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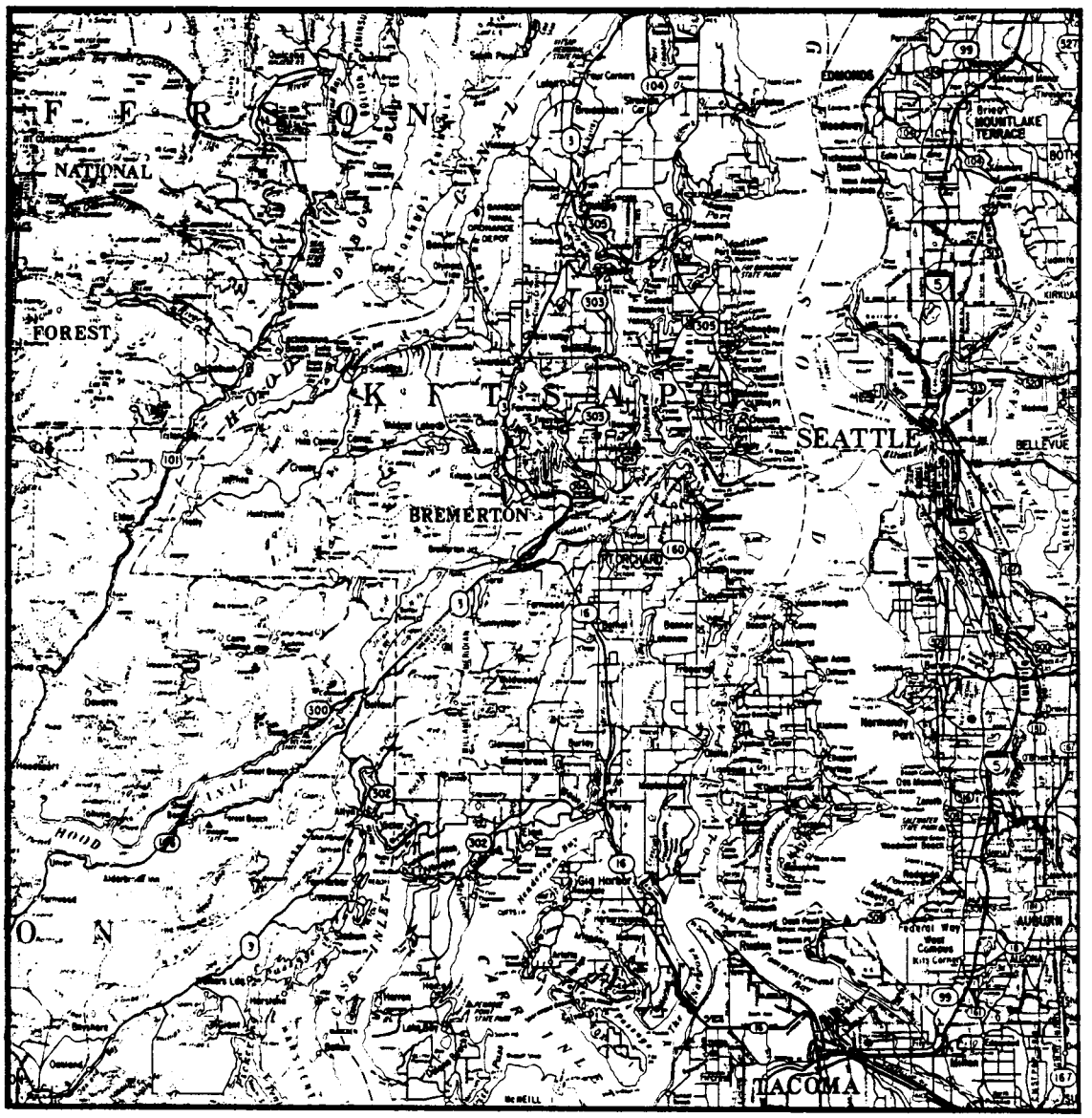

RECONNAISSANCE SURVEY OF EIGHT BAYS

IN PUGET SOUND

Final Report

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to

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EXECUTIVE SUMMARY

Over the past two years, Pacific Northwest Laboratory's (PNL) Marine Research Laboratory (MRL) has conducted a research program in Puget Sound with the objective of developing a better understanding of the toxic contamination problems in selected urban-industrialized bays.

Two major tasks comprised this effort. First, screening surveys and analyses were conducted in four urban-industrialized embayments (Bellingham Bay, Port Gardner - Everett Harbor, the Fourmile Rock - Elliott Bay dump site vicinity, Sinclair Inlet) and in four baseline embayments (Samish Bay, Case Inlet, Dabob Bay, Sequim Bay) to serve as a guide for more detailed technical analyses. The second task involved conducting detailed surveys and analyses in the same bays, but at a limited number of stations.

The results of these surveys are given in this report: a description of each urban and baseline bay in terms of its sediment chemistry, sediment toxicity, numerically dominant benthic infauna, and incidence of fish and shellfish disease. Each biological parameter (benthic infauna, incidence of fish and shellfish disease, amphipod and oyster larval bioassay) is discussed in relation to the physical and chemical properties of associated sediments. Present research findings are compared with results of historical surveys. Finally, the embayments and the stations in each embayment that currently show signs of degraded sediment quality are identified.

The organization of this study was such that the U.S. Environmental Protection Agency (EPA), Region 10 Laboratory; the National Oceanic and Atmospheric Administration (NOAA), Northwest and Alaska Fisheries Center; and Pacific Northwest Laboratory, Marine Research Laboratory participated in developing material included in this report. The EPA, Region 10 Laboratory staff assisted in conducting screening surveys in the fall of 1983, and conducted the detailed surveys in the spring of 1984 themselves. They also analyzed for priority pollutants and performed amphipod bioassays. The NOAA Northwest and Alaska Fisheries Center conducted independent surveys in the same urban-industrialized and

undeveloped embayments to obtain fish and shellfish for analyses of neoplasia and other diseases. The MRL assisted in conducting the screening surveys, performed range-finding chemistry for both the screening and detailed surveys, analyzed benthic infauna, and conducted oyster larval bioassays. With the exceptions of the NOAA neoplasm/disease and EPA amphipod studies, MRL also statistically treated and interpreted all data, and prepared this final report.

It should be understood that these results represent a reconnaissance of the problems and are not always rigorously quantitative. Hypothesis testing was not always possible and selected statistical tests were sometimes exploratory and intended to detect patterns and trends in the data. The strength of our strategy was in the performance of several chemical analyses, biological analyses, and bioassays on the same fresh (unfrozen) sediment sample. The development of a broad based (summary) index of degraded sediment quality also facilitated detection of a wider range of sediment toxicity that would not have been evident from applying any single analysis or bioassay technique.

The major findings resulting from this report are summarized as follows.

Bathymetry and Sediment Characteristics

The eight bays demonstrated different depth and sediment characteristics. The Fourmile Rock - Elliott Bay dump site vicinity and Dabob Bay were distinctly deeper (>100 m) and contained sediments that, on the average, were sandier and of lower total organic carbon (TOC) content than the rest. Samish and Bellingham Bays were the shallowest and the muddiest of the eight bays. Bellingham Bay showed the second highest TOC level. Case Inlet, Sinclair Inlet, and Sequim Bay were of intermediate depth and had intermediate percentages of silt and clay and TOC. These three bays exhibited sediment types ranging from sandy-silt to silty-mud. Port Gardner - Everett Harbor was distinct from the other bays because it had a significantly higher mean TOC level.

Contaminant Levels

Urban bay sediments, particularly in Sinclair Inlet and at the Fourmile Rock - Elliott Bay dump site vicinity, were found to be contaminated by Ag, As, Cu, Cd, Hg, Pb, and Zn. Other metals (Sb, Be, Cr, Ni, Se, Ti) were found at about the same concentration in all bays; however, a few urban sediments in Bellingham Bay and Sinclair Inlet were enriched in Sb, Cr, and Ni. Baseline bays generally contained lower metals concentrations, many of which were found at approximately the same concentrations as in pre-1900 sediments.

Urban bay sediments, particularly those from Sinclair Inlet and Port Gardner - Everett Harbor, also contained significant quantities of aromatic hydrocarbons and PCB-1254. Other organic compounds occasionally found in urban bays included phthalates and PCB-1260. However, no PCBs were detected in baseline bays, and only a few stations in Samish Bay contained detectable quantities of phthalates and aromatic hydrocarbons.

Benthic Infauna

It was determined that in all sediment types, TOC levels between 4 and 16% were associated with a disturbed infauna dominated by "organic enrichment opportunists." This shift to enrichment opportunists, primarily nematodes and *Capitella capitata*, occurred at almost all stations in Port Gardner - Everett Harbor and at several stations in Bellingham Bay. The extent of this shift was less in sandy sediments when compared to silty sediments.

After omitting the stations with high TOC, the numerically dominant infauna were shown to vary more with sediment type than by bay, except that Case Inlet and Samish Bay had distinctly different infaunas than all other bays. Also, omitting these same stations did not reveal a distinctly different set of numerically dominant infaunal species in urban bays when compared with baseline bays.

Fish and Shellfish Pathology

Flatfish species were not collected in four of the eight bays (Bellingham Bay, Samish Bay, Dabob Bay, Sequim Bay) because of seasonal scarcity, so the resulting pathology data were not used in a summary statistical exercise to determine signs of degraded sediment quality. However, these data served to corroborate the results of other approaches used in the summary exercise (chemistry, infaunal analyses and amphipod and oyster larval bioassays) indicating that urban bays were generally more severely impacted than baseline bays.

English sole caught in Sinclair Inlet demonstrated the highest incidence of liver, kidney, and gill lesions. Also, two types of lesions in shellfish were found only in animals from urban bays. Hydropic degeneration/membrane lysis in the hepatopancreas of Dungeness crab and degenerative disorders in the antennal gland of two shrimp species were observed in Bellingham Bay and at the Fourmile Rock - Elliott Bay dump site vicinity. And, although the incidences of serious liver, kidney, and gill lesions in English sole from Case Inlet were low compared to incidences detected in sole from other urban bays (Duwamish Waterway), these lesions were not detected in sole from Eliza Island (substituted baseline station).

Amphipod Survival

Based on the amphipod survival data alone, the urban bays were not always clearly distinguishable from baseline bays. The Fourmile Rock - Elliott Bay dump site vicinity, representative of urban bay conditions, and Sequim Bay, a baseline bay, demonstrated the highest and second highest mean survivals of 17.3 and 17.0 respectively. Similarly, Case Inlet, a baseline bay, and Port Gardner - Everett Harbor, an urban bay, demonstrated the second lowest and lowest mean survivals of 13.0 and 12.3 respectively.

Correlation analyses illustrated significant relationships between the amphipod bioassay and various physical and chemical properties of sediments. Of particular importance were the relationships of amphipod survival to sediment grain size, percent water, organic content (percent volatiles and TOC), and burden of organic compounds (PCB-1254, aromatic hydrocarbons). All such properties were interpreted as accounting for lowered amphipod survival. These analyses also revealed that lowered amphipod survival correlated with increased abundance of organic enrichment infaunal species in Port Gardner - Everett Harbor.

Oyster Larval Bioassay

Results of the oyster larval bioassay generally paralleled those of amphipod bioassays. The analyses, when taken alone, indicated that urban bays were not always different from baseline bays. Although a majority of stations in Bellingham Bay and Sinclair Inlet, both urban bays, exhibited significantly higher mean percentages of abnormal larvae when compared with controls, the Fourmile Rock - Elliott Bay dump site vicinity, which was also considered representative of urban bay conditions, exhibited no differences when compared with controls. Similarly, although Sequim Bay, Samish Bay, and Case Inlet (all baseline bays) exhibited no differences among mean percentages of abnormal larvae when compared with controls, Dabob Bay, (another baseline bay) exhibited higher mean percentages of abnormal larvae at two of four stations.

Correlation analyses showed a significant relationship between percent abnormal oyster larvae and amphipod survival. The trend to higher percentages of abnormal oyster larvae become more evident as amphipod survival decreased.

Summary Statistics

On the basis of the summary exercise which evaluated all data, chemical and biological, urban bays were significantly more impacted than

baseline bays. The greatest impacts were found in Port Gardner - Everett Harbor, Sinclair Inlet, and Bellingham Bay. The Fourmile Rock - Elliott Bay dump site vicinity appeared to be the least impacted of the urban-industrialized sampling areas. Not surprisingly, Sequim Bay was the least impacted of all bays. However, Case Inlet and Samish Bay, both baseline bays, showed some indication of degraded sediment quality. All sediments in both bays resulted in lower than control amphipod survival. The amphipod and oyster larval bioassay data from Dabob Bay also suggest some degree of impact.

It was also observed that the most adversely impacted stations in Port Gardner - Everett Harbor were located in the East Waterway. For Sinclair Inlet, they were located in proximity to the Puget Sound Naval Shipyard. In Bellingham Bay, they were associated with the inner harbor.

Relationship of Urban to Baseline (Reference) Bay Concept

On the basis of chemical contaminants alone, the baseline or "reference bay" concept appears to be valid. Sequim Bay or Dabob Bay, with relatively low metals burdens and no detectable organic compounds, seem to be excellent choices for reference bays.

Biological variables, however, do not always clearly distinguish between what we hypothesized at the outset of the study to be urban bays and baseline bays. Rather, the biological findings better describe a continuum of responses consistent with a gradient of contaminants existing between regions of higher and lower contamination in Puget Sound.

This continuum is described here: 1) Port Gardner - Everett Harbor, Bellingham Bay, and Sinclair Inlet occupy one end of the continuum, representing water bodies now showing the most evidence of degraded sediment quality. 2) Sequim Bay is a baseline bay located at the opposite end of the continuum. It presently shows the least indication of degraded sediment quality of all the bays sampled. 3) The Fourmile Rock - Elliott Bay dump site vicinity is located intermediate on the continuum but closer

to the position occupied by the urban bays. However, clearly the dump site is somewhat anomalous because not one of the eight stations sampled during the detailed surveys showed any sign of degraded sediment quality when evaluated by either the amphipod or oyster larval bioassay. 4) Case Inlet and Samish Bay are also located intermediate on the continuum but closer to the baseline bay position occupied by Sequim Bay. However, both bays are close enough to Commencement Bay and Bellingham Bay respectively to show some signs of degraded sediments. 5) Dabob Bay is also located intermediate on the continuum but closest to the baseline bay position occupied by Sequim Bay. The finding of degraded sediment quality based again on the bioassay results is perhaps attributed to circulation of contaminants from the main basin of Puget Sound.

In summary, the results of our study support the "reference bay" concept; however, our results also suggest that the process to select reference bays needs refinement. It is evident that selection should be based on chemical, biological, and physical data. Also, the 1983 screening survey was particulary useful in providing relevant and recent data on which to base the selection process. Ideally, a reference bay should contain little or no chemical contamination, demonstrate few or no impacts when evaluated by infaunal analyses and sediment bioassays, but should also possess physical characteristics (depth and grain size) that closely approximate the urban bay(s) to be examined. Our results perhaps suggest that "reference stations" may be a more appropriate concept than "reference bays", particularly when time and funds limit the scope of studies performed in support of regulatory action.

There is a need to bring together a forum of scientists and regulators to refine this concept and its intended use. Also, our strategy of selecting equal numbers of the most contaminated stations within each urban bay does not address issues concerning the full range of contaminated sediments within each bay. Clearly, the present study would

have benefitted from the addition of stations both in the urban and baseline bays. Consequently, our results apply only to the sediments (stations) sampled in each bay and do not likely represent all similiar depositional sediments in each bay.

Usefulness of the Reconnaissance Survey Concept

The 1983 screening survey of 181 stations from eight bays was particularly useful in appropriately focusing the 1984 detailed survey. The strategy of selecting the most contaminated stations from the urban bays and the least contaminated stations from the baseline bays permitted limited resources to be directed toward identifying those stations most likely to reveal degraded sediment quality. Moreover, the screening survey data allowed the station selection process for the detailed surveys to be based on data that was recently acquired rather than on historical data that was collected in support of other goals. The variability encountered in biological indices subsequently revealed the wisdom of the selection strategy applied. Another selection strategy would have likely facilitated less clear-cut biological findings. Consequently, we have learned that sampling strategies must be developed specifically for each study; and if faced with another large sediment survey, we would again recommend that a preliminary or reconnaissance level effort be undertaken to determine which sampling strategy best addressed the study goals within available resources.

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RECONNAISSANCE LEVEL ASSESSMENT OF SELECTED SEDIMENTS FROM PUGET SOUND

1.0 INTRODUCTION

Puget Sound is one of the most biologically productive and recreationally important fjord-like estuaries in the continental United States. Although most of Puget Sound is considered clean, healthy, and productive, serious water quality problems exist in certain areas, particularly in the more urbanized embayments. In addition, as the region continues to grow and develop, the potential cumulative effects of waste discharges on the environmental quality of Puget Sound as a whole become a concern. This type of cumulative effect markedly reduced the productivity of Chesapeake Bay during the 1960s. Major economic uses of Puget Sound, such as commercial and sport fisheries, tourism and recreation, depend on maintaining a high level of water quality. Accordingly, existing and potential water quality degradation represent a serious threat to these important uses of Puget Sound.

The eastern shore of Puget Sound from Tacoma to Everett is the most densely populated area of the Pacific Northwest. The waters of Puget Sound (see Figure 1) receive the wastes of approximately 2.5 million people, as well as wastes from the outfalls of numerous industrial facilities associated with pulp and paper, other forest products, food processing, metals and petroleum refining, and chemical manufacturing. In addition to these point sources of pollution, nonpoint sources of pollution diffuse into Puget Sound from groundwater, surface runoff from adjacent land areas, return flow from irrigated fields, and atmospheric fallout. These many sources of pollution to Puget Sound combine to alter water and sediment quality (Pavlou and Dexter 1979; Riley et al. 1980, 1981), impact or potentially impact biological communities (Bingham 1978; Swartz et al. 1982), and limit beneficial uses of surface waters (Malins

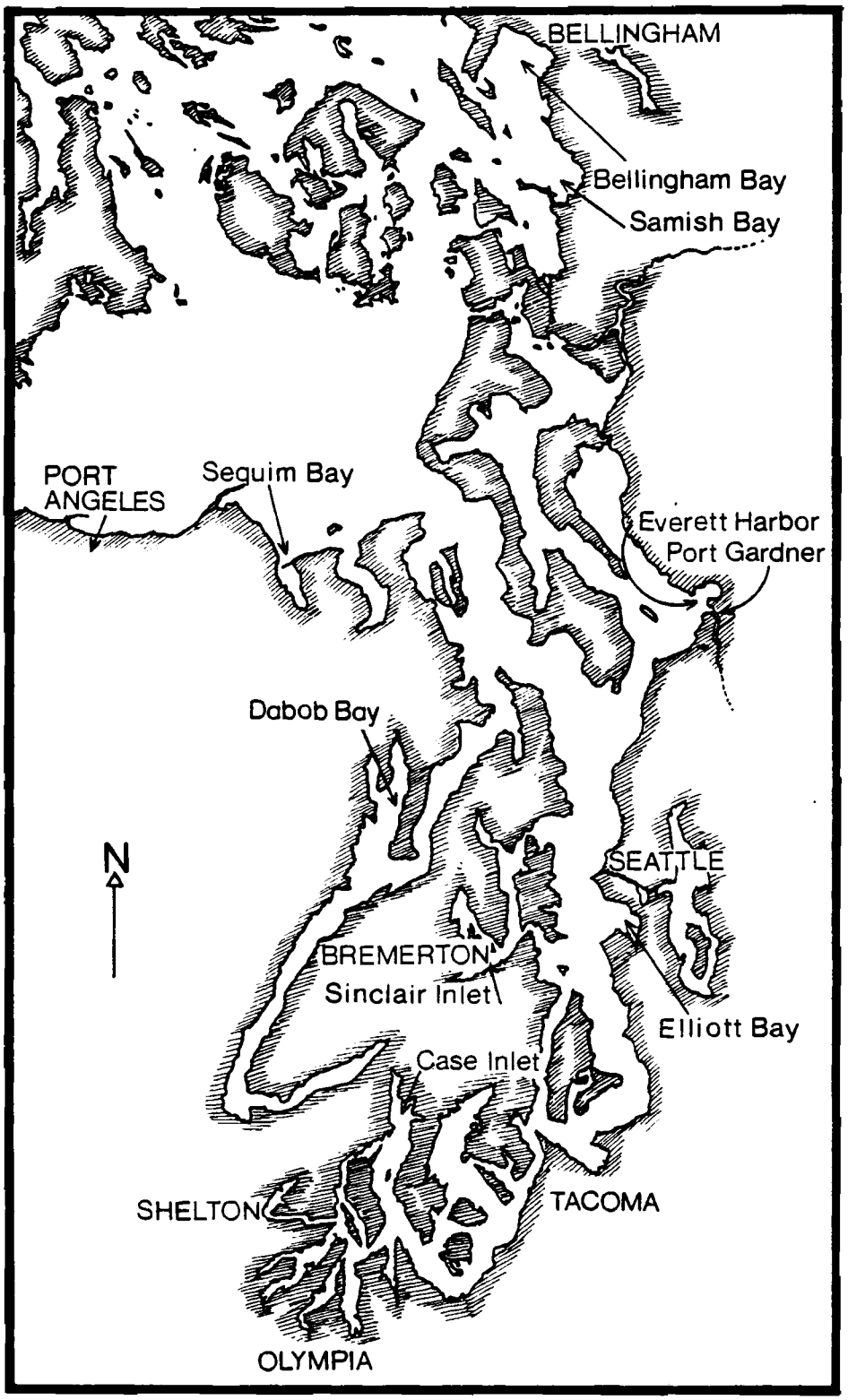

FIGURE 1. Puget Sound.

et al. 1980, 1982). These sources of pollution and concern for vulnerable resources emphasize the need to reevaluate the procedures in place for managing Puget Sound water quality.

The U.S. Environmental Protection Agency (EPA) and the Washington Department of Ecology (WDOE), as primary environmental regulatory agencies in the Puget Sound area, recognized this need and, in 1983, designed and implemented a program to focus attention and interagency resources on solving the most pressing Puget Sound water quality problems.

With the consent of WDOE, EPA contracted with Pacific Northwest Laboratory (PNL) on July 9, 1983, to conduct a study to better define the significance of pollution in certain urban-industrial bays in Puget Sound (Bellingham Bay, Port Gardner - Everett Harbor, Sinclair Inlet, and the Fourmile Rock - Elliott Bay dump site vicinity). The EPA and WDOE envisioned that this could best be achieved by first defining reference levels of contamination in several relatively undeveloped or baseline embayments in Puget Sound (Samish Bay, Case Inlet, Dabob Bay, Sequim Bay). At the same time, EPA and WDOE asked PNL to estimate the mass loading of contaminants to Port Gardner - Everett Harbor and to determine the rate of accumulation of contaminants in the local sediments so as to direct possible corrective actions (report submitted). Also, as part of this contract, PNL studied existing institutional arrangements and procedures for water quality management of Puget Sound (Morris et al. 1984).

1.1 OBJECTIVES AND SCOPE

The objective of this effort was to develop a better understanding of the toxic contamination problems in selected urban-industrialized bays of Puget Sound. Understanding these problems will aid in developing a basis for additional regulatory action.

Two major tasks comprised this effort. The first was to conduct preliminary or screening surveys and analyze sediment samples collected from 101 stations distributed in four urban-industrialized embayments (Bellingham Bay, Port Gardner - Everett Harbor, Fourmile Rock - Elliott

Bay dump site vicinity, and Sinclair Inlet) and at 80 stations distributed in four baseline embayments (Samish Bay, Case Inlet, Dabob Bay, Sequim Bay). The screening surveys were used as a guide for more detailed technical analyses. The second task involved detailed surveys and analyses of the same bays, but at a limited number of stations (32 in urban embayments, 16 in baseline bays). The 1984 sediment samples selected for detailed analyses were the "cleanest" of the clean and the "dirtiest" of the dirty as determined by the 1983 sediment chemical analyses, and within restrictions imposed by sediment type.

It should be understood that these studies represent a reconnaissance of the problem and are not always rigorously quantitative. Hypothesis testing was not always possible and selected statistical tests were exploratory and designed to detect patterns and trends in the data. The strength of our strategy was the conduct of several chemical analyses, biological analyses, and bioassays on the same fresh (unfrozen) sediment sample.

Based on the results of these surveys, this final report describes the sediment chemistry, sediment toxicity, numerically dominant benthic infauna, and incidence of fish and shellfish disease in each urban and baseline bay. Each biological parameter (benthic infauna, incidence of fish and shellfish disease, amphipod and oyster larval bioassay) is discussed in relation to the physical and chemical properties of associated sediments. Present research findings are also compared with those of historical surveys. Finally, the embayments and the stations in each embayment that currently show signs of degraded sediment quality are identified.

The EPA Region 10 Laboratory, the Northwest and Alaska Fisheries Center of NOAA, and the Marine Research Laboratory (MRL) of PNL participated in developing material included in this report. The EPA Region 10 Laboratory staff helped conduct screening surveys in the fall of 1983 and conducted the detailed surveys in the spring of 1984. They also performed priority pollutant analyses and amphipod bioassays. The NOAA Northwest and Alaska Fisheries Center conducted independent surveys in the same urban and baseline embayments to obtain fish and shellfish for

analyses of neoplasia and other diseases. The MRL helped conduct the screening surveys, performed range-finding chemistry for both the screening and detailed surveys, analyzed benthic infauna, and conducted oyster larval bioassays. With the exceptions of the NOAA neoplasia/disease and EPA amphipod studies, MRL also statistically treated and interpreted the data, and prepared the final report.

2.0 METHODS AND MATERIALS

2.1 FIELD COLLECTION PROCEDURES

This section describes the procedures used to conduct the screening and detailed surveys.

2.1.1 Screening Surveys

2.1.1.1 Sampling Areas and Locations

The locations of most sampling stations in urban bays were chosen on the basis of their proximity to point sources of pollutants, or on the basis of previously performed chemical analyses of bottom sediments from these areas. Other locations in urban bays were chosen on the basis of sediment type; that is, depositional sediments with a high mud to sand ratio were selected for sampling. Such depositional sediments often act as "sinks" for industrial chemicals (Wildish et al. 1980). Sampling stations in baseline bays were selected solely on the basis of sediment type. Again, sediments with a high mud to sand ratio were selected for sampling.

The locations of 101 sampling stations in the urban-industrialized bays of Puget Sound (Bellingham Bay, Port Gardner - Everett Harbor, Fourmile Rock - Elliott Bay dump site vicinity, and Sinclair Inlet) are shown in Figures 2-6. Figures 7-10 show the locations of the 80 sampling stations in the baseline bays (Samish Bay, Case Inlet, Dabob Bay, and Sequim Bay). Detailed descriptions (latitude, longitude, and depth) of each sampling station are given in Appendix A. Other stations sampled, in both urban and baseline bays, to determine incidence of fish and shellfish disease are described in Tables 1 and 2. An adequate sample size of fish or shellfish could not always be obtained at preselected sampling stations.

