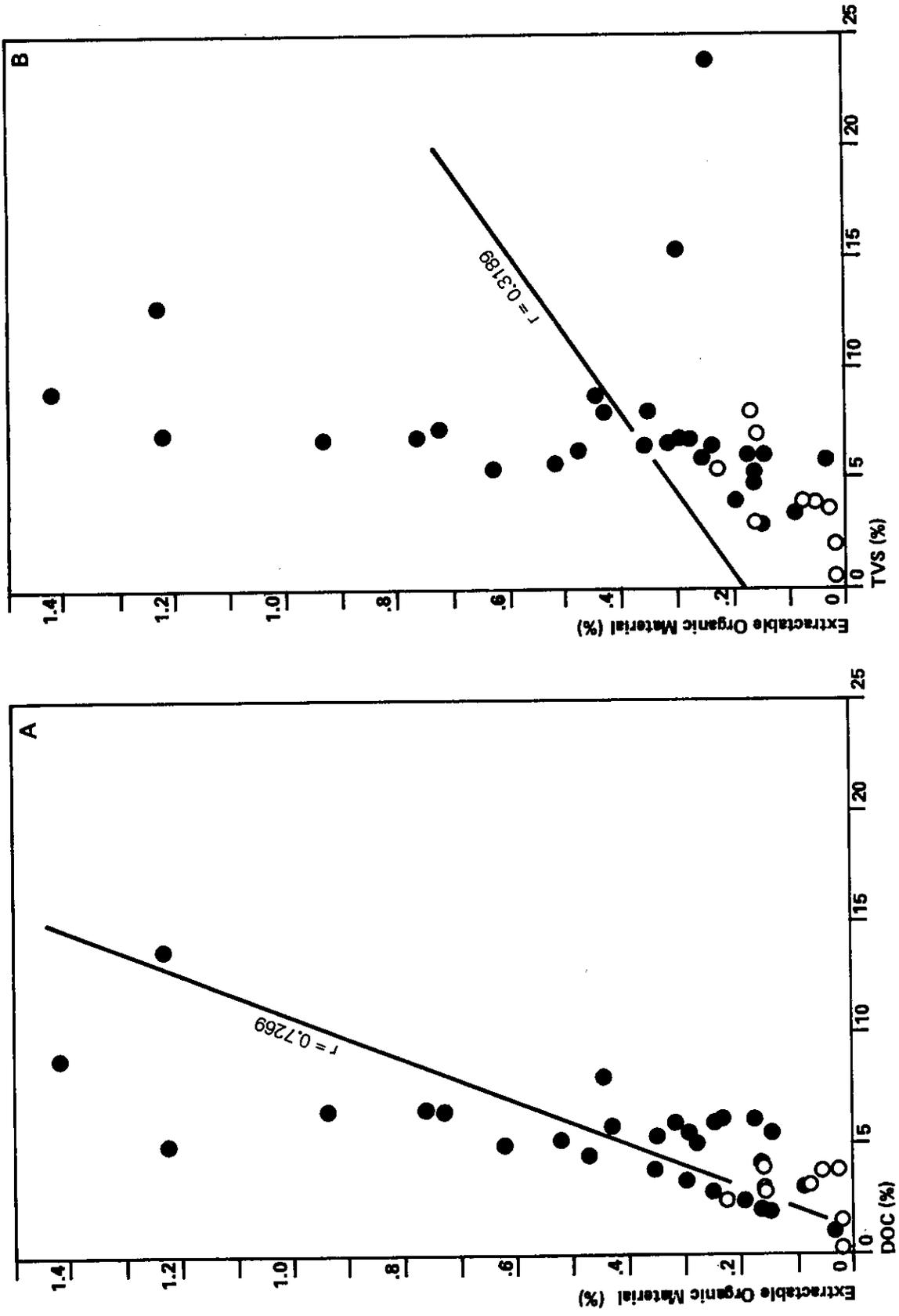


Figure 17. Plots of the Concentrations of Extractable Organic Matter in the Sediments of Commencement Bay (○) and the Waterways of Commencement Bay (●) versus Concentrations of (A.) DOC and (B.) TVS. Lines represent linear correlation analysis.

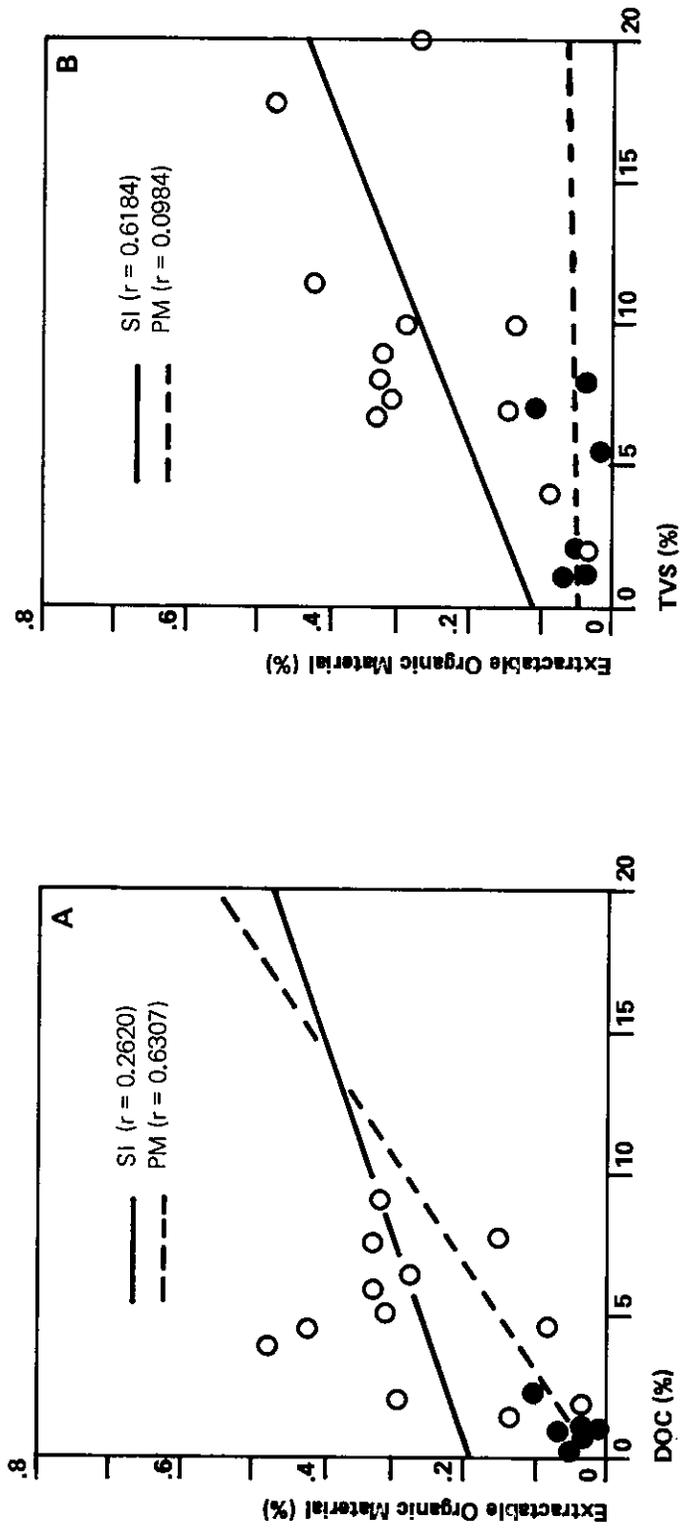


Figure 18. Plots of the Concentrations of Extractable Organic Matter in the Sediments of Sinclair Inlet (O) and Port Madison (●) versus Concentrations of (A.) DOC and (B.) TVS. Lines represent linear correlation analysis.

**Table 4**  
**Comparisons of DOC, TVS, and Extractable Organic Matter**  
**in Sediments from Selected Areas of Puget Sound**

<u>AREA</u>	<u>DOC</u>	<u>ORGANIC PARAMETERS, %</u>	
		<u>TVS</u>	<u>EXTRACTABLES</u>
<b>ELLIOTT BAY</b>			
Outer & Inner Bay (1,2,3,8,13-17) <sup>a</sup>	2.2 ± 1.7 <sup>b</sup>	6.1 ± 5.6	0.13 ± 0.15
Denny Way (4-7)	6.4 ± 3.8	7.3 ± 2.7	0.47 ± 0.19
Pier 54 (9-12)	7.8 ± 6.1	8.8 ± 2.4	0.73 ± 0.98
Duwamish River (9-12)	3.7 ± 2.0	6.8 ± 2.2	0.39 ± 0.18
<b>COMMENCEMENT BAY</b>			
Outer & Inner Bay (38-40,53,62,66,71-74)	2.6 ± 1.3	4.4 ± 2.3	0.09 ± 0.08
Hylebos Waterway (41-52)	6.2 ± 2.7	8.1 ± 3.1	0.62 ± 0.35
Blair Waterway (54-61)	3.7 ± 1.8	5.1 ± 1.5	0.18 ± 0.06
Sitcum Waterway (63-65)	5.7 ± 0.5	7.0 <sup>c</sup>	0.28 ± 0.04
City Waterway (67-70)	6.4 ± 1.9	7.4 ± 1.8	0.68 ± 0.54
<b>SINCLAIR INLET</b> (78-86)	5.3 ± 2.6	10.7 ± 4.6	0.30 ± 0.10
<b>PORT MADISON</b> (87-92)	1.1 ± 0.7	4.3 ± 3.1	0.05 ± 0.04
<b>BIRCH BAY</b> (93-97)	3.5 ± 2.2	3.9 ± 2.5	0.01 ± 0.01

- a Numbers in parentheses indicate stations included in average  
b Values presented as means ± one standard deviation  
c Station 64, TVS = 23.7%, was deleted as questionable

Table 5  
Water Physical and Chemical Parameters

Station Number	Salinity (% )	Temperature (oC)	Extractable Organics (mg/l)	Soluble <sup>a</sup> (%)
12	31.0	13.4	1.13	93
29	26.8	-b	0.74	152
43	27.0	12.2	2.91	28
65	32.0	11.9	1.34	167
70	31.5	11.4	1.71	61
82	26.0	11.0	1.46	113
96	19.9	9.9	1.67	61

- a Expressed as percent of extractable organic matter  
b Data not recorded

Appendix A) and their lower salinities may be due to freshwater input from seasonal runoff. Birch Bay was sampled by hand near the surface (wading depth) and the sample probably included a surface brackish layer.

Water temperatures were within normal seasonal ranges (Dexter et al., 1981), and tended to show normal decreases with time. Elliott Bay was sampled first followed by Commencement Bay, Sinclair Inlet and, finally, Birch Bay.

The apparent concentrations of extractable organic matter, about 1.5 mg/L, were reasonably constant among all samples. A slightly lower concentration was noted for the Duwamish River sample (Station 29) than observed in most of the samples, while Station 43, from the Hylebos Waterway, exhibited the greatest concentration. Insufficient data were available to determine the significance of these differences. Also included in Table 5 are data on the fraction of extracted organic matter which was soluble in the anaphase aberration testing. As noted earlier, this measurement was added to the experimental sequence after the sample handling was completed and is of low precision. Specifically, the very low quantities of organic matter obtained from the water samples led to high variability in this measurement, with more than half of the sample containers weighing the same as, or slightly less than, the originally tared tubes upon return for weighing, resulting in soluble extract values in excess of 100%.

## 3.2 Toxicity Testing

### 3.2.1 Acute Lethal Bioassays

Bioassay data sheets for the three test species are shown in Appendix C. The results are summarized, by species, below.

#### 3.2.1.1 Eogammarus confervicolus

In only one instance were survivals of less than 85% recorded for the combined replicated amphipod tests after 10 d: Station 4, 60%.

#### 3.2.1.2 Monopylephorus cuticulatus

All bioassays with oligochaetes had a greater than 85% survival.

#### 3.2.1.3 Gasterosterus aculeatus

All bioassays with stickleback had a greater than 90% survival rate.

### 3.2.2 Sublethal Respiration Rate Tests

Respiration data sheets are shown in Appendix D and the results are summarized in Table 6. Control respiration values are given in Table 7.

Table 6

Respiration Rate Data Summary for 97 Sediment  
Samples and 7 Water Samples

<u>Station</u>	<u><math>\bar{X}</math> Respiration (<math>\mu\text{L O}_2/\text{mg dry wt/h}</math>)</u>	<u>S.D.</u>	<u>Sample Size</u>	<u>PO<sub>2</sub> Range (mm Hg)</u>	<u>Level of Significance</u>
1	0.28	0.07	6	141-125	n.s.
2	0.28	0.18	5	141-116	n.s.
3	0.20	0.12	6	130-101	n.s.
4	0.19	0.03	6	131-104	**
5	0.17	0.05	8	131-110	***
6	0.18	0.04	8	128-102	***
7	0.23	0.06	6	130-110	n.s.
8	0.24	0.04	6	127-104	n.s.
9	0.22	0.03	6	142-108	n.s.
10	0.23	0.05	8	139-111	n.s.
11	0.23	0.06	8	115- 86	n.s.
12	0.16	0.05	8	124-103	***
13	0.22	0.03	8	119- 89	n.s.
14	0.17	0.04	6	121- 96	***
15	0.38	0.08	8	140-123	***
16	0.22	0.04	6	130-111	n.s.
17	0.28	0.09	8	126-104	n.s.
18	0.21	0.06	6	123-102	n.s.
19	0.19	0.11	7	135-102	n.s.
20	0.24	0.07	6	125-112	n.s.
21	0.15	0.05	8	124- 97	***
22	0.25	0.08	6	122-108	n.s.
23	0.24	0.04	6	127-110	n.s.
24	0.18	0.05	8	126-111	**
25	0.28	0.11	8	136- 98	n.s.
26	0.26	0.11	6	126- 85	n.s.
27	0.25	0.05	8	134-113	n.s.
28	0.27	0.12	6	139-127	n.s.
29	0.29	0.06	8	134-126	**
30	0.20	0.04	6	130-117	*
31	0.17	0.03	7	141-124	***
32	0.28	0.09	8	132-120	n.s.
33	0.20	0.02	8	132-117	*
34	0.25	0.04	5	139-129	n.s.
35	0.27	0.06	8	136-123	n.s.