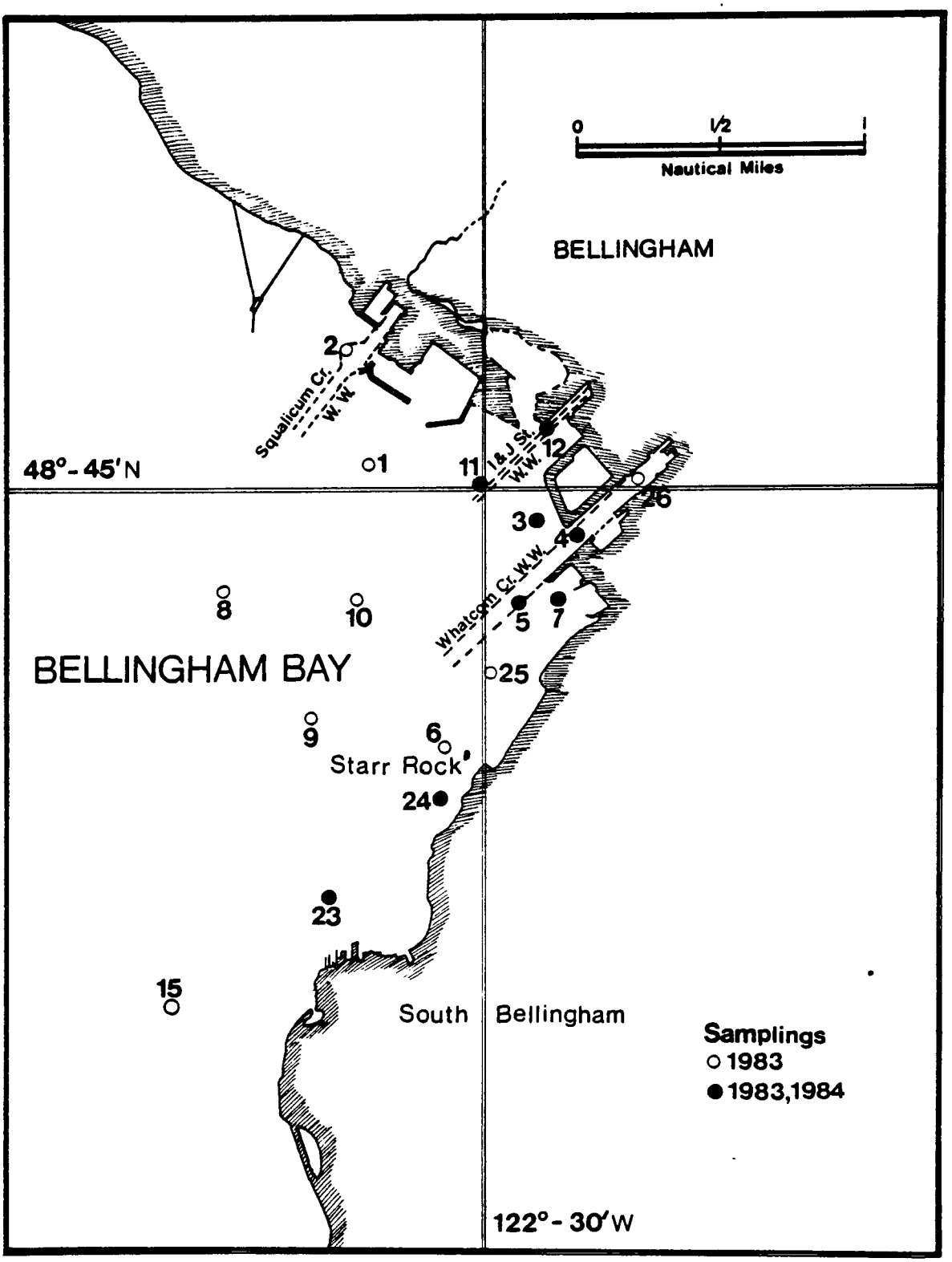

FIGURE 2. Bellingham Bay Sampling Stations (Inner Harbor)

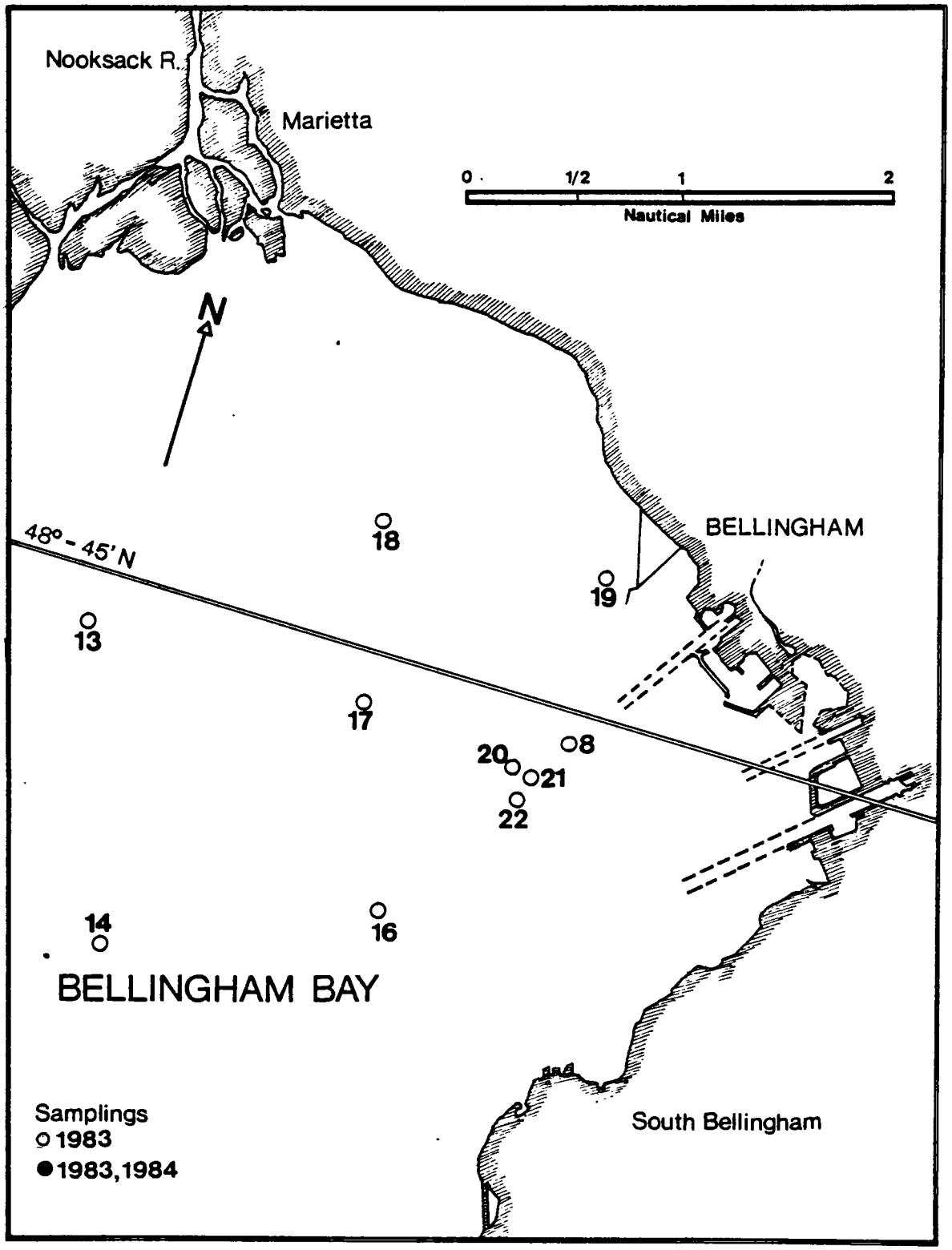

FIGURE 3. Bellingham Bay Sampling Stations (Outer Harbor)

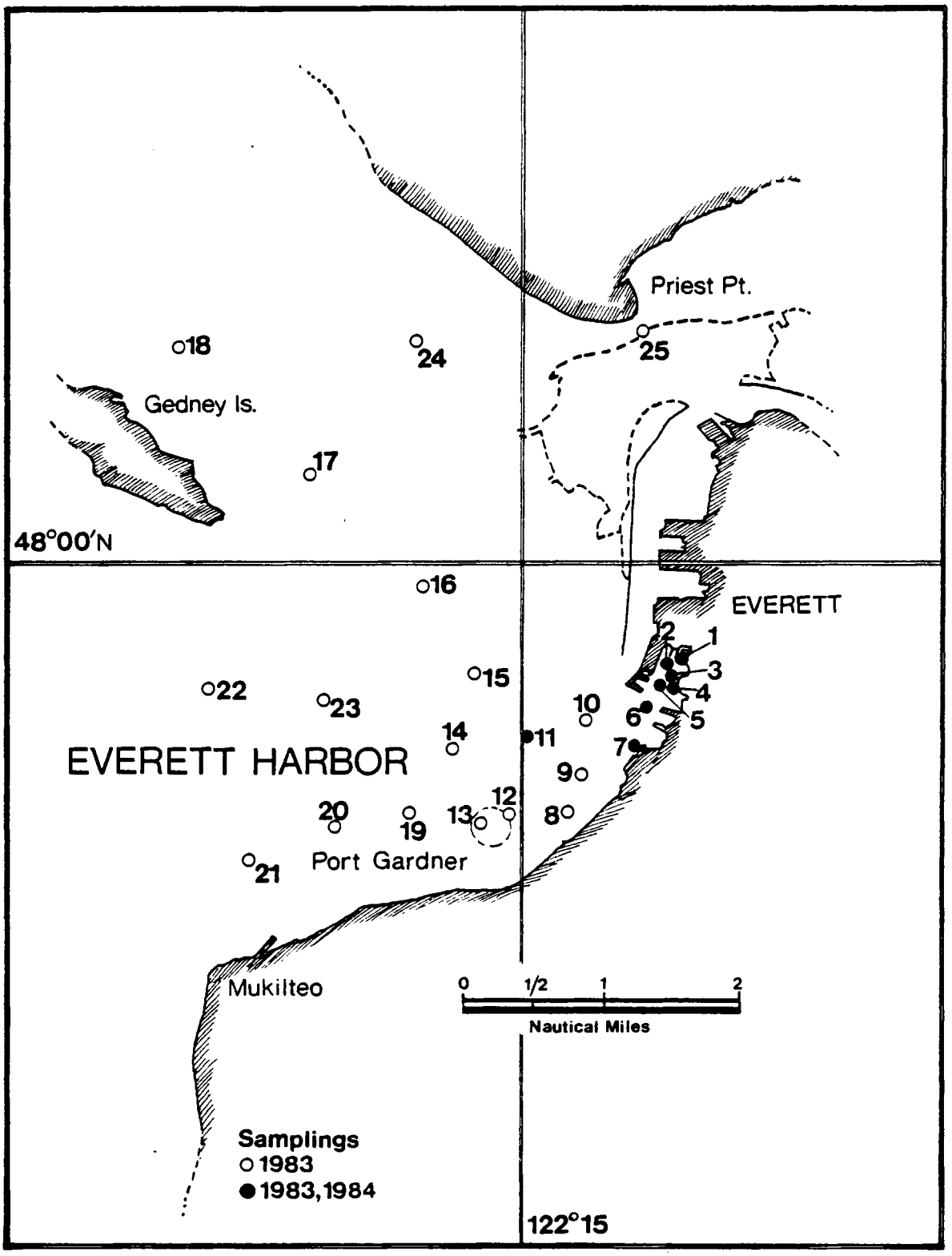

FIGURE 4. Port Gardner - Everett Harbor Sampling Stations

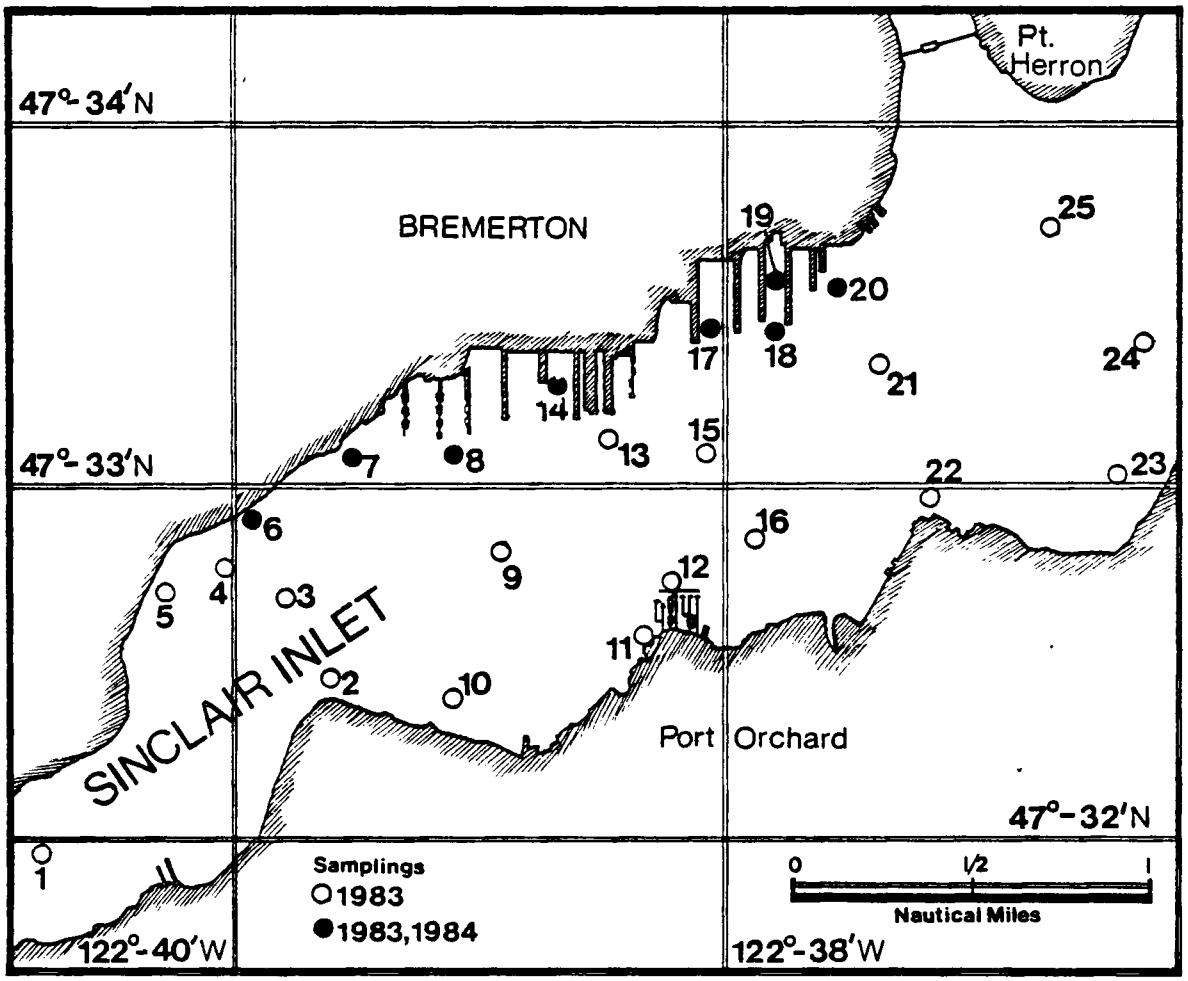

FIGURE 5. Sinclair Inlet Sampling Stations

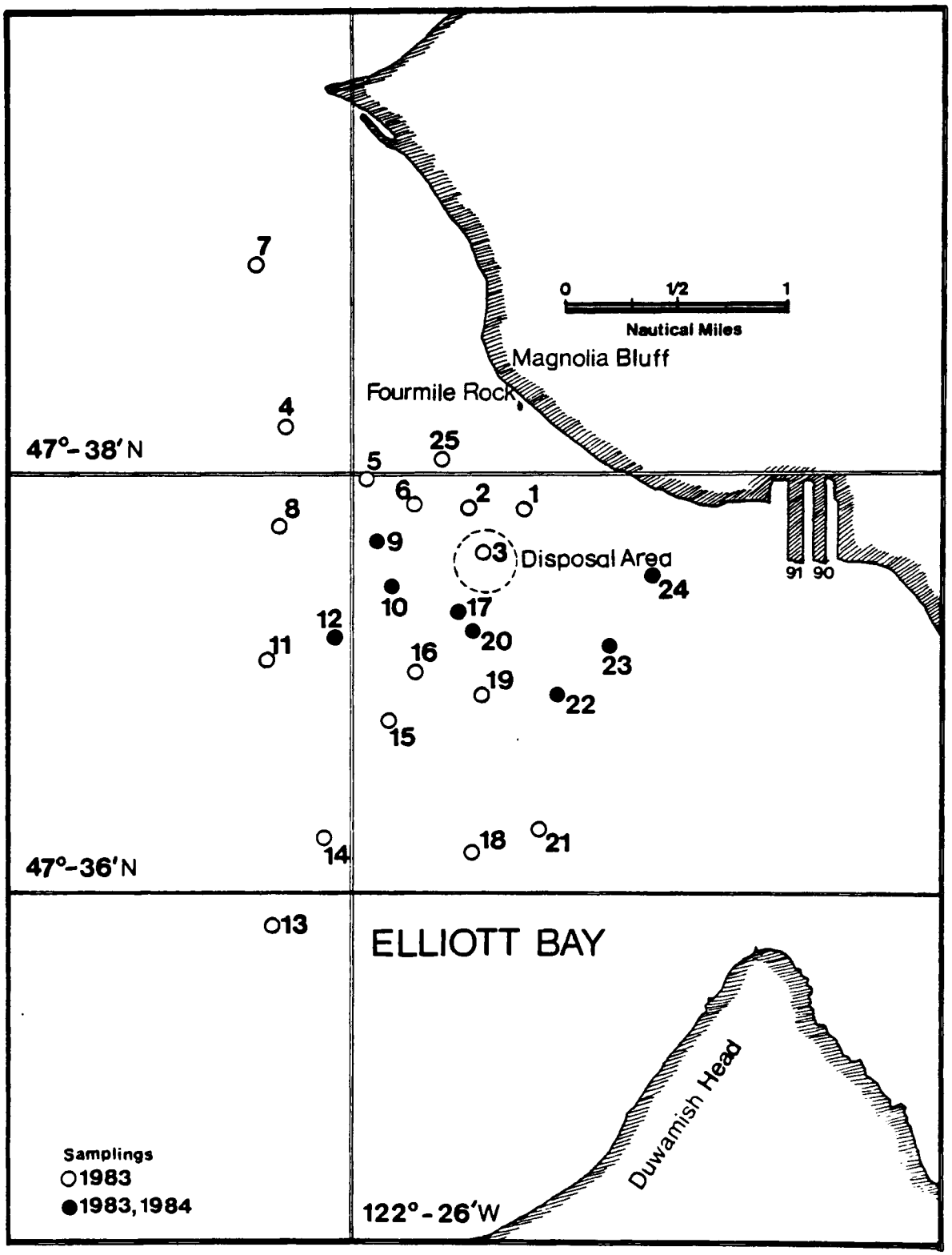

FIGURE 6. Fourmile Rock - Elliott Bay Dump Site Vicinity Sampling Stations

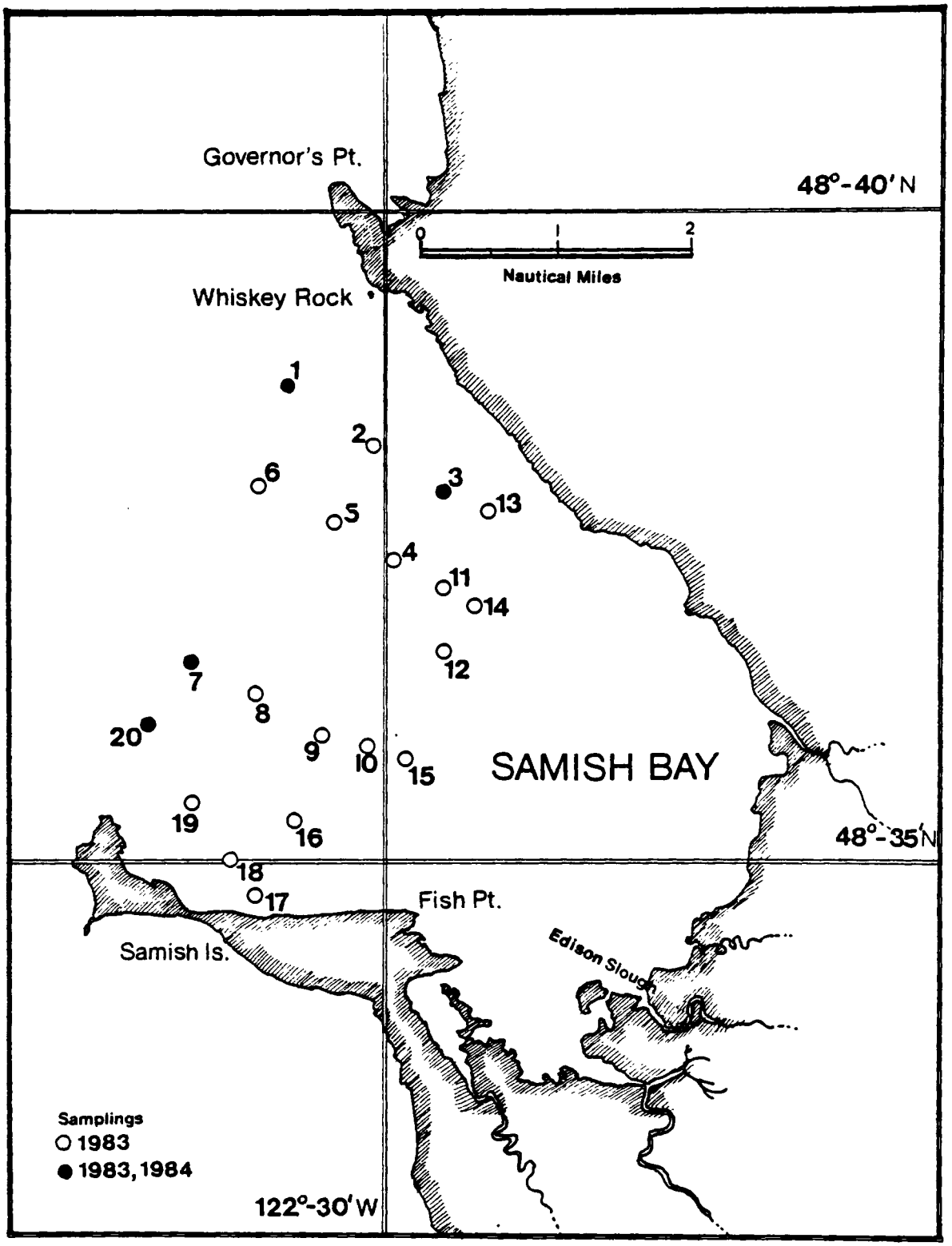

FIGURE 7. Samish Bay Sampling Stations

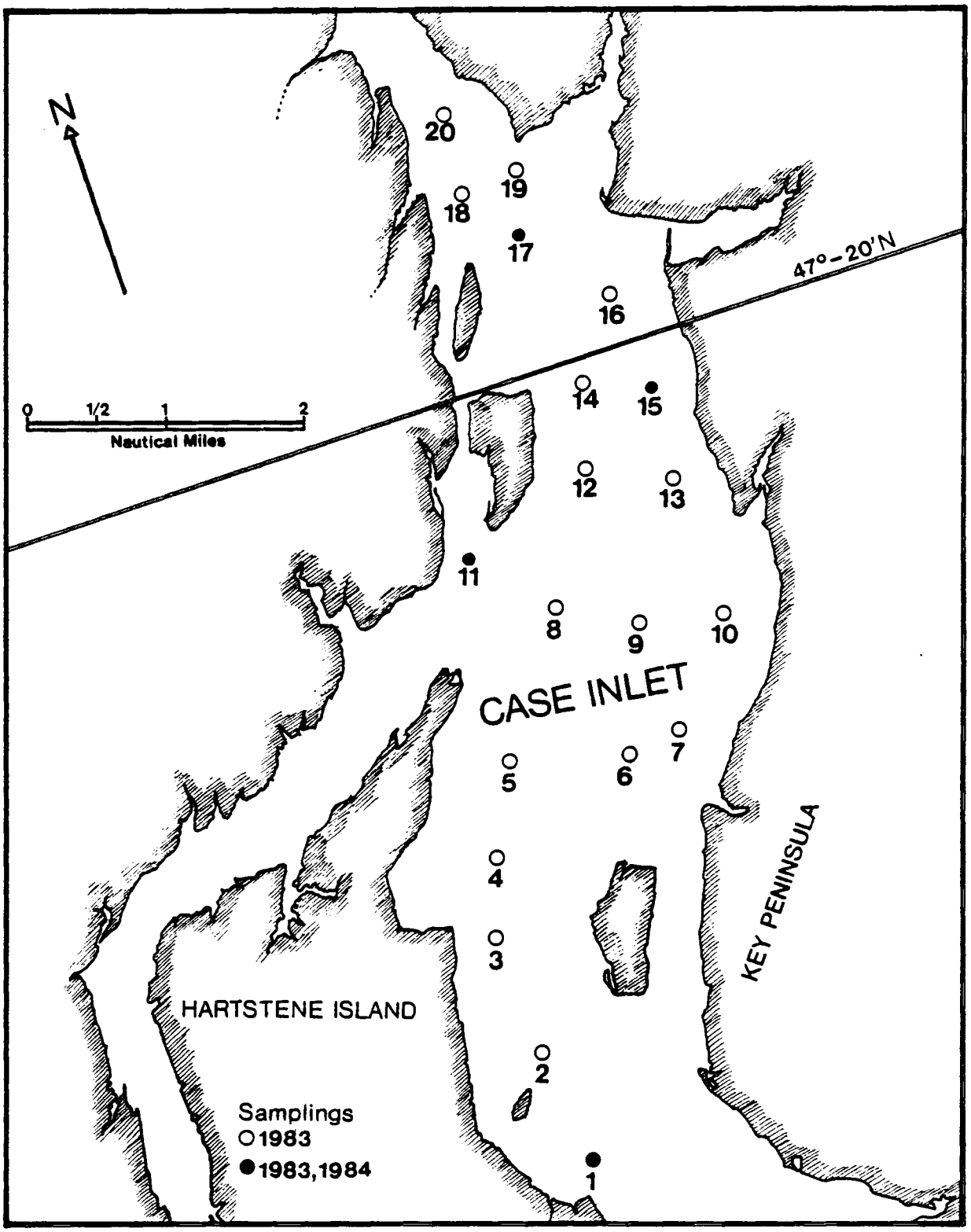

FIGURE 8. Case Inlet Sampling Stations

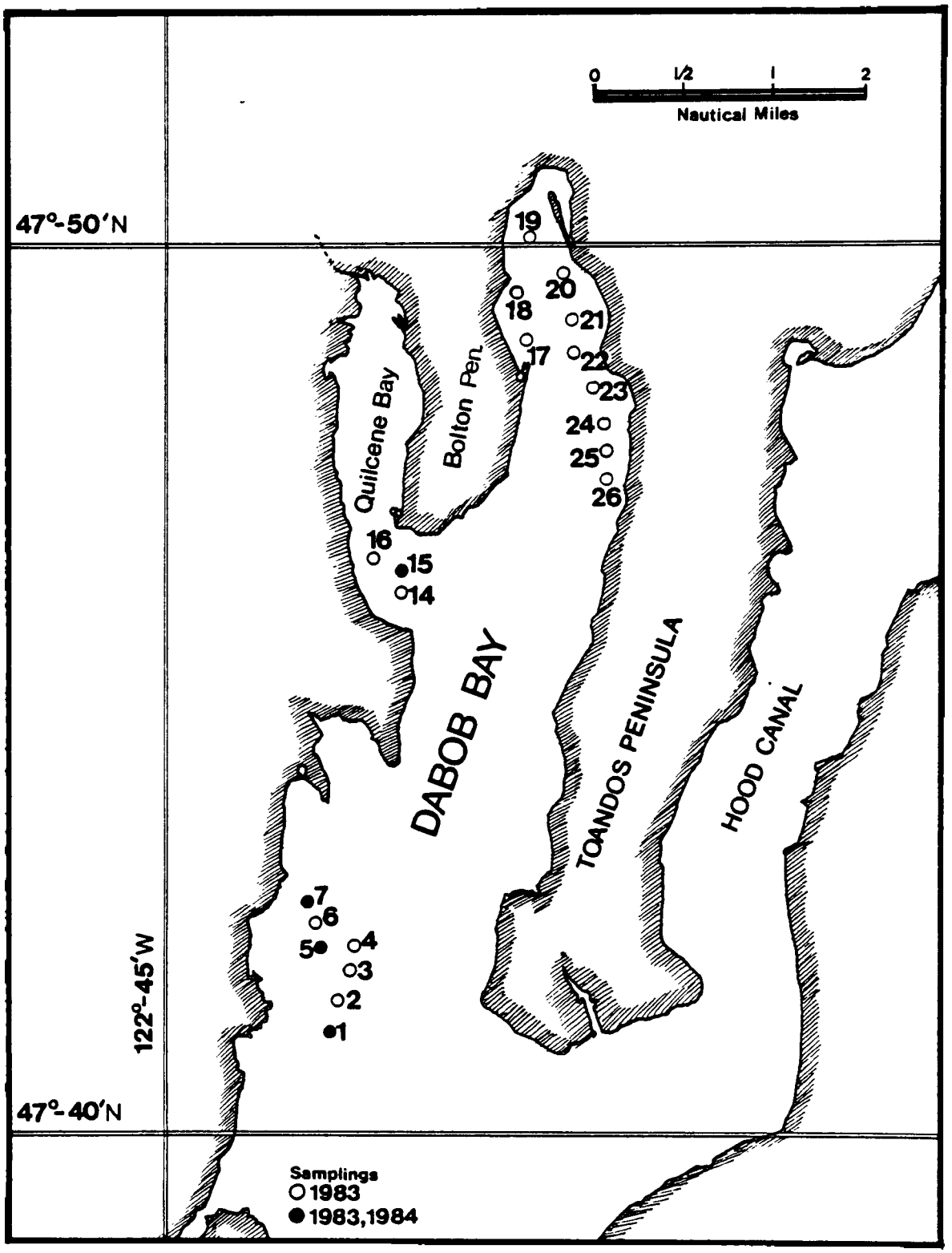

FIGURE 9. Dabob Bay Sampling Stations

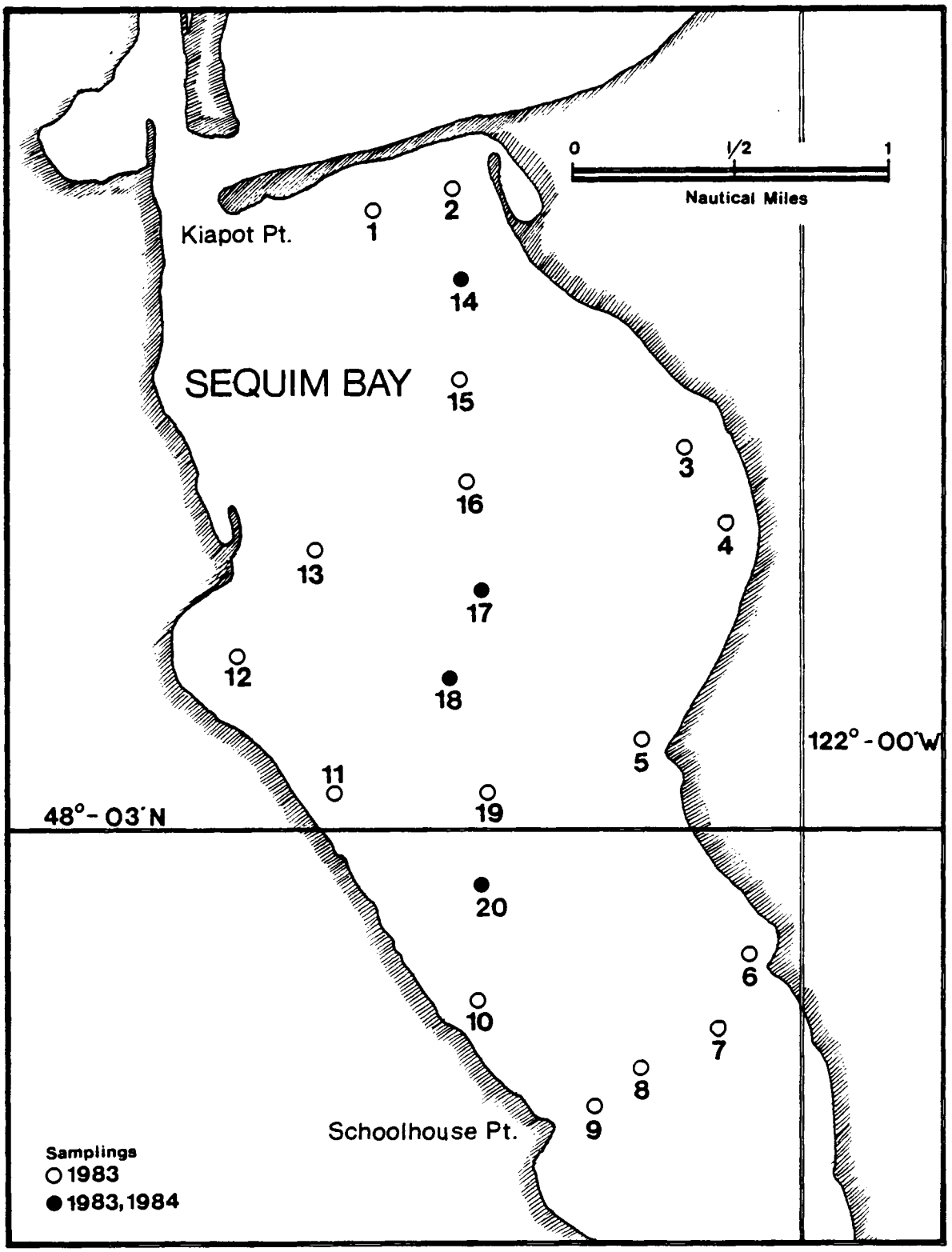

FIGURE 10. Sequim Bay Sampling Stations

TABLE 1. Locations of Stations at Which Target Fish Species Were Captured and Necropsied, March 1984

Area	Location	ID #	NODC[1] Station #	English Sole (*Parophrys vetulus*)	Dover Sole (*Microstomus pacificus*)
Case Inlet	7°17'20"N X 122°50'34"W	Case 5	12134	30	---
Bellingham-Samish Bays (Eliza Island)	48°17'20"N X 122°50'34"W	NA[2]	4015	28	---
Dabob Bay	47°46'21.0"N X 122°51'12.6"W	Dabob 15	7026	1	---
Sinclair Inlet	47°32'54.9"N X 122°39'56.9"W	Sinclair 6	8010	30	---
	47°33'27.1"N X 122°38'03.2"W	Sinclair 17	8009	7	---
	47°33'25.5"N X 122°37'50.4"W	Sinclair 18	8006	23	---
Elliott Bay[3]	47°33'25.5"N X 122°25'12.1"W	Elliott 17	10061	5	30
	47°37'10.8"N X 122°24'08.9"W	Elliott 23	10063	12	20
	47°40'00"N X 122°25'55"W	NA[2]	10065 (West Pt.)	2	30
	47°35'38"W X 122°23'51"W	NA[2]	10064 (Duwamish Head)	-	3
			Total Fish Examined	138	83

[1] National Ocean Data Center.
[2] NA - Not applicable.
[3] Dump site vicinity.

TABLE 2. Locations of Stations at which Target Crab and Shrimp Species were Captured and Necropsied, March 1984

Area	Location	ID #	Depth (ft)	NODC[1] Station #	*Cancer magister*	*Cancer gracilis*	*Pandalopsis dispar*	*Pandalus platyceros*
Case Inlet	47° 18' 38.0" N x 122° 50' 08.0" W x	Case 11	40	12137		23		
Bellingham Bay	48° 44' 39.0" N x 122° 29' 50.0" W	Bellingham 4-5	35	4205	30	4		
	48° 45' 06.2" N x 122° 29' 51.2" N	Bellingham 11-12	35	4210	30			
	48° 43' 32.8" N x 122° 29' 51.2" W	Bellingham 23-24	55	4027	17			
Samish Bay	48° 38' 29.0" N x 122° 30' 45.0" W	Samish 1	50	4250	30			
	48° 36' 00.0" N x 122° 32' 33.4" W	Samish 20	90	4036	30			
Sinclair Inlet	47° 33' 08.0" N x 122° 39' 28.0" W	Sinclair 7	40	8010		28		
	47° 33' 27.1" N x 122° 38' 03.2" W	Sinclair 17	35	8009		9		
	47° 33' 25.5" N x 122° 37' 50.4" W	Sinclair 18	50	8006		8		
	47° 33' 34.1" N x 122° 37' 47.9" W	Sinclair 19	35	8006		1		
	47° 33' 38.3" N x 122° 37' 27.1" W	Sinclair 20	50	8006				
Dungeness Spit	48° 10' 45.0" N x 122° 06' 50.0" W		40	2021	11			
Elliott Bay[2]	-47° 33' 25.5" N x 122° 06' 12.1" W	Elliott 17	500	10061			23	21
Dabob Bay	47° 41' 32.0" N x 122° 52' 33.0" W	Dabob 5	300	7024				34
	47° 46' 21.0" N x 122° 51' 12.6" W	Dabob 15	350	7026			30	

[1] National Ocean Data Center.
[2] Dump site vicinity.

2.1.1.2 Logistics and General Sampling Procedures

All 181 of the screening sediment samples were collected during an extended cruise over the period from August 2, 1983, through September 15, 1983. The research vessel, Cathlamet Bay, was leased by Battelle and manned by MRL and EPA staff. The EPA was responsible for operating the vessel and documenting sampling location. The MRL staff was responsible for sample acquisition and documentation.

Each sampling site was located using a precision navigational system (Mini-Ranger®), and was represented by a single composite sample collected and prepared on the research vessel. The samples were collected through multiple casts (2-5) with a modified 0.1-m^2 van Veen grab sampler to acquire a 26-L composite sample suitable for analysis. The following sampling procedure was used. The material from each cast was immediately examined visually while in the sampler. Samples not achieving a minimium penetration depth of 10 cm, containing boulders, or excessive amounts of gravel or foreign objects (tires, wood, metal) were rejected and the cast retaken. Acceptable casts were emptied into a sample compositing chamber (a schedule-316, stainless steel container with orifices for decanting). After the appropriate volume was collected and allowed to stand for 5 min, seawater was decanted from the sediment. The sediment was then mixed manually with a contaminant-free stainless steel paddle. The assumption that the sample was well mixed was validated by replicate analysis for selected subsamples, as follows: (1) three replicate subsamples for one sample from each bay for analysis for carbon tetrachloride (CCl_4) extractable organics, sediment grain size and percent volatiles; (2) three replicate subsamples for one sample from each urban bay for metals analysis.

To prevent contamination between samples, the van Veen grab sampler, the composite sample chamber, and mixing paddle were cleaned thoroughly after collection of each 26-L sample. The samples were maintained in the dark and on ice at a temperature of 4°C. As an additional quality control measure paper wipes were collected at the beginning of each day to

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ensure the cleanliness of the composite sampling chamber and sampling containers, respectively. Control of other potential sources of variation are addressed in the specific analytical protocols.

The subsample aliquots were placed into appropriately labeled containers on board the research vessel as a part of the sampling protocol. The containers were marked with a serial number representing a bay code number (1 through 8), a station code number (1 through 26) and a sample replicate code number (e.g., 1-2-3 where 1 = Bellingham Bay; 2 = Station No. 2 coordinates, 48° 45' 28.2"N, 122° 30' 47.0"W; and 3 = replicate number). During the course of the cruise day, the appropriate size aliquots were taken from the samples and the containers holding aliquots were sealed.

The EPA prepared and provided the appropriate containers for priority pollutant analyses and amphipod bioassay. All other containers were provided and prepared by the MRL.

2.1.1.3 Sample Custody

During the cruise day the samples were in custody of the MRL Senior Scientist on board the vessel. At the close of the cruise day, collection data were inscribed on each aliquot container, the subsamples were signed by the Collector, and a list of samples and subsamples taken were logged in MRL official laboratory record book(s) assigned to the project. The laboratory record book also provided a record of the disposition of the subsample. At this point, custody of subsamples for priority pollutant analysis and amphipod bioassay was given to EPA. The remaining subsamples were delivered to the MRL in Sequim, Washington. There, they were given to the appropriate Task Leaders who logged the receipt of these samples into the appropriate laboratory record books. As analyses were completed, the data pertaining to those samples were also logged into the Task Leaders' laboratory record books.

2.1.2 Detailed Surveys

2.1.2.1 Sampling Areas and Locations

Table 3 presents the 48 stations that were revisited and resampled for the detailed surveys. A protocol for choosing stations was developed that selected the most contaminated stations from the urban bays and the least contaminated stations from the baseline bays, based on the chemical data derived from the screening surveys. The procedures and results of applying this protocol to physical and chemical data generated during the screening surveys are presented in Appendix A.

TABLE 3. Stations Selected for Detailed Surveys

Station Numbers							
Bellingham	Elliott	Everett	Sinclair	Samish	Dabob	Sequim	Case
3	9	1	6	1	1	14	1
4	10	2	7	3	5	17	11
5	12	3	8	7	7	18	15
7	17	4	14	20	15	20	17
11	20	5	17				
12	22	6	18				
23	23	7	19				
24	24	11	20				

2.1.2.2 Logistics and General Sampling Procedures

All 48 samples from the detailed surveys were collected during the period from April 23 to May 29, 1984. The EPA furnished the vessel and conducted all sampling operations except those scheduled in Sequim Bay. Staff from the MRL were responsible for sampling Sequim Bay and used their own boat and gear.

Each sampling site was located with the precision navigational system (Mini-Ranger); or in Sequim Bay, by sightings with a hand-bearing compass. The samples were collected in two casts with a 0.1-m^2 van Veen grab sampler to obtain a 5-L composite sample. Excess seawater was then decanted. Care was exercised to ensure a minimum penetration depth of 10 cm was achieved and that the samples were free of boulders and foreign materials (tires, pieces of wood, rusted metal), and contained less than 20% gravel. Samples not achieving the minimum pentration depth, or containing foreign materials were rejected and the cast retaken. Rejection of the cast was appropriately documented. Cross contamination of samples was controlled as described in Section 2.1.1.2.

As each grab sample was brought aboard the research vessel, three (3) cores (5-cm diameter x 15-cm long) were taken from the undisturbed surface of the sample for benthic infaunal analyses. The cores were gently extruded into labeled sample jars containing buffered 10% formalin. Each jar was capped and shaken to ensure thorough mixing of the preservative with the sample.

After taking cores for benthic infaunal analyses, the remaining sediment in each of the two grab samples was removed in vertical sections from the top to the bottom of the sampler and placed into acid-cleaned, prelabeled, 1-gal glass carboys. During this step, care was taken not to collect any sediment that was in contact with the inside surfaces of the van Veen sampler. The carboys were capped with appropriate liner (aluminum or Teflon®), and iced. There was no mixing or further subsampling in the field.

At the EPA Region 10 Laboratory, each sample was emptied into a chemically cleaned glass aquarium and mixed manually for 20 min with an acid-cleaned, hard plastic spoon. After mixing, subsamples were taken for (1) priority pollutant analysis (minimum volume 1 L), (2) range-finding chemistry (minimum volume 0.5 L), (3) amphipod bioassay (minimum volume sample 3 L), and (4) oyster larvae bioassay (minimum volume sample 0.5 L). EPA staff performed both mixing and subsampling.

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For four (4) samples collected in Sequim Bay, MRL staff were responsible for transporting the samples to the Region 10 Laboratory for mixing and subsampling.

2.2 PHYSICAL AND CHEMICAL (RANGE FINDING) SEDIMENT ANALYSES (PERFORMED BY MRL)

2.2.1 Grain Size

Grain-size analysis was performed to estimate percent fractions for gravel ($\geq$2 mm), sand ($\leq$2 mm $\geq$62 μm), silt ($\leq$62 μm $\geq$4 μm) and clay ($\leq$4 μm). A wet weight sample of approximately 50 g was wet sieved through 2-mm and 62-μm screens. Material retained on the screens was dried (100°C for 24 h) and weighed to determine total sample weights of gravel and sand, respectively. Material washed into the pan was transferred to a 1-L graduated cylinder and distilled water was added to bring the total volume to 1 L. The graduated cylinder was stoppered, and the sample was mixed by inverting for 1 min and then allowed to stand for 29 sec. Ten-milliliter pipet samples were taken from 10 cm for silt plus clay and from 5 cm after 61 min and 29 sec for clay. These subsamples were dried (100°C for 24 h) and the residue weighed. The weight of the residue was multiplied by 100 to calculate the total weight of silt and clay in the sample. Percent contribution of each fraction was based on the aggregate weight of all fractions.

2.2.2 Total Extractable Hydrocarbons Determined by Infrared Spectrophotometry

2.2.2.1 Sampling

Twenty grams of sediment, 20 g of anhydrous sodium sulfate, and 25 mL of carbon tetrachloride (CCL_4) were placed into a labeled 250-mL bottle with a ground glass stopper. The bottle was placed on a shaker and

agitated overnight (16 h). The CCL_4 containing the extractable organic material received infrared analysis using a modification of the procedure described by Simard et al. (1951).

2.2.2.2 Analytical Procedures

Simard's procedure (Simard et al. 1951) was modified because sediments rather than water were extracted. Analyses were run on a Beckman[1] Acculab 4 spectrophotometer by comparing the amount of absorbance obtained at 2930 cm^{-1} to a calibration curve of Prudhoe Bay crude oil. Recoveries of total oil from CCL_4 extracts of oil-spiked sediments were found to be >90%. Results were reported as ppm dry weight.

2.2.2.3 Calibration Procedures and Frequency

The infrared (IR) spectrophotometer was calibrated weekly using a Beckman polystyrene wavelength/wavenumber calibrator for IR spectrophotometers, Part No. 80785; Polystyrene film versus air.

2.2.3 Water Content

The sediment samples were stirred to homogenize, and then 20 to 50 g were placed in a prepared 60-mL polystyrene bottle and weighed to the nearest 10 mg. The samples were frozen to -80°C and freeze-dried (10 millitorrs) to constant weight. Results were reported as percent water, which was calculated as follows.

$$\% \text{ water} = \frac{A-B}{A} \cdot 100$$

where A = wet weight
B = dry weight.

[1] Beckman Instruments, Inc., Berkeley, California.

2.2.4 Volatile Solids

Evaporating dishes were ignited for at least 60 min at a temperature of no less than 550°C, then cooled in a desiccator, weighed to the nearest 10 mg and kept in the desiccator until ready for use. Five- to fifteen- (5 to 15) gram aliquots of freeze-dried sediment were then placed in evaporating dishes and weighed to the nearest 10 mg to establish sample dry weights. The evaporating dishes and their contents were then ignited for 60 min at 550°C, again cooled in a desiccator and reweighed to the nearest 10 mg to establish the sample dry weights less the volatiles. Results were reported as percent volatiles, which was calculated as follows.

$$\% \text{ volatiles} = \frac{B-C}{B} \cdot 100$$

where B = dry weight
C = furnace weight

2.2.5 Total Organic Carbon

Total organic carbon was determined using a Leco[1] Model WR-12 carbon analyzer. An 150- to 250-mg aliquot of dried sediment sample was homogenized in a ceramic crucible. The sample was washed twice with 6N HCl to remove carbonate carbon. If the reaction was vigorous on the second wash, a third treatment with HCl was conducted. Following decarbonation, the sample was rinsed with distilled water until neutral and dried at 45°C. The carbon analyzer was calibrated daily with freshly prepared standards. Copper and zinc accelerators were added to the sample crucibles and combusted with an induction furnace. The CO_2 evolved was scrubbed of water, halide, and sulfur and the percent total organic carbon was calculated. Typically, two duplicates and one blank were run for every 25 samples.

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[1] Leco Corporation, St. Joseph, Missouri.

2.2.6 Metals Analyses

2.2.6.1 Sample Handling

Sediment samples analyzed for trace metals consisted of 30-g aliquots subsampled from well-homogenized primary samples. Aliquots were taken with a Teflon®-coated spatula and placed into acid-cleaned polystyrene jars. On return to the laboratory, the trace-metal aliquots were frozen at -80°C in the jars until analysis.

Before analysis, the samples were freeze-dried to constant weight with a Virtis[1] Freeze-Mobile 12, and then homogenized on a Spex[2] 8000 mixer-mill. All of the samples were handled in the containers that they were collected in to prevent contamination. This procedure has been shown to yield no contamination for any trace metal measured.

2.2.6.2 Sediment Digestion Procedures

Before analyzing by atomic absorption spectroscopy, the samples were digested in a 5:2:5 mixture of HNO_3, H_2SO_4, and H_2O (Bloom 1983). This procedure oxidized all organic matter and released all anthropogenic Pb, Hg, and Ag. All digestates were diluted to 1.0 g of sediment (dry weight) to 100 mL of solution. This dilution ensured greater reproducibility in graphite furnace analysis and eased data reduction.

2.2.6.3 Atomic Absorption Procedures: Lead and Silver

Both Pb and Ag were measured using a Zeeman graphite furnace atomic absorption spectrophotometer (GFZAAS). By using a state-of-the-art Perkin-Elmer[3] Zeeman background corrector and Model 5000

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[1] Virtis Co., Inc., Gardiner, New York.

[2] Spex Industries, Inc., Edison, New Jersey.

[3] Perkin-Elmer Corporation, Norwalk, Connecticut.

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spectrophotometer, accurate measurements were provided even in such complex matrices as digested sediments (Riley et al. 1980; 1981). The detection limits, based on twice the standard deviation of triplicate samples near the detection limit were as follows: Ag, 0.02 μg/g dry weight, and Pb, 0.5 μg/g dry weight. These limits were at least ten times lower than any values that were encountered in Puget Sound sediments.

2.2.6.4 Atomic Absorption Procedures: Mercury

Mercury was measured using the cold-vapor atomic absorption technique in conjunction with dual cold traps (Bloom and Crecelius 1983). A Laboratory Data Control[1] ultraviolet monitor with 30-cm cell was used to perform the analysis. With this technique, a detection limit of 0.005 μg/g dry weight was attained. This limit was ten times lower than any value measured in Puget Sound sediments.

2.2.6.5 Calibration Procedures and Frequency

All atomic absorption calibration was done by the method of standard additions according to manufacturer's instructions. A calibration curve was prepared each day and all samples were analyzed under conditions that gave a response in the linear range. Ten percent of the samples analyzed were standards in the linear range. Standards were prepared from commercially available (1000 mg/L) atomic absorption standards.

2.2.6.6 Detection Limits

Detection limits for metals in sediments were in accordance with EPA guidelines and are presented in Table 4.

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[1] LDC/Milton Roy, Sunnyvale, California.

TABLE 4. Detection Limits, Blanks, and Sediment Standards Analyzed by MRL (ppm dry weight)

Element	Detection Limits	Blanks	NBS 1646 MRL Value	Certified Value
Pb	0.02	1.0	22	28
Hg	0.005	0.01	0.074	0.063
Ag	0.02	0.02	0.072	none

2.2.6.7 Precision, Accuracy, and Completeness

The precision and accuracy of the methods were determined in accordance with EPA guidelines for assessing and reporting quality for environmental measurements. All other quality control (QC) data were recorded and maintained in MRL laboratory record books.

2.2.6.8 Standard Materials (Accuracy)

Five percent of the samples analyzed were a standard material to ensure the accuracy of the method. These materials included National Bureau of Standards (NBS) estuarine sediment, National Research Council of Canada (NRCC) estuarine sediment, and NBS air particulate matter. Measured values deviated from the established value by more than one standard deviation no more than 10% of the time. When this deviation occurred, the parameters were reexamined and the standards rerun. If the deviating value was repeated, in-depth investigation of the equipment and sample were undertaken.

Note: For lead, the analysis may have consistently reported NBS standard estuarine sediments 20% to 30% low because of the high crustal/anthropogenic ratio in these clean sediments; therefore, we also intercalibrated 5% of the enriched samples collected by nondestructive x-ray fluorescence spectroscopy. These values were within ±10% of the atomic absorption measurements.

2.2.6.9 Blanks

Ten percent of the samples analyzed were procedural blanks. Blanks for the three metals (Table 4) were consistently low and were routinely subtracted from the sample concentrations. These values were always recorded in MRL laboratory record books.

2.3 PRIORITY POLLUTANT SEDIMENT ANALYSES (PERFORMED BY EPA)

2.3.1 Procedures for Organic Compounds

Samples of thoroughly mixed wet sediments were dosed with surrogate standards and extracted with acetone using a Soxhlet extractor. The extract was concentrated, added to organic-free water, and extracted with methylene chloride. This extraction was performed three times at pH 6 to 8 and three times at pH 2. The combined extracts were dried, concentrated, and divided into equal portions for analysis of pesticide/chlorinated hydrocarbon and semivolatile compounds.

2.3.1.1 Acid/Base-Neutrals

The portion to be analyzed using gas chromatography-mass spectrometry (GC-MS) was further concentrated to 1.0 mL. The sample was analyzed with a Finnigan[1] 5100 and SuperIncos data system using a DB-5 fused silica column and splitless injection. The gas chromatograph (GC) was held at 35°C for 2 min during injection and then programmed to 50°C at 5°C per minute. After that, it was programmed from 50°C to 320°C at 8°C per minute. Total time for analysis was 45 min. The mass spectrophotometer scanned the mass range of 35 to 450 amu. The sample constituents were quantified using the internal standard method. Six internal standards were used for the entire range of the sample.

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[1] Finnigan MAT, San Jose, California.

The internal standards were d_4-1,4-dichlorobenzene, d_8-naphthalene, D_{10}-acenaphthalene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-benzo-a-pyrene. All except the last compound are listed in the Statement of Work (SOW) for Organics Analysis printed by the EPA Contract Laboratory Program. The procedure for using internal standards for quantification is described in the SOW and also in Method 625 of the Federal Register, Vol. 49, No. 209, October 26, 1984.

2.3.1.2 Pesticides/PCBs/PCBDs

The solvent for the pesticide, PCB (polychlorinated biphenyl) and PCBD (polychlorinated butadiene) determinations was changed from methylene chloride to petroleum ether. The sample volume was reduced to 1.0 mL by volatilization of the solvent with nitrogen gas, and then placed on a Florisil column. Two hundred milliliters of pentane were used to elute PCBs, PCBDs, and DDE. A 200-mL mixture of equal volumes of diethyl ether and petroleum ether was used to elute the remaining pesticides. The volume of each eluate was reduced to 1.0 mL.

The EPA Pesticides and Industrial Chemicals Repository at Research Triangle Park, North Carolina, is the source for all pesticide, PCB and PCBD standards used in this study.

The PCBs were identified and quantified based on the calculation of peak height of all major peaks (at least 6 to 8) and comparison to a PCB standard. When evidence indicated that erroneous data would result from the interference of extraneous compounds such as DDT analogs, only those peaks that were free of such interferences were used for quantification.

The Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples (EPA-600/8-80-038), 1980, Section 9 was used as the reference.

For the PCBD analysis, 5 μL of the pentane fraction were injected on a 3% OV-225 column at 40°C, isothermally. The detector used for

quantification was a Hall® 700A Microconductivity Detector. Confirmation was achieved using a 1% OV-1/2.4% OV-225 column at 40°C isothermally with a ^{63}Ni electron capture (EC) detector. Chromatography was completed in 15 min.

A qualitative standard containing PCBDs was obtained from NOAA. Peak identification/assignments were determined by GC-MS using a 3% OV-225 packed column which is the same column used for analysis with the Hall 700A microconductivity detector. The Hall detector was calibrated using hexachlorobutadiene and equivalent response factors were determined for trichloro-, tetrachloro-, and pentachlorobutadiene.

For the regular pesticide/PCB analysis, the primary column was a 6-ft x 2-mm packed column that contained 1.5% SP2250/1.95% SP3401 as the stationary phase. The secondary column contained 4% SE-30/6% OV-210. The temperature of both columns was 200°C. Chromatography was completed in 30 min. The primary detector used was a ^{63}Ni EC detector, and the confirming detector was a ^{3}H EC detector. The GC used was a Tracor[1] Model 222. Three levels of standards were injected daily before samples were analyzed. Percent recoveries of o, p'-DDE, the surrogate spike, were calculated, but the results were not adjusted by these recoveries.

2.3.1.3 Gas Chromatography/Mass Spectrometry Analysis-Volatile Organic Analysis (VOA)

Approximately 2 g of sediment were added to a 16-mm x 125-mm screw-top culture tube that had previously been cleaned in a muffle furnace at 410°C. The tube was sealed with a screw cap containing a Teflon liner, and stored at 4°C until analysis. Just before purging, 5 mL of organic-free water, containing the internal and surrogate standards, were added to the sample in the culture tube. The volatile organic analyses were spiked with 50 ng of each compound, except for acrylonitrile, which was spiked with 100 ng. The tube was connected to a Tekmar[2] LSC-3 purge

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® Hall is a registered trademark of Tracor Inc., Austin, Texas.

[1] Tracor, Inc., Austin, Texas.

[2] Tekmar Company, Cincinnati, Ohio.

and trap instrument and sparged onto a trap containing one-half Tenax and one-half coconut charcoal in series. The volatile compounds were desorbed for 4 min at 180°C into a DB-5, fused silica, capillary column (1.0-μ/m film thickness, 30-m long). The flow was split in a 1:10 ratio. A loop of the column at the front portion was immersed in a thick slurry of solid powdered carbon dioxide and methanol at least 1 min before desorption, and kept there for the entire desorption period. The GC-MS was initiated at the same time as desorption. The CO_2/methanol cold trap was removed immediately after desorption and the GC oven kept at 30°C for an additional 6 min. The oven was then programmed to increase from 30°C to 120°C at a rate of 6°C per min and held for 2 min or until the final compound, 1,4-bromofluorobenzene, had eluted. The analysis was performed on a Finnigan GC-MS 3200 with an INCOS data system.

2.3.2 Procedures for Metals

The sediment samples were dried at 105°C, pulverized and screened through No. 20 mesh or equivalent nylon screen. One gram of sample was digested with a mixture of nitric acid and hydrogen peroxide. The sample was filtered through a 0.8-μm membrane filter, and the volume was adjusted to 100 mL.

The metals were determined by atomic absorption spectrophotometry using a Zeeman flame furnace following EPA methods. Mercury was determined on a wet sample by the manual cold vapor technique according to EPA Method 245.5. Wet weight values were transformed into dry weight values on the basis of percent solids data.

The mean concentrations of Ag, Hg, and Pb in sediments collected in 1983 and 1984 were determined by MRL. The concentrations of these same metals were also determined by the EPA Region 10 Laboratory and both sets of results are presented in Appendix B (Tables B-9 to B-16). For the most part, results from the two laboratories compare well; however, a few discrepancies exist (e.g., Hg at Fourmile Rock - Elliott Bay dump site vicinity; Ag in Sinclair Inlet). Unfortunately, these few discrepancies cannot be resolved because the EPA Laboratory did not analyze standard reference sediments.

2.3.3 Procedures for Solids (Percent)

Samples were dried at 103°C to 105°C according to EPA methods.

2.3.4 Procedures for Phenolics and Cyanides

Phenolics and cyanides were distilled and the distillate analyzed by automated spectrophotometry following EPA methods.

2.3.5 Detection and Quantitation Limits

The limit of detection of a compound is defined as an analyte signal, three standard deviations above the peak-to-peak noise level of the instrument in the region of the expected analyte peak. Any signal not exceeding this minimum response would be judged as not indicating the presence of the analyte. The limit of quantitation is defined as the analyte signal that is 10 standard deviations above the noise level of the instrument. Between these two limits is a region of detection in which a compound can be identified, but where the concentration is too low to allow an accurate estimate of its value.

The reviewer should refer to "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry," Anal. Chem. 52, p. 2241 (1980).

In Appendix B (Tables B-17 to B-26), when the symbol "μ" is used, the value preceding it is the quantitation limit for the particular substance. It means that the concentration, if any, falls below the detection limit. The symbol "m" indicates that the concentration of the substance falls between its detection limit and its quantitation limit.

The quantitation limits (QL) of the acid/base-neutral compounds are given as a range. The quantitation limits are not all the same because the samples had a varying percent solids value. A high percent solids means a lower quantitation limit and vice versa. Also the ranges are not all alike because some compounds are more sensitive to detection than others. Most of the VOA compounds had a similar quantitation limit, so that an average was given for them. The range of the QL for all but acrolein and acrylonitrile was 4.1 to 18 μg/kg, dry weight. The

pesticides were also affected by the percent solids value, but the variation was always between 0.3 and 1.0 μg/kg. Therefore, their QL is reported to the nearest whole number.

The quantitation limits of all target organic compounds are listed in Table 5. The quantitation limits of the VOA compounds are generally lower than the acid/base-neutral compounds because the purge and trap procedure eliminates many matrix problems. Detection limits for metals are reported in Table 6.

2.3.6 Precision, Accuracy, and Completeness

The accuracy of the methods was determined in general accordance to the EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories (1979). The average recovery and standard deviation (σ) for many spiked samples was determined, and the control limits were calculated to be $\pm 3\sigma$ about the mean. The Statement of Work for Organics Analysis for the EPA Contract Laboratory Program (CLP) (Rev. Jan. 1985) outlines a detailed QA procedure, but unfortunately, it was not available at the time this survey was performed. For the purpose of comparison of QC limits, CLP-suggested spiking compounds that were used for the QA of this study will be examined more closely.

2.3.6.1 Organic Parameters

The quality of the Puget Sound sediment data was determined with the use of quality control and surrogate spiking compounds. Table 7 summarizes the scope of the QC work.

2.3.6.2 VOA Fraction

The surrogate spiking compounds used were 1,2-dichloroethene-d_4, d_6-benzene, d_8-toluene, and 1,4-bromofluorobenzene. The average recovery of each surrogate compound is presented in Table 8.

TABLE 5. Average Quantitation Limits of Organic Compounds Analyzed in Puget Sound Sediment Samples[1]

Organochlorine Compounds (ppb)

Aldrin	1	Endrin	1	Hexachlorobenzene	1
Chlordane	1	Endrin Aldehyde	1	Polychlorinated Butadiens	2
Dieldrin	1	Heptachlor	1	PCB 1016	20
4,4'-DDT	1	Heptachlor Epoxide	1	PCB 1221	20
4,4'-DDE	1	Alpha BHC	1	PBC 1232	20
4,4'-DDD	1	Beta BHC	1	PCB 1242	20
Alpha Endosulfan	1	Lindane	1	PCB 1248	20
Beta Endosulfan	1	Delta BHC	1	PCB 1254	20
Endosulfan Sulfate	1	Toxaphene	30	PCB 1260	20

Base Neutral Compounds (ppb)

Acenaphthene	40-200	4-Chlorophenylphenylether	100-250	Diethylphthalate	40-200
1,2,4-Trichlorobenzene	80-200	4-Bromophenylphenylether	200-500	Dimethylphthalate	40-200
Hexachlorobenzene	400-1000	bis(2-Chloroisopropyl)ether	40-200	Benzo(a)anthracene	40-100
Hexachloroethane	100-250	bis(2-Chloroethoxy)methane	40-200	Benzo(a)pyrene	100-250
bis (2-Chloroethyl)ether	40-200	Hexachlorobutadiene	160-400	Benzo(k)fluoranthene	40-100
2-Chloronaphthalene	40-200	Hexachlorocylopentadiene	600-1500	and/or Benzo(b)	
1,2-Dichlorobenzene	40-200	Isophorone	40-200	Chrysene	40-100
1,3-Dichlorobenzene	40-200	Naphthalene	40-200	Acenaphthylene	40-200
1,4-Dichlorobenzene	40-200	Nitrobenzene	40-200	Anthracene	40-200
2,4-Dinitrotoluene	100-500	N-Nitrosodiphenylamine	1000-2500	Benzo(ghi)perylene	200-800
2,6-Dinitrotoluene	100-500	N-Nitrosodi-n-propylamine	600-1500	Fluorene	40-200
1,2-Diphenylhydrazine	100-250	bis(2-Ethylhexyl)phthalate	100-1100	Phenanthrene	40-100
(as azo benzene)		Butyl benzylphthalate	40-200	Dibenzo(a,h)anthracene	200-800
Fluoranthene	40-250	Di-n-butylphthalate	40-200	Indeno (1,2,3-c,d)pyrene	200-800
		Benzidine	1000-2500	Pyrene	40-100
		3,3'-Dichlorobenzidine	100-500	Benzyl Alcohol	160-400
		Dibenzofuran	40-100	2-Methylnaphthalene	40-200

[1] Dry weight basis.