Table 6 (Contd)

<u>Station</u>	<u>X Respiration</u> <u>(<math>\mu\text{L O}_2/\text{mg dry wt/h}</math>)</u>	<u>S.D.</u>	<u>Sample</u> <u>Size</u>	<u>PO<sub>2</sub> Range</u> <u>(mm Hg)</u>	<u>Level of</u> <u>Significance</u>
36	0.20	0.06	6	133-124	n.s.
37	0.16	0.06	6	130-121	***
38	0.20	0.03	6	134-121	*
39	0.18	0.04	8	133-123	***
40	0.27	0.09	8	135-120	n.s.
41	0.30	0.06	8	140-122	**
42	0.22	0.05	8	122-101	n.s.
43	0.20	0.03	8	122-104	*
44	0.26	0.09	8	132-106	n.s.
45	0.24	0.03	8	132-113	n.s.
46	0.31	0.06	5	133-108	**
47	0.36	0.07	6	130-113	***
48	0.36	0.07	8	130-111	***
49	0.28	0.05	8	127-110	*
50	0.24	0.04	6	117-102	n.s.
51	0.22	0.11	6	120- 89	n.s.
52	0.37	0.08	6	130- 94	***
53	0.39	0.08	6	132-110	***
54	0.24	0.04	6	139-115	n.s.
55	0.33	0.12	6	141-125	**
56	0.27	0.10	6	123-107	n.s.
57	0.37	0.10	8	130-105	***
58	0.26	0.04	6	132-110	n.s.
59	0.25	0.07	8	116-103	n.s.
60	0.39	0.10	8	130-117	***
61	0.42	0.11	8	128-117	***
62	0.26	0.10	8	130-117	n.s.
63	0.31	0.09	6	121-111	**
64	0.24	0.04	8	127-107	n.s.
65	0.21	0.05	8	126-106	n.s.
66	0.26	0.07	6	118-112	n.s.
67	0.18	0.01	8	122-109	***
68	0.21	0.08	6	130-119	n.s.
69	0.18	0.02	6	127-118	**
70	0.31	0.05	6	124-112	***

Table 6 (Contd)

<u>Station</u>	<u><math>\bar{X}</math> Respiration (<math>\mu\text{L O}_2/\text{mg dry wt/h}</math>)</u>	<u>S.D.</u>	<u>Sample Size</u>	<u>PO<sub>2</sub> Range (mm Hg)</u>	<u>Level of Significance</u>
71	0.40	0.08	6	120-106	***
72	0.24	0.03	6	124-113	n.s.
73	0.32	0.05	6	119-110	***
74	0.22	0.04	8	129-117	n.s.
75	0.20	0.05	6	126-119	*
76	0.22	0.08	8	133-118	n.s.
77	0.23	0.04	6	130-116	n.s.
78	0.23	0.07	7	134-125	n.s.
79	0.25	0.07	8	128-118	n.s.
80	0.32	0.07	8	122-107	***
81	0.19	0.05	8	117-108	**
82	0.36	0.09	6	131-120	***
83	0.48	0.09	6	123-109	***
84	0.33	0.06	8	118- 85	***
85	0.27	0.07	6	126-106	n.s.
86	0.26	0.04	5	114-101	n.s.
87	0.26	0.07	7	131-119	n.s.
88	0.28	0.06	8	130-116	*
89	0.25	0.08	8	129-113	n.s.
90	0.22	0.06	6	136-128	n.s.
91	0.23	0.06	6	132-123	n.s.
92	0.28	0.07	6	131-108	n.s.
93	0.27	0.10	6	131-112	n.s.
94	0.26	0.05	6	140-125	n.s.
95	0.27	0.04	6	134-111	n.s.
96	0.27	0.06	6	127-114	n.s.
97	0.26	0.06	6	124-113	n.s.
H <sub>2</sub> O 12	0.21	0.06	8	150-131	n.s.
H <sub>2</sub> O 29	0.17	0.05	8	149-137	***
H <sub>2</sub> O 43	0.23	0.09	7	146-130	n.s.
H <sub>2</sub> O 65	0.29	0.05	6	165-147	**
H <sub>2</sub> O 70	0.21	0.05	8	152-134	n.s.
H <sub>2</sub> O 82	0.31	0.10	6	142-123	**
H <sub>2</sub> O 96	0.23	0.09	5	149-136	n.s.
Controls (4 separate tests)	0.24	0.04	29	138- 78	

n.s. = not significant; \* =  $0.05 > P > 0.01$ ; \*\* =  $0.01 > P > 0.001$ ; \*\*\* =  $P < 0.001$

Table 7  
Control Respiration Rate Values

<u>Control Replicate</u>	<u><math>\bar{X}</math> Respiration (<math>\mu\text{L O}_2/\text{mg dry wt/h}</math>)</u>	<u>S.D.</u>	<u>Sample Size</u>	<u>PO<sub>2</sub> Range (mm Hg)</u>	<u>Date</u>
1	0.24	0.07	6	111- 78	October 18, 1981
2	0.24	0.02	8	138-132	November 20, 1981
3	0.25	0.05	7	132-117	February 02, 1982
4	0.23	0.03	8	127-109	March 02, 1982

All testing was carried out at high PO<sub>2</sub> ranges (165-85 mm Hg for test solutions; 138-78 for controls). Mean respiration rates (in  $\mu\text{L O}_2$  /mg dry weight/h) ranged from 0.23-0.25 in controls and from 0.16-0.48 in test solutions. Significant respiratory anomalies compared to controls were detected in 13 of 37 sediments from Elliott Bay and the Duwamish, 20 of 37 sediments from Commencement Bay, 6 of 12 sediments from Sinclair Inlet, 1 of 6 sediments from Port Madison, and none of 5 sediments from Birch Bay. Three of 7 water samples tested showed significant respiratory anomalies.

Control values (Table 7) showed insignificant variation in response over the 6 months of testing. Test values with high standard deviations were routinely re-run for confirmation of results. Thus, for example, the high variability (S.D.) in respiration rate for Station 2 (Table 6) is repeatable and appears to be a real response to that sample.

Of the significantly different respiration rates recorded, as compared to controls, approximately equal numbers were depressed (44%) as those elevated (56%). Significantly depressed rates were noted at all stations near the Denny Way CSO showing effects, while elevated rates were noted at almost all stations showing effects in Hylebos, Blair and Sitcum Waterways. No clear trends were noted for other areas.

Of the seven water samples tested, only Stations 29, 65, and 82 were significantly different from controls. Comparing the results for sediments and waters from the same stations, only at Stations 29, 82 and 96 were similar results obtained; water samples from Stations 12, 43, and 70 did not significantly affect respiration rates in contrast to the corresponding sediment samples; and Station 65 showed significant respiration effects in the water but not in the sediment sample.

### 3.2.3 Sediment Spike

Bioassay data sheets for acute LT50 tests with the three test species and respiration data sheets are shown in Appendix E and the results are summarized in Table 8. Results of the chemical analysis of waters and sediments in the stickleback aquaria after 10 d are shown in Table 9.

Stickleback were, as anticipated, the most sensitive species in the LT50 tests conducted with spiked sediments. Amphipods and oligochaetes respectively demonstrated intermediate sensitivity and tolerance. Significant respiration increases were noted in both sediment spikes. Analytical results indicated that very little pentachlorophenol was retained in the sediments, most occurring in the water column. Highest chlorophenol levels in both sediments and water were noted in sample 50, which is in accord with the bioassay results. These high levels appear to be due to the muddy nature of the sediments from Station 50 which retained the NaPCP spike better than sediments from Station 95.

Table 8

Sediment Spike Bioassay Data

Station	Acute Lethality				Oligochaetes		Sublethal Respiration Effects	
	Stickleback		Amphipods		LT50 (h)	% Survival at 10 d	$\bar{X}$ Respiration Rate ( $\mu\text{L O}_2/\text{mg dry wt/h}$ )	Level of Significance
50	LT50 (h)	% Survival at 10 d	LT50 (h)	% Survival at 10 d	LT50 (h)	% Survival at 10 d	0.37	P < 0.001
95	6.5	0	13.5	0	n/a <sup>a</sup>	100	0.32	P < 0.001
	160.	20	n/a	100	n/a	100		

<sup>a</sup>n/a = not applicable

Table 9

Sediment Spike Chemical Analysis Data for Water and Sediment (parts per billion)  
(from stickleback test aquaria)

Station	2,3,4,6-tetrachlorophenol (ppb)	pentachlorophenol (ppb)
50 - sediment	0.36	3.5
- water	2.1	81.0
95 - sediment	< 0.01	0.01
- water	0.40	3.6

### 3.2.4 Anaphase Aberration Tests

#### 3.2.4.1 Range-finding Titration (Toxicity)

A titration was carried out on each sample to determine the maximum concentration of sediment extract which could be tolerated by the cultured fish cells and still would permit proliferation (mitosis) to occur. Cells were exposed for 72 h to five concentrations of extract ranging from less than 1  $\mu\text{g/ml}$  to 100  $\mu\text{g/ml}$  of culture medium. The maximum tolerable (non-toxic) concentration of sediment extract was determined by the presence of actively dividing cells (mitotic figures), as seen in the living cultures when examined with a phase contrast microscope. This maximum nontoxic concentration as well as one lower dilution was used for the final cell exposures and anaphase aberration scoring.

The concentrations of sediment extracts which exhibited inhibitory effects on the proliferation of cultured fish cells ranged from 0.75  $\mu\text{g/ml}$  to 40  $\mu\text{g/ml}$ , with 76% of the extracts showing inhibitory activity between 1 and 15  $\mu\text{g/ml}$ . All extracts were toxic and lethal over 40  $\mu\text{g/ml}$ .

A comparison of the various areas of Puget Sound from which sediment extracts were tested shows that Stations 1-7, 15-18, 22, 29, 30, 35, 38, 43, 53-55, 58, 65, 67, 71, 72, 89, 90, 92, 93, 95, and 97 appeared to have the greatest inhibitory activity on cell proliferation (0-5  $\mu\text{g/ml}$ ), Stations 8-11, 21, 26, 36, 68-70, 76-79, 81, and 82 had the least inhibitory activity (over 15  $\mu\text{g/ml}$ ) and the remaining stations were intermediate in their toxic effect. Table 10 summarizes the relative inhibitory effects of the various sediment extracts on a scale ranging from 0 to 5.

#### 3.2.4.2 Anaphase Controls

The classification of abnormal anaphases is the same as that presented by Nichols et al. (1977) and subsequently used by Kocan et al. (1982). Since the significance of the various forms of aberration is not known, we have presented a rough grouping of the two major types of abnormalities observed (i.e., chromosomal fragments and bridges) in Table 10. Figure 19 shows some of the various types of abnormalities which were observed in cells exposed to Puget Sound sediment and water extracts.

Baseline levels of abnormalities (negative controls) were determined by examining 100 anaphase cells from 10 separate untreated or solvent treated cultures which were run in parallel with the sediment-extract treated cells. From these 1000 cells a mean abnormality frequency of 11.5% was determined with a standard deviation (S.D.) of  $\pm 3.02$ . By using 2 S.D.s to represent a 0.05 confidence limit and 3 S.D.s to represent a confidence limit of 0.01, predictions could be made as follows: anaphase abnormality frequencies of 18-20% ( $P < 0.05$ ) were significant and frequencies of 21% or greater ( $P < 0.01$ ) were highly significant.

Table 10

Genotoxic Effect of Puget Sound Sediment and Water Extract on Rainbow Trout Gonad Cells In Vitro

<u>Station</u>	<u>Concentration Tested µg/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>	
1	0.46		18	4	22	0.01	*b
	2.3	4	28	1	29	0.01	
2	4.0		34	1	35	0.01	*
	8.0	3	28	5	33	0.01	
3	2.3		7	1	8	n.s.	
	4.7	4	6	3	9	n.s.	
4	0.28		14	7	21	0.01	*
	1.4	4	11	5	16	n.s.	
5	0.39		16	3	19	0.05	*
	1.9	4	20	7	27	0.01	
6	0.81		14	3	17	n.s.	*
	4.0	4	17	1	18	0.05	
7	0.07		13	1	14	n.s.	
	0.75	5	12	1	13	n.s.	
8	16.7		11	1	12	n.s.	*
	33.5	0	6	2	8	n.s.	
9	5.8		11	1	12	n.s.	
	29.0	0	21	4	25	0.01	
10	4.6		9	4	13	n.s.	*
	23.0	0	10	4	14	n.s.	
11	7.2		14	3	17	n.s.	
	18.0	1	18	6	24	0.01	
12	2.5		10	2	12	n.s.	
	6.2	3	11	1	12	n.s.	
13	2.1		12	3	15	n.s.	*
	10.5	2	26	3	29	0.01	
14	2.7		12	4	16	n.s.	*
	13.5	2	17	5	22	0.01	
15	1.6		20	1	21	0.01	*
	3.3	4	22	5	27	0.01	
16	2.5		20	1	21	0.01	*
	5.0	4	19	4	23	0.01	
17	0.68		11	2	13	n.s.	*
	3.4	4	21	3	24	0.01	

Table 10 (Contd)

<u>Station</u>	<u>Concentration Tested µg/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>
18	0.9		15	4	19	0.05
	4.5	4	16	3	19	0.05 *
19	4.0		10	1	11	n.s.
	10.0	3	21	2	23	0.01 *
20	2.3		5	1	6	n.s.
	11.5	2	10	5	15	n.s.
21	6.5		12	2	14	n.s.
	32.5	0	9	0	9	n.s. *
22	2.1		10	2	12	n.s.
	4.3	4	8	2	10	n.s.
23	6.0		11	2	13	n.s.
	15.0	2	14	3	17	n.s.
24	2.4		7	1	8	n.s.
	12.0	2	16	2	18	0.05 *
25	2.8		18	2	20	0.05
	14.0	2	11	3	14	n.s. *
26	8.0		11	2	13	n.s.
	40.0	0	17	1	18	0.05
27	2.7		10	1	11	n.s.
	13.5	2	16	3	19	0.05 *
28	5.5		11	1	12	n.s.
	11.0	2	19	3	22	0.01 *
29	1.6		8	1	9	n.s.
	3.2	4	19	3	22	0.01 *
30	1.8		10	3	13	n.s.
	3.7	4	13	1	14	n.s.
31	4.4		11	3	14	n.s.
	11.0	2	17	1	18	0.05 *
32	3.5		3	0	3	n.s.
	7.0	3	13	13	26	0.01 *
33	3.2		7	1	8	n.s.
	8.0	3	13	3	16	n.s.
34	5.7		5	1	6	n.s.
	11.5	2	6	2	8	n.s.

Table 10 (Contd)

<u>Station</u>	<u>Concentration Tested µg/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>
35	1.3	4	9	1	10	n.s.
	2.7		16	3	19	0.05 *
36	7.0	1	11	1	12	n.s.
	17.5		10	0	10	n.s. *
37	5.4	2	10	3	13	n.s.
	13.5		44	14	58	0.01 *
38	0.14	4	11	1	12	n.s.
	1.4		17	2	19	0.05 *
39	2.7	3	8	0	8	n.s.
	5.5		8	2	10	n.s.
40	4.0	3	15	0	15	n.s.
	10.0		19	3	22	0.01 *
41	3.3	3	10	3	13	n.s.
	8.2		12	0	12	n.s.
42	2.5	3	11	3	14	n.s.
	6.2		12	1	13	n.s.
43	0.6	4	9	4	13	n.s.
	1.5		8	5	13	n.s.
44	3.5	3	12	7	9	n.s.
	8.7		9	3	12	n.s.
45	2.8	3	13	1	14	n.s.
	7.0		23	2	25	0.01 *
46	Data not recorded		10	0	10	n.s.
	Data not recorded		8	1	9	n.s.
47	10.5	0	14	5	19	0.05
	21.0		23	8	31	0.01
48	12.0	0	8	2	10	n.s.
	24.0		16	3	19	0.05
49	6.25	2	25	1	26	0.01
	12.5		26	6	32	0.01 *
50	12.25	0	13	6	19	0.05
	24.5		39	5	44	0.01

Table 10 (Contd)

<u>Station</u>	<u>Concentration Tested ug/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>
51	2.5	3	14	4	18	0.05
	6.2		27	6	33	0.01 *
52	10.0	1	12	2	14	n.s.
	20.0		17	6	23	0.01
53	0.29	4	9	3	12	n.s.
	2.9		13	3	16	n.s.
54	0.44	4	10	0	10	n.s.
	4.4		36	5	41	0.01 *
55	0.4	4	18	2	20	0.05
	4.0		15	1	16	n.s. *
56	4.7	2	16	2	18	0.05
	11.7		26	0	26	0.01 *
57	6.5	2	19	4	23	0.01
	13.0		26	8	24	0.01 *
58	2.0	4	32	3	35	0.01
	4.0		38	10	48	0.01 *
59	15.5	0	21	8	29	0.01
	31.0		23	5	28	0.01
60	6.0	2	7	5	13	n.s.
	15.0		12	2	14	n.s.
61	11.25	0	23	1	24	0.01
	22.5		26	1	27	0.01
62	3.1	3	4	0	4	n.s.
	6.2		14	5	19	0.05
63	3.8	3	9	3	12	n.s.
	9.5		10	2	12	n.s.
64	5.4	2	7	5	12	n.s.
	13.5		16	4	20	0.01 *
65	0.65	4	8	2	10	n.s.
	3.2		4	1	5	n.s.
66	2.8	3	6	2	8	n.s.
	5.7		17	3	20	0.05

Table 10 (Contd)

<u>Station</u>	<u>Concentration Tested µg/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>
67	0.49	4	5	2	7	n.s.
	4.9		18	3	21	0.01 *
68	14.5	0	8	3	11	n.s.
	29.0		21	7	28	0.01
69	10.7	0	17	0	17	n.s.
	21.5		23	2	34	0.01
70	12.5	0	12	5	17	n.s.
	25.0		21	5	26	0.01
71	0.53	4	8	3	11	n.s.
	2.6		6	1	7	n.s.
72	0.42	4	13	1	14	n.s.
	2.1		4	1	5	n.s.
73	5.3	2	4	0	4	n.s.
	13.2		14	2	16	n.s.
74	5.0	3	18	5	23	0.01
	10.0		13	3	19	0.05 *
75	5.7	2	9	1	10	n.s.
	11.5		8	2	10	n.s.
76	12.5	0	7	1	8	n.s.
	25.0		19	4	77	0.01 *
77	17.0	0	30	3	33	0.01
	34.0		tox	tox	tox	tox <sup>c</sup>
78	16.0	0	23	3	26	0.01
	32.0		tox	tox	tox	tox
79	10.2	0	14	2	16	n.s.
	20.4		35	0	35	0.01
80	4.0	1	17	2	81	0.05
	20.0		20	1	79	0.01
81	14.25	0	9	3	12	n.s.
	28.5		tox	tox	tox	tox
82	7.7	1	24	0	24	0.01
	15.5		21	1	23	0.01
83	5.0	2	12	2	86	n.s.
	13.0		14	4	82	0.05

Table 10 (Contd)

<u>Station</u>	<u>Concentration Tested µg/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>
84	4.9 12.2	2	11 40	4 15	15 55	n.s. 0.01 *
85	2.6 5.2	3	14 10	1 0	15 10	n.s. n.s.
86	3.0 6.0	3	13 15	1 2	86 83	n.s. n.s.
87	4.3 8.0	2	10 14	0 3	10 17	n.s. n.s.
88	5.8 14.5	2	15 tox	3 tox	18 tox	0.05 tox
89	0.73 3.6	4	9 10	0 2	9 12	n.s. n.s.
90	2.2 4.4	4	12 12	0 2	12 14	n.s. n.s.
91	2.8 5.7	3	19 12	0 3	19 15	0.05 n.s.
92	0.77 3.8	4	15 15	0 2	15 17	n.s. n.s.
93	0.89 2.2	4	13 9	2 1	15 90	n.s. n.s.
94	5.5 13.7	2	8 15	2 2	10 17	n.s. n.s.
95	0.44 2.2	4	17 13	1 2	18 15	0.05 n.s.
96	5.0 10.0	3	13 18	3 3	19 21	0.05 0.01 *
97	2.3 4.7	4	15 18	0 0	15 18	n.s. 0.05
W-12	10 25	n/a	7 16	0 0	7 16	n.s. n.s.
W-29	1 5	n/a	14 14	0 1	14 15	n.s. n.s.
W-43	50 100	n/a	13 20	0 3	13 23	n.s. 0.01

Table 10 (Contd)

<u>Station</u>	<u>Concentration Tested µg/ml</u>	<u>Relative<sup>a</sup> Toxicity</u>	<u>% Fragments</u>	<u>% Bridges</u>	<u>Total % Abnormalities</u>	<u>p &lt;</u>
W-65	10	n/a	14	0	14	n.s.
	25		17	0	17	n.s.
W-70	10	n/a	11	5	16	n.s.
	25		21	3	24	0.01
W-82	10	n/a	3	5	18	0.05
	25		16	3	19	0.05
W-96	50	n/a	12	15	17	n.s.
	100		13	1	14	n.s.

Controls: 10 cultures; 100 anaphase cells/culture = 1,000 cells  
 Mean = 11.5 abnormalities  
 S.D. = 3.02

<sup>a</sup>Toxic concentrations of sediment extract were scored on the following scale:

0 - 1 µg/ml	= 5 - Highly inhibitory
1.1 - 5 µg/ml	= 4 - " "
5.1 - 10 µg/ml	= 3 - " "
10.1 - 15 µg/ml	= 2 - Inhibitory
15.1 - 20 µg/ml	= 1 - Slightly inhibitory
20.1 - 40 µg/ml	= 0 - Questionable effect

<sup>b</sup>The asterisk (\*) denotes positive correlation between inhibitory effect (toxicity) and genetic damage. Remainder exhibit no or negative relationship.

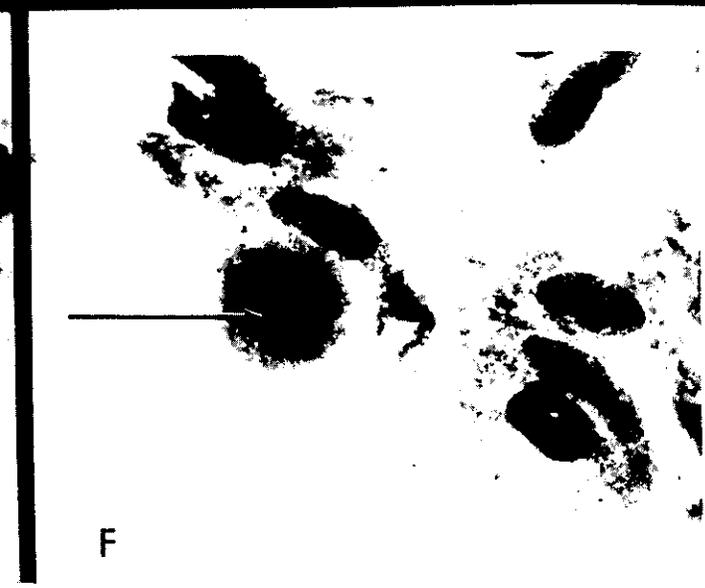
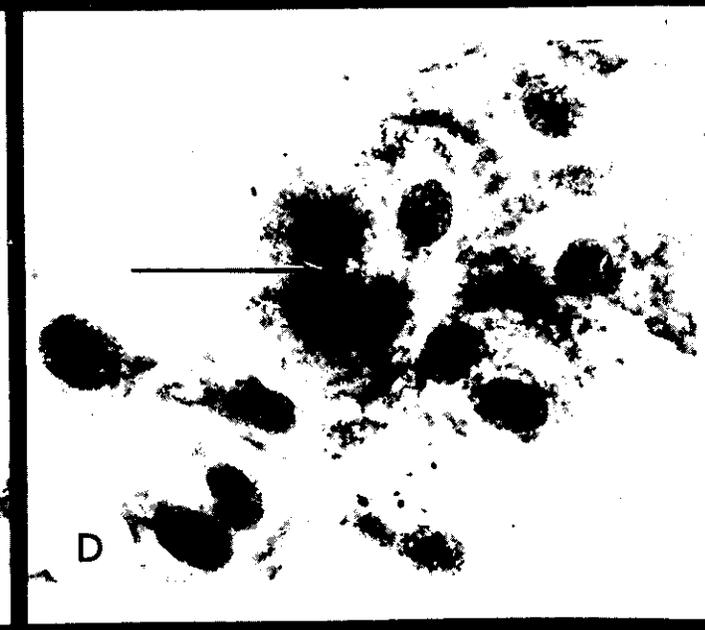
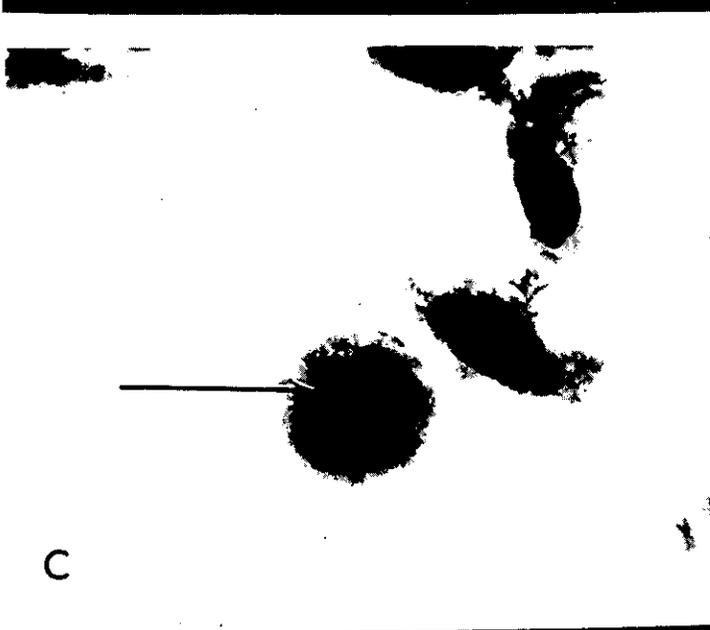
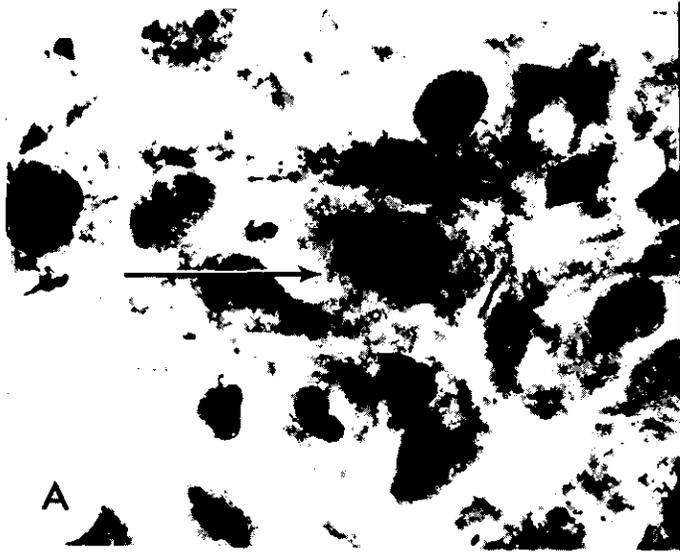
<sup>c</sup>tox = toxic (inhibitory)

**Figure 19**

**Normal and Aberrant Anaphases**

(Photos of cells exposed to samples from the present study)

A. Normal Anaphase. B. Acentric chromosome fragment. C. Whole chromosomes, fragments, non-disjunctions. D. Attached fragments. E. Multiple acentric fragments. F. Bridges and fragments.