TABLE 5. Average Quantitation Limits of Organic Compounds Analyzed in Puget Sound Sediment Samples (Continued)

Acid Compounds (ppb)

2,4,6-Trichlorophenol	200-500	2,4-Dimethylphenol	100-250	4,6-Dinitro-o-cresol	1000-2500
p-Chloro-m-cresol	100-250	2-Nitrophenol	200-500	Pentachlorophenol	600-1500
2-Chlorophenol	40-200	4-Nitrophenol	600-1500	Phenol	40-200
2,4-Dichlorophenol	200-500	2,4-Dinitrophenol	1000-2500	Benzoic Acid	600-1500
		2-Methylphenol	100-250	4-Methylphenol	100-250
		2,4,5-Trichlorophenol	200-500		

VOA Compounds (ppb)

Acrolein	240	Chloroethane	12	Methyl Bromide	12
Acrylonitrile	120	Chloroform	12	Bromoform	12
Benzene	12	1,1-Dichloroethylene	12	Bromodichloromethane	12
Carbon Tetrachloride	12	1,2-trans-Dichloroethylene	12	Trichlorofluoromethane	12
Chlorobenzene	12	1,2-Dichloropropane	12	Dibromochloromethane	12
1,2-Dichloroethane	12	cis-1,3-Dichloropropene	12	Tetrachloroethylene	12
1,1,1-Trichloroethane	12	trans-1,3-Dichloropropene	12	Toluene	12
1,1-Dichloroethane	12	Ethylbenzene	12	Trichloroethylene	12
1,1,2-Trichloroethane	12	Methylene Chloride	12	Vinyl Chloride	12
1,1,2,2-Tetrachloroethane	12	Methyl Chloride	12	2-Chloroethyl Vinyl Ether	12
Acetone	12	2-Butanone	12	2-Hexanone	12
4-Methyl-2-Pentanone	12	Carbon Disulfide	12	Styrene	12
o-Xylene	12				

TABLE 6. Detection Limits of Metals in Sediments (1.0 g/100 mL)

Element	Detection Limit ppm (dry weight)
Antimony	0.1
Arsenic	0.1
Beryllium	0.02
Cadmium	0.02
Chromium	0.05
Cobalt	0.05
Copper	0.05
Lead	0.02
Mercury	0.001
Nickel	0.05
Selenium	0.1
Silver	0.01
Thallium	0.1
Zinc	0.2

TABLE 7. Quality Control Samples Analyzed

Fraction	Samples Analyzed	Blanks	Duplicates	Spikes	Duplicate Spikes
Acid/Base-Neutral	48	4	1	5	4
VOA	48	13	0	5	5
Pesticide/PCB	48	4	1	3	3

TABLE 8. Average Percent Recovery of Surrogate Compounds of VOA Fraction

Type	1,2-Dichloro-ethene-d_4	d_6-Benzene	d_8-Toluene	1,4-Bromo-fluorobenzene
Sample	102.7 ± 13.0	97.1 ± 13.4	100.0 ± 15.5	73.0 ± 15.0
Blanks	96.5 ± 13.6	100.8 ± 10.5	102.8 ± 11.2	95.0 ± 13.4
Matrix Spikes	105.3 ± 22.3	101.8 ± 5.1	99.4 ± 7.0	76.0 ± 11.3

The internal surrogate standards were added to 5 mL of organic-free water, which was then added to the sediment sample just before sparging in the gas purge device.

All of the VOA compounds were in the matrix and duplicate matrix spikes. They were added to the organic-free water that was mixed with sediment before sparging. Table 9 lists the total recovery for all compounds as well as the five VOA compounds recommended by CLP, and the Region 10 QC limits.

Table 9 shows that all but one of the recovery data for all compounds and the five CLP compounds were within the Region 10 control limits. Only two compounds were not within CLP control limits, but they were for different compounds in different samples.

The number of duplicate spikes was insufficient to prepare a Region 10 control chart for precision. However, the relative percent difference (RPD) of two recovery values from the CLP could be used to obtain an approximate measure of precision. The RPD is calculated as follows:

$$RPD = \frac{D_1 - D_2}{(D_1 + D_2)/2} \times 100$$

where D_1 = first spike value
and D_2 = second spike value (duplicate).

TABLE 9. Percent Recovery of VOA Spikes

Sample No.	Average Recovery Matrix Spike	CLP-Suggested Compound Recovery				
		1,1-Dichloro-ethene	Trichloro-ethene	Chloro-benzene	Toluene	Benzene
17008MS	92.8	69	95	89	138	107
17008MSD	96.5	89	95	87	162	109
19058MS	114.4	137	92	84	92	100
19058MSD	105.4	107	96	90	97	104
20057MS	101.7	88	104	80	104	104
20057MSD	114.5	103	94	82	100	101
21057MS	122.6	139	121	88	116	125
21057MSD	125.5	145	132	95	124	140
22058MS	109.9	57	112	83	101	106
22058MSD	132.3	82	121	103	122	122
CLP Recovery QC Limits	*	59-172	62-137	60-133	59-139	66-142
Region 10 Recovery QC Limits	67-151	13-178	70-136	50-127	48-159	53-163

*CLP does not give control limits for the above parameter.

The precision of the spikes is shown in Table 10.

TABLE 10. Precision of VOA Spikes (RPD)

Sample No.	1,1-Dichloroethene	Trichloroethene	Chlorobenzene	Toluene	Benzene
17008	25	0	2	16	2
19058	24	4	7	5	0
20057	16	10	2	4	3
21057	4	9	8	7	11
22058	52	8	21	19	14
CLP RPD QC Limits	22	24	21	21	21

From Table 10, values for 1,1-dichloroethene seem to be high, but the other compounds indicate that the precision of the spikes appear to be valid, and, except for one point, the dichloroethene problem is not too severe.

The VOA blanks were generally free of halogenated compounds. The only compounds that appeared regularly were acetone and methylene chloride. Acetone concentrations were generally 10 μg/kg or less, and the CH_2Cl_2 concentrations were 1 μg/kg or less. Other compounds present were 2-butanone, 2-hexanone, 4-methyl-2-pentanone, toluene, ethylbenzene, and ortho-xylene. These compounds were not present in more than 40% of the blanks and concentrations were 20 μg/kg or less.

2.3.6.3 Acid/Base-Neutral Fraction

Table 11 gives the results of the average recoveries and percent relative standard deviations (% RSD) of the surrogate standards in all of the samples, blanks, and spikes.

TABLE 11. Average Recovery of Acid/Base-Neutral Surrogate Standards

	d_5-Phenol	Pentafluorophenol	d_5-Nitrobenzene	d_{10}-Pyrene
Mean Percent Recovery	77.7%	48.9%	63.0%	43.9%
σ	± 26.8%	± 28.4%	± 23.2%	± 14.9%
Percent RSD	22%	59%	38%	34%

Table 12 lists the recovery of the acid and base-neutral compounds in the matrix spikes. The samples were spiked with all 11 acid compounds, but some of the base-neutral spiking solutions were depleted during the sampling and analysis period, and could not be replaced. At that time, the EPA Laboratory was also in the process of switching to new spiking standards. Ten acid and base-neutral spiking compounds that were available for the whole year are presented in the table. The average

TABLE 12. Matrix Spike Percent Recovery of the Acid/Base-Neutral Fraction

Matrix Spike Compound	17008Y	17008YJ	18050Y	18050YJ	19059Y	20059Y	20059YJ	22051Y	22051YJ	Region 10 Control Limits	CLP Control Limits
Average recovery Acid & B/N cmpds.	137	120	56	56	89	59	74	86	87	0-180	1
Average recovery Acid compounds	120	120	66	59	97	54	76	90	91	7-157	1
4-Chloro-3-Methylphenol	128	118	94	81	141	71	84	82	88	0-218	26-103
2-Chlorophenol	99	102	34	8	76	78	87	78	81	0-171	25-102
4-Nitrophenol	470	430	0	155	119	0	93	183	247	0-456	11-114
Pentachlorophenol	0	15	59	72	22	65	62	39	29	0-119	17-109
Phenol	102	99	57	31	77	88	85	75	72	0-192	26-90
1,2,4-Trichlorobenzene	99	103	41	0	99	106	108	105	104	0-190	38-107
1,4-Dichlorobenzene	56	73	0	0	51	65	63	60	61	0-134	28-104
Hexachlorobutadiene	46	78	12	0	50	49	47	60	62	0-141	1
Nitrobenzene	78	54	22	0	74	77	77	86	90	0-167	1
Diethyl phthalate	80	58	73	61	82	57	57	79	87	0-159	1

[1] CLP does not give control limits for these parameters or compounds.

recovery of all compounds, and the acid compounds only, in each matrix spike is presented in the table. The average recoveries for all compounds are not an accurate indicator of precision, because the number and kinds of base-neutral compounds varied over the analysis period. The acid compound recoveries accurately represent the spiking of each matrix spike.

The Region 10 Laboratory control limits were ± 3σ about the mean recovery. The control limits are not symetrical about the mean because, most of the time, the value of 3σ exceeded the mean. Therefore, the lowest recovery limit used was 0%.

When the Region 10 control limits are used, 10 values out of 90, or 11% of the values, are at, or beyond, the limits, including 8 compounds that had 0% recovery. When the CLP control limits are applied, 21 out of the same 90 values, or 23% of the values, are beyond the CLP limits. Two reasons could explain why more sample values are beyond the CLP limits. First, the CLP range is narrower than the Region 10 range, indicating that perhaps a larger number of values were used when calculating the average or the values used were more precise. Second, the CLP values indicate low average recoveries. The largest recovery possible for the acid compounds that still is within the limits is 114%. Some of the spike values were not unreasonably higher than 100%, but were still out of the CLP range. The compound that gave the most difficulty was 4-nitrophenol. The Region 10 control limits for this compound are unrealistically large, as are some of the listed recoveries. The extraction procedures should be closely followed to be sure that they are not the cause of the variation. Matrix spike duplicate 18050YJ had four values each beyond Region 10 and CLP limits, including four base-neutral values that were zero. Its duplicate had values that were better, so the problem probably was not due to matrix effects.

The number of duplicate spike pairs was insufficient to calculate Region 10 RPD limits for precision. The CLP limits were used as indicators of precision, and the results obtained are given in Table 13. The samples that show an RPD of 200 are those in which one of the duplicate recovery values is zero. Nitrophenol, again, appears to be a recurring problem, and the spikes of 18050 also appear to be

unsatisfactory. The zero RPD for 1,4-dichlorobenzene for 18050 is not meaningful, because the percent recovery for this compound in both duplicates is zero. A total of 7 RPD values out of 28, or 25%, are unsatisfactory.

TABLE 13. Precision of Acid/Base-Neutral Spikes (RPD)

Compound	Sample No. 17008	18050	20059	22051	CLP RPD Limits
4-Chloro-3-methylphenol	8	8	17	7	33
2-Chlorophenol	3	124	11	4	50
4-Nitrophenol	9	200	200	30	50
Pentachlorophenol	200	20	5	29	47
Phenol	3	59	3	4	35
1,2,4-Trichlorobenzene	4	200	2	1	23
1,4-Dichlorobenzene	26	0	3	2	27

Four acid/base-netural solvent blanks were prepared simultaneously with the samples. Except for occasional phthalates, the blanks were free of detectable amounts of target compounds.

2.3.6.4 Pesticide Fraction

The pesticide recovery efficiency was monitored by using σ, p'-DDE as a surrogate standard. Some samples contained high levels of PCBs that interfered with the quantitation of the pesticide surrogate spike. But for the 38 samples, spikes and blanks that did have quantifiable σ, p'-DDE, the average recovery was 91.6% ± 15.8%. The CLP suggests dibutylchlorendate as the surrogate standard, with an acceptable range of 46% to 136%. If a similar range was assumed to be reasonable for σ, p'-DDE, then the average surrogate recovery is satisfactory. In fact, all individual surrogate recoveries were in this range.

One of the samples, 21056, was analyzed in duplicate. No detectable amount of pesticide or PCB was found in either duplicate.

Three other samples were spiked with known amounts of pesticide compounds. Unfortunately, two of these spikes had high levels of PCB that interfered with the recovery quantitation. As a consequence, only one sample was effectively spiked. For all 16 pesticide compounds, the percent recoveries of the duplicates were 117% ± 25% and 116% ± 18%. The CLP recommends six pesticides to monitor spike recovery. The recommended recovery range of the CLP compounds averages from 35% to 133%. The average recovery of all 12 values of the duplicates was 116%. Two of the values, both for 4,4'-DDT, were one percentage point beyond the recommended upper limit. All of the other individual values were within their specific range. Precision between duplicate values was good. The RPD for the six pesticide compounds ranged from 31% to 50%, with an average of about 43%. None of the RPDs for each pesticide spiking duplicate exceeded this range of its specific RPD limit. The RPD of each pesticide was, in fact, always one-half or less of its limit. Although the Standard Reference Material for "PCBs in sediment" were available, they were not used, because of an oversight. (They have been used successfully in the past.)

2.4 BENTHIC INFAUNAL ANALYSES (PERFORMED BY MRL)

2.4.1 General Procedures

Preserved samples (six cores per station, each core 5 cm x 15 cm) were placed in enamel pans and a quantity of freshwater was added. The contents were gently agitated. The water and suspended organisms were then poured through a 1.0-mm mesh screen, and the organisms retained were transferred to a water-filled petri dish. This "panning" process tended to break down large clumps of sediment and suspended relatively light and fragile organisms such as gammarid amphipods and juvenile polychaetes. This process was usually repeated six times to remove virtually all types of organisms from the sediment with minimum damage. Sediments remaining in the pan were then placed in a 1.0-mm mesh sieve and washed with freshwater, entraining the heavier organisms such as bivalves and tube-dwelling polychaetes on the screen.

All organisms from a sample were then placed in freshwater in a petri

dish, examined under a dissecting scope, and sorted into major taxa. Common, easily identified species were enumerated at this time. More difficult groups were set aside for more detailed examination. All specimens were transferred to 70% isopropyl alcohol.

Each major taxonomic group was identified to the generic/specific level if possible, but at the discretion of the taxonomic specialist. If organisms were damaged or immature, identification was to the lowest possible taxonomic ranking. Organisms were identified using stereo (dissecting) and compound microscopes. Identification and enumeration were always conducted in the "blind"; that is, the specialist conducting the analyses did not know the origin (bay, station) of the particular sample being quantified. And, as an additional quality assurance measure, the taxonomic identifications from 10% of all benthic samples (5 of 48 stations) were verified by a second (independent) specialist, Mr. Jack Q. Word of Evans-Hamilton, Inc. After being identified all samples were stored in 70% isopropyl alcohol with approximately 20% glycerol added to prevent desiccation.

The general taxonomic keys of Kozloff (1976), Smith and Carlton (1975), Hobson and Banse (1981), and Banse and Hobson (1974) were used together with specific supplementary publications for precise identifications (i.e., Austin and Haylock 1973; Holleman 1972; McCauley and Carey 1967).

2.4.2 Statistical Treatment

Infaunal data were tabulated by abundance (number of individuals per station) and richness (number of taxa per station). Correlation analyses (see Section 2.8) were performed with these data to determine relationships with physical and chemical properties of the sediments.

2.5 FISH AND INVERTEBRATE PATHOLOGY (PERFORMED BY NOAA)

2.5.1 Fish Capture and Necropsy

The target fish species, English sole (*Parophrys vetulus*) and Dover sole (*Microstomus pacificus*) were collected from the RV *Harold W. Streeter*

with an otter trawl having a 7.5-m opening, a 10.8-m total length, 3.8-cm mesh in the body of the net, and a 0.64-cm mesh liner in the cod end. Individual trawls were for 5 min and covered a distance of approximately 0.2 nautical miles. At each sampling station (Table 1), English sole were sorted first by gender; then each group was separated into five size classes. When possible, up to six individuals of each size class, up to a total of 30 fish, were selected for necropsy. English sole less than 150 mm in total length (0+ year class) were not sampled because this age group previously has been found to possess no neoplastic lesions and very few idiopathic lesions of the liver.

Total sample size at each station was determined on the basis of differences in incidences of neoplasms determined in previous studies of Puget Sound. Because neoplasms are relatively rare among the important idiopathic lesions of interest, detecting significant differences in the incidence of this lesion would require a large sample size. Fish from significantly contaminated sites, such as the Duwamish River, have been found to exhibit neoplasms in more than 15% of the population sampled, while fish from reference areas have been found to have a very low (near 0%) incidence of neoplasia. For a two-way comparison of incidence (α = 0.05, power = 0.80, two-tailed test), at least 30 fish are required to detect significant differences between an incidence rate near zero and incidence rates greater than or equal to 15%. Consequently, a total sample size of 30 fish per site was selected for necropsy.

Fish to be necropsied were measured for total length (mm), weighed (g) and assigned an individual field number. The spinal cord was then severed and otoliths were collected to determine fish age (year-class). The gender was determined and tissues were excised from the following organs: liver, kidney, spleen, gall bladder, small intestine, gonad, heart, and gill. Any grossly visible lesions were noted and recorded and tissue specimens were collected. Tissues were placed in labeled cassettes and immersed immediately in Dietrich's fixative (Gray 1954).

2.5.2 Fish Histologic and Diagnostic Procedures

Tissue specimens were stored in fixative for a minimum of 48 h, then they were dehydrated and paraffin-infiltrated with an automated tissue processor, embedded in paraffin and sectioned at a thickness of 5 to 6 μm. Sections were routinely stained with Mayer's hematoxylin and eosin-phloxine (Armed Forces Institute of Pathology 1968). All slides were examined by light microscopy employing a "blind" system, in which the examining histopathologist had information only on the species, length, weight, sex, and description of grossly visible lesions, with no knowledge of the location from which the fish was collected. Diagnoses were then performed and the diagnostic information was coded on National Ocean Data Center (NODC) File type 13, and placed on computer tape along with information on gross pathology and other biological characteristics of each fish. Data management and lesion incidence analyses were performed using Minitab version 81.1 (Ryan et al. 1981) and SPSS (Statistical Program for Social Sciences) versions 8.1 and 9.1 (Nie et al. 1975) on a Burroughs 7800 computer.

2.5.3 Crab and Shrimp Capture and Necropsy

Crab (*Cancer magister* and *Cancer gracilis*) and shrimp (*Pandalus platyceros* and *Pandalopsis dispar*) were collected using methods identical to those used for target fish species at sampling stations shown in Table 2. Some crab were also caught with baited crab ringnets set in the sampling areas. Crab and shrimp to be necropsied were examined for externally visible anomalies. Crab were measured for carapace width and shrimp for carapace length (mm).

Some specimens of both crab and shrimp were necropsied immediately after capture, while others were held in seawater-filled ice chests until they could be processed. Necropsy of crabs was initiated by clipping off all walking legs and then carefully separating the body carapace from the underlying epidermis, enabling the prosector to then remove the internal organs. Tissues were then excised for examination: antennal gland, bladder, cardiac and pyloric stomachs, epidermis, esophagus, eye, gill,

gonad, hemopoietic tissue, hepatopancreas, hindgut, midgut, and midgut ampulla. Crab tissues were fixed in Helly's fixative (Humason 1979).

Shrimp were first injected by syringe with Davidson's fixative (Humason 1979) directly into the hepatopancreas and body cavity. The legs, antennae, and abdomen were then cut off and the carapace split open to expose the internal organs. Whole shrimp were then placed in Davidson's fixative. Tissues taken for examination were: antennal gland, bladder, cardiac and pyloric stomach, epidermis, esophagus, eye, gill, gonad, heart, hepatopancreas, hemopoietic tissue, midgut, and nervous tissue.

2.5.4 Crab and Shrimp Histological and Diagnostic Procedures

Crab and shrimp tissues were fixed for 48 to 72 h and then stored in 70% ethanol until processed in the same manner as described for fish tissues. Tissues were routinely stained with Harris' hematoxylin and eosin-B-phloxine (Armed Forces Institute of Pathology 1968). All tissues were examined by light microscopy using the same "blind" system as used to examine fish tissues.

2.5.5 Statistical Treatment

The G-statistic (Zar 1974; Sokal and Rohlf 1969) was used to test for differences in lesion incidence between stations within each size class of English sole. For other fish and invertebrate species, the G-statistic was used to simply identify differences in lesion incidence between stations. In cases where the incidence patterns were significantly heterogeneous ($p < 0.05$), the individual areas or stations contributing to the heterogeneity were identified by inspection.

2.6 AMPHIPOD BIOASSAY (PERFORMED BY EPA)

2.6.1 Strategy

The acute toxicity of Puget Sound sediments to the marine amphipod, *Rhepoxynius abronius*, was determined by the general method of Swartz

et al. (1984). This approach involved exposing amphipods to test and control sediments for 10 days at 15°C, under continuous illumination and aeration. Sediment toxicity was based on measurements of amphipod survival, emergence, and reburial.

2.6.2 Test Organisms and Control Sediments

Amphipods and control sediments were collected weekly from West Beach, Whidbey Island, Washington using a stainless steel benthic dredge. The amphipods were kept in their natural sediment substrate (control sediment) and transported on ice to the EPA Region 10 Laboratory, Manchester, Washington, the day of collection. At the laboratory, the amphipods were placed in a flow-through seawater system and slowly acclimated to water temperatures (5°C per day) approaching the test temperature (15°C). Control sediments were obtained by first wet sieving West Beach sediments through a polypropylene screen having a mesh size of 1.0 mm. The sediment was then wet sieved through a 0.5-mm mesh, polyethylene screen into seawater (salinity, 28.0 °/₀₀) and kept at 4°C until distributed for testing.

2.6.3 Screening Surveys

Using a sediment toxicity screening approach similar to the one followed by Swartz et al. (1982) in Commencement Bay, test sediments (with the exception of control sediments) collected during screening surveys (August 2 - September 18, 1983) were assayed without replication. Control sediments were assayed using five laboratory replicates. While testing single replicates limited the statistical treatment of the data generated, this approach provided the broadest screening coverage achievable within the time allotted.

Three hundred grams (wet weight) of well-mixed sediment were added to 1-L glass beakers and brought up to 850 mL with filtered, Clam Bay seawater. Interstitial water salinities associated with each sediment sample were measured with a refractometer before the sediments were added to the test beakers. The salinity of the seawater used to overlay the

sediments was adjusted to 28.0 °/₀₀ with distilled-demineralized water before filtration.

Sediment and seawater preparations were covered with watch glasses and plastic wrap, then incubated overnight (without amphipods) at 15°C under continuous aeration and "cool white" fluorescent illumination (10-60 ft-c). The next day, 20 amphipods were randomly added to each test beaker and the contents of the beakers brought up to 950 mL. The "seeded" beakers were then incubated for 10 days as described above.

The pH, dissolved oxygen concentration, and salinity of the overlaying water were measured for small samples collected from each sediment preparation just before adding the amphipods and again at the end of the test. Floating amphipods were counted daily then gently tapped down with a pipet tip. Amphipods surviving the 10-day exposure period were counted and their ability to rebury in control sediment determined.

2.6.4 Detailed Surveys

Those test sediments collected during detailed surveys (April 23 - May 29, 1984) were assayed using five laboratory replicates. Otherwise, all methods were applied as described in the previous section.

In addition to the routine assay of non-toxic control sediment, the response of each lot of amphipods to a reference toxicant was also measured. This approach was used to determine the potential for a "batch" effect on our assay. The toxicant, sodium pentachlorophenate (PCP), was included in the test protocol in an effort to detect changes in the sensitivity of the amphipods during the 6-week sediment testing period. Sealed ampules of PCP were obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. (Sodium Pentachlorophenate; Toxicant Concentrate #4; 3210 mg/L; Ampule Serial Numbers 153, 157, 159, 170, 174, 176; October 15, 1979.) Fresh PCP concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/L) and a control (0.0 mg/L) were prepared in seawater just before each assay. The seawater diluent was drawn from the same batch of seawater used to overlay the sediment preparations described earlier. The sediment-free test preparations were assayed in triplicate by first adding 200 mL of test solution to each of three 250-mL glass beakers. Each beaker was then randomly seeded with 10

amphipods, loosely covered with a plastic petri dish, and incubated at 15°C under dark plastic for four days (96 h). No aeration was provided.

The pH, dissolved oxygen concentration, and salinity of each test concentration was measured at the beginning and end of the test. Floating amphipods were counted daily, then gently tapped down with a pipet tip.

2.6.5 Statistical Treatment

2.6.5.1 Screening Surveys

Results of the amphipod bioassay in screening survey sediments were used in a qualitative reconnaissance sense. Samples were not replicated which allowed analysis of more stations within bays. This approach was used to gain a more representative spatial sampling. Because there were no replicates, certain statistical comparisons could not be made, but samples (stations) of concern were identified if mortality exceeded the control range. Swartz et al. (1984) indicated that for *R. abronius*, survival of less than 17 of 20 individuals would indicate possible toxicity.

2.6.5.2 Detailed Surveys

An analysis of variance (ANOVA) was performed to compare mean survival and reburial in controls with that of treatments (sediment sources). Before an ANOVA was performed, however, it was necessary to apply Cochran's Test (HOMOV) (EPA/COE 1977) to determine whether the variances of the data sets were homogeneous. The homogeneity of the variances was determined by calculating the C-value, defined as the ratio of the largest variance to the sum of all variances. In the situation where variances were found to be heterogeneous, it was necessary to transform the data. The transformation was performed by obtaining the arcsin $\sqrt{X}$, where X was the datum.

To determine potential differences among treatment means (when ANOVA F-value exceeded tabulated value), the Student-Newman-Keuls (SNK) Multiple Range Test (Sokal and Rohlf 1969) was applied. Dunnett's Test (Steel and

Torrie 1960) was performed to compare the control to each treatment mean. This procedure requires a single difference for judging the significance of observed differences.

In reference toxicant tests, amphipod survival at the end of the 96-h exposure period was used to calculate the LC_{50} for each PCP assay. The moving average-angle technique was used to make these calculations (Bennett 1952). In addition, log-linear analyses (Fienberg 1977) of the resulting dose response curves and a chi-square analysis of a 2 x 6 contingency table were conducted to determine the presence or absence of a batch effect.

2.7 OYSTER LARVAL BIOASSAY (PERFORMED BY MRL)

2.7.1 Strategy

In general, we followed the American Society for Testing and Materials (ASTM) Standard Procedure, Designation E 724-80 (ASTM 1980). The technique had to be modified, however, to perform sediment testing. These modifications pertained mainly to the method of exposing the larvae to the potentially toxic metals/chemicals associated with test sediments (ASTM E 724-80 was not designed to test sediments).

2.7.2 Screening Surveys

For the 1983 oyster larval bioassay series, sediments were collected by MRL staff as described in Section 2.1.1.2. Sediments were stored (48 to 96 h) at 4°C until used. Ripe oysters, *Crassostrea gigas*, were spawned at either MRL or at a local oyster hatchery, and the embryos were delivered within 2 h of spawning. Sediments were weighed at 0.01, 0.10, 1.0, 10.0, and 100.0 g (each replicated three times) and placed into glass beakers and the volume in each beaker brought up to 1 L. The sediments were resuspended by stirring for 5 min, allowed to settle for 2 h, and then innoculated with ~30,000 developing oyster embryos. Sand-filtered Sequim Bay seawater was used a diluent. Salinity, dissolved oxygen, temperature, and pH of the seawater and sediment mixtures were measured at the start and completion of each experiment, and at an interim

point in some cases. Sediments assayed were collected from two stations at the Fourmile Rock - Elliott Bay dump site vicinity, two stations in Sinclair Inlet, and one station each in Port Gardner - Everett Harbor and Sequim Bay (see Appendix A for detailed station descriptions). Four control replicates were run with each sediment sample assayed. Controls consisted of sand-filtered Sequim Bay seawater without sediment. Sequim Bay sediment from Station 14 was used in a sediment control. Station 14 is classified as sandy-silt consisting of 58.2% fines (the sum of silt and clay fractions), and ranked as the cleanest station in Sequim Bay.

Experiments were terminated between 46 and 50 h after introduction of the oyster larvae. The liquid portion of each experimental beaker was then siphoned into a flask. From this flask, two 10-mL samples for scoring were withdrawn and preserved with an equal volume of buffered 8% formalin. The remaining suspended oyster larvae were screened and preserved similarly. The pH of the preserved specimens was 7.5. Samples were scored in a 10-mL counting chamber at 40x and 100x magnification. Samples were sheathed with a blind-coded label and not specifically identified until the counts had been completed. Samples were enumerated for percent normal and percent abnormal larvae. Normal larvae were those that were fully shelled. It should be noted that some of those called normal were slightly bean shaped and undersized, but still fit Woelke's normal classification (Woelke 1960; 1972).

2.7.3 Detailed Surveys

The 1984 oyster larval bioassays were conducted following the same general procedures as outlined in the preceding section. In most cases, however, sediments were collected and processed by EPA Region 10 Laboratory staff, and delivered the following day to the MRL for bioassay. The only exception was the Sequim Bay sediments, which were collected by MRL staff. Also, a single dilution of sediment (100.0 g/L) was tested instead of a range of dilutions.

Sediments assayed were collected from eight stations in each urban bay and four stations in each baseline bay (see Appendix A for detailed station descriptions).

2.7.4 Statistical Treatment

2.7.4.1 Screening Surveys

The objective of the oyster larval bioassays in sediments from the screening surveys was to calibrate our methods and, in particular, test various modifications of ASTM E 724-80 (ASTM 1980) to adapt the technique to sediment testing. Therefore, rigorous statistical comparisons were not conducted, nor were they intended.

2.7.4.2 Detailed Surveys

An analysis of variance (ANOVA) was performed to compare the proportion of abnormal larvae in controls with the proportion of abnormal larvae in treatments (sediment sources). Before performing the ANOVA, however, all data were transformed by obtaining the arcsin $\sqrt{X}$, where X was the proportion of abnormal larvae.

Dunnett's Test (Steel and Torrie 1960) was performed to compare the control to each treatment mean. This procedure requires a single difference for judging the significance of observed differences.

2.8 GENERAL STATISTICAL PROCEDURES (PERFORMED BY MRL)

2.8.1 Discriminant Analysis

Stepwise discriminant analysis was used to build linear classification functions to discriminate among samples from bays using both physical and chemical data from the 1984 detailed survey.

The linear discrimination functions are built by examining each variable in the observation vector and comparing "between bay" variation to "within bay" variation. The variable that discriminates the most is assigned to the classification function and the variance associated with that variable is removed. The remaining variables are then examined again for the same purpose. When a group of variables is highly correlated, and one of the group is chosen to be included in the function, the discriminating ability of the rest of the group is correspondingly lower.