These values do, in fact, correspond with t-test values obtained from dose response tests that were performed with various known mutagenic/carcinogenic compounds and with this same fish cell line (Kocan et al., 1982). Abnormality frequencies of 17% or less were not considered significantly different from controls.

To insure that the cell culture test system was performing satisfactorily, the polycyclic aromatic hydrocarbon benzo(a)pyrene (B(a)P) was used as a positive control compound. B(a)P is a known environmental contaminant with mutagenic/carcinogenic effects, but requires metabolic conversion by the mixed function oxidase (MFO) enzyme system before it becomes active. By using this compound as a positive control, testing could be done for both the effects of a known genotoxic agent and the presence of an active MFO system. Without an active MFO system, many of the promutagens/procarcinogens which require activation would have gone undetected. The result of exposure of fish cell systems for 48 h to B(a)P was an anaphase aberration frequency of 50-65% at a concentration of 0.25 µg/ml of culture medium.

#### 3.2.4.3 Test Results

Table 10 summarizes the data obtained from exposure of cells to sediment and water extracts. Significant ( $P < 0.05$ ) and highly significant ( $P < 0.01$ ) increases in mitotic (anaphase) damage were observed in 58 sediment samples (17 significant; 41 highly significant). No changes were observed in 39 of the samples. Comparison of the low and high concentrations of each sample generally showed an increased dose response. Five samples exhibited a reverse relationship (Stations 4, 25, 55, 74, 95) and one was inhibitory (toxic) at the higher concentration (Station 81). This latter effect could be attributed to the higher concentration of extract being sufficiently toxic to inhibit mitosis, thereby partially or totally reducing the number of quantifiable cells. This would be the result of having exceeded the optimum genotoxic concentration in the system.

Of the seven water samples tested, only Stations 43, 70, and 82 showed significant activity. Comparing the results from sediment and water extracts from the same stations, only at Stations 12, 65, and 70 were similar results obtained; Stations 29, 82, and 96 showed decreased activity in the water over that seen in the corresponding sediment samples while Station 43 showed increased activity in the water.

Consideration of the above results must take into account the fact that only a small fraction (generally less than 1%) of the total sediment mass was exposed to the trout gonad cells. This fraction was determined by the extraction procedure which recovered only those chemicals which were solvent-soluble. While a full characterization of this fraction has not been made, most of the organic compounds of direct biological concern (e.g. able to penetrate biological membranes) would be included. However, very polar substances such as some

unproteinated phenols and other organic acids and bases would not be included, and high molecular weight natural and polar organic compounds (e.g. humic acids, proteins, anthropogenic polymers) would have limited recovery in the extracts. In addition, because most trace metals exist primarily in ionic form, they would not be extracted unless methylated. No data are available on the extent of methylation of metals in Puget Sound sediments.

Elemental sulfur, which exists in relatively large quantities in anoxic sediments, is efficiently extracted and was present in sufficient quantities to crystallize in some of the samples as the solvent was removed. A large amount of additional colored material was recovered in some samples, probably representing degraded pigments, fulvic acids, and other material of natural origin. The colored material and sulfur constituted the greater fraction of material recovered.

A variable quantity of the compounds extracted was dissolved in DMSO. No attempt was made to determine whether further fractionation (e.g. preferential dissolution) of the extracted compounds occurred. However, based on the correspondence between the color of the DMSO solution and that of the dried extracts, it does not appear that major fractionation occurred.

It is entirely possible that biologically active compounds - toxic and genotoxic - were present in the samples but were not recovered in the sample extracts and hence not tested by the procedure employed. It is also possible that compounds of natural origin present in the material recovered had toxic or genotoxic activity. However, this particular test procedure was not intended as an exhaustive analysis of all possibilities of eliciting genotoxic responses, but rather was meant to provide an initial screening assessment of genotoxicity in sediments. This purpose was successfully achieved.

## 4.0 DISCUSSION

### 4.1 Sediment Physical/Chemical Characteristics

The physical characteristics of the sediments sampled in this study conform with other studies (Malins et al., 1980; Riley et al., 1980; Dexter et al., 1981; Enkeball, 1981). The data indicate that all of the inner harbor areas, i.e., upper Duwamish River, upper Commencement Bay waterways, and Sinclair Inlet, are depositional areas with fine-grained, organic-rich sediments. These areas are also industrialized and receive pollutant loadings from effluents, runoff, overland drainage, and bank seepage (Dexter et al., 1981).

The only area along the Seattle waterfront exhibiting grain size characteristics not expected for that relatively exposed area was the Denny Way CSO where large quantities of fine sediments were noted. These data agree with a previous intensive study of the CSO area (Tomlinson et al., 1980) and may reflect the dominance of local inputs from the CSO over normal processes, or may result from a presently undocumented current and/or wave "shadow" in northeastern Elliott Bay.

The presence of readily apparent living organisms in many sediments was a clear demonstration that the majority of the sediments were not acutely toxic to most species. Even the samples from the vicinity of the Denny Way CSO, which appeared on inspection to be polluted (i.e., were black, apparently anoxic, and malodorous) had some live organisms. Only a few samples from the Hylebos and City Waterways, Stations 51 and 70 in particular, showed coincident strong odor, no readily apparent organisms, and high organic matter levels.

The organic matter parameters (DOC, TVS, and extractables) did not provide a clear distinction among samples. The levels of all parameters tended to be higher near known sources. Grain size of the sediments associated with these areas was also smaller. DOC was, on the average, about 65% of the TVS from the same sample. Variations among samples were not consistent and did not follow any apparent trend. The samples from Birch Bay were apparently biased toward high DOC and TVS values in comparison with other sediments of comparable grain size through the inclusion of fragments of floatable plant matter (kelp and eelgrass) present on the beach and in the surf zone.

Neither DOC nor TVS values appeared to be good indicators of the amount of extractable matter in the sample. However, between the two, TVS is probably more reliable simply due to the simplicity of the measurement and, hence, greater analytical precision.

The levels of extractable matter appeared to reflect the impact of local inputs as well as grain size, being generally higher in the finer material. The "background" stations at Port Madison and Birch Bay, as well as those at Alki Point, West Point, and outer Elliott and Commencement Bays were low in extractable matter compared to the other stations.

The fractions of extracted matter observed as soluble in the anaphase testing were not originally intended to be measured and, as a result, the values recorded were not of high accuracy. In particular, losses of material from the sample containers were probably such that many of the values indicate greater dissolution than actually occurred. None of the samples exhibiting large differences in the dissolved fraction compared to the majority of the samples were anomalous in other characteristics. As a result, it appears that these data probably do no more than indicate that some 25 to 50% of the organic matter extracted from the sediments was soluble. A variety of toxic substances previously measured in the study areas (polynuclear aromatic hydrocarbons, PCBs and other chlorinated hydrocarbons) are soluble and extracted readily from the insoluble residue; however, the significance of this fact in terms of bioavailability is unknown.

#### 4.2 Water Physical/Chemical Characteristics

The observed physical characteristics of the water samples were within the expected ranges for normal autumn conditions in Puget Sound. Station 43 in the Hylebos Waterway had a lower salinity than noted in the other waterways of Commencement Bay; however, the

water from the Puyallup River can move northeast along the waterfront (Loehr et al., 1981) and enter the waterways with the tidal flows.

The water samples were generally much more turbid than surface waters at each station, based on visual observations. This turbidity may reflect either the presence of a strong turbid layer near the bottom (samples were collected within 0.3 m of the sediment) or may be the result of disturbance of the top sediment layer by the sampler. In either case, the extractions of organic matter probably recovered more material from these fine sediments than would be present in the bulk of the water column. The calculated extractable concentrations are, therefore, probably overestimates of the general water column values, but may accurately reflect the levels experienced by organisms which reside in or on the surficial sediments.

#### 4.3 Acute Lethal Bioassays

Both E. confervicolus and G. aculeatus are relatively sensitive species as shown both from a review of the literature (Section 2.3) and from comparative testing conducted as part of the present study, but there was a significant acute lethal effect recorded at only one site: 60% of E. confervicolus exposed to sediment from Station 4 died after 10 d.

Previous studies have shown that very sensitive species are killed following exposure to sediments from various sites in Elliott and Commencement Bays. Shuba et al. (1978) found that Palaemonetes, the grass shrimp, suffered significant mortalities after exposure to Duwamish sediments. Swartz et al. (1979) noted similar results for the clam Macoma inquinata and the amphipod Rhepoxynius abronius exposed to Elliott and Commencement Bay sediments. In a comprehensive study of Commencement Bay, Swartz et al. (1982) documented a variety of toxic areas, using R. abronius as a bioassay organism. The significance of this latter study is discussed in relation to respiration and anaphase aberration test results in Section 4.6 of this report. Recent work by Ott et al. (in prep.), confirms that R. abronius mortalities occur following exposure to sediments from a variety of areas in Puget Sound.

Recent work (Chapman and Swartz unpub. data, 1982) has shown that R. abronius is more sensitive and likely to exhibit lethal effects on exposure to contaminated sediments than any of the three species tested herein. However, Swartz et al. (1982) have also shown that this amphipod is normally only found in areas with clean, fine sands whereas the other three species used in this study are found in and around "contaminated" Puget Sound sites. Thus, the bioassay results indicated that the sediments tested are generally not acutely lethal to the resident fauna. Even at Station 4, the grab sample was noted to contain a variety of fauna including: two species of polychaetes, a crab, and algae (see Station Logs, Appendix A).

#### 4.4 Sublethal Respiration Rate Tests

Respiration is a physiological process that is responsive to sublethal effects and relatively easy to monitor in live animals. The value of using respiration to detect sublethal stress has been confirmed by a number of authors working with a variety of taxa (e.g. MacInnes and Thurberg, 1973; Thurberg et al., 1973; Calabrese et al., 1975; Saliba and Vella, 1977; Cantelmo et al., 1978; Laughlin and Neff, 1979; Chapman et al., 1982c; Brinkhurst et al., 1983).

Previous studies (summarized by Chapman and Brinkhurst, 1983) have shown that significant changes (elevation or depression) in respiration rate of *M. cuticulatus* can be caused by exposure to sublethal levels of metals and organic toxicants. Thus, it appears likely that significant changes in respiration rate, recorded following exposure to sediment elutriates and water samples from various Puget Sound stations, are indicative of stress and, therefore, have sublethal (adverse) effects.

The data collected during this study indicated that a number of stations in Puget Sound are capable of sublethal stress effects.

Although care must be taken in assessing specific geographical areas, given that both positive and negative results were noted in the same area, and given the known patchiness of sediment toxic effects, particularly in the Commencement Bay waterways (Swartz et al., 1982), a general classification of particular subareas can be made by comparing relative numbers of positive responses. Such a subjective classification has been attempted in Table II, using respiratory stress as an indicator of potential sublethal toxic effects. From these data, (which are not normalized for different numbers of stations sampled), the most toxic areas were near the Denny Way CSO, outer Commencement Bay, Hylebos Waterway and City Waterway. The least toxic areas were outer and inner Elliott Bay, inner Commencement Bay, the Pier 54 area, the East and West Duwamish Waterways, Sitcum Waterway, Port Madison and Birch Bay. Available information does not permit a definition of the ultimate effects of exposure of an organism to the most toxic areas, but chronic effects might include reduced viability, altered life-cycle, altered growth, or behavioral effects (e.g. slow burrowing response in contaminated sediments resulting in increased predation and, hence, species mortality).

#### 4.5 Anaphase Aberration Tests

The data obtained during this study clearly indicate that extracts of sediments collected from a number of locations in Puget Sound are capable of causing chromosomal (DNA) damage in cultured fish cells, as measured by the anaphase aberration test. Since earlier studies (e.g. Kocan et al., 1982) have demonstrated that anaphase aberrations result from the exposure of actively dividing cells to genotoxic agents (capable of causing chromosomal damage), it is a reasonable assumption that the damage reported here was caused by similar agents present in Puget Sound sediment. The high positive correspondence between induction of aberrations by known genotoxic agents implies that the test does indeed measure adverse effects on DNA. It should, therefore, be presumed that any organism which is

Table II

Rating of Puget Sound Subareas Based on the Number of  
Significant Respiration Effects Per Site

<u>Location</u>	<u>% Not Significant</u>	<u>% Significant</u>	<u>Subarea<sup>a</sup> Rating</u>
ELLIOTT BAY			
Outer Bay (1,2,14,16,17) <sup>b</sup>	80	20	+
Inner Bay (3,8,13,15)	80	20	+
Denny Way (4-7)	25	75	+++
Pier 54 (9-12)	75	25	+
Duwamish River (W. Waterway; 18-21)	75	25	+
Duwamish River (E. Waterway; 22-26)	80	20	+
Duwamish River (upper river; 27-37)	55	45	++
COMMENCEMENT BAY			
Outer Bay (38,39,71,74)	33	67	+++
Inner Bay (40,53,62,66)	75	25	+
Hylebos Waterway (41-52)	42	58	+++
Blair Waterway (54-61)	50	50	++
Sitcum Waterway (63-65)	67	33	+
City Waterway (67-70)	25	75	+++
SINCLAIR INLET (75-86)	50	50	++
PORT MADISON (87-92)	83	17	+
BIRCH BAY (93-97)	100	0	0

Table 11 (Contd)

<u><sup>a</sup>Rating</u>	<u>Description</u>
0	negative
+	slightly positive (1 or 2 stations significant)
++	positive (several stations significant)
+++	highly positive (most stations significant)

<sup>b</sup>Numbers in parenthesis indicate stations included in subarea designation.

chronically exposed to agents which cause anaphase aberrations may, in some way, be adversely affected. The nature and extent of the adverse effects would have to be determined on an individual species and site basis.

Attempts to relate cell toxicity (inhibition of proliferation) to genotoxicity (anaphase aberration) revealed that 42% of the stations showed a positive correspondence between toxicity and genetic damage. From the available data it must be assumed that two distinct cause and effect relationships have been measured, and that no association between the two can be made without further study. Thus, in assessing the relative toxicity of the various sediment extracts, the two were considered separately.

Care must also be taken in assessing the overall genotoxicity of a specific geographic area (e.g. Elliott Bay, Commencement Bay, Port Madison). Examination of the data (Table 12) shows that certain subareas contain a high proportion of significant genotoxic sample stations, while others are predominantly negative. In all cases there is a mixture of reactions ranging from negative (not significant) to highly genotoxic (highly significant). The toxic assessment of each area is best achieved by comparing the relative numbers of each type of reaction observed. Such relative classification of the areas was developed and is presented in Table 12. From these data it can be seen that the highest genotoxic responses were found in outer Elliott Bay, near the Denny Way CSO, in inner Commencement Bay, and in Blair and City Waterways. The lowest genotoxic responses were found in inner Elliott Bay, around Pier 54, the Duwamish West Waterway, outer Commencement Bay, Sitcum Waterway and Port Madison.

Significant genotoxic response was noted at Birch Bay, the control site. However, a number of storm drains (attached to roadside culverts and collecting agricultural and residential runoff) discharge into the Bay, and during sample collection a foamy discharge was emanating from them. The genotoxic responses noted most likely reflect these inputs. In addition, Birch Bay samples were taken intertidally while all other samples were taken subtidally and the present results may not be indicative of subtidal sediments in the area. However, although unlikely, the presence of natural sediment components capable of causing positive genotoxic responses cannot be ruled out.

The ultimate long-term effect of exposure of an organism to sublethal chromosome damage could manifest itself as reduced growth rate, reduced life span, or possibly neoplasia. The effect on germ cells may be reduced fecundity, dominant lethal mutations or birth defects, while exposure of the developing embryo could produce juvenile mortality, teratogenicity, low birth weight or ultimately tumor development in adult animals. It would be a formidable task to relate any of the above responses to specific sites; at this point it can only be stated that the type of chromosome alterations noted in this study are known to be detrimental to the normal development and health of most organisms. In addition, due to the extraction procedures, the

Table 12

Genotoxicity Rating of Puget Sound Subareas  
Based on the Number of Positive Samples Per Site

<u>Location</u>	<u>% Not Significant</u>	<u>% Significant</u>	<u>Subarea<sup>a</sup> Rating</u>
<b>ELLIOTT BAY</b>			
Outer Bay <sup>b</sup> (1,2,14,16,17)	0	100	+++
Inner Bay (3,8,13,15)	50	50	+
Denny Way (4-7)	25	75	+++
Pier 54 (9-12)	50	50	+
Duwamish River (W. Waterway; 18-21)	50	50	+
Duwamish River (E. Waterway; 22-26)	40	60	++
Duwamish River (upper river; 27-37)	36	64	++
<b>COMMENCEMENT BAY</b>			
Outer Bay (38,39,71-74)	67	33	+
Inner Bay (40,53,62,66)	25	75	+++
Hylebos Waterway (41-52)	42	58	++
Blair Waterway (54-61)	12	88	+++
Sitcum Waterway (63-65)	67	33	+
City Waterway (67-70)	0	100	+++
<b>SINCLAIR INLET</b> (75-86) (+ 1 toxic)	27	73	++
<b>PORT MADISON</b> (87-92)	67	33	+
<b>BIRCH BAY</b> (93-97)	40	60	++

Table 12 (Contd)

<u><sup>a</sup>Rating</u>	<u>Description</u>
0	negative
+	slightly genotoxic (1 or 2 positives) (1-49%)
++	genotoxic (several positives) (50-74%)
+++	highly genotoxic (most stations positive) (>75%)

<sup>b</sup>Numbers in parentheses indicate stations included in subarea designation.

present genotoxicity tests only portray the effects of organic compounds. The genotoxicity of metals and of physical agents is a distinct, but untested possibility.

#### 4.6 Combined Test Results

Summary bar diagrams of biological effects at each station tested are presented in Figs. 20-26, and an overall rating of different geographical subareas based on combined biological effects testing is given in Table 13. Although there is not always agreement on the response of individual stations in terms of both genotoxicity and respiration tests, the results for entire geographic subareas show remarkably good agreement. However, there are four subareas where totally different ratings are provided by these two tests. Outer Elliott Bay and inner Commencement Bay rated very high in terms of genotoxicity, but not respiration. The reverse occurred in the case of outer Commencement Bay and Birch Bay, where samples exhibited genotoxic, but not respiration response. These differences between the two tests are probably indicative of different active toxicants in each area. The respiration test, using sediment elutriates, was potentially responsive to both metals and organic toxicants; the anaphase aberration tests involved extraction of organic components and did not include possible genotoxic effects of metals.

Based on combined test results (Table 13), the tested subareas can be ranked in terms of biological toxicity as follows:

1. Denny Way CSO
2. City Waterway
3. Blair and Hylebos Waterways
4. upper Duwamish, Sinclair Inlet, outer Elliott Bay, outer and inner Commencement Bay
5. East Duwamish Waterway
6. Sitcum Waterway, Pier 54, inner Elliott Bay, West Duwamish Waterway, Port Madison and Birch Bay.

##### 4.6.1 Relationship to Chemical Data

The above ordering, in general, corresponds with the concentrations of metals and organic compounds which have been observed by others in the sediments, water, biota and/or discharges to the various subareas. Table 14 summarizes the bulk of this information within broad chemical classes and over wide spatial areas. 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). Many compounds falling into the latter two classes have not yet been positively identified. Quiescent areas in proximity to known or likely sources generally have exhibited the highest concentrations of these four classes of compounds, but the compounds

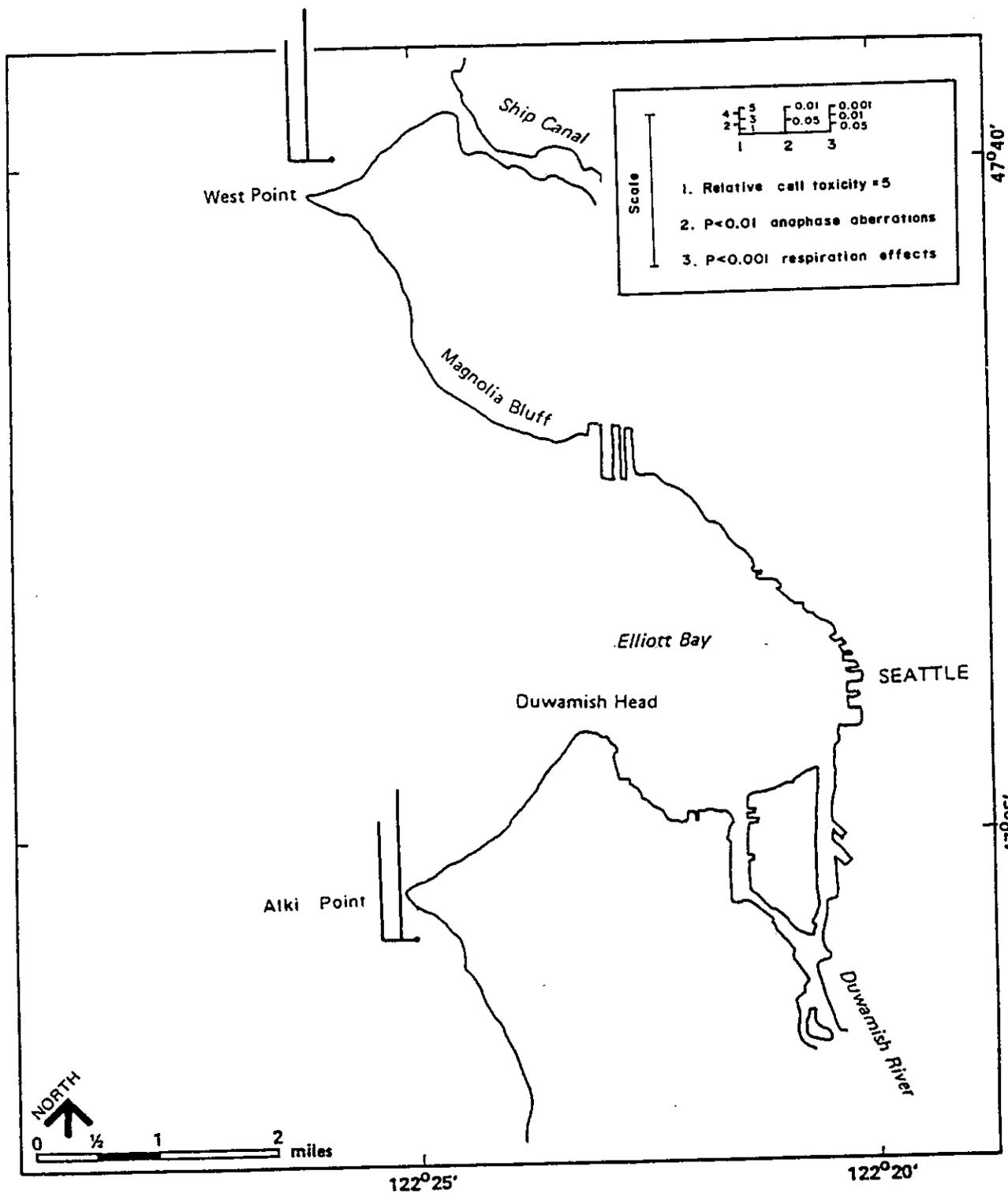


Figure 20. Bar Diagrams of Biological Effects at West Point and Alki Point. (see Tables 6 and 10 for explanation of scales)

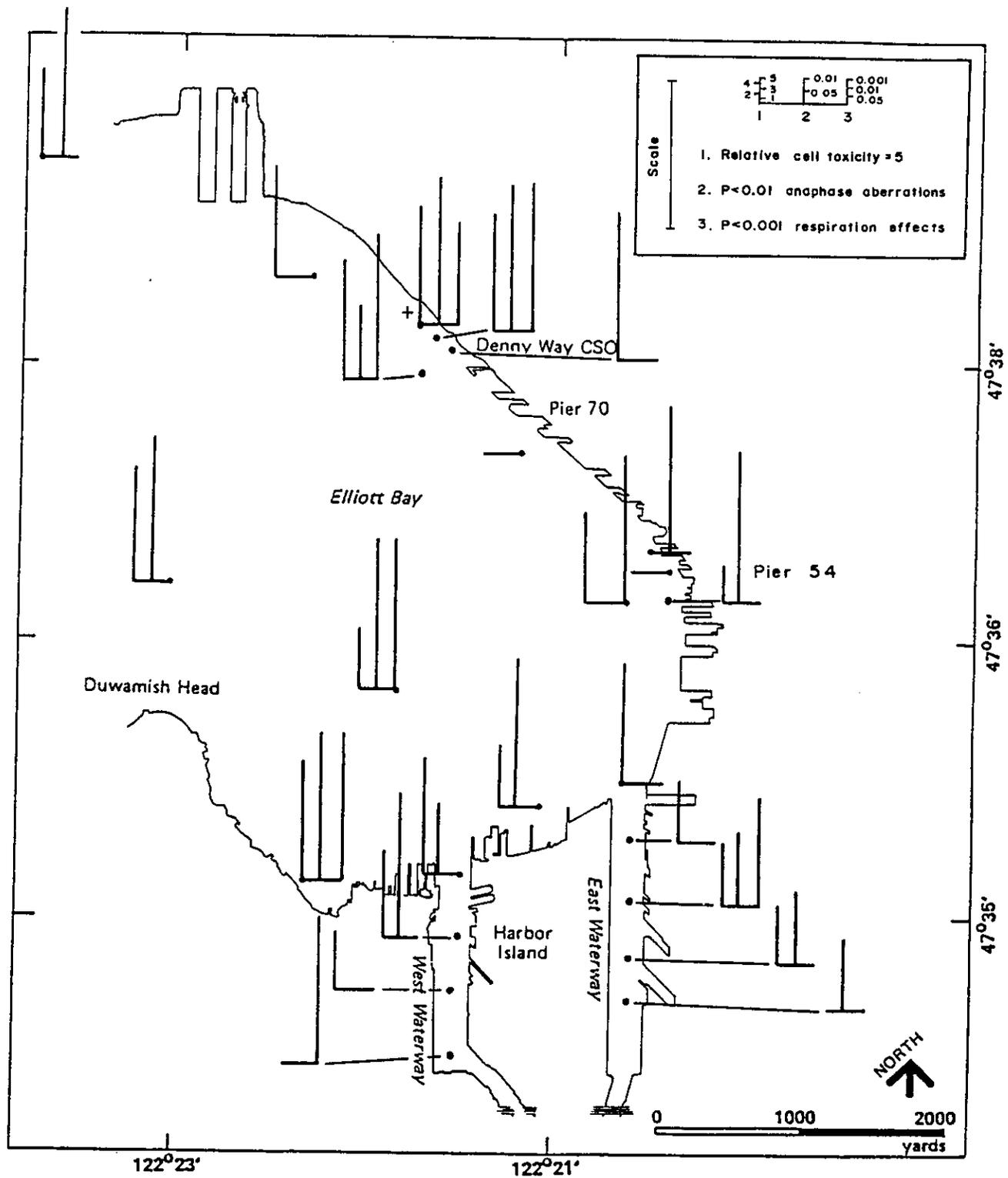


Figure 21. Bar Diagrams of Biological Effects in Elliott Bay and the Lower Duwamish River. A plus sign (+) indicates significant lethal effects among amphipods exposed in laboratory bioassays to sediments.