Thus, a rather large vector of observations may be reduced to relatively few classification functions based on a reduced number of variables. The process is described in more detail by Morrison (1976).

The technique has two limitations from the regulatory standpoint. One, the discriminant function may turn out to be data-set dependent, and so should be verified with independent data. Two, the variables chosen are those which discriminate among all bays. Thus, variables that are uniform across a number of bays (e.g., PCB-1254 in the baseline bays), are often not powerful discriminators.

The computer program P7M from the Biomedical Computer Program P-Series developed at the Health Sciences Computing Facility, University of California at Los Angeles was used for the analysis. The Health Sciences Computing Facility is sponsored by the National Insitute of Health Special Research Resources Grant RR-3. The 1982 revision of the program is available on the VAX® 11-780 computer, located at PNL, Richland, Washington, which was used for the computation.

2.8.2 Factor Analysis

Factor analysis was also performed on the 1984 detailed survey physical and chemical data. Factor analysis presupposes that a few driving forces are behind the values of a number of variables. By discerning these underlying factors, one may well be able to obtain a less complicated picture of the data structure.

The final data matrix for the 1984 detailed survey (48 stations) included the following variables measured by the MRL: sediment grain-size (average phi); percent water; percent volatiles; concentrations of silver, mercury, lead, IR, and total organic carbon. The data generated by EPA included concentrations of PCB-1254, aromatic hydrocarbons, arsenic, copper, and zinc.

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A factor analysis performed on the matrix used the covariance structure of the data to reduce the matrix to a smaller number of so-called common factors. In the words of Morrison (1976), "Under the factor model, each response variate will be represented as a linear function of a small number of unobservable common-factor variates and a single latent specific variate. The common factors generate the covariances among the observable responses, while the specific terms contribute only to the variances of their particular responses." It may thereby be possible to obtain a less complicated picture of the data structure that may enhance understanding of the occurrence of the variables.

The computer program P4M from the Biomedical Computer Program P-Series (University of California at Los Angeles) was used for the analysis.

2.8.3 Correlation Analysis

Correlation analyses were performed on the sediment data to determine the relationships among physical, chemical, and biological variables. The resulting matrix contains 300 pairwise correlations. We recognize that with this many correlations, the chance of a spurious correlation existing is greater than the $\alpha = 0.05$ level we reported. We have, nonetheless, reported pairwise α levels simply to assist in making judgments about the relative strengths of the relationships.

The final data matrix presented in Appendix E includes 25 variables derived from the 1984 detailed survey (48 stations). These are BAY, STATION, AGMRL (silver analyzed by MRL), HGMRL (mercury analyzed by MRL), PBMRL (lead analyzed by MRL), ASEPA (arsenic analyzed by EPA), CUEPA (copper analyzed by EPA), ZNEPA (zinc analyzed by EPA), DEPTH, PCH2O (percent water), GRAVEL (percent gravel), SAND (percent sand), SILT (percent silt), CLAY (percent clay), FINES (percent fines), PCB, AH, IRMRL (IR analyzed by MRL), PCVOL (percent volatiles), TOC, AVGPHI (average Phi), NEMATOD (nematodes), CAPITELL (*Capitella capitata*), AMPHIPOD (amphipod survival), and OYSLAR (percent abnormal oyster larvae).

Computer program BMD P8D, Missing Value Correlation was used for the analyses. The 1982 revision of the program is available on the PNL VAX® 11-780 VAX computer, which was used for the computation.

2.8.4 Tolerance Intervals

Tolerance intervals were used to develop summary statistics useful in determining which of the eight bays currently show signs of degraded sediment quality (see Section 2.8.5). Tolerance intervals are used when it is desirable to know whether a single observation falls outside the bounds of some sample distribution. Because one is not examining distributions of means, tolerance intervals are much wider than confidence intervals. Tolerance intervals are established by taking the sample mean, plus or minus a tolerance factor, times the sample standard deviation. Tolerance factors for the normal distribution are available from the CRC Handbook of Tables for Probability and Statistics (Beyer 1976). To attain a tolerance factor from the table, three items need to be considered:

1. n, the number of observations in the sample distribution
2. P, the percentage of the sample distribution to include
3. α, the probability that a given observation will fall in the given percentage of the sample distribution.

Accordingly, tolerance intervals are usually presented as "the probability is α that P percent of the observations of the given sample distribution fall within the tolerance interval."

2.8.5 Summary Statistics

Summary statistics presented in Section 3.7 were developed as an aid in determining which of the eight bays currently showed signs of degraded sediment quality. Important chemical, biological, and physical variables as well as their critical values were selected to make this discrimination.

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Six metals, (Ag, Hg, Pb, As, Cu, and Zn), were selected as variables representing metals contamination. It was assumed that all six metals were of equal importance as indicators of environmental contamination. The metals concentrations were first standardized to the same scale by dividing each by the largest concentration of that metal found in the sample. The resulting scores were added and the sum divided by six to ensure that the summary metals score was on a scale of zero to one.

A similar approach was taken with a second set of variables used to represent levels of organic pollution. Again, it was assumed that each organic variable was of equal importance as an indicator of environmental contamination. The variables selected were PCB, AH, and IR. Each score was standardized to the same scale by dividing by the largest. The resulting numbers were added and the sum divided by three to ensure that the summary score was on a scale of zero to one.

The next step was to calculate a critical value for the metals and/or organics, which if exceeded would signify degraded sediment quality. Scores from the baseline bays were assumed to be from areas of low metals and/or organic contamination. The means and standard deviations for these 16 scores and a tolerance interval for each distribution were then calculated using the methods described in Section 2.8.4

The three biological variables selected were amphipod mortality, oyster larvae abnormality, and the number of capitellids at each station. The critical value for amphipod mortality, as established in Section 3.5 was approximately 0.20. This was the level used to identify a station with higher than expected mortality.

In the case of abnormal oyster larvae, an across-the-board critical value could not be established. The oyster larval bioassays were not constant in the abnormality observed in the control conditions. A level not significantly different from control in one test may well be significant in another. For this reason, those stations found in Section 3.6 as showing a greater percentage of abnormal larvae when compared to control stations were noted as indicators of degraded sediment quality.

The number of capitellids in the benthic infauna sampling were included as a biological variables because these organisms are classified as organic enrichment opportunists. No attempt was made to develop a quantitatively defensible critical value for this variable; however, finding a quantity of these animals in an infauna sample could indicate a shift away from the uncontaminated environment. It was assumed that a finding of six or more of these animals at a station indicated a condition different from the uncontaminated or baseline condition. This was based on the finding of no more than one capitellid at any baseline bay station, and the finding of six or more capitellids at seven urban bay stations.

The physical variable selected was the proportion of fines in the sediment. There is, of course, no critical value for this variable, but it was included to further understand the data.

Because flatfish species were not caught in four of the eight bays (Bellingham Bay, Samish Bay, Dabob Bay, Sequim Bay) because of seasonal scarcity, the resulting pathology data were not used in the summary statistical exercise.

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3.0 RESULTS

3.1 SEDIMENTARY CHARACTERISTICS (PERFORMED BY MRL)

3.1.1 Screening Surveys

During the summer of 1983, surface sediment samples were collected at 181 stations in the eight bays (Section 2.1). These sediments were analyzed for physical and chemical characteristics including grain size, percent water, percent loss on ignition (percent volatile solids), Ag, Hg, Pb, and IR (oil and grease). These "range-finding" data and the amphipod toxicity data were used to select 48 stations, which were resampled in 1984 for additional chemical analyses and biological studies. The results of the 1983 range-finding data are included in Table 14 and in Appendix B.

3.1.1.1 Grain Size

A variety of sediment types were sampled in each urban bay. The most common types of sediments were sands and silts. Gravel was usually a minor component with only four stations containing more than 20% gravel. Sediments collected from greater depths usually contained greater amounts of clay than sediments collected from shallower waters.

The sediment sampling stations in the baseline bays were selected so that the sediment types and water depth were similar to those sampled in the urban bays. The sediment grain size charts prepared by Roberts[1] were used to select sampling stations. The types of sediments collected in baseline bays were almost identical to those in urban bays. Most of these sediments were sands or muds and a few stations contained gravel. The finest grain sediments were from the deeper stations in Case Inlet.

3.1.1.2 Water Content

Water content of sediments collected in urban bays ranged from 15% to 71%. The lower values were from sandy sediments, and the higher values were from sediments with high silt and clay contents. The water

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[1] Personal communication 1979. Original charts at the Department of Oceanography, University of Washington, Seattle.

TABLE 14. Mean Sediment Characteristics and Contaminant Levels for 48 Stations Sampled During Screening (1983) and Detailed Surveys (1984) Determined by MRL

Bay	Year	Water	Volatiles	Gravel	Sand	Silt	Clay	Ag	Hg	Pb	IR
		Percent						ppm			
Samish	83	45	5.0	0.0	30	40	30	0.07	0.09	13	141
	84	55	6.0	0.3	16	59	25	0.11	0.10	15	92
Dabob	83	54	6.0	0.5	27	45	28	0.11	0.08	14	22
	84	47	8.0	1.3	53	25	21	0.09	0.06	10	45
Sequim	83	53	6.0	0.2	22	53	25	0.15	0.08	12	48
	84	63	10.0	0.2	22	44	33	0.20	0.08	13	89
Case	83	65	7.0	0.1	20	59	21	0.26	0.14	22	139
	84	65	10.0	2.8	25	38	34	0.33	0.13	26	91
Bellingham	83	54	7.0	1.4	13	51	34	0.26	1.20	28	1052
	84	68	11.0	1.8	11	59	28	0.25	1.00	24	925
Fourmile Rock-Elliott Bay[1]	83	49	6.0	1.2	41	39	18	0.60	1.70	165	470
	84	49	8.0	1.1	46	31	22	0.55	2.80	134	304
Port Gardner-Everett Harbor	83	63	19.0	7.2	38	40	16	0.36	0.28	50	4687
	84	62	20.0	4.6	32	44	19	0.30	0.24	40	1852
Sinclair	83	55	8.0	0.7	18	54	27	1.73	2.40	184	1350
	84	60	10.0	1.8	24	45	29	2.19	2.10	170	1219
Overall Mean	83	55	8.4	1.8	26	47	25	0.54	1.00	76	1289
	84	58	10.9	1.9	29	44	26	0.61	1.10	67	743

1 Dump site vicinity.

content in baseline bays ranged from as low as 19% in sandy sediments to 75% in the finest grained sediments of Case Inlet. Of the 20 stations sampled in Case Inlet, 15 had water contents greater than 65%. In the other bays, no more than five stations per bay showed water contents greater than 65%.

3.1.1.3 Volatile Solids

The volatile solids of urban bays ranged from 1% to 27%; however, most of the values were in the range of 2% to 8%. Generally, volatile solids are organic matter such as wood, bark, and marine detritus. The volatile solids of baseline bays ranged from 0.5% to 8% with the exception of one station in Case Inlet which showed a value of 11%. High volatile solids are usually associated with high percent water and low percent sand.

3.1.1.4 Infrared Spectrophotometer

The infrared spectrophotometer (IR) analysis measures the absorption of infrared light due to the carbon-hydrogen bond in organic matter extracted from sediment. The analysis is used as a gross measurement of oil and grease contamination. The IR spectrophotometer was calibrated with Prudhoe Bay crude oil, so the results, expressed in ppm dry weight of sediment, are an indicator of petroleum content. Note, however, that nonpetroleum sources of hydrocarbon material can also contribute to the signal.

The IR results for urban bays ranged from 4 to 15,990 ppm. The lower values were usually from either sandy sediments or sediments collected away from the industrial waterways. The highest IR values were from stations located near industrial activities.

The IR concentrations in the baseline bays were generally much lower than in the urban bays. The IR range was 14 to 246 ppm in the baseline bays with one value of 409 ppm found in Samish Bay.

3.1.2 Detailed Surveys

During the detailed surveys (spring 1984), 48 stations were revisited and resampled (see Section 2.1). These sediments were analyzed for essentially the same physical and chemical characteristics that were included in the screening surveys, and additionally for EPA priority pollutants. The results of the 1984 detailed survey analyses are shown in graphic form in Figures 11 through 14 and in tabular form in Table 14 and in Appendix E.

3.1.2.1 Grain Size

The sediment grain size data are presented in three different formats: (1) percent gravel, sand, silt and clay; (2) percent silt plus clay; and (3) mean phi[1] for each bay. The percentage of silt plus clay is shown in Figure 11 for the 48 stations. The percentage of silt plus clay ranged from approximately 15% to 98%, however, the mean for each bay was in the range of 55% to 86% which would be classified as silty mud (50% to 90% silt and clay). The grand mean for the eight bays was 71% silt plus clay. Mean sand content of the bays ranged from 12% to 44%. The gravel content was usually not more than a few percent. When the mean grain size was expressed as mean phi, the range was 4.2 to 5.9 phi units.

3.1.2.2 Water Content

The water content of the sediment averaged 58% for all 48 stations. The mean for each of the eight bays ranged from 47% to 65%. The water content of sediments increased with decreasing grain size.

3.1.2.3 Volatile Solids

The volatile solids content is a measure of the weight loss when dry sediment is ashed in a muffle furnace at 550°C. Most of the volatile

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[1] The unit phi is the grain size expressed by the negative log to the base 2 of the mean diameter in millimeters. Phi units increase with decrease in grain size. Each unit of change, for example, 4.0 to 5.0, results in a factor of 2 decrease in mean grain size, for example, 0.062 to 0.031 mm.

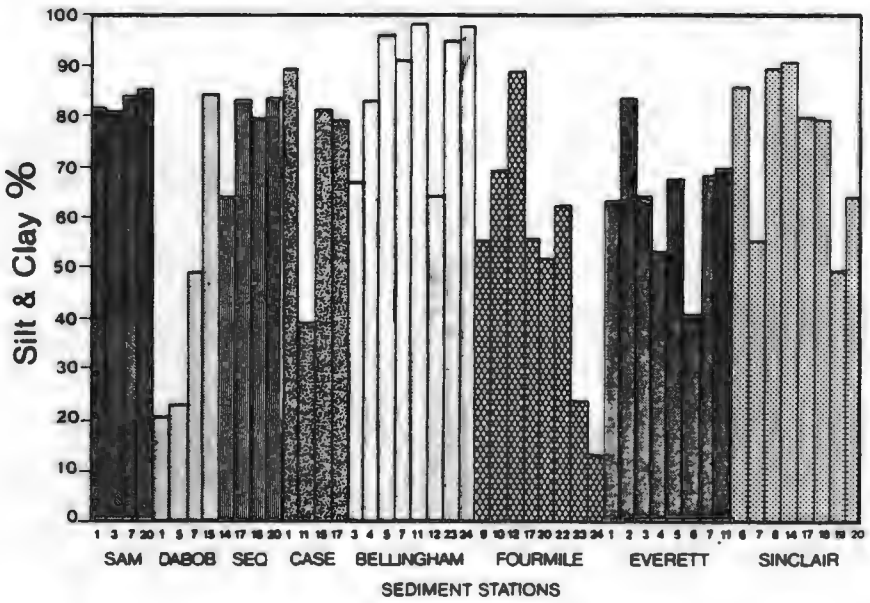


FIGURE 11. Percent of Silt and Clay in Sediments

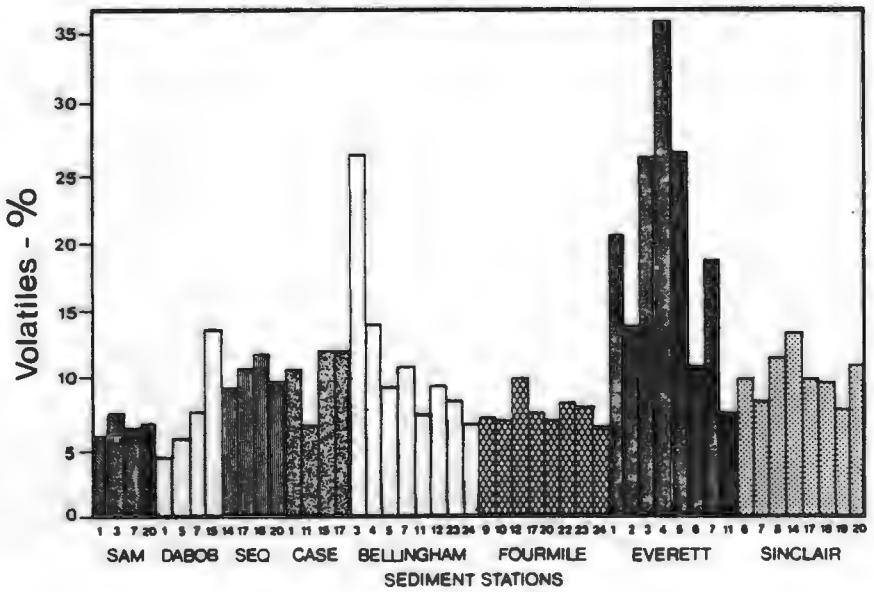


FIGURE 12. Percent of Volatiles in Sediments

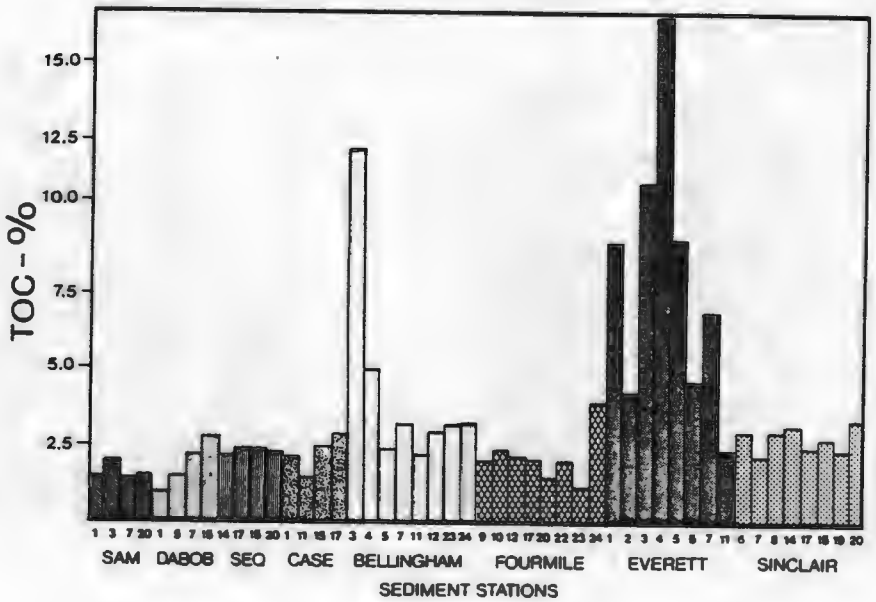


FIGURE 13. Percent of TOC in Sediments

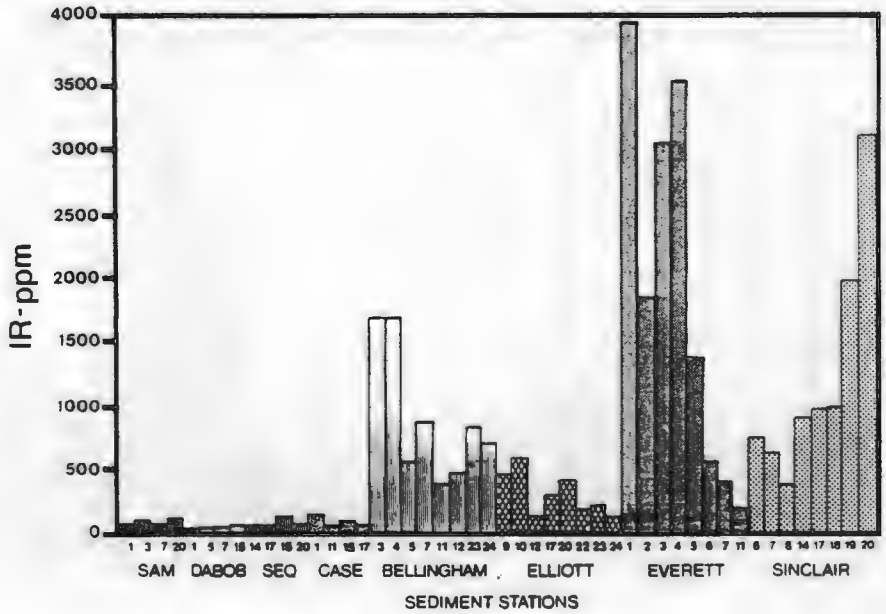


FIGURE 14. IR Results for Organics in Sediments

material was organic matter, therefore, TOC content was closely correlated with volatile solids. The mean volatile solids for each bay ranged from 6.5% to 19.5%. The mean for all of the bays was 10.9%. The volatile solids contents of the 48 stations are shown in Figure 12. Most stations were in the range of 5% to 15%. One station in Bellingham and five stations in Port Gardner - Everett Harbor exceeded 15% because of wood and tree bark in the sediments, undoubtedly derived from the local forest products industries.

3.1.2.4 Total Organic Carbon

The pattern of TOC in sediments followed that of volatile solids (Figure 13). The TOC content in sediments was generally in the range of 2% to 4%. A few stations from Bellingham Bay and Port Gardner - Everett Harbor contained concentrations of TOC in the range of 5% to 16%.

3.1.2.5 Infrared Spectrophotometer

The results from the IR analyses clearly showed that baseline sediments contained relatively low concentrations of hydrocarbon compounds compared to most of the urban sediments (Figure 14). The mean IR values for baseline bays were between 45 and 92 ppm, while those for urban bays ranged from 304 ppm for the Fourmile Rock - Elliott Bay dump site vicinity to 1852 ppm for Port Gardner - Everett Harbor.

3.2 CONTAMINANT LEVELS (PERFORMED BY BOTH EPA AND MRL)

3.2.1 Screening Surveys

The 181 sediment samples collected in 1983 were analyzed by the MRL for Ag, Hg, and Pb. Results are presented in Table 14 and in Appendix B. These three heavy metals were selected for the screening survey because they are usually the metals that show the greatest enrichment in Puget Sound sediments compared to baseline sediments. Silver enters Puget Sound in sewage effluents (Romberg et al. 1984); lead enters Puget Sound from many sources including sewage outfalls, rivers, industrial outfalls and atmospheric fallout; mercury enters the Sound from industry, sewage, and

rivers. These three metals are usually found enriched together in sediments that are also contaminated with other metals, petroleum hydrocarbons, and chlorinated organics.

3.2.1.1 Urban Bays

The concentration of Ag ranged from below the detection limit of 0.014 to 2.96 ppm. Most of the values were in the range of 0.1 to 1.0 ppm. The sediments with the highest concentrations were from Sinclair Inlet and the lowest concentrations were from Bellingham Bay.

The Hg concentrations ranged from 0.006 to 4.9 ppm. Most values were in the range of 0.1 to 0.6 ppm. Several values greater than the 1.0 ppm were measured in Bellingham Bay, at the Fourmile Rock - Elliott Bay dump site vicinity, and in Sinclair Inlet. Sinclair Inlet was the most contaminated urban bay. Bellingham Bay was also highly contaminated as a result of past industrial discharges of Hg (Bothner 1973). The Fourmile Rock - Elliott Bay dump site vicinity revealed five sediment samples that contained more than 1 ppm Hg. These same samples also contained the highest Pb concentrations in the area, suggesting that past dredge spoil disposal activities could have created several local hot spots. However, many of the sediments from the dump site vicinity did not contain high concentrations of metals when compared to other main basin sediments. This wide range of concentrations indicated that the area surrounding the Fourmile Rock - Elliott Bay dump site was not uniformly contaminated, and several hot spots existed, presumably as a result of specific disposal events.

The Pb concentrations ranged from 3 to 338 ppm. Sinclair Inlet was the most contaminated, and the dump site vicinity also indicated high sediment concentrations. Both Port Gardner - Everett Harbor and Bellingham Bay showed Pb contamination, but showed much less than the other two urban areas.

3.2.1.2 Baseline Bays

The majority of sediments in the baseline bays were contaminated with Ag, Hg, and Pb, but to a much lesser degree than the urban bays. The

concentrations of Ag, Hg, and Pb in fine-grained or muddy sediments deposited in Puget Sound during the 1800s are on the order of 0.04, 0.04, and 6 ppm, respectively (Romberg et al. 1984). Sandy sediments generally contain lower concentrations than muds.

The concentrations of Ag in sediments from the baseline bays ranged from less than 0.014 to 0.4 ppm. Case Inlet was the most contaminated baseline bay, presumably from particulate transport from the central basin (Riley et al. 1983). The sources of Ag deposited in Dabob and Sequim Bays have not been identified. Possible sources include local sewage outfalls and transport from the main basin.

The Hg concentrations in baseline bays ranged from 0.006 to 0.2 ppm. Again, Case Inlet was the most contaminated. In Samish Bay, Hg levels were generally higher than Ag. This was not the case for the other baseline bays or for the urban bays except Bellingham. The ratio of Ag to Hg in Case Inlet and Sequim Bay was approximately 2:1, similar to the ratio in the main basin of Puget Sound. In Bellingham Bay, the ratio was 1:3 because of a local Hg source. Presumably, Samish Bay is influenced by contaminants in Bellingham, and Case Inlet is influenced by the main basin of Puget Sound. The sources of contaminants to Dabob Bay and Sequim Bay are not clear.

The Pb concentrations ranged from 2 to 32 ppm in the baseline bays. Again, Case Inlet was the most contaminated because of its proximity to major Pb sources. Atmospheric fallout could be a significant source of Pb to all the baseline bays. As with the other metals, Pb concentrations in sediments were related to grain sizes. Within each baseline bay, Pb concentrations ranged approximately an order of magnitude range in Pb concentrations. Also, Pb concentrations correlated with water content and volatiles, and inversely with sand.

3.2.2 Detailed Surveys

The 48 sediments collected in 1984 were analyzed by the EPA Region 10 Laboratory for priority pollutants, which included 13 metals

and approximately 100 organic compounds. Battelle analyzed the same samples for Ag, Hg, and Pb. The EPA results are presented in graphic form in Figures 16, 17, and 19 through 25, and in tabular form in Appendix B. The Battelle MRL data are reported in Figures 15, 18, and 19, and in tabular form in Table 14.

3.2.2.1 Urban Bays

Metals. Urban bay sediments were generally significantly contaminated with Ag, As, Cu, Cd, Hg, Pb, and Zn. Sinclair Inlet sediments demonstrated the highest average concentrations of these metals. The other six metals (Sb, Be, Cr, Ni, Se, and Tl) were found at about the same concentration in all bays, however, a few urban sediments were enriched in Sb, Cr, and Ni.

The concentrations of Ag generally ranged from 0.1 to 0.5 ppm except for five stations in the Fourmile Rock - Elliott Bay dump site vicinity. All eight Sinclair Inlet stations demonstrated concentrations greater than 0.5 ppm (Figure 15).

Urban bays were only slightly contaminated with As, although Sinclair Inlet showed concentrations in the range of 25 to 67 ppm at three stations (Figure 16).

Cu concentrations in all urban bays were elevated, with values as high as 800 ppm recorded in Sinclair Inlet (Figure 17). The mean concentration in Sinclair Inlet was 305 ppm, which was three times the concentration in other urban bays.

All stations in Bellingham Bay, at the Fourmile Rock - Elliott Bay dump site vicinity, and in Sinclair Inlet were elevated in Hg. Port Gardner - Everett Harbor, however, exhibited relatively low concentrations of Hg similar to the baseline bays (Figure 18).

The mean concentrations of Pb in all eight bays were elevated compared to a "baseline" concentration of 6 ppm for fine-grained sediment (Romberg et al. 1984). All Sinclair Inlet stations and several from the Fourmile Rock - Elliott Bay dump site vicinity were found to contain more than 98 ppm Pb (Figure 19).

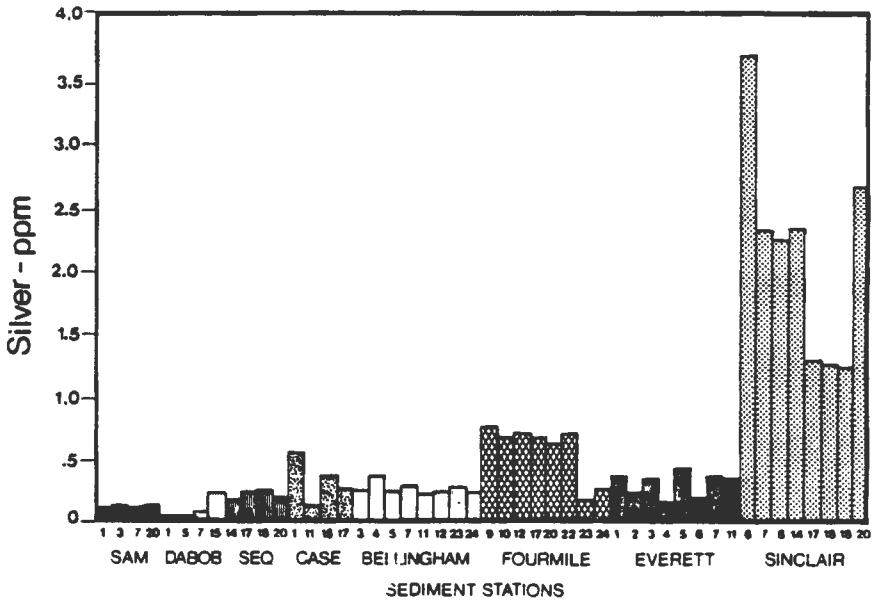


FIGURE 15. Silver Concentrations in Sediments

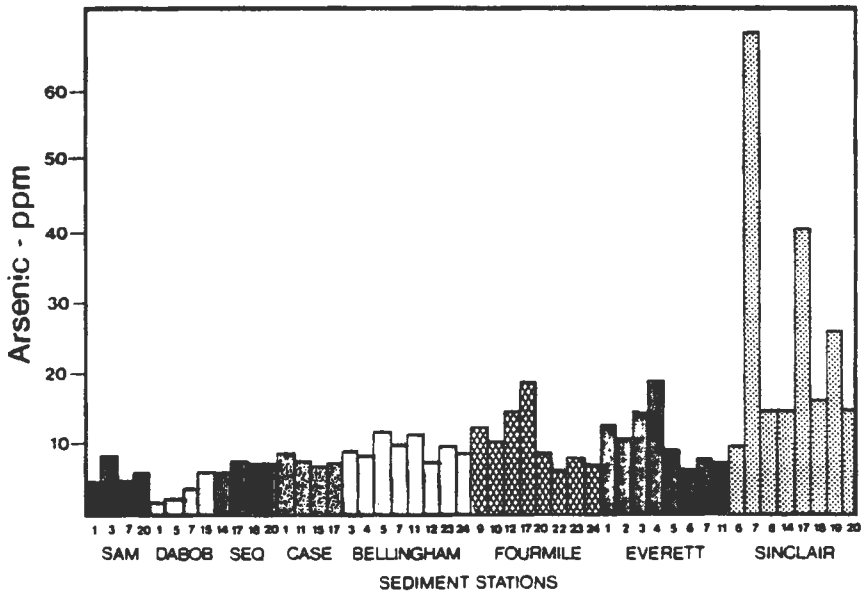


FIGURE 16. Arsenic Concentrations in Sediments

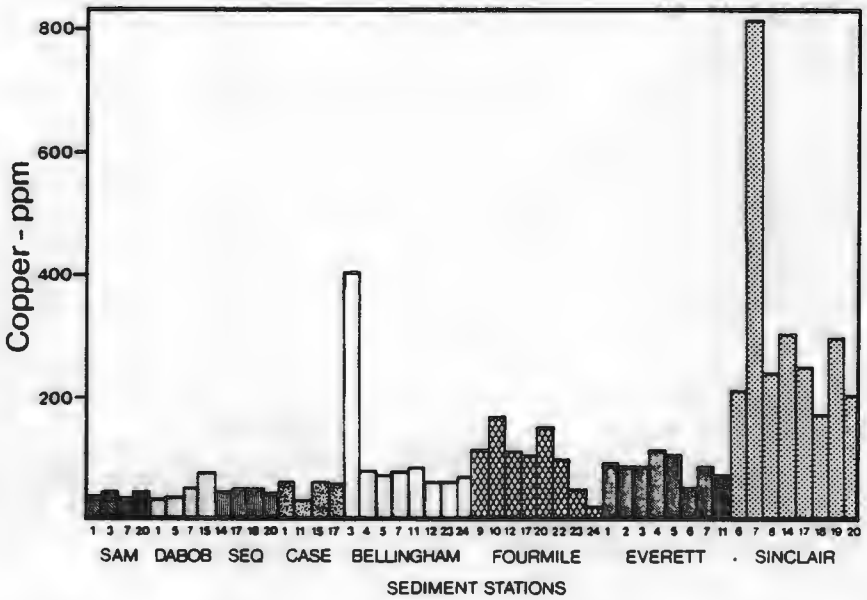


FIGURE 17. Copper Concentrations in Sediments

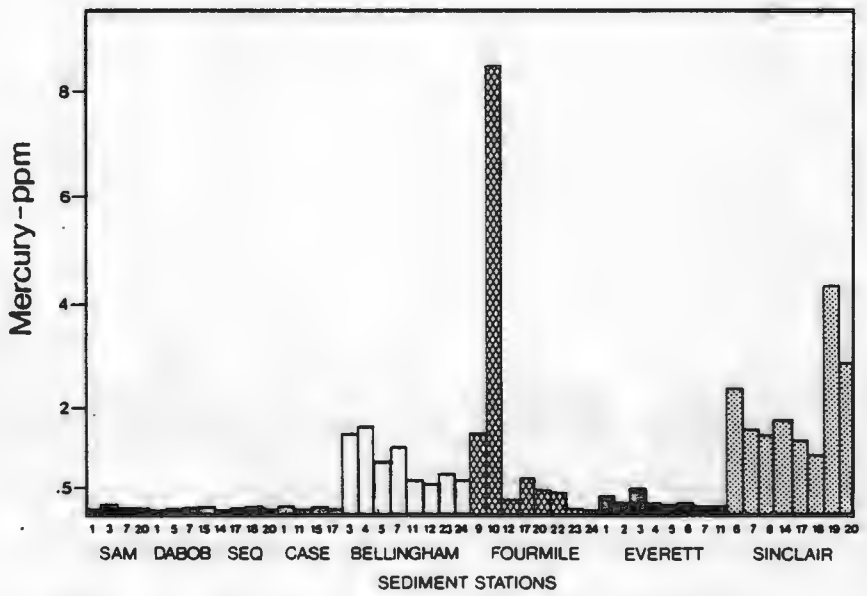


FIGURE 18. Mercury Concentrations in Sediments

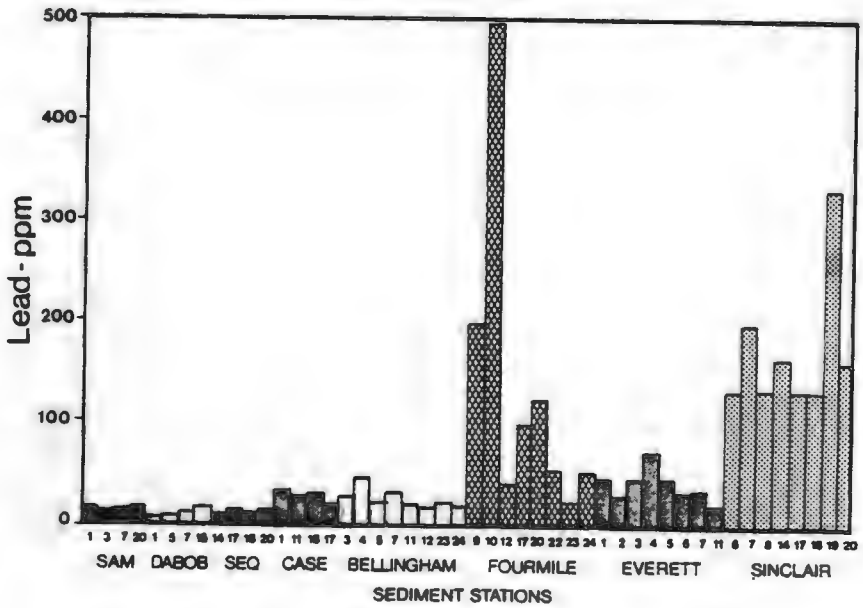


FIGURE 19. Lead Concentrations in Sediments

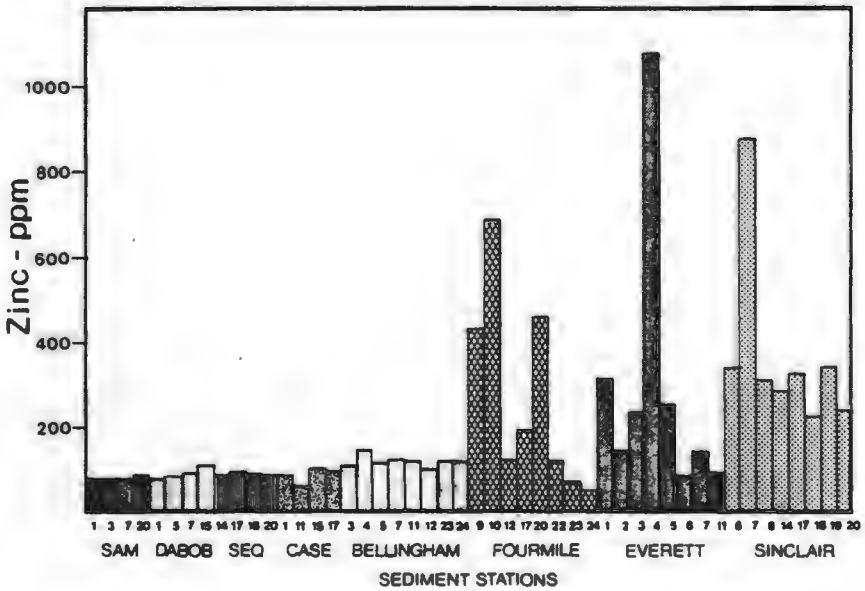


FIGURE 20. Zinc Concentrations in Sediments

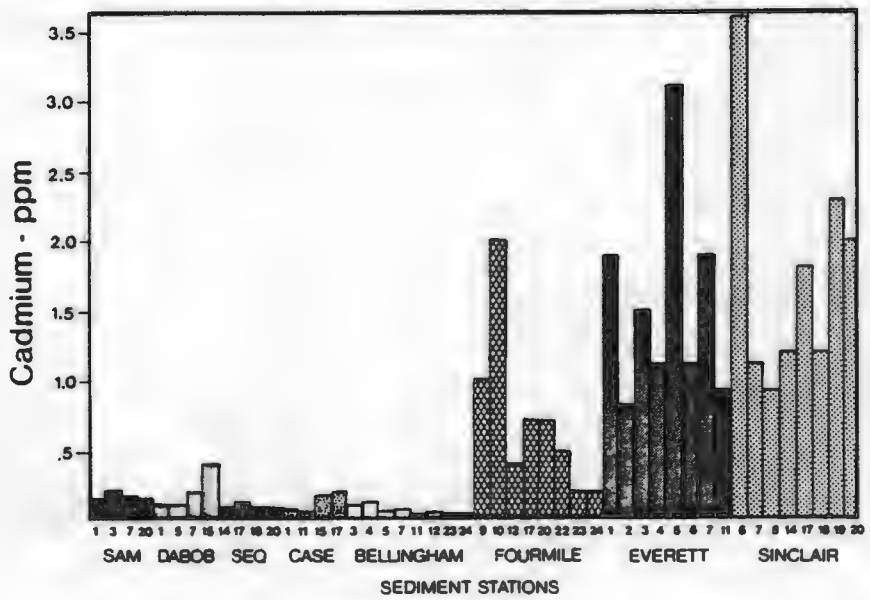


FIGURE 21. Cadmium Concentrations in Sediments

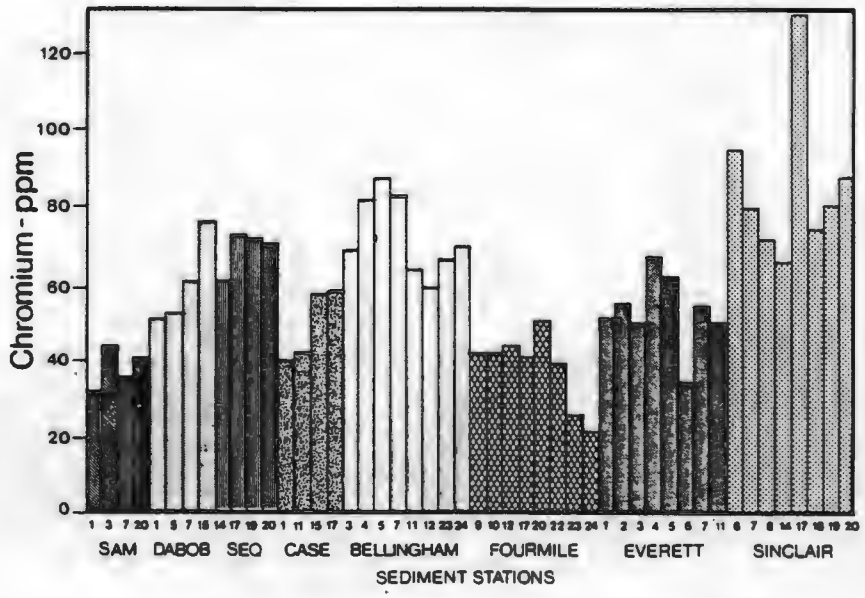


FIGURE 22. Chromium Concentrations in Sediments

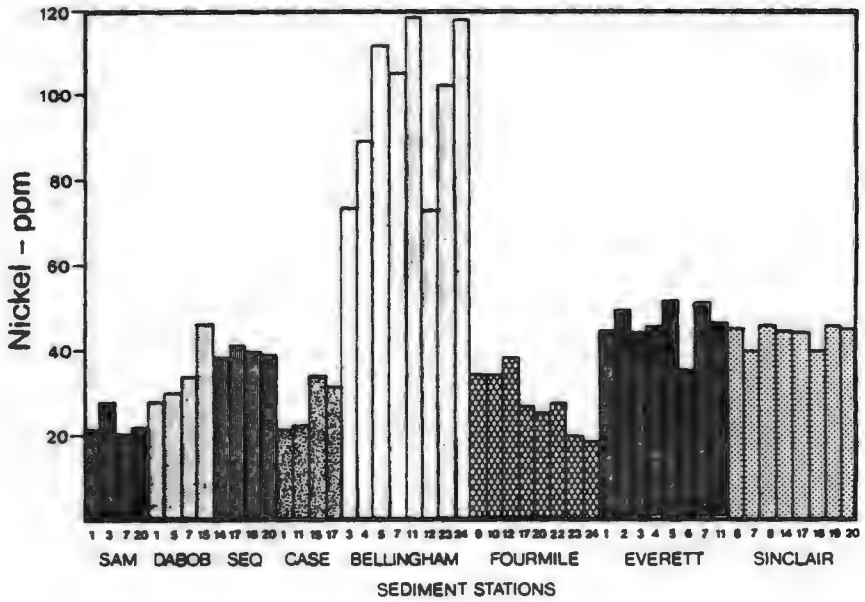


FIGURE 23. Nickel Concentrations in Sediments

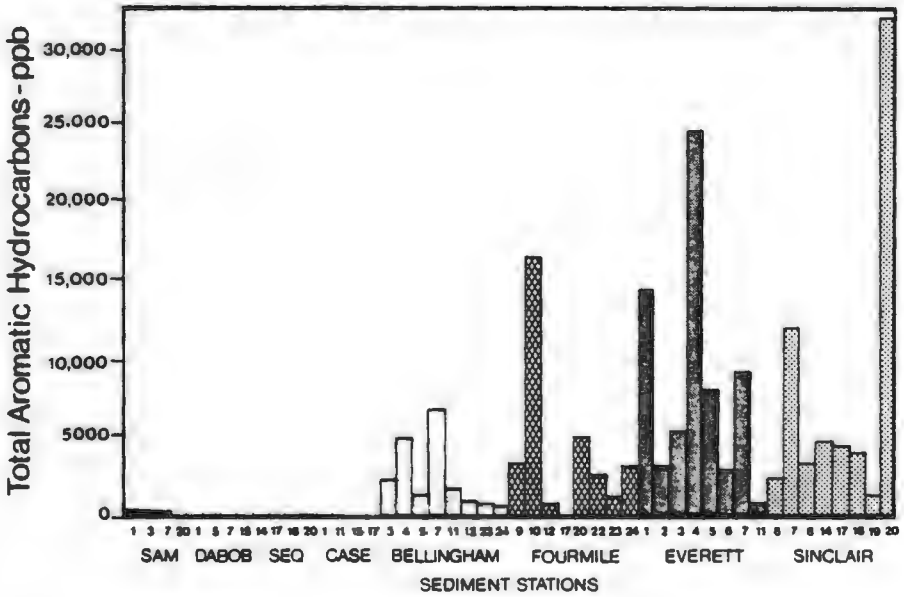


FIGURE 24. Aromatic Hydrocarbons Concentrations in Sediments

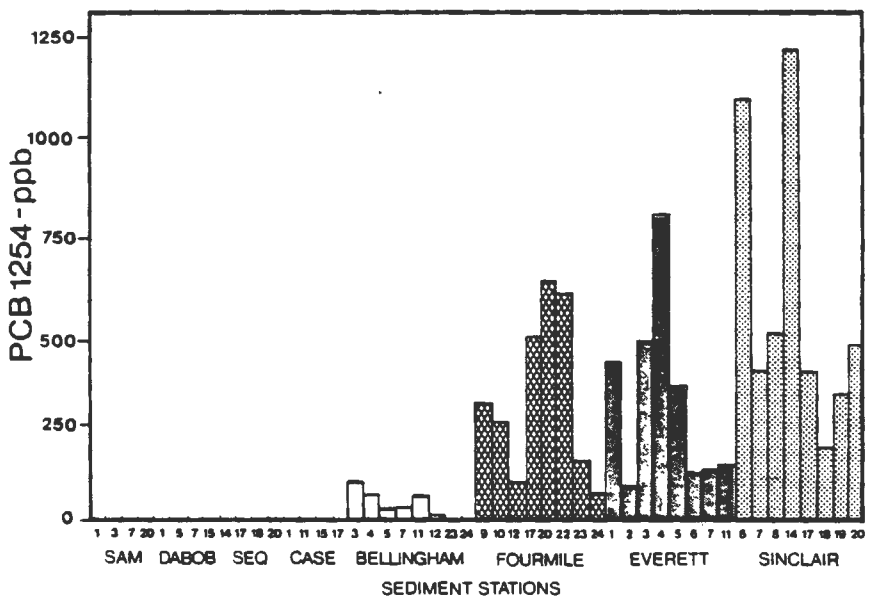


FIGURE 25. PCB-1254 Concentrations in Sediments

Zinc concentrations in sediments from Bellingham Bay did not appear to be significantly elevated (Figure 20). However, several stations from the Fourmile Rock - Elliott Bay dump site vicinity, Port Gardner - Everett Harbor and all Sinclair Inlet stations were elevated with respect to Zn.

The pattern of Cd enrichment was similar to that of Zn (Figure 21). Cadmium concentrations in the range of 0.1 to 0.5 ppm are not considered enriched. As expected, all sediments from Port Gardner - Everett Harbor, Sinclair Inlet, and several from the Fourmile Rock - Elliott Bay dump site vicinity showed some contamination with Cd.

Two other metals that also showed little enrichment were Cr and Ni (Figures 22 and 23). One station in Sinclair Inlet contained 129 ppm Cr compared to a range of 40 to 80 ppm for Cr in most of the bays. Nickel concentrations were in the range of 20 to 50 ppm for all bays except Bellingham Bay where concentrations were in the range of 80 to 120 ppm. Possibly, these high nickel concentrations were due to the mineralogy of the Nooksack River drainage because since there was little or no indication of a local source of pollution.

The quality of the analytical results for Be, Tl, Sb, and Se made it difficult to ascertain differences among bays. There was no evidence for contamination of sediments by Be or Tl. Many of the sediments had undetectable concentrations of these elements. The concentrations of Sb and Se were often undetectable at 0.1 ppm, however, Sb levels of 2.0 ppm were detected in two sediments from Sinclair Inlet. Previous analyses for Sb in Puget Sound sediments showed that Sb was enriched in urban sediments of Puget Sound and correlated strongly with As (Crecelius et al. 1975).

No attempt was made in the present study to normalize the metals data with sediment grain size. Most of those stations selected for sampling contained fine grained sediments. We also chose to apply correlation analyses to reveal relationships among physical, chemical, and biological data. These results are presented in Section 3.2.3.2 and discussed in Section 4.1.1.

Organics. The only organic compounds that were consistently quantified in the urban bay sediments were the aromatic hydrocarbons and

PCB-1254. Other compounds occasionally quantified in urban bay sediments included phthalates and PCB-1260. For the purpose of this report, the aromatic hydrocarbon (AH) data were presented as total aromatic hydrocarbons for each sediment sample. Total AHs were calculated by summing each of the individual aromatic compounds that were detected. Usually between 3 to 10 AH compounds were detected in each urban bay sediment.

The concentrations of total AH in sediments ranged from below the detection limit of 60 ppb to a high of 37,000 ppb. Only three sediments from baseline bays contained detectable AH concentrations (68 to 334 ppb). Almost all urban bay sediments contained detectable concentrations of AHs, but the concentrations varied greatly within each bay. Concentrations greater than 10,000 ppb were present in one or more samples from the Fourmile Rock - Elliott Bay dump site vicinity, Port Gardner - Everett Harbor, and from Sinclair Inlet (Figure 24).

The concentration of PCB-1254 varied from less than 20 ppb in the baseline bays to a mean of 579 ppb in Sinclair Inlet (Figure 25). Bellingham Bay contained the lowest concentrations with a mean of 42 ppb. The other urban bays each contained one or more stations with concentrations greater than 500 ppb.

3.2.2.2 Baseline Bays

Metals. In general, baseline bay sediments contained significantly less metals. The concentrations of Ag ranged from 0.1 to 0.5 ppm except for one station in Case Inlet (Figure 15). The concentrations of As in baseline bays ranged from 1.9 to 8.5 ppm (Figure 16). These are typical concentrations for As in marine sediments (Crecelius et al. 1975). Copper concentrations in baseline bays (Figure 17) were in the range of 28 to 74 ppm, which suggests only a slight increase above the 23 ppm measured in sediments deposited in the 1800s (Romberg et al. 1984). Mercury concentrations were relatively low in all baseline bays (Figure 18). However, the mean concentrations for lead in all baseline bays (Figure 19) were higher than a background concentration of 6 ppm for fine-grained

sediment (Romberg et al. 1984). Neither zinc nor cadmium concentrations appeared to be elevated in any baseline bay (Figures 21 and 22).

3.2.2.2.2 Organics. Of the baseline bays, only Samish Bay contained detectable concentrations of phthalates and AHs. (Figure 24).

3.2.3 Statistical Treatment

3.2.3.1 Discriminant Analyses

Results of discriminant analyses are graphically presented in Figures 26 and 27. The variables which produced the greatest amount of separation (discrimination) were found to be Ag, IR, and As. The locations of each bay or station in the respective graphs are plotted in two-dimensional space by multiplying the concentrations of Ag, IR, and As for that bay or station by the coefficients shown in Table 15.

Both plots seem to show that the baseline bays do not discriminate from each other; they are all closely clustered. However, some discrimination is observed among the urban bays, and particularly for Sinclair Inlet. Silver is the variable in Sinclair Inlet that makes this bay so much different than the other bays. Infrared (IR) response is the variable that tends to make Port Gardner - Everett Harbor different.

TABLE 15. Coefficients for Canonical Variables Resulting from Discriminant Analyses Performed on Physical and Chemical Characteristics of Sediments Collected at 48 Stations

Variable	Coefficients for Canonical Variables		
	X	Y	Z
Ag	-2.76219	0.45734	0.80779
IR	-0.00014	-0.00129	0.00017
As	-0.05677	0.00009	-0.09816

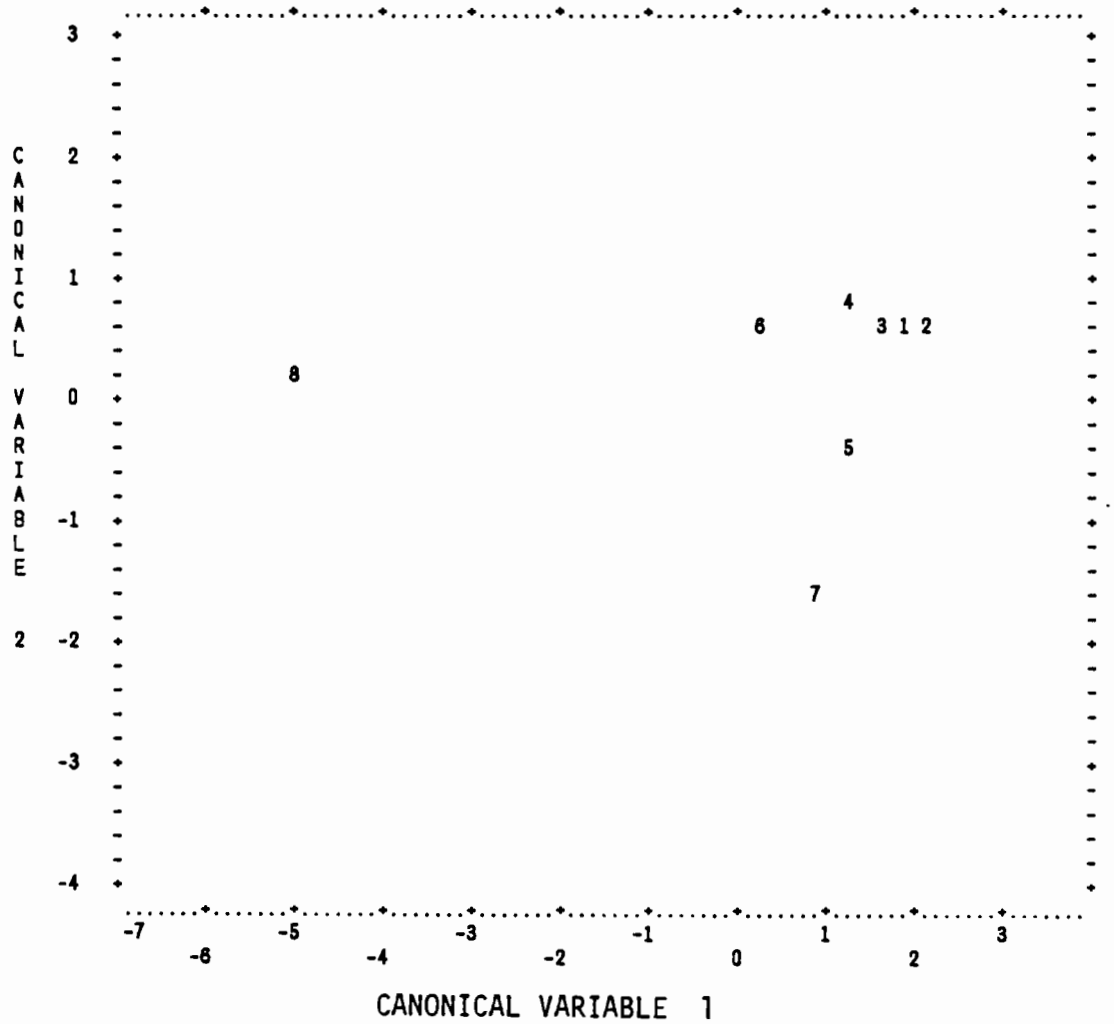


FIGURE 26. Discriminant Analysis of 8 Bays Using the Canonical Variables Ag, IR, and As, where 1 is Samish Bay; 2 is Dabob Bay; 3 is Sequim Bay; 4 is Case Inlet; 5 is Bellingham Bay; 6 is Fourmile Rock - Elliott Bay Dump Site Vicinity; 7 is Port Gardner - Everett Harbor; and 8 is Sinclair Inlet

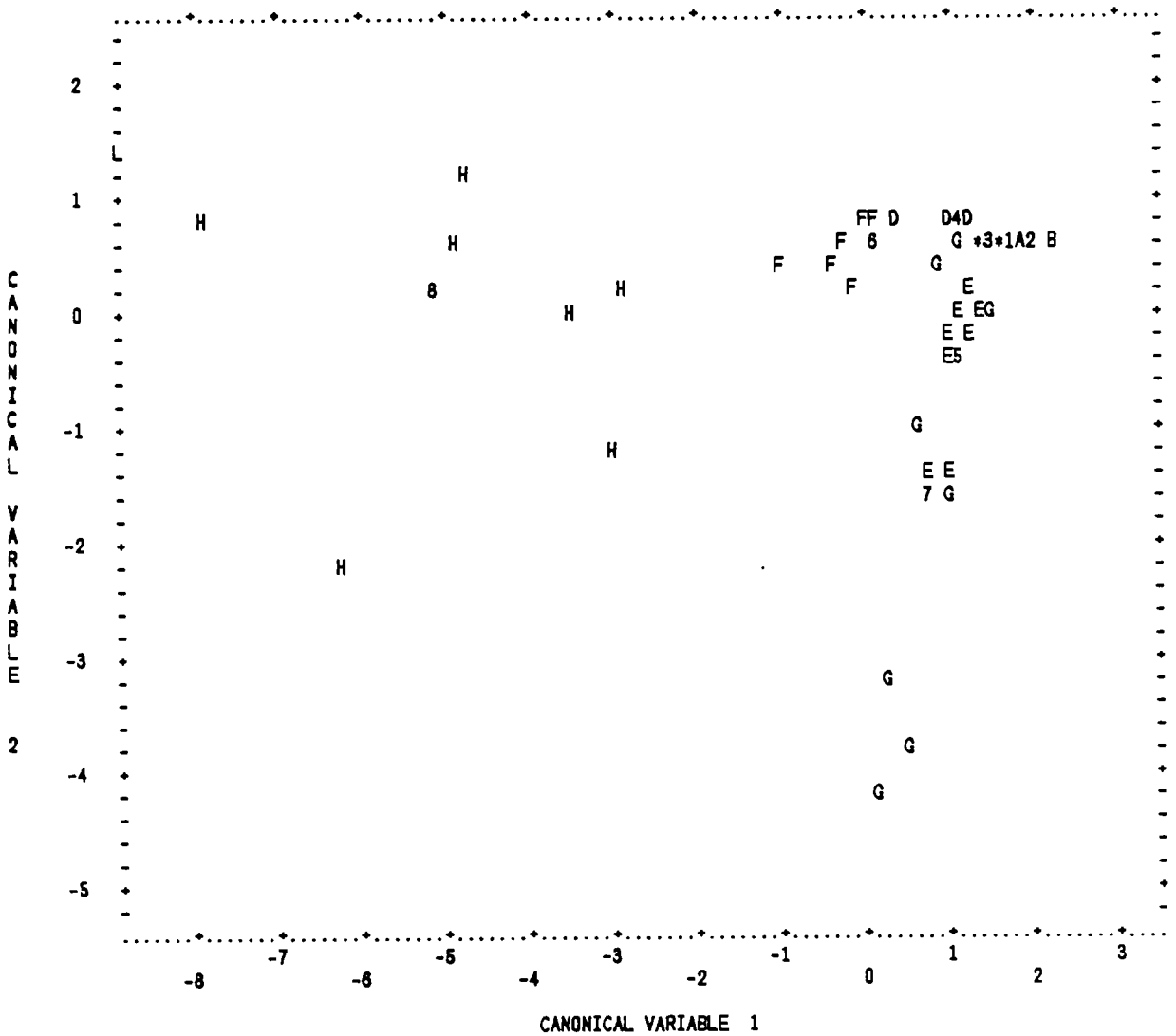


FIGURE 27. Discriminant Analysis of 48 Stations from 8 Bays Using the Canonical Variables Ag, IR, and As, where A are Stations from Samish Bay; B are Stations from Dabob Bay; C are Stations from Sequim Bay; D are Stations from Case Inlet; E are Stations from Bellingham Bay; F are Stations from Fourmile Rock - Elliott Bay Dump Site Vicinity; G are Stations from Port Gardner - Everett Harbor; and H are Stations from Sinclair Inlet. Stations Overlapping are Plotted as an Asterisk (*).

3.2.3.2 Correlation and Factor Analyses

As an aid to the interpretation of the relationships among contaminants and physical properties of the sediments, a correlation coefficient matrix was constructed and R-mode factor analysis applied to the 48 sediments collected in 1984. Data on 13 variables from both urban and baseline bays were included in the factor analysis. These variables included the six metals (Ag, Hg, Pb, As, Cu, and Zn), PCB-1254, AH, IR, TOC, volatile solids (PCVOL), grain size (in phi units) and percent water (PCH2O). Correlation coefficients are shown in Table E.2.

Three major relationships were expressed by the correlation coefficients: 1) the strong correlation between grain size and percent water, 2) the strong correlations among many metals, and 3) the strong correlations among organic contaminants, IR and TOC.