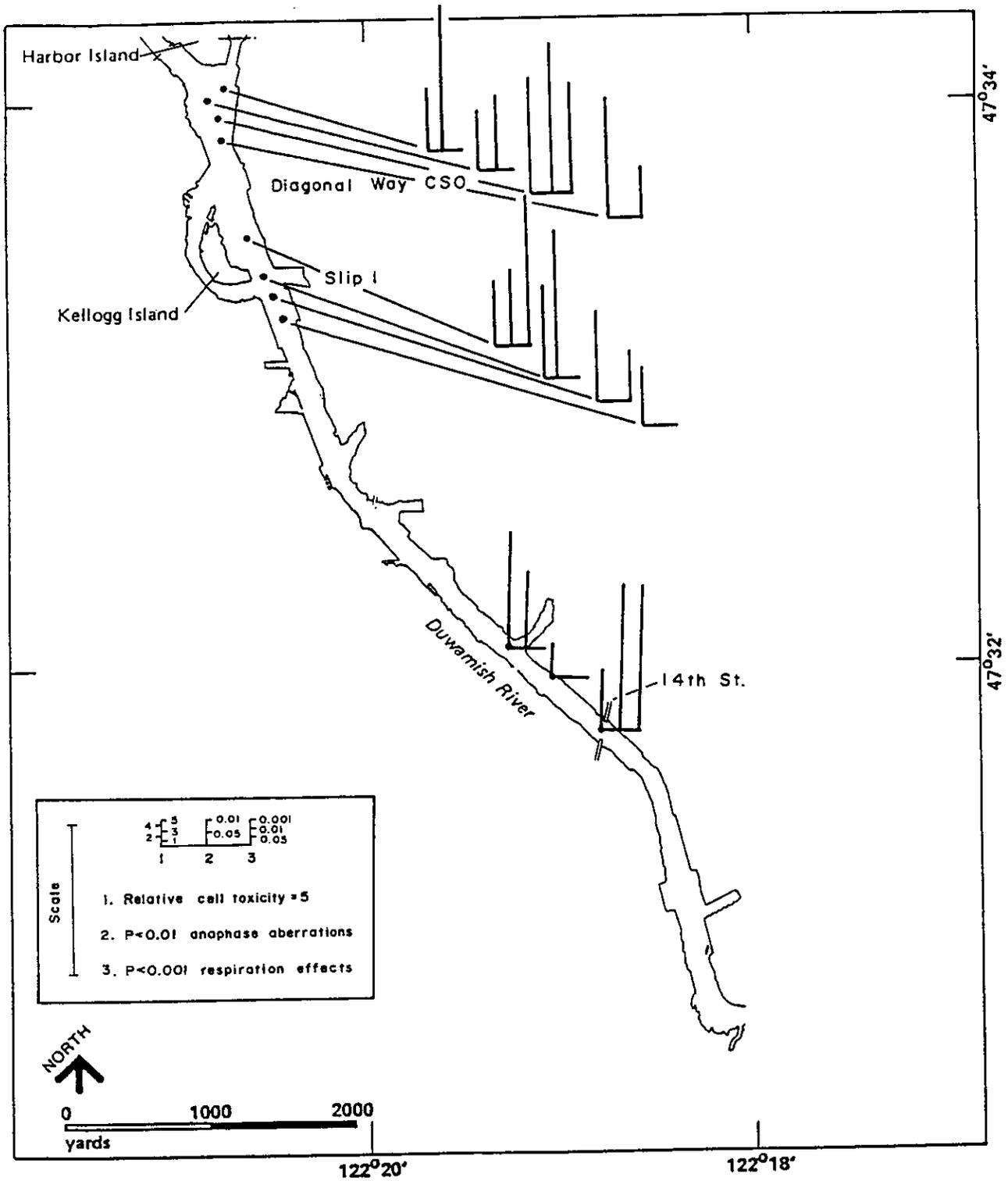


Figure 22. Bar Diagrams of Biological Effects in the Upper Duwamish River.

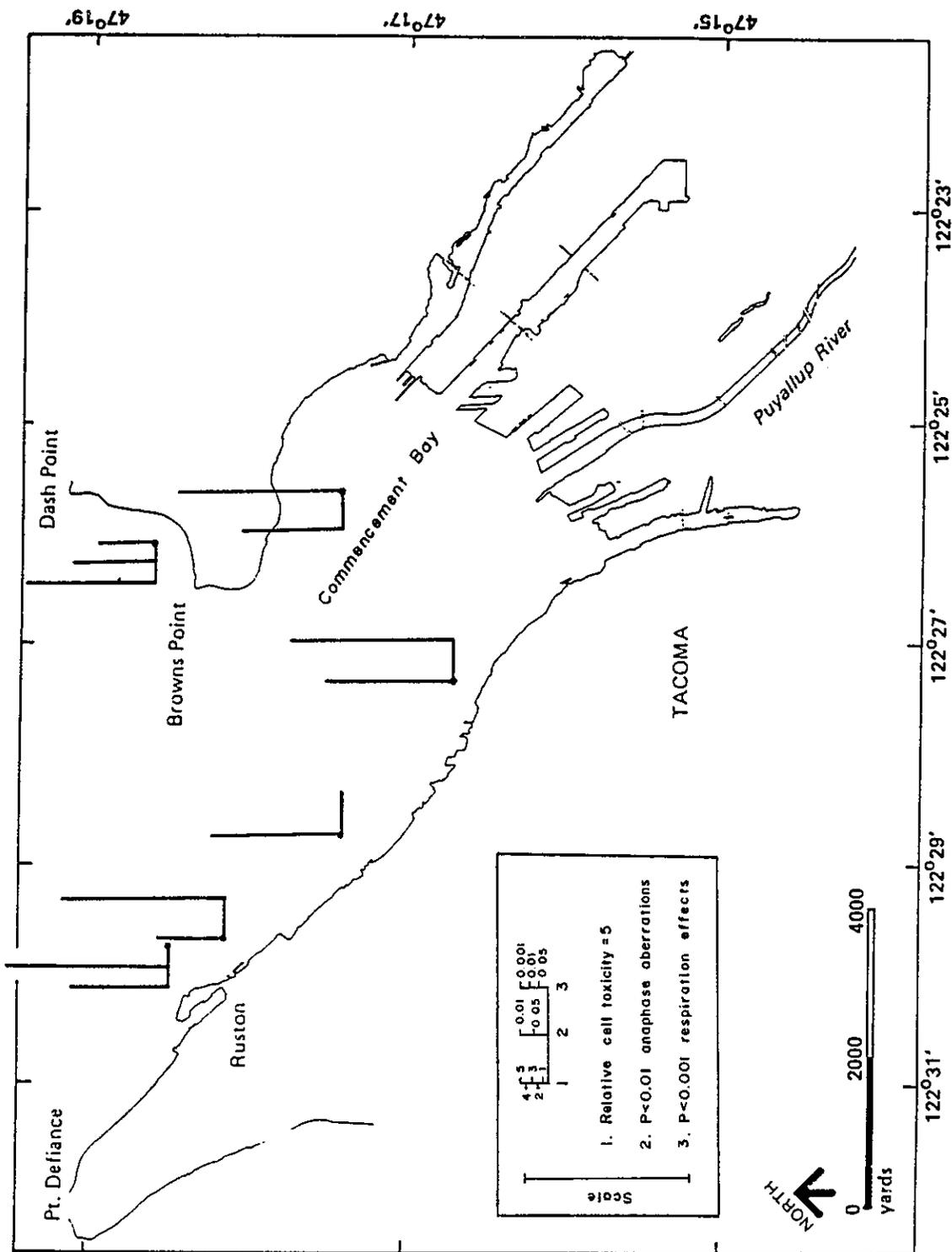


Figure 23. Bar Diagrams of Biological Effects in Commencement Bay.

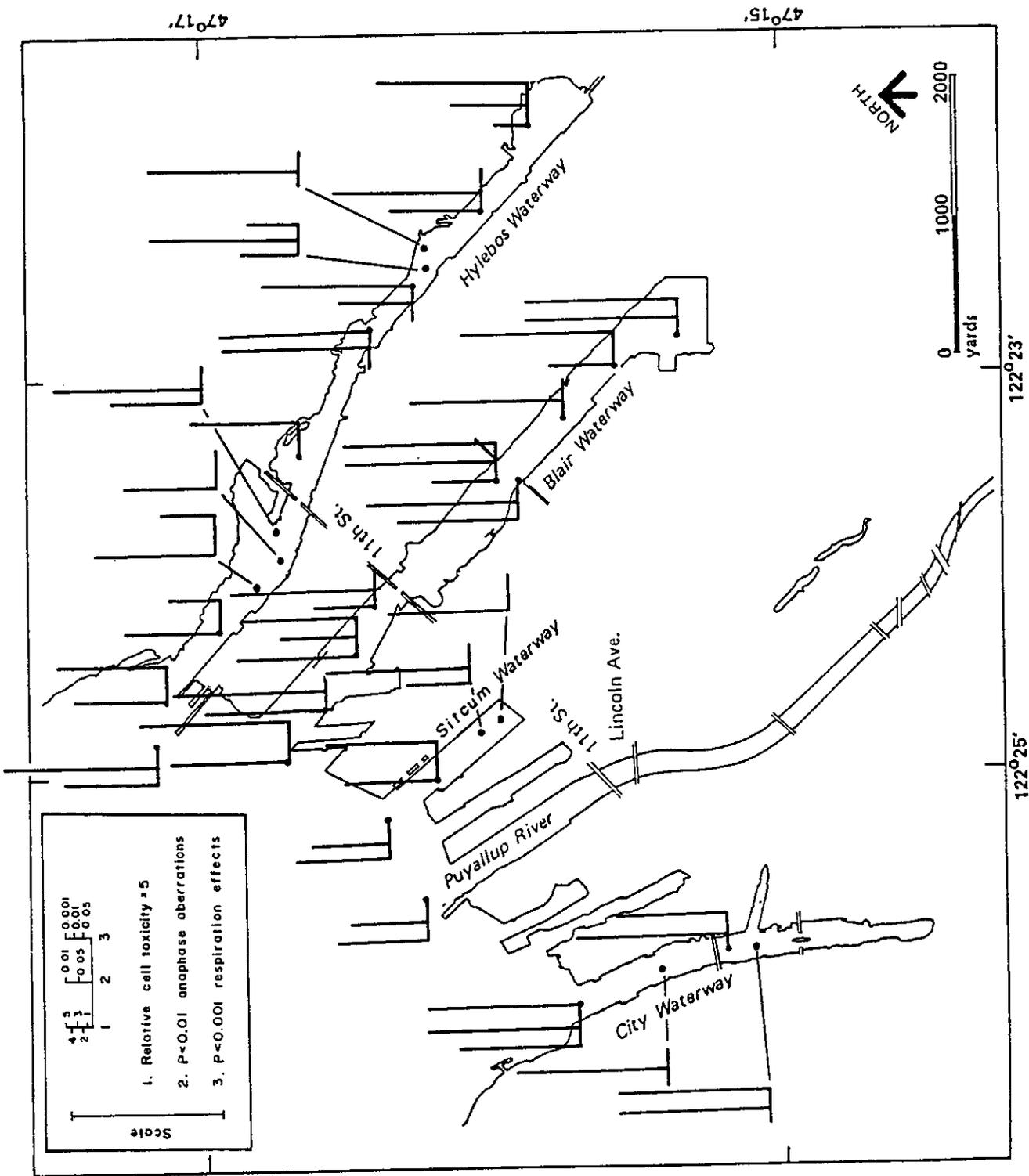


Figure 24. Bar Diagrams of Biological Effects in the Waterways of Commencement Bay.

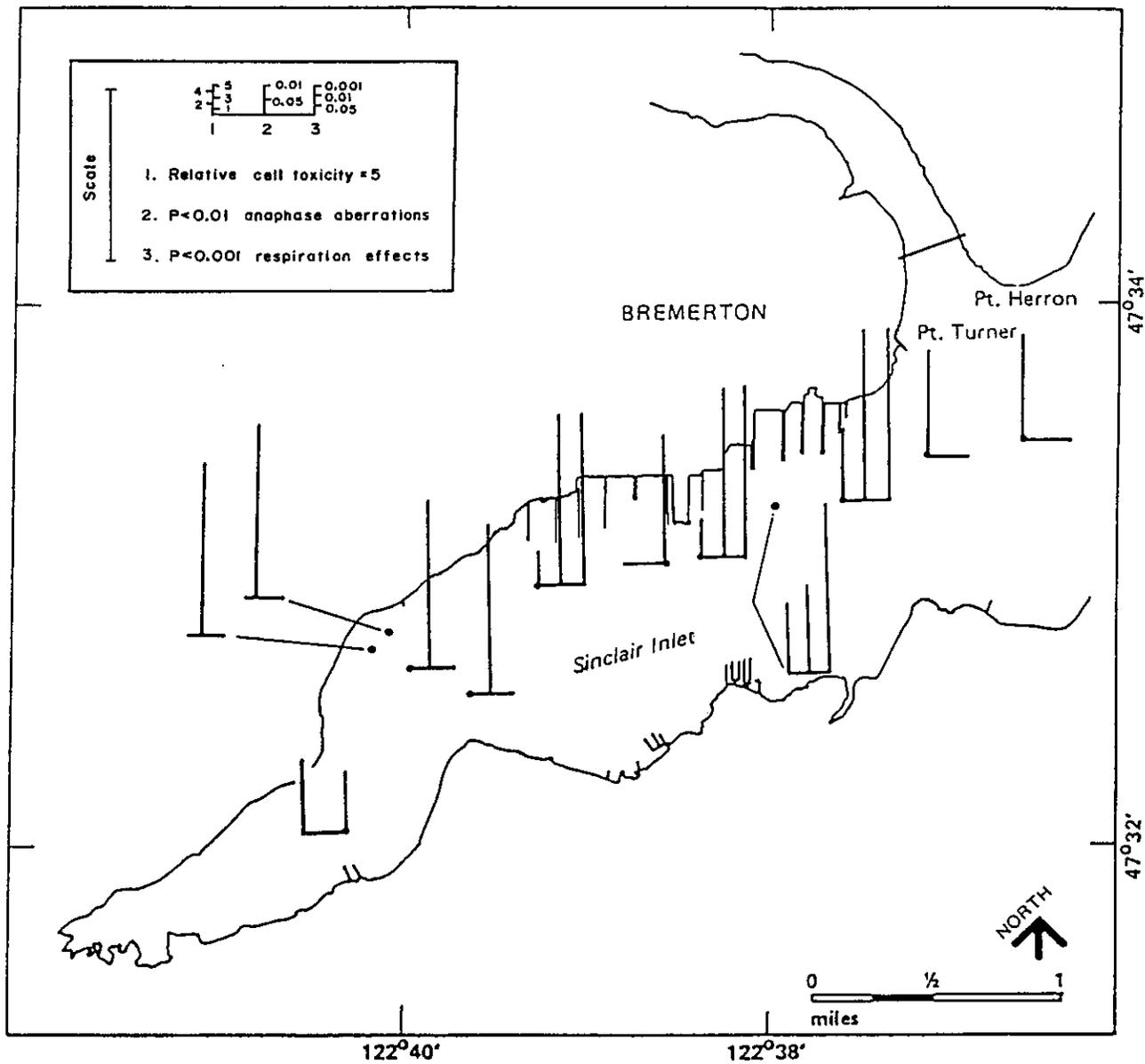


Figure 25. Bar Diagrams of Biological Effects in Sinclair Inlet.

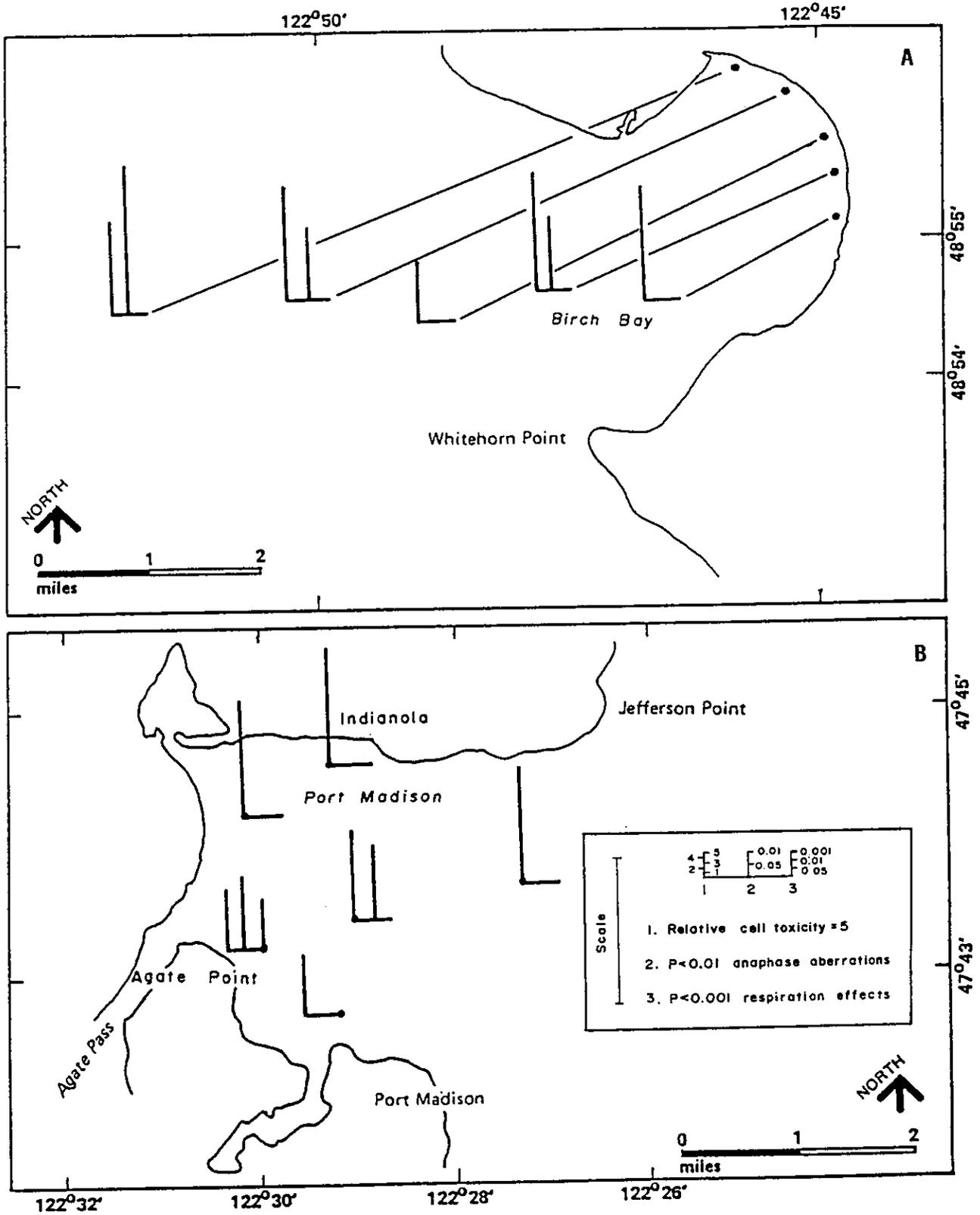


Figure 26. Bar Diagrams of Biological Effects in (A) Birch Bay and (B) Port Madison.

Table 13

Overall Rating of Puget Sound Subareas Based on Combined Lethality, Respiration and Genotoxicity Test Results with Sediments

<u>Location</u>	<u>Individual Ratings<sup>a</sup></u>			<u>Overall Ratings (No. of Positives)</u>
	<u>Lethality</u>	<u>Respiration</u>	<u>Genotoxicity</u>	
ELLIOTT BAY				
Outer Bay	0	+	+++	4
Inner Bay	0	+	+	2
Denny Way	+	+++	+++	7
Pier 54	0	+	+	2
Duwamish:				
W. Waterway	0	+	+	2
E. Waterway	0	+	++	3
upper river	0	++	++	4
COMMENCEMENT BAY				
Outer Bay	0	+++	+	4
Inner Bay	0	+	+++	4
Hylebos Waterway	0	+++	++	5
Blair Waterway	0	++	+++	5
Sitcum Waterway	0	+	+	2
City Waterway	0	+++	+++	6
SINCLAIR INLET	0	++	++	4
PORT MADISON	0	+	+	2
BIRCH BAY	0	0	++	2

<u><sup>a</sup>Rating</u>	<u>Description</u>
0	negative
+	slightly positive (1 or 2 toxic stations)
++	positive (several toxic stations)
+++	highly positive (most stations toxic)

Table 14

Summary of Contaminant Distribution Data Related to the Study Area.

<u>SITE (STATION NO.)</u>	<u>POSSIBLE POLLUTANT INPUTS</u>	<u>PRIMARY POLLUTANTS IDENTIFIED a</u>	<u>REFERENCES AND COMMENTS</u>
West Point (1)	Municipal sewage discharge, discontinued; possible advective transport from present sewage discharge, and from Ship Canal	Very high levels of PAHs	Malins et al., 1980 Sample from old sewage sludge deposit.
Elliott Bay Outer Bay (2,3,16)	Advective transport	Generally low levels of all pollutants	Malins et al., 1980 Dexter et al., 1981
Seattle Waterfront (8,13,15)	Advective transport; possibly some local industrial/commercial inputs; some local CSO inputs	Elevated levels of PAHs, metals and PCBs	Malins et al., 1980 Dexter et al., 1981
Denny Way CSO (4-7)	Municipal CSO discharge from predominantly commercial area	Limited analyses available. Elevated metals, PCBs	Dexter et al., 1981 Tomlinson et al., 1980 Sludge deposit present in area.
Pier 54 (9-12)	Municipal CSO discharge from predominantly commercial area	Very high levels of PAHs, elevated metals and PCBs; some CHs	Malins et al., 1980 Riley et al., 1980
Corps Dump Site (14)	Contaminated sediments from the upper Duwamish Estuary	Very high PCBs; elevated PAHs	Malins et al., 1980 Dexter et al., in preparation.
Duwamish Estuary West Waterway (18-21)	Shipyards storm drains; CSO discharge; riverine transport	Very high metals, particularly lead and copper; high PCBs; high PAHs	Malins et al., 1980 Dexter et al., 1981 Storm drains carry contamination from lead smelter.

Table 14 (Contd.)

<u>SITE (STATION NO.)</u>	<u>POSSIBLE POLLUTANT INPUTS</u>	<u>PRIMARY POLLUTANTS IDENTIFIED <sup>a</sup></u>	<u>REFERENCES AND COMMENTS</u>
East Waterway (22-26)	CSO discharges; possibly local industrial input, riverine transport	Elevated levels of metals, PCBs, and PAHs	Malins et al., 1980 Dexter et al., 1981
South Harbor Island (27-30)	CSO and storm drain discharges from industrial areas; riverine transport; no local industrial inputs have been identified	Elevated levels of metals, PCBs, and PAHs	Malins et al., 1980 Dexter et al., 1981
Slip 1 (31-34)	Riverine transport; no local industrial inputs have been identified	Elevated levels of metals, PCBs, and PAHs	Malins et al., 1980 Dexter, et al., 1981 Site of 1974 PCB spill; subsequently cleaned-up.
Upper Duwamish (35-37)	CSO and storm drain inputs; no local industrial inputs have been identified	Generally low pollutant levels	Malins et al., 1980 Dexter et al., 1981 Very high PCB levels were observed in 1976; section of the river was subsequently dredged and placed at site near station 14.
Alki Point (17)	Municipal sewage treatment plant discharge; advective transport	Generally low pollutant levels	Malins et al., 1980 Coarse nonaccumulating sediments.

Table 14 (Contd.)

SITe (STATION NO.)	POSSIBLE POLLUTANT INPUTS	PRIMARY POLLUTANTS IDENTIFIED a	REFERENCES AND COMMENTS
Commencement Bay Browns Point (38)	Advective transport; small local municipal sewage discharge	Generally low pollutant levels	Malins et al., 1980 Commencement Bay water probably exits around Browns Point regularly; high PCB and CIH levels observed in fish; very coarse, nonaccumulating sediments sampled
Northern Bay (39)	Advective transport	Elevated levels of metals, PCBs, and CIHs	Malins et al., 1980 Receives transport from water- ways; high organic, accumulating mud due to log boom storage.
Inner Bay (40,41,53,62,66)	Advective transport	Elevated levels of metals, PCBs, and CIHs	Malins et al., 1980
Southeastern Shore (71-74)	Advective transport (71); municipal sewage plant discharge (72); smelter discharges, effluent and slag (73 and 74)	Very high metals particu- larly arsenic and copper, near 73 and 74. Elevated metals, PCBs and CIHs at others	Malins et al., 1980 Dexter et al., 1981 WDOE 1981 - 1982 Samples were sandy and thus nonaccumulating. High-metals slag (73 and 74) probably refractory and not toxic.
Hylebos Waterway (42-52)	Numerous industrial point and non-point inputs: chemical manufacturing, oil refining, metal fabricating and shipyards	Very high CIHs: chlor- butadiens, chlorobenzenes, many other unidentified chlorinated compounds. Elevated metals, PCBs and PAHs	Malins et al., 1980 Riley et al., 1981 WDOE 1981 - 1982 U.S. EPA 1980

Table 14 (Contd)

<u>SITE (STATION NO.)</u>	<u>POSSIBLE POLLUTANT INPUTS</u>	<u>PRIMARY POLLUTANTS IDENTIFIED a</u>	<u>REFERENCES AND COMMENTS</u>
Blair Waterway (54-61)	Numerous industrial point and non-point inputs: chemical manufacturing, shipyards, oil refining	Elevated metals, PCBs, PAHs and CHs	Malins et al., 1980 Riley et al., 1981 WDOE 1981 - 1982 U.S. EPA 1980
Sitcum Waterway (63-65)	Probable industrial point and non-point discharges; none identified at present time	Very high metals	Malins et al., 1980
City Waterway (67-70)	Probable industrial point and non-point discharges, including significant storm/CSO inputs; none identified at present time	Elevated metals, PCBs, PAHs, and CHs	Malins et al., 1980
Sinclair Inlet Inner Inlet (75,76)	Advection transport	Elevated levels of metals, PCBs and PAHs	Malins et al., 1980 Fine organic-rich sediments from quiescent, depositional area.
Sewage Discharge (77-79)	Municipal sewage discharge		
Bremerton Waterfront (80-84)	Shipyard point and non-point discharges	Elevated metals, particularly mercury; elevated PCBs and PAHs	Malins et al., 1980 Riley et al., 1980

Table 14 (Contd.)

SITE (STATION NO.)	POSSIBLE POLLUTANT INPUTS	PRIMARY POLLUTANTS IDENTIFIED <sup>a</sup>	REFERENCES AND COMMENTS
Outer Inlet (85,86)	Advective transport	Elevated PAHs	Malins et al., 1980 Higher current-speed area; coarser sediments.
Port Madison (87-92)	Advective transport from outside of embayment; small sewage discharges near 89 and 90	Generally low pollutant levels	Malins et al., 1980. Most pollutants examined were low but readily detectable indicating significant long range transport in Puget Sound.
Birch Bay (93-97)	Possible storm drains; none currently identified	Limited analyses available.	Coarse, sandy sediments, far from any known major pollution source; expected to have low background levels of all pollutants. Low PAHs in sediment and mussels (Brown et al., 1979).