The sorted rotated factor loadings for the 13 variables in 48 sediments showed that 83% of the variance in these data was explained by four factors (Table 16). Factor loadings of less than 0.250 have been replaced by zero. Factor 1 was characterized by high loadings for TOC, PCVOL, IR, AHs and smaller loadings of Zn, PCB-1254, and PCH2O. This factor reflected the association of these contaminants in sediments. The positive correlation coefficients among such contaminants indicated that these contaminants were bound together in contaminated sediments. Factor 1 explained 39% of the variance in the data. Factor 2, which explained 22% of the variance in the data, represented primarily an organic matter factor characterized by high loadings of As, Cu, Ag, Zn, and PCB. Factor 3 accounted for 13% of the variance and was associated with high loadings of Pb and Hg. These heavy metals were highly correlated, apparently because several sediments at the Fourmile Rock - Elliott Bay dump site vicinity were highly enriched in Pb and Hg. The fourth factor, which contributed only 9% of the variance, represented a grain-size factor reflecting both grain size and water content.

TABLE 16. Sorted Rotated Factor Loadings for 13 Sediment Characteristics and Contaminants for 48 Stations

	Factor 1	Factor 2	Factor 3	Factor 4
ASEPA	0.000	0.903	0.000	0.000
CUEPA	0.000	0.893	0.000	0.000
AGMRL	0.000	0.697	0.383	0.000
ZNEPA	0.456	0.623	0.398	0.000
PCB-1254	0.339	0.607	0.269	0.000
TOC	0.960	0.000	0.000	0.000
PCVOL	0.935	0.000	0.000	0.000
IRMRL	0.842	0.000	0.000	0.000
AH	0.647	0.252	0.478	0.000
HGMRL	0.000	0.000	0.938	0.000
PBMRL	0.000	0.392	0.878	0.000
AVGPHI	0.000	0.000	0.000	0.948
PCH2O	0.470	0.000	0.000	0.832

3.3 BENTHIC INFAUNA (PERFORMED BY MRL)

3.3.1 Bathymetry and Sediment Types

To understand the results of the benthic infaunal survey, it is helpful to understand how the general physical characteristics of the eight bays varied. Because the eight bays differed in depth and sediment type, the stations drawn from the bays did not all derive from the same benthic habitat.

Table 17 shows how sediment types, depth, and other physical-chemical characteristics varied among the bays. Based on the means for these physical characteristics, the eight bays were clustered into four groups (Figures 28, 29, and 30). First, the Fourmile Rock - Elliott Bay dump site vicinity and Dabob Bay were distinctly deeper (both over 100 m) and had sediments with higher sand content and lower TOC than those of all the rest. Second, Samish and Bellingham bays were the most shallow and had the highest mud content of the eight. Bellingham Bay had the second highest average TOC level. Third, Case Inlet, Sinclair Inlet, and Sequim Bay clustered together with intermediate mean values for depth, percentages of silt and clay and TOC. These three bays possessed shallow to moderate depths and sediment types of both sandy-silt and silty-mud. Fourth, Port Gardner - Everett Harbor was distinct from the other bays in its considerably higher mean TOC level. Among the bays of shallow to moderate depth, Port Gardner - Everett Harbor contained the least number of stations with silty-mud. Figure 30 summarizes the major differences of depth and sediment characteristics among the eight bays.

3.3.2 Richness and Abundance

Bay means for the number of taxa per station (richness) and the number of individuals per station (abundance) appear in Table 18. For several reasons, the values for number of taxa reported here should be considered a minimum. First, the sampling technique did not obtain sufficient material from sediments with high clay content to fully characterize the typically sparse infauna of such sediments. Second, the sampling techniques could have undersampled the small crustacean component

TABLE 17. Summary of the Distribution of Sediment Types, Depth, and Other Characteristics of the Eight Bays (48 Stations)

Bay	Number of Stations by Sediment Type					Depth (m)		Silt and Clay	
	Sand	Silty Sand	Sandy Silt	Silty Mud	Clayey Mud	Mean	Range	Mean (%)	TOC (%)
Samish	0	0	0	4	0	19	10-30	84	1.5
Dabob	1	2	0	1	0	102	88-113	46	1.8
Sequim	0	0	2	2	0	23	19-26	77	2.2
Case	0	1	1	2	0	29	21-40	72	2.1
Baseline	1	3	3	9	0	43	10-113		
Bellingham	0	0	2	6	0	10	6-17	87	4.0
Fourmile Rock-Elliott Bay[1]	1	1	5	1	0	166	121-189	53	2.0
Port Gardner-Everett Harbor	0	1	6	1	0	25	10-98	63	9.6
Sinclair	0	1	4	3	0	14	7-18	74	2.7
Urban	1	3	17	11	0	54	7-189		

[1] Dump site vicinity.

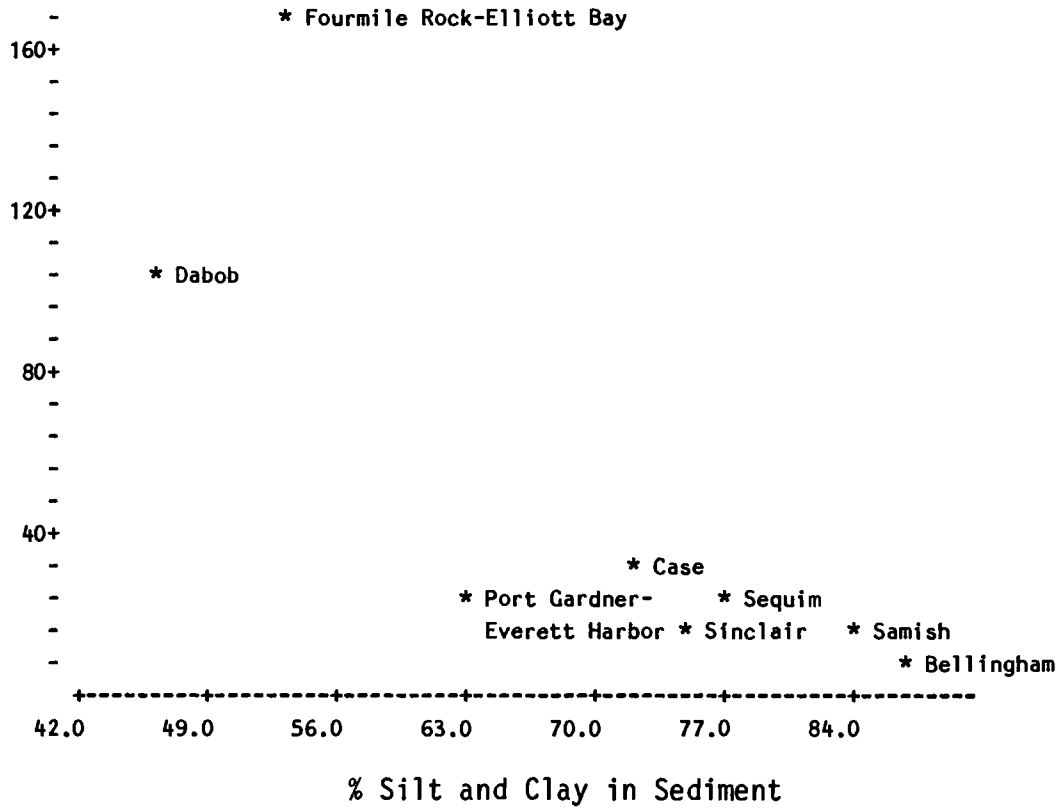


FIGURE 28. Mean Depth Versus Mean Percent Silt and Clay by Bay (48 Stations). The Fourmile Rock - Elliott Bay stations were in and around the dump site.

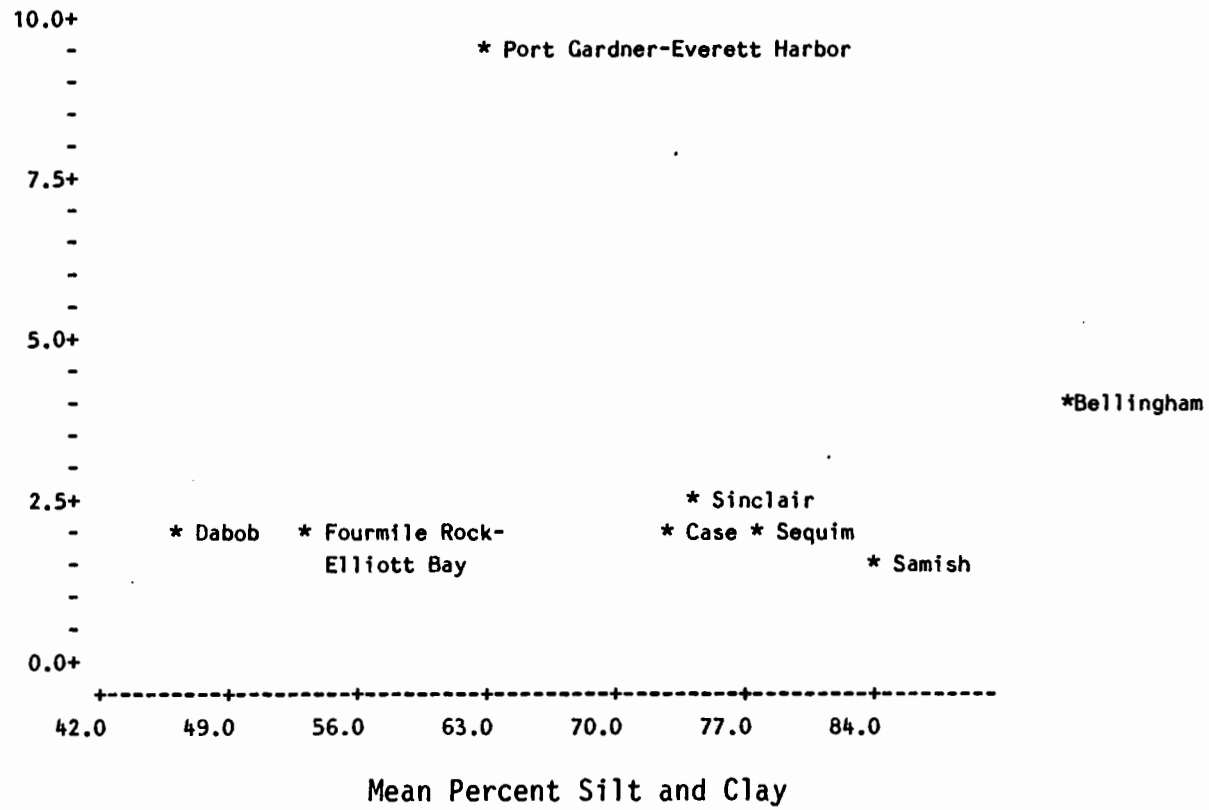


FIGURE 29. Mean TOC Versus Mean Percent Silt and Clay (48 Stations). The Fourmile Rock - Elliott Bay stations were in and around the dump site.

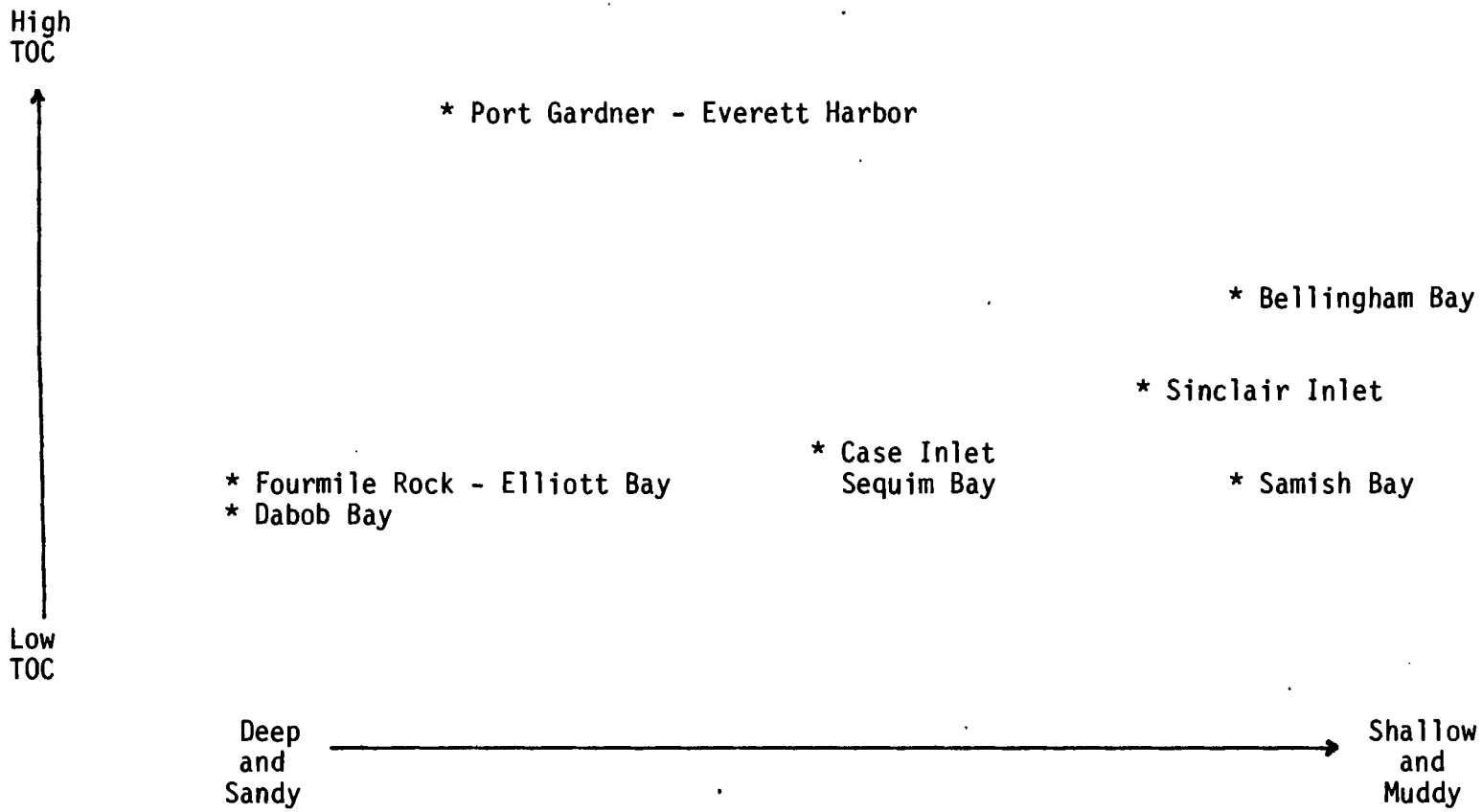


FIGURE 30. Differences Among Bays According to Depth, Sediment Type and TOC (48 Stations). The Fourmile Rock - Elliott Bay stations were in and around the dump site.

TABLE 18. Mean Values for Number of Taxa and Number of Individuals per Station from 48 Stations Within 8 Puget Sound Embayments

Bay	Mean per Station	
	Number of Taxa	Number of Individuals
Baseline		
Samish Bay	11.3	19.3
Dabob Bay	11.3	18.0
Sequim Bay	13.8	113.5
Case Inlet	7.8	13.5
All Baseline	11.0	41.0
Urban		
Bellingham Bay	7.8	71.0
Fourmile Rock - Elliott Bay[1]	9.0	17.5
Port Gardner - Everett Harbor	7.3	113.6
Sinclair Inlet	9.9	82.6
All Urban	9.0	71.0

[1] Dump site vicinity.

of the infauna. Third, for some taxonomic groups (e.g., the polychaete family Cirratulidae), more extensive and specialized examination under a compound microscope is required to completely distinguish genera and species within the group. Because richness was probably underestimated, the benthic infauna data will not support the calculation of diversity indices.

The benthic infauna data are best viewed as supporting their intended role: another measure for reconnaissance rather than a definitive data set able to support sophisticated statistical analysis. These data indicate the numerically dominant taxa and their relative abundance and provide a qualitative picture of the relative richness of the infauna at selected sites in the eight bays. These data likely do not represent conditions in all similar depositional sediments in each bay.

Based on richness and abundance, the eight bays can be classified into four or perhaps five groups (Figure 31): 1) Sequim Bay was characterized by the highest mean number of taxa and the second highest mean abundance; 2) Samish and Dabob Bays were characterized by the second highest number of taxa and low numbers of individuals; 3) Sinclair Inlet and Bellingham Bay were characterized by intermediate to low numbers of taxa and intermediate numbers of individuals; 4) Case Inlet and the Fourmile Rock - Elliott Bay dump site vicinity were characterized by low numbers of taxa and numbers of individuals; 5) Port Gardner - Everett Harbor had the lowest number of taxa and the highest value for number of individuals.

3.3.3 General Infaunal Patterns

At least 120 taxa of benthic infaunal organisms were present at the 48 stations from the 8 bays. For comparison, a survey by Lie (1968), including a wider range of sediment types, and in different areas of Puget Sound, found a total of 157 species from 48 stations. Of the 120 taxa, 52% were polychaetes, 22% mollusks, 15% crustaceans, and 10% other groups. Following Simenstad et al. (1979), 44% were classified as detritivores, 21% as carnivores, 7% as herbivores, 19% as suspension feeders, and 15% as undetermined or other.

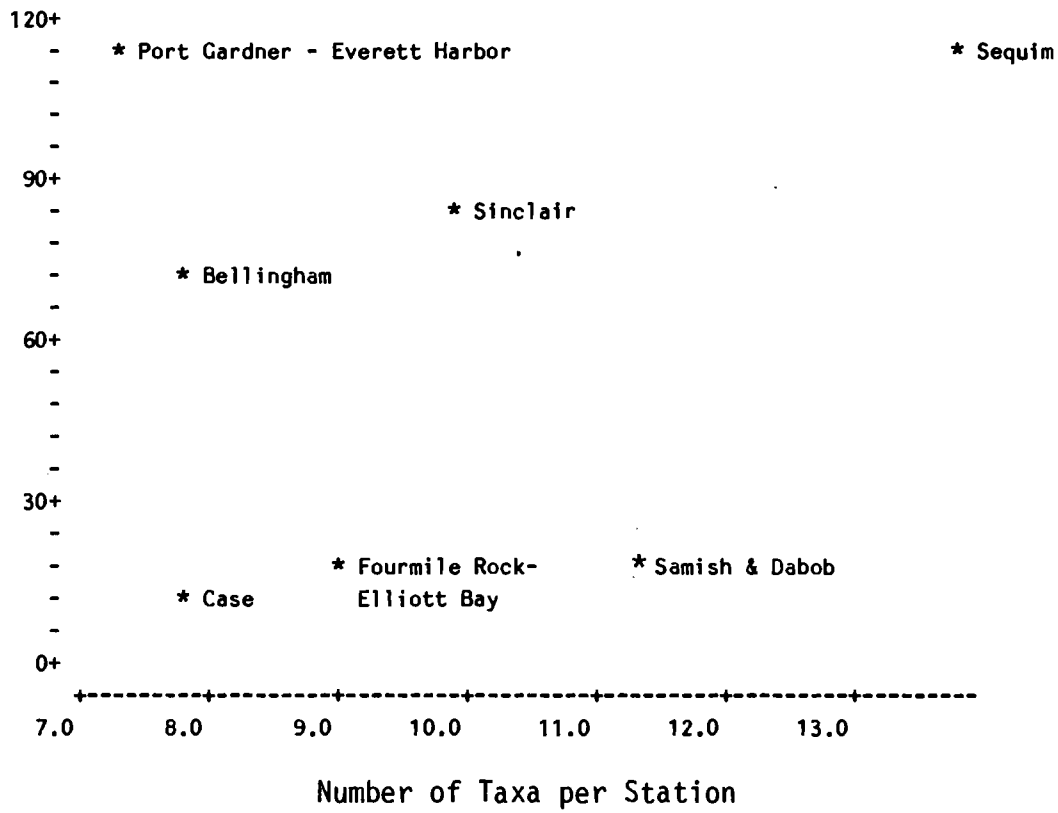


FIGURE 31. Mean Number of Individuals per Station Versus Mean Number of Taxa per Station (48 Stations). The Fourmile Rock - Elliott Bay stations were in and around the dump site.

From all 48 stations, the total number of individuals found was 2941. Based on abundance, polychaetes and detritivores were the dominant taxonomic and trophic groups in all the bays except Port Gardner - Everett Harbor. In Port Gardner - Everett Harbor, one taxon, nematodes, contributed the majority (almost 73%) of the number of individuals per station. Of the mean number of individuals per station, polychaetes were 19% in Port Gardner - Everett Harbor and ranged in the other bays from 47% (Samish Bay) to 95% (Sequim Bay). Mollusks and suspension feeders exceeded 20% of the mean number of individuals in Samish Bay, Dabob Bay, and the Fourmile Rock - Elliott Bay dump site vicinity, the shallow muddy bay and the two deep sandier bays, respectively. With the exceptions just noted, groups other than polychaetes contributed little to the number of individuals.

Over all bays, 10 taxa accounted for 85% of the total number of individuals; 5 taxa, for 79% (Table 19).

TABLE 19. The Ten Most Abundant Taxa for All Bays

Code	Species Name	No. Stations Present	Percent Occur (n/48)	No. Indiv.	Percent Total (n/2941)	Cum. Percent
009	Cirratulids	28	58	1206	41.0	41.0
001	Nematode	8	17	753	25.6	66.6
006	*Capitella capitata*	12	25	157	5.3	71.9
073	*Axinopsida serricata*	22	46	113	3.8	75.7
024	*Nephthys cornuta francisca*	33	69	103	3.5	79.2
021	*Lumbrinereis* spp. A	12	25	40	1.4	80.6
020	*Lumbrinereis* spp. B	4	8	34	1.2	81.8
057	*Polydora* spp.	7	15	34	1.2	83.0
011	*Dorvillea moniloceras*	4	8	29	1.0	84.0
058	*Paraprionspio pinnata*	11	23	28	1.0	85.0

The free-living cirratulid polychaetes (Tharyx spp. plus Chaetozone spp.) occurred at 58% of the 48 stations and accounted for 41% of the total number of individuals. The second most abundant taxon was a nematode found only in two bays, Port Gardner - Everett Harbor and Bellingham Bay. The third most abundant taxon, the capitellid polychaete (Capitella capitata), was found in four bays and achieved large numbers only in Port Gardner - Everett Harbor in association with the nematodes. The fourth most abundant taxon, the mollusk, Axinopsida serricata, occurred in low numbers in all bays except Samish Bay. The fifth most abundant taxon, the nephtyid polychaete, Nephtys cornuta fransciscana, was the most ubiquitous species and occurred in low numbers in all bays at 69% of the 48 stations.

3.3.4 Infaunal Patterns in Each Bay

3.3.4.1 Samish Bay

The four stations in Samish Bay were at shallow to intermediate depths (13-30 m) and contained sediments of silty-mud with TOC levels below 2%. Stations 7 and 20 were more than twice as deep as Stations 1 and 3.

The infauna at all stations (Table 20) were characterized by a wide range of richness (6 to 15 taxa per station) and by low abundance (9 to 26 individuals per station). At the two shallowest stations, polychaetes were the most abundant group, but there were no distinctly dominant species. At the two deepest stations, the sea cucumber, Molpadia intermedia, was the most or second-most abundant species. These latter stations were the only ones of all 48 stations where echinoderms were an important component of the infauna. Samish Bay also had the highest percentage of mollusks in its faunal list (31%).

3.3.4.2 Dabob Bay

The four stations from Dabob Bay were from depths of 88 to 113 m and held sediments with the highest sand content found in all 48 stations. The TOC levels were generally low (<3%). The four Stations 1, 5, 7, and

TABLE 20. Comparison of Stations in Bays by Dominant Benthic Infauna

Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Samish	01	11	19	13	20	19	1.32	*Nephtys cornuta f.*	4	21	*Sternapsis fossor*	4	21
Samish	03	6	9	10	26	19	1.89	*Prionospio cirrifera*	3	33	*Amphipolis* sp.	2	22
Samish	07	13	23	24	27	15	1.32	*Nephtys cornuta f.*	5	22	*Molpadia intermedia*	4	17
Samish	20	15	26	30	26	12	1.39	*Molpadia intermedia*	8	31	*Acila castrensis*	4	15
Dabob	01	17	34	113	8	80	0.83	*Axinopsida serricata*	8	24	*Axiothella rubricincta*	3	9
Dabob	05	13	23	88	10	76	1.39	*Axinopsida serricata*	5	22	*Axiothella rubricincta*	3	13
Dabob	07	12	12	98	24	51	2.14	--			--		
Dabob	15	3	3	110	40	10	2.65	--			--		
Sequim	14	12	96	24	29	36	2.09	Cirratulids	60	63	*Lumbrinereis* spp. B	13	14
Sequim	17	14	96	26	35	17	2.3	Cirratulids	65	68	*Lumbrinereis* spp. B	7	7
Sequim	18	10	153	25	34	21	2.28	Cirratulids	131	86	*Paraprionospio pinnata*	8	5
Sequim	20	19	109	19	35	16	2.23	Cirratulids	72	66	*Lumbrinereis* spp. B	10	9
Case	01	11	17	40	35	11	2.01	*Aricidea* sp.	3	18	*Sigambri bassi*	3	18
Case	11	10	19	21	18	62	1.36	*Aricidea* sp.	6	32	*Axiothella rubricincta*	2	11
Case	15	8	16	28	38	17	2.42	*Aricidea* sp.	4	25	*Haploscoloplos elongatus*	3	19
Case	17	2	2	24	47	22	2.69	--			--		
Bellingham	03	10	66	6	17	33	12.15	*Dorvillea monilaris*	26	39	Cirratulids	22	33
Bellingham	04	10	65	12	25	16	4.83	Nematode	39	60	*Capitella capitata*	9	14
Bellingham	05	10	87	12	25	4	2.34	Cirratulids	68	78	*Nephtys cornuta f.*	8	9
Bellingham	07	1	1	10	28	8	3.15	--			--		
Bellingham	11	5	102	7	37	2	2.07	Cirratulids	95	93	*Macoma* sp.	3	3
Bellingham	12	11	57	6	28	36	3.69	Cirratulids	41	72	*Eteone pacifica*	4	7
Bellingham	23	9	14	17	28	5	2.01	Cirratulids	5	36	*Glycinde armigera*	2	14
Bellingham	24	6	177	11	32	3	2.09	Cirratulids	151	85	*Eteone pacifica*	9	5
Fourmile Rock-Elliott Bay[1]	09	6	12	173	24	44	1.77	*Axinopsida serricata*	4	33	*Prionospio cerrifera*	3	25
Fourmile Rock-Elliott Bay[1]	10	7	11	184	27	31	2.35	*Axinopsida serricata*	3	27	*Nephtys cornuta f.*	2	18
Fourmile Rock-Elliott Bay[1]	12	0	0	189	38	11	2.14	--			--		

[1] Dump site vicinity.

TABLE 20. Comparison of Stations in Bays by Dominant Benthic Infauna (continued)

Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Fourmile Rock-Elliott Bay[1]	17	6	9	171	22	44	1.92	*Nephtys cornuta f.*	3	33	*Axinopsida serricata*	2	22
Fourmile Rock-Elliott Bay[1]	20	11	25	174	18	48	1.39	*Axinopsida serricata*	14	56	*Macoma* sp.	2	8
Fourmile Rock-Elliott Bay[1]	22	11	19	179	26	38	1.93	*Axinopsida serricata*	7	37	*Paraonis gracilis*	2	11
Fourmile Rock-Elliott Bay[1]	23	12	29	122	11	76	1.11	*Axinopsida serricata*	10	34	*Nephtys cornuta f.*	6	21
Fourmile Rock-Elliott Bay[1]	24	19	35	137	7	86	3.8	*Megacrenella columbiana*	4	11	*Nephtys cornuta f.*	3	9
Port Gardner-Everett Harbor	01	5	130	17	20	37	8.98	Nematode	109	84	*Capitella capitata*	16	12
Port Gardner-Everett Harbor	02	6	86	17	21	17	4.17	Nematode	64	74	*Capitella capitata*	17	20
Port Gardner-Everett Harbor	03	5	97	13	23	37	11	Nematode	84	87	*Capitella capitata*	11	11
Port Gardner-Everett Harbor	04	5	169	14	20	46	16.42	Nematode	122	72	*Capitella capitata*	43	25
Port Gardner-Everett Harbor	05	4	335	10	22	33	9.22	Nematode	279	83	*Capitella capitata*	50	15
Port Gardner-Everett Harbor	06	16	33	12	12	60	4.51	*Capitella capitata*	6	18	Nematode	5	15
Port Gardner-Everett Harbor	07	11	30	17	19	32	6.67	Crustacean	12	40	*Macoma* sp.	5	17
Port Gardner-Everett Harbor	11	6	33	98	18	31	2.29	*Axinopsida serricata*	23	70	*Macoma* sp.	3	9
Sinclair	06	8	50	7	26	13	2.93	Cirratulids	36	72	*Nephtys cornuta f.*	5	10
Sinclair	07	12	202	8	25	44	2.12	Cirratulids	142	70	*Polydora brachycephala*	28	14
Sinclair	08	8	61	13	38	10	2.87	Cirratulids	50	82	*Lumbrinereis* spp. A	4	7
Sinclair	14	8	81	15	33	8	3.04	Cirratulids	60	74	*Nephtys cornuta f.*	7	9
Sinclair	17	9	73	15	33	21	2.4	Cirratulids	60	82	*Nephtys cornuta f.*	3	4
Sinclair	18	10	40	17	32	22	2.58	Cirratulids	15	38	*Nephtys cornuta f.*	10	25
Sinclair	19	14	137	16	19	51	2.33	Cirratulids	111	81	*Lumbrinereis* spp. A	9	7
Sinclair	20	9	18	18	29	32	3.28	*Axinopsida serricata*	4	22	*Nephtys cornuta f.*	4	22

15 constituted a gradient in sediment types from sand to silty-mud.

The characteristics of the infauna in Dabob Bay (Table 20) changed along the sand-to-mud continuum. The sandy stations had high richness, but low to moderate abundances. Moving from stations with the most to the least sand, the richness and abundance decreased dramatically from 17 taxa and 34 individuals per station at Station 1, to only 3 species and 3 individuals at Station 15. Station 1 was the third highest of the 48 stations in terms of richness, while Station 15 was one of the four lowest in terms of both infaunal richness and abundance. Of all 48 stations, Station 1 had the second highest sand content, while Station 15 had the second highest clay content.

At the two sandiest stations, the bivalve mollusk, Axinopsida serricata, was the most abundant species with the polychaete, Axiothella rubrocincta, second in abundance. The abundances at the other two stations were too low to distinguish any dominant species.

3.3.4.3 Sequim Bay

The four stations in Sequim Bay were of moderate depth (19 to 26 m). Two stations had sandy-silt, and two had silty-mud sediments.