<sup>a</sup> PAHs = polynuclear aromatic hydrocarbons  
PCBs = polychlorinated biphenyls  
ClHs = other chlorinated organic compounds

have been detected in all areas of Puget Sound. Some areas, e.g., the lower Duwamish River and the Hylebos and Blair Waterways, are sufficiently documented to assume that most of the samples taken for this study contained elevated concentrations of toxic metals and organic compounds. For other areas, e.g., the Denny Way CSO site and the City Waterway, limited chemical data are available but the proximity of the sites to probable sources was consistent with the positive toxic responses observed. The toxicological data agree with the general assessments presented in Table 14.

The previously collected chemical data clearly demonstrated that short-range spatial variations in the concentrations of any of the types of compounds can be as great as the differences among areas. Further, many compounds known to be toxic have received limited or no study, e.g., aliphatic and chlorinated phenols. As a result, it was not possible to estimate the types or the levels of specific toxic compounds or classes of compounds to their biological activity in the samples collected in this study. The toxicological data showed considerable ranges of responses among closely spaced samples and attempts to statistically correlate these data revealed no dependence, even within regions, on any sediment chemical or physical factor. These results were somewhat surprising because previous research had demonstrated that, in an area exposed to similar pollutant loading, the finer, organic-rich sediments accumulated greater concentrations of most toxic substances (Dexter et al., in prep.). As a result, it was expected that some dependence of toxic response to grain-size and/or organic matter would be found. Pending further testing of both biological responses and chemical content, the spatial breakdown and source and chemical ratings presented in Table 14 probably represent the extent of reasonable comparisons between chemical and toxicological data.

Industrial chlorinated hydrocarbons, including chlorinated butadienes and benzenes, have been detected at high levels only in subareas of Commencement Bay. This distribution constitutes the only clearly established chemical distinction among regions since metals, PCBs and PAHs are present in comparable concentrations in all of the industrialized areas. The only toxicological difference observed in this study that might reflect the presence of these compounds was the dominance of elevated respiration (above controls) from Commencement Bay samples compared to predominantly decreased respiration observed with Elliott Bay samples. However, the majority of samples from Sinclair Inlet also caused increased respiration, but with much lower CIH concentrations than observed in Commencement Bay.

The very high metal levels noted in previous studies (Malins et al., 1980; Dexter et al., 1981) near Stations 73 and 74 (and possibly in the Sitcum Waterway) are associated with smelter slag which has been reported to be very refractory and hence should not be toxic. The

sediments from Stations 73 and 74 were coarse and would not be expected to accumulate organic contaminants if they were present. However, sediments from Station 73 caused increased respiration while sediments from Station 74 produced significant anaphase aberrations.

The Denny Way CSO site warrants a number of specific comments. Exposure to sediments from this subarea resulted in significant amphipod mortalities, oligochaete respiration responses, and fish cell chromosomal aberrations, making it clearly a highly toxic subarea. Although the chemical data from the area are limited, it is one of the few areas where a single dominant source of contamination can be readily and unmistakably identified, presenting significant possibilities for further study in evaluating and identifying the causes of the toxic responses and establishing actual cause/effect relationships. The apparent high toxicity which may be associated with the CSO discharge raises the question of whether currently unidentified toxic substances are normal components of municipal sewage and/or street runoff and if so, what risk is involved in allowing other discharges to marine or fresh waters without treatment. More explicit work would be required to answer this question.

#### 4.6.2 Toxic Areas

Acute toxicity tests are generally regarded as unsurpassed for toxicity screening and as comparative tools (Buikema et al., 1982), and when they are added to tests that measure sublethal responses, they provide the essential tools in any biomonitoring study. In Puget Sound, although high chemical contaminant levels have been found in particular areas (e.g. Malins et al., 1980, 1982; Pavlou et al., 1978; EPA, 1980; Riley et al., 1980; Dexter et al., in prep.), it appears that only the most sensitive species are actually killed directly and rapidly by exposure to contaminated sediments. For example, the three species used for lethality testing herein, G. aculeatus, E. confervicolus and M. cuticulatus, were virtually unaffected. Observations of the presence of benthic infauna at contaminated sites supports this observation and conclusion.

Although the Hylebos Waterway exhibited a variety of sublethal toxic effects, Meyer and Vogel (1978) found large numbers of gammarid amphipods and harpacticoid copepods living around the mouth. These species are important food sources for salmonids and apparently survive well in an area classified as among the most contaminated in Puget Sound (Malins et al., 1980). Swartz et al. (1982) noted that Commencement Bay waterways were not devoid of benthic macroinvertebrate life in areas toxic to R. abronius though phoxocephalid amphipods were missing. Similarly, in the present study, an apparently diverse fauna was observed in grab samples from Station 4, the only station exhibiting significant direct lethality.

An unexpected result in the present study was the determination of biological effects (both respiration and genotoxicity) in some bottom water samples. Although some of these effects may be ascribed to contamination from associated toxic sediments (many water samples were turbid, containing a large amount of particulates), in two cases effects were noted in bottom water samples but not in the associated sediments. Respiratory effects were noted in water samples from Station 65, at the head of Sitcum Waterway, an area noted for high arsenic levels; and genotoxic effects were obtained from the water collected at Station 43 in Hylebos Waterway, an area noted for high metals and organic contaminants input (EPA, 1980). These results indicated that toxic effects may be important in the near bottom water and toxicity is not restricted to sediments.

Another unexpected result was the determination of toxicity at Port Madison (respiration and genotoxic effects) and Birch Bay (genotoxicity). Malins et al. (1980) have noted that Port Madison is not entirely free of contaminants such as chlorinated organics. Limited chemical testing done at Birch Bay revealed only low levels of PAHs (Brown et al., 1979) however, since the Birch Bay samples were collected intertidally, surface/floatable materials may have been included which were different from those present in the other (subtidal) samples. Malins et al.'s (1980) supposition that no area in Puget Sound is completely free of chemical contaminants, is supported by the present study results.

Comprehensive biological toxicity testing of Commencement Bay and associated waterways was undertaken by Swartz et al. (1982), who measured survival of the marine infaunal amphipod Rhepoxynius abronius during 10 d exposure to test sediments from a variety of sites. These authors noted, as in the present study, that there was a great deal of variability in toxicity at different sites, leading them to question the use of single station results as representative of entire areas. We agree with their assessment that extrapolation from single samples can lead to erroneous conclusions, depending on whether the sample is truly representative. Thus, we have taken the approach of establishing broad-scale toxicity patterns related to chemical data. Future in-depth studies should be conducted at particular sites, to more precisely delimit toxic subareas and biologically active constituents.

Swartz et al. (1982) conducted most of their testing in Hylebos and Blair Waterways. In areas of overlap, there is good correspondence between their designation of toxic subareas and those determined in the present study. In this regard, the following stations are sited in toxic subareas: 42-44, 48-51, and 57-58. Swartz et al. (1982) tested the central, deeper portions of Commencement Bay and found these to be non-toxic in contrast to the present study in which toxic effects

were noted along the shoreline. Although these researchers stated that their tests of acute, lethal effects might not be sensitive to chronic/sublethal impacts, the high degree of correspondence between the two studies reinforces the present prioritization of toxic subareas.

An earlier study by Swartz et al. (1979) conducted in the Duwamish River in the area of Slip 1 (near our Stations 31-33) and the Elliott Bay dumpsite (near our Station 14) also showed good correspondence with the present study. Swartz et al. (1979) found sediments from the area of Slip 1 caused mortalities in the clam Macoma inquinata and in R. abronius; in the present study significant respiration and anaphase aberration responses were noted at 2 of the 3 stations in this area. Swartz et al. (1979) also found R. abronius were killed by sediments from the dumpsite. In the present study, Station 14 (dumpsite) was positive in both respiration and anaphase aberration tests. A study by Shuba et al. (1978) also recorded mortalities for the grass shrimp Palaemonetes, exposed to sediments from the dumpsite.

The results of recent acute lethality tests conducted with R. abronius by Ott et al. (in prep.) also showed good correspondence with present results. Amphipod mortalities at our Stations 6 (Denny Way CSO) and 29 (Harbor Island south, Duwamish River) corresponded with both genotoxic and respiratory effects. At Station 83 (Pier 4, Sinclair Inlet) amphipod mortalities corresponded with respiratory (but not genotoxic) effects; similar correspondence was noted in the case of significant amphipod morbidity at Station 71 (outer Commencement Bay). A major difference was noted between the two studies only at our Station 20 (Duwamish West Waterway) where Ott et al. (in prep.) observed that these sediments caused the most significant amphipod mortalities. However, in the present study this station (and indeed the whole Duwamish West Waterway) exhibited a low level of biological effects. This difference points out both the variability in toxicity at different sites, and the problems associated with categorizing whole areas on the basis of single samples.

Previous studies have indicated that subtle adverse effects are manifest in Puget Sound in such forms as increased frequency of neoplasia and changes in benthic community structure (Malins, 1980; Dexter et al., 1981). A higher incidence of mitotic abnormalities in cells from the blood of English Sole caught in the Duwamish has been noted (Stromberg et al., 1981). The results of the present study have served to provide direct evidence linking specific geographic areas with toxic biological effects.

Testing was conducted in the laboratory under specified experimental conditions to attain the primary objective of establishing one or more biological responses. Changes in these conditions might have served to increase or decrease toxic responses. For instance, increased lethality and respiratory effects might have been noted in Duwamish and Commencement Bay Waterways if sample testing had been

conducted at lower salinities; and, higher temperatures with these and with other samples might also have increased responses. Both lowered salinity and increased temperature can increase the susceptibility of organisms to some (but not all) toxicants (Chapman et al., 1982b). The relationship between toxicant levels and biological responses is extremely complex, including interactions with sediments and their constituents, environmental factors, toxicant combinations and concentrations, and biological factors.

The identity and source of toxicants causing biological effects cannot be determined from the results of the present study. However, particular subareas of concern have been determined. Based on the location of these subareas, suspect sources could be determined for further intensive study related to mitigation and possible clean-up.

Although chemical toxicant data for tested sediments were not available for inclusion in this report, a review of previous studies indicated good correspondence between areas of greatest biological effects and areas of greatest chemical contamination. In the present study a subjective rating system (Tables 11-13) was used to categorize 16 different geographic subareas. This rating was not normalized as the same number of stations were not sampled in each area, and each subarea may be subject to the small-scale variability in toxicity demonstrated by Swartz et al. (1982). However, the fact that this rating of different areas using multiparametric biological responses showed such good correspondence with available chemical data demonstrated its value in prioritizing areas for adverse environmental effects on the basis of their lethal, sublethal and genotoxic characteristics.

## 5.0 CONCLUSIONS

1. In only one case, sediment from Station 4 (near the Denny Way CSO), was an acute lethal response recorded, specifically significant mortalities of the amphipod Eogammarus confervicolus. This result, coupled with the presence of readily apparent live organisms in tested sediments (including Station 4), suggests that only the most sensitive species are directly and rapidly killed by exposure to contaminated sediments.
2. Sublethal tests using oligochaete respiration indicated that sediments from a number of stations in Puget Sound are capable of causing sublethal toxic effects.
3. Genotoxicity testing indicated that sediments from a number of stations in Puget Sound are capable of inducing mitotic abnormalities in Rainbow Trout gonad cells.

4. Both respiration and genotoxicity tests showed positive results with some bottom water samples, indicating that toxic effects are not restricted to sediments.
5. There was good agreement between the results of the respiration and genotoxicity testing for entire geographic areas. Differences between these two tests may indicate the presence of different active toxicants.
6. The results of the present study fit well with toxicity studies conducted previously in Puget Sound by various investigators.
7. Based on the study results, a subjective ranking system was used to categorize the relative toxicity of 16 different geographic subareas. The ranking, in decreasing order, is as follows:
  - i. Denny Way CSO (Elliott Bay)
  - ii. City Waterway ( Commencement Bay)
  - iii. Blair and Hylebos Waterways (Commencement Bay)
  - iv. Upper Duwamish, Sinclair Inlet, outer Elliott Bay, outer and inner Commencement Bay
  - v. East Duwamish Waterway
  - vi. Sitcum Waterway (Commencement Bay), Pier 54 (Elliott Bay), inner Elliott Bay, west Duwamish Waterway, Port Madison and Birch Bay.
8. Although chemical contaminant data were not available for the tested sediments, a review of previous studies indicated good correspondence between areas of greatest biological effects and areas of greatest chemical contamination.
9. The results of this study provide direct evidence linking specific geographic subareas to toxic biological effects.

## 6.0 RECOMMENDATIONS

1. Further biological effects testing should be undertaken to extend and define the areas of most concern described herein. Such testing would serve to refine the present study results, and would provide additional information related to Puget Sound fauna. We recommend that additional tests be used and suggest that reproductive effects testing be considered.
2. In-depth studies should be undertaken to determine small-scale variations in toxicity at the most toxic subareas: near the Denny Way CSO, and in City, Blair and Hylebos Waterways.

Such studies would serve to more accurately define high priority areas for possible clean-up. Particular attention should be directed towards the following stations, some of which are not included in the above areas: 4-6, Denny Way CSO; 14-15, Elliott Bay; 24, 29, 31, 37, Duwamish River; 38, Commencement Bay; 47-50, 52, Hylebos Waterway; 55, 57, 61, Blair Waterway; 67, 69-70, City Waterway; 82, 84, Sinclair Inlet.

3. Chemical analyses should be undertaken on archived sediment samples from these and other sites to relate chemical contaminant levels to toxic biological effects.
4. The sources of contamination related to specific toxic subareas should be determined. This information is essential to allow mitigation of present adverse environmental effects in Puget Sound.

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