Of all of the bays, Sequim Bay demonstrated the highest mean number of taxa per station. However, the mean number of individuals in Sequim Bay was second to the highest. The shallowest station had the highest number of taxa and individuals (Table 20). The stations with clay contents of 34% to 35% possessed a diverse and abundant fauna.

Species composition varied little among the four stations. The Cirratulid polychaetes, primarily Tharyx spp., dominated all four stations. The next most abundant species in order were Lumbrinereis spp., Paraprionospio pinnata, and Nephtys cornuta franciscana. Reisch (1959, 1971) listed Prionospio pinnata (now called Paraprionospio pinnata) as characteristic of healthy bottoms.

3.3.4.4 Case Inlet

The four stations from Case Inlet were of moderate depth (21 to 40 m) with sediments ranging from sandy-silt to silty-mud. In contrast to other bays, even the sandy-silt sediment had a relatively high clay content (47%).

Case Inlet showed moderate to low numbers of taxa and consistently low abundances (Table 20). Station 17, with the highest clay content (47%) of the 48 stations sampled, was represented by only two species with a total of two individuals.

Because of the low abundances, a clearly dominant species was not evident in Case Inlet. The polychaetes, *Aricidea* spp. and *Sigambri bassi*, were slightly more abundant than the other species at Stations 1, 11, and 15, which had lower clay content.

As determined during the screening surveys, Case Inlet had the highest mud content of all bays. In selecting the 48 stations to revisit during detailed surveys, our approach for choosing stations in the baseline bays led to selection of the sandier stations in Case Inlet.

3.3.4.5 Bellingham Bay

Bellingham Bay was the shallowest bay (depth 6 to 17 m) and contained six stations with silty-mud and two with sandy-silt sediments. Thus, in addition to Samish Bay, Bellingham Bay exhibited one of the highest mud contents. Although Bellingham Bay did not show stations with clay contents above 37%, five stations did have sand and gravel contents of less than 10%. Accordingly, Bellingham Bay sediments demonstrated the lowest sand content of all eight bays.

In Bellingham Bay (Table 20), three kinds of stations were distinguished: 1) those with sparse infauna, 2) those with infauna dominated by organic enrichment opportunist species, and 3) those with infauna similar to that of Sequim Bay. Station 7, where only one individual was observed, was included in the first category. Station 4, dominated by nematodes and *Capitella capitata*, and Station 3 with some

nematodes and dominated by the polychaete, Dorvillea monilaris, were included in the second category. Stations 5, 11, 12, 23, and 24 with Cirratulid polychaetes in high abundance, but with no nematodes or Capitellid polychaetes, were included in the last category.

A number of polychaete species have been classified as opportunists, because they achieved high abundances where the bottom had been disturbed (dredging, dumping). Of such opportunists, however, only a few of the species appeared to be associated with successions following organic enrichment (Pearson and Rosenberg 1978). Pearson and Rosenberg (1978) reserved the term enrichment opportunist for Capitella capitata, Streblospio spp. and the dorvilleid polychaetes. Stations 3 and 4 showed infauna characteristic of organic enrichment, and both stations had elevated TOC levels. Although Station 3 had a higher TOC level, Station 4 had a greater shift to opportunistic infauna. Station 3 had high TOC, but it also had a high sand content. At higher levels of TOC, less infaunal change occurred at those stations with higher sand and less clay content.

At the remaining stations, the infauna were similiar to that of Sequim Bay. The Cirratulid polychaetes dominated the stations in Bellingham Bay with TOC levels below 4%. Mollusks were more important in Bellingham Bay than elsewhere, and this greater importance was probably related to shallower depths.

3.3.4.6 Fourmile Rock - Elliott Bay

The deepest stations sampled were in the Fourmile Rock - Elliott Bay dump site vicinity, which revealed the most heterogeneity in sediment type among its stations. The dump site vicinity also had the highest sand contents of the urban bays, and only one station possessed silty-mud.

The general characteristics of the infauna (Table 20) varied considerably. Station 12, with silty-mud, was the most barren of all 48 stations, revealing no observed infauna. In contrast, Station 24, with sand, showed the highest number of taxa of the 48 stations and moderate abundance.

The mollusk, Axinopsida serricata, was the most abundant species sampled, and dominated the stations with sandy-silt and silty-sand. A large number of polychaete species occurred at the Fourmile Rock - Elliott Bay dump site vicinity, but none in high abundance. Unlike the other bays where polychaetes dominated, a polychaete was the most abundant species at only one station, No. 17. This polychaete was Nephtys cornuta franciscana found as a common but not a dominant species in the other bays.

The other bay with relatively deep stations was Dabob Bay, which also contained sediments with high sand content. At the sand and silty-sand stations, the Fourmile Rock - Elliott Bay dump site area had a species composition similar to Dabob Bay, but with lower richness and abundance. In silty-mud, stations in both bays had the lowest richness and abundance of species of the 48 stations.

3.3.4.7 Port Gardner - Everett Harbor

The eight stations in Port Gardner - Everett Harbor were of moderate depth (17 to 60 m) and possessed sediments of sandy-silt with relatively low clay content. What distinguished Port Gardner - Everett Harbor from the other bays was its high TOC levels at seven out of the eight stations. Total organic carbon ranged from 2.29% to 16.42%.

Low richness and high abundance occurred at six of the eight stations in Port Gardner - Everett Harbor (Table 20). Five stations were dominated by nematodes and Capitella capitata, the organic enrichment opportunist. Station 6 had both an elevated TOC and a high sand content. Although the nematodes and Capitella capitata did occur there, their numbers did not reach the high levels observed elsewhere in the bay. Swartz et al. (1982) observed that Capitella capitata did not colonize an area recovering from dredging until finer sediment had accumulated. At Station 11 with sandy-silt and TOC <3%, the mollusk, Axinopsida serricata, appeared as the most abundant species, and the nematodes and Capitella capitata were not observed.

Crustaceans were a more important component of the infauna in this bay than elsewhere. Except for one station with sandy-silt and low TOC, mollusks were absent.

Port Gardner - Everett Harbor had the most stations of all eight bays with the most obvious change in benthic infauna.

3.3.4.8 Sinclair Inlet

Sinclair Inlet was one of the more shallow bays (7 to 18 m) and contained sediments ranging from silty-sand through sandy-silt to silty-mud. The infauna (Table 20) were characterized by moderate richness (8 to 14 taxa per station) and moderate to high abundance (18 to 202 individuals per station). Silty-sand supported the highest richness and highest abundance.

As in Sequim Bay, Cirratulid polychaetes were the most abundant taxon at most stations. This taxon generally dominated all stations that were characterized by silty-mud, sandy-silt, or silty-sand, and achieved their highest abundance at stations with higher sand contents (Nos. 19 and 7). Station 20, however, was an exception in that it was dominated by *Axinopsida serricata*.

The station with highest IR and metal levels revealed the least rich and abundant fauna; whereas, the next most contaminated station was one of the richest in species and abundance. Station 20 in sandy-silt sediment exhibited the lowest abundance of all infauna including Cirratulids, but contained a very high IR (3191 ppm) signal. Station 19, with a similarly high IR (1973), but low clay and high sand content, yielded high richness and abundance. At stations with high sand content, the effect of an elevated contaminant on infauna may have been less than at stations with similiar contaminant levels but high silt and clay content. High clay by itself appeared to support an infauna of lower numbers of taxa and individuals.

3.3.5 Infaunal Characteristics by Sediment Type

Placing all 48 stations into three major sediment groups 1) silty-mud, 2) sandy-silt, 3) silty-sand or sand (Table 21), helps us understand the relationships between infauna and sediment type. The sediment types were not evenly distributed among the bays or by depth (Table 17 and Figure 28). For example, Samish Bay contained only silty-mud while Dabob Bay contained sediments with higher sand contents. In moving from one end of this continuum to the other, the percentage of sand in the sediment ranged from as little as 3% to an observed maximum of 86%. In general, the deepest stations had higher sand contents, while the most shallow stations had higher mud contents.

Comparing all stations within a sediment type regardless of bay (Table 21) showed that the stations with silty-sand or sand had distinctly different infaunal characteristics than those with the other two sediment types. Stations with silty-sand or sand generally had a higher number of taxa, while stations with silty-mud and sandy-silt possessed a lower number of taxa. Of all 48 stations, the two stations with the highest sand content also had the highest and second highest number of taxa. Stations with silty-sand or sand had the lowest mean abundance, while those with sandy-silt had the highest abundance. Stations located in sediments of silty-sand or sand showed consistently low abundances except for one station in Sinclair Inlet. This station (No. 19) was considerably more shallow than the rest of the stations in sandy sediments.

The sediment types supported different species compositions. In silty-mud, the species compositions in all bays except Case Inlet and Samish Bay were similiar. The Cirratulid polychaetes, primarily *Tharyx* spp., were the most frequent dominant (45% of the silty-mud stations) and most abundant taxon. In silty-mud, nematodes were in high abundance at only 10% of the stations. In sandy-silt, Cirratulids were not as frequent a dominant (30% of the stations) as in silty-mud. Nematodes had the highest abundance followed by Cirratulids. In sandy-silt, the mollusk, *Axinopsida serricata*, was dominant at 30% of the stations, but it

TABLE 21. Comparison of the Dominant Benthic Infauna by Sediment Type

Silty Mud

Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Samish	01	11	19	13	20	19	1.32	*Nephtys cornuta f.*	4	21	*Sternapsis fossor*	4	21
Samish	03	6	9	10	26	19	1.89	*Prionospio cirrifera*	3	33	*Amphipolis* sp.	2	22
Samish	07	13	23	24	27	15	1.32	*Nephtys cornuta f.*	5	22	*Molpadia intermedia*	4	17
Samish	20	15	26	30	26	12	1.39	*Molpadia intermodia*	8	31	*Acila castrensis*	4	15
Dabob	15	3	3	110	40	10	2.65	--			--		
Sequim	17	14	96	26	35	17	2.3	Cirratulids	65	68	*Lumbrinereis* spp. B	7	7
Sequim	20	19	109	19	35	16	2.23	Cirratulids	72	66	*Lumbrinereis* spp. B	10	9
Case	01	11	17	40	35	11	2.01	*Aricidea* sp.	3	18	*Sigambri bassi*	3	18
Case	15	8	16	28	38	17	2.42	*Aricidea* sp.	4	25	*Haploscoloplos elongatus*	3	19
Bellingham	04	10	65	12	25	16	4.83	Nematode	39	60	*Capitella capitata*	9	14
Bellingham	05	10	87	12	25	4	2.34	Cirratulids	68	78	*Nephtys cornuta f.*	8	9
Bellingham	07	1	1	10	28	8	3.15	--			--		
Bellingham	11	5	102	7	37	2	2.07	Cirratulids	95	93	*Macoma* sp.	3	3
Bellingham	23	9	14	17	28	5	2.01	Cirratulids	5	36	*Glycinde armigera*	2	14
Bellingham	24	6	177	11	32	3	2.09	Cirratulids	151	85	*Eteone pacifica*	9	5
Fourmile Rock-Elliott Bay[1]	12	0	0	189	38	11	2.14	--			--		
Port Gardner-Everett Harbor	02	6	86	17	21	17	4.17	Nematode	64	74	*Capitella capitata*	17	20
Sinclair	06	8	50	7	26	13	2.93	Cirratulids	36	72	*Nephtys cornuta f.*	5	10
Sinclair	08	8	61	13	38	10	2.87	Cirratulids	50	82	*Lumbrinereis* spp. A	4	7
Sinclair	14	8	81	15	33	8	3.04	Cirratulids	60	74	*Nephtys cornuta f.*	7	9
TOC >3.8	N= 2	8	75.5	15.0	23	16.5	4.5						
TOC <3.8	N=18	8.6	49.5	32.2	31.5	11.1	2.23						
Baseline	N= 9	11.1	35.3	33.3	31.3	15.1	1.95						
Urban	N=11	6.5	65.8	28.2	30.1	8.8	2.88						
All Bays	N=20	8.6	52.1	30.6	30.7	11.7	2.46						

[1] Dump site vicinity.

TABLE 21. Comparison of the Dominant Benthic Infauna by Sediment Type (continued)

Sandy Silt

Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Sequim	14	12	96	24	29	36	2.09	Cirratulids	60	63	*Lumbrinereis* spp. B	13	14
Sequim	18	10	153	25	34	21	2.28	Cirratulids	131	86	*Paraprionospio pinnata*	8	5
Case	17	2	2	24	47	22	2.69	--			--		
Bellingham	03	10	66	6	17	33	12.15	*Dorvillea monilaris*	26	39	Cirratulids	22	33
Bellingham	12	11	57	6	28	36	3.69	Cirratulids	41	72	*Eteone pacifica*	4	7
Fourmile Rock-Elliott Bay[1]	09	6	12	173	24	44	1.77	*Axinopsida serricata*	4	33	*Prionospio cirrifera*	3	25
Fourmile Rock-Elliott Bay[1]	10	7	11	184	27	31	2.35	*Axinopsida serricata*	3	27	*Nephtys cornuta f.*	2	18
Fourmile Rock-Elliott Bay[1]	17	6	9	171	22	44	1.92	*Nephtys cornuta f.*	3	33	*Axinopsida serricata*	2	22
Fourmile Rock-Elliott Bay[1]	20	11	25	174	18	48	1.39	*Axinopsida serricata*	14	56	*Macoma* sp.	2	8
Fourmile Rock-Elliott Bay[1]	22	11	19	179	26	38	1.93	*Axinopsida serricata*	7	37	*Paraonis gracilis*	2	11
Port Gardner-Everett Harbor	01	5	130	17	20	37	8.98	Nematode	109	84	*Capitella capitata*	16	12
Port Gardner-Everett Harbor	03	5	97	13	23	37	11	Nematode	84	87	*Capitella capitata*	11	11
Port Gardner-Everett Harbor	04	5	169	14	20	46	16.42	Nematode	122	72	*Capitella capitata*	43	25
Port Gardner-Everett Harbor	05	4	335	10	22	33	9.22	Nematode	279	83	*Capitella capitata*	50	15
Port Gardner-Everett Harbor	07	11	30	17	20	32	6.67	Crustacean	12	40	*Macoma* sp.	5	17
Port Gardner-Everett Harbor	11	6	33	98	18	31	2.29	*Axinopsida serricata*	23	70	*Macoma* sp.	3	9
Sinclair	07	12	202	8	25	44	2.12	Cirratulids	142	70	*Polydora* spp.	28	14
Sinclair	17	9	73	15	33	21	2.4	Cirratulids	60	82	*Nephtys cornuta f.*	3	4
Sinclair	18	10	40	17	32	22	2.58	Cirratulids	15	38	*Nephtys cornuta f.*	10	25
Sinclair	20	9	18	18	29	32	3.28	*Axinopsida serricata*	4	22	*Nephtys cornuta f.*	4	22
TOC >3.8	N= 6	6.7	137.8	12.8	20.1	36.3	10.74						
TOC <3.8	N=14	8.7	53.6	74.9	28.1	33.6	2.34						
Baseline	N= 3	8	83.7	24.3	36.7	26.3	2.35						
Urban	N=17	8.1	78	62.2	23.8	35.8	5.35						
All Bays	N=20	8.1	78.9	56.8	25.8	34.4	4.90						

TABLE 21. Comparison of the Dominant Benthic Infauna by Sediment Type (continued)

Silty Sand and Sand Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Dabob	05	13	23	88	10	76	1.39	*Axinopsida serricata*	5	22	*Axiothella rubricincta*	3	13
Dabob	07	12	12	98	24	51	2.14	--			--		
Case	11	10	19	21	18	62	1.36	*Aricidea* sp.	6	32	*Axiothella rubricincta*	2	11
Fourmile Rock-Elliott Bay[1]	23	12	29	122	11	76	1.11	*Axinopsida serricata*	10	34	*Nephtys cornuta f.*	6	21
Port Gardner-Everett Harbor	06	16	33	12	12	60	4.51	*Capitella capitata*	6	18	Nematode	5	15
Sinclair	19	14	137	16	19	51	2.33	Cirratulids	111	81	*Lumbrinereis* spp.	9	7
Sand													
Dabob	01	17	34	113	8	80	0.83	*Axinopsida serricata*	8	24	*Axiothella rubricincta*	3	9
Fourmile Rock-Elliott Bay[1]	24	19	35	137	7	86	3.8	*Megacrenella columbiana*	4	11	*Nephtys cornuta f.*	3	9
TOC >3.8	N= 1	16	33	12	12	60	4.51						
TOC <3.8	N= 7	13.9	41.3	85.0	13.9	68.9	1.85						
Baseline	N= 4	13	22	80.0	15	67.3	1.43						
Urban	N= 4	15.3	58.5	71.8	12.3	68.3	2.94						
All Bays	N= 8	14.1	40.3	75.9	13.6	67.8	2.18						

occurred in low numbers. In silty-sand or sand, the mollusk, Axinopsida serricata, was the most frequent dominant (38% of the stations).

The infaunas of Case Inlet and Samish Bay did not follow this general pattern of change with sediment type. Case Inlet stations consistently demonstrated the lowest richness and abundance within each sediment type. The polychaete, Aricidea spp., was the most frequent dominant in both silty-mud and silty-sand. Of all of the stations in silty-sand, those in Case Inlet also exhibited the lowest number of taxa. Each station in Samish Bay revealed a different dominant species and low abundance. In both Case Inlet and Samish Bay, the most abundant species contributed less than 33% to the total number of individuals regardless of sediment type. In the other seven bays, the most abundant species typically contributed 50% to 90% of the total number of individuals at a station.

In all sediment types, high TOC was associated with lower richness and elevated abundance, and a shift in species composition to organic enrichment opportunists. Such shifts occurred in all sediment types, but were observed more often in sandy-silt than in silty-sand and silty-mud.

Table 22 shows how the infauna differed with sediment type without the influence of elevated TOC. In the table those stations with TOC levels greater than 4% (the mean TOC level of all 48 stations was 3.8%) and those stations from Case Inlet and Samish Bay are omitted. After partitioning-out the influence of high TOC and the distinctly different infauna of Samish Bay and Case Inlet, a consistent pattern of variation in infaunal characteristics with sediment type became more evident. Richness decreased consistently over a continuum from sand through silt to mud. Abundance was much lower in silty-sand and sand than in the other sediment types. The more sandy stations would have demonstrated even lower abundance except for one station from Sinclair Inlet. This station was by far the most shallow of all stations and had a high sand content. Silty-mud and sandy-silt stations had equivalent abundances when TOC levels were partitioned-out.

TABLE 22. Comparison of the Dominant Benthic Infauna by Sediment Type (Stations with High TOC and Stations from Case Inlet and Samish Bay omitted)

Silty Mud

Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Dabob	15	3	3	110	40	10	2.65	--			--		
Sequim	17	14	96	26	35	17	2.3	Cirratulids	65	68	*Lumbrinereis* spp. B	7	7
Sequim	20	19	109	19	35	16	2.23	Cirratulids	72	66	*Lumbrinereis* spp. B	10	9
Bellingham	05	10	87	12	25	4	2.34	Cirratulids	68	78	*Nephtys cornuta f.*	8	9
Bellingham	07	1	1	10	28	8	3.15	--			--		
Bellingham	11	5	102	7	37	2	2.07	Cirratulids	95	93	*Macoma* sp.	3	3
Bellingham	23	9	14	17	28	5	2.01	Cirratulids	5	36	*Glycinde armigera*	2	14
Bellingham	24	6	177	11	32	3	2.09	Cirratulids	151	85	*Eteone pacifica*	9	5
Fourmile Rock-Elliott Bay[1]	12	0	0	189	38	11	2.14	--			--		
Sinclair	06	8	50	7	26	13	2.93	Cirratulids	36	72	*Nephtys cornuta f.*	5	10
Sinclair	08	8	61	13	38	10	2.87	Cirratulids	50	82	*Lumbrinereis* spp. A	4	7
Sinclair	14	8	81	15	33	8	3.04	Cirratulids	60	74	*Nephtys cornuta f.*	7	9
Baseline	N= 3	12	69.3	51.7	36.7	14.3	2.4						
Urban	N= 9	6.1	63.7	31.2	31.7	7.1	2.5						
All Bays	N=12	7.6	65.1	36.3	32.9	8.9	2.5						

[1] Dump site vicinity.

TABLE 22. Comparison of the Dominant Benthic Infauna by Sediment Type (Stations with High TOC and Stations from Case Inlet and Samish Bay Omitted) (Continued)

Sandy Silt													
Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Sequim	14	12	96	24	29	36	2.09	Cirratulids	60	63	*Lumbrineris* spp. B	13	14
Sequim	18	10	153	25	34	21	2.28	Cirratulids	131	86	*Paraprionospio pinnata*	8	5
Bellingham	12	11	57	6	28	36	3.69	Cirratulids	41	72	*Eteone pacifica*	4	7
Fourmile Rock-Elliott Bay[1]	09	6	12	173	24	44	1.77	*Axinopsida serricata*	4	33	*Prionospio cirrifera*	3	25
Fourmile Rock-Elliott Bay[1]	10	7	11	184	27	31	2.35	*Axinopsida serricata*	3	27	*Nephtys cornuta f.*	2	18
Fourmile Rock-Elliott Bay[1]	17	6	9	171	22	44	1.93	*Nephtys cornuta f.*	3	33	*Axinopsida serricata*	2	22
Fourmile Rock-Elliott Bay[1]	20	11	25	174	18	48	1.39	*Axinopsida serricata*	14	56	*Macoma* sp.	2	8
Fourmile Rock-Elliott Bay[1]	22	11	19	179	26	38	1.93	*Axinopsida serricata*	7	37	*Paraonis gracilis*	2	11
Port Gardner-Everett Harbor	11	6	33	98	18	31	2.29	*Axinopsida serricata*	23	70	*Macoma* sp.	3	9
Sinclair	07	12	202	8	25	44	2.12	Cirratulids	142	70	*Polydora* spp.	28	14
Sinclair	17	9	73	15	33	21	2.4	Cirratulids	60	82	*Nephtys cornuta f.*	3	4
Sinclair	18	10	40	17	32	22	2.58	Cirratulids	15	38	*Nephtys cornuta f.*	10	25
Sinclair	20	9	18	18	29	32	3.28	*Axinopsida serricata*	4	22	*Nephtys cornuta f.*	4	22
Baseline	N= 2	11	124.5	24.5	31.5	28.5	2.2						
Urban	N=11	8.9	45.4	94.8	25.6	35.5	2.3						
All Bays	N=13	9.2	57.5	84.0	26.5	34.5	2.3						

TABLE 22. Comparison of the Dominant Benthic Infauna by Sediment Type (Stations with High TOC and Stations from Case Inlet and Samish Bay Omitted) (Continued)

Silty Sand and Sand Bay	Station	No. Taxa	No. Indiv.	Depth (m)	Percent Clay	Percent Sand and Gravel	Percent TOC	Most Abundant Species	No. Indiv.	Percent Total Indiv.	Second Most Abundant	No. Indiv.	Percent Total Indiv.
Dabob	05	13	23	88	10	76	1.39	*Axinopsida serricata*	5	22	*Axiothella rubricincta*	3	13
Dabob	07	12	12	98	24	51	2.14	--			--		
Fourmile Rock-Elliott Bay[1]	23	12	29	122	11	76	1.11	*Axinopsida serricata*	10	34	*Nephtys cornuta f.*	6	21
Sinclair	19	14	137	16	19	51	2.33	Cirratulids	111	81	*Lumbrinereis* spp. A	9	7
Sand Dabob	01	17	34	113	8	80	0.83	*Axinopsida serricata*	8	24	*Axiothella rubricincta*	3	9
Sand Fourmile Rock-Elliott Bay[1]	24	19	35	137	7	86	3.8	*Megacrenella* sp.	4	11	*Nephtys cornuta f.*	3	9
Baseline	N= 3	14	23	99.7	14	69	1.5						
Urban	N= 3	15	67	91.7	12.3	71	2.4						
All Bays	N= 6	14.5	45	95.7	13.2	70	1.9						

Without the influence of high TOC, species composition in silty-mud was primarily dominated by Cirratulid polychaetes, primarily Tharyx monilaris. In sandy-silt, Cirratulids were the most abundant taxon at half of the stations, whereas Axinopsida serricata was the most abundant species in the other half. Within sandy-silt, Cirratulids were dominant at stations with the highest clay and lowest sand content, whereas Axinopsida serricata was dominant at stations with higher sand contents. In silty-sand and sand, Axinopsida serricata was the common dominant. Cirratulids were the most abundant taxon at the most shallow sandy station. From Table 22, Nephtys cornuta franciscana was commonly the second-most abundant species found in urban bays. Its contribution to the total number of individuals was greater in sandy-silt than in silty-sand and sand, and smallest in silty-mud.

In summary, along the continuum from shallow muddy stations to deep sandy stations, the species composition of those stations without high TOC changed from one dominated heavily by Cirratulid polychaetes in silty-mud to one dominated by Axinopsida serricata in sand. Along the same continuum, the number of taxa increased while the number of individuals decreased.

3.3.6 Statistical Treatment

3.3.6.1 Correlation Analyses

The correlation matrix presented in Appendix E was again used to interpret relationships among biological indices and physical and chemical properties of the sediments. As before, data on 25 variables from both urban and baseline bays were included.

The station abundances of nematodes and Capitata capitella were positively correlated with water content, grain size, and AH. The strongest positive correlations ($p < 0.001$) of abundance of opportunists occurred with percent volatile solids and TOC. The

abundances of the opportunist infauna were also strongly, but negatively, correlated ($p < 0.001$) with amphipod survival (i.e., opportunist abundances were high at stations where amphipod survival was lowest).

3.4 FISH AND SHELLFISH PATHOLOGY (PERFORMED BY NOAA)

3.4.1 Fish Pathology

A total of 138 English sole (*Parophrys vetulus*) and 83 Dover sole (*Microstomus pacificus*) were collected and examined from 10 stations. A broad spectrum of pathologic conditions were observed in each species. These lesions were classified as either infectious (lesions associated with infectious agents, such as a virus, rickettsia, or a protozoan, mesozoan, or metazoan parasite) or idiopathic (lesions having no apparent association with infectious organisms). Because the results of previously conducted studies (Malins et al. 1982, 1984; McCain et al. 1982) on diseases of wild flatfish populations in Puget Sound have indicated that the liver, kidney, and gill are most often affected with lesions of an idiopathic nature, the focus of the present study was on idiopathic conditions of these organs. These lesions may be caused by chemical contaminants, nutritional imbalances, genetic disorders, microorganisms (not distinguishable at the light microscope level), trauma, or as yet undefined environmental factors.

3.4.1.1 English Sole

Liver Lesions. Microscopic lesions of the liver were categorized into eight broad classifications based on histopathologic features. These classifications were 1) neoplastic conditions, 2) preneoplastic conditions (hyperplasia/foci of cellular alteration), 3) specific degenerative lesions, 4) storage disorders, 5) nonspecific degenerative lesions, 6) vascular disorders, 7) proliferative disorders, and 8) inflammation (Malins et al. 1982, 1984). The incidences of these liver lesions in

English sole are shown by area and station in Tables 23 and 24. Hepatic neoplasms were detected only in sole from Sinclair Inlet and Case Inlet. The incidence of hepatic neoplasms is not significantly higher than the overall incidence of neoplasms in English sole from all areas sampled in this study. Similarly, preneoplastic conditions were found only in Sinclair and Case inlets, with the incidence of these lesions at Station 6 in Sinclair Inlet being significantly higher than at any of the other stations. English sole with specific degenerative hepatic lesions were found only in the Fourmile Rock - Elliott Bay dump site vicinity and Sinclair Inlet; the incidence was highest at Station 23 in Elliott Bay. Storage disorders in the liver were generally found to be widely distributed throughout the sampling sites, with significantly lower incidences at the baseline stations in Case Inlet and offshore Eliza Island and significantly higher incidences at the stations in Sinclair Inlet. The incidences of the other five types of hepatic lesions in English sole were either generally similar at a number of sampling stations, or the incidences were very low (Tables 23, 24).

Because the incidence of some liver lesions in English sole has been found to increase with age, the fish from each station were classified into five size classes that corresponded approximately to five age categories ranging from 1+ year class to >5+ year class. Table 24 gives the number of fish examined and the incidences of liver lesions for each size class by groups of stations (i.e., all reference sites, all Elliott Bay sites including West Point, and all Sinclair Inlet sites). One instance of significantly greater lesion incidence was found among the >5 size class. Fish in this size class from the Sinclair Inlet stations were found to have more preneoplastic conditions than fish of comparable size from either the reference group or the Elliott Bay stations. On the other hand, four instances of significantly lower lesion incidences in individual size classes were found. In size class 2, significantly lower incidences of preneoplasms were found among fish from the reference group when compared to the Elliott Bay and Sinclair Inlet groups. Among the

TABLE 23. Incidences (%) of Idiopathic Liver Lesions in English Sole from Stations Sampled in Puget Sound, March 1984

		Lesion Types (%)								
I.D. (NODC #)	Sample Size	Neoplasms	Preneoplastic Lesions	Specific Degenerative/ Necrotic	Storage	Non-Specific Degenerative/ Necrotic	Vascular Disorders	Proliferative Disorders	Inflammations	Any Idiopathic Liver Lesions
Case 5 (12134)	30	3.3	3.3	0.0	6.7(L)[1]	43.3	0.0	0.0	3.3	46.7
Eliza Is. (04015)	28	0.0	0.0(L)	0.0	7.1(L)	7.1	0.0	0.0	0.0(L)	14.3(L)
Dabob 15 (07026)	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fourmile Rock-Elliott Bay 17[2] (10061)	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0(L)
Fourmile Rock-Elliott Bay 23[2] (10063)	12	0.0	0.0	25.0(H)	16.7	16.7	0.0	0.0	0.0	41.7
West Pt. (10065)	2	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	50.0
Sinclair 17 & 18 (08009 & 08006)	30	0.0	10.0	0.0	46.7(H)	33.3	3.3	0.0	16.7	70.0(H)

[1] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.
[2] Dump site vicinity.

TABLE 23. Incidences (%) of Idiopathic Liver Lesions in English Sole from Stations Sampled in Puget Sound, March 1984 (Continued)

		Lesion Types (%)								
I.D. (NODC #)	Sample Size	Neoplasms	Preneo-plastic Lesions	Specific Degenera-tive/ Necrotic	Storage	Non-Specific Degenera-tive/ Necrotic	Vascular Disorders	Prolif-erative Disorders	Inflam-mations	Any Idiopathic Liver Lesions
Sinclair 6 (08010)	30	6.7	30.0(H)	10.0	53.3(H)	46.7	6.7	3.3	20.0	73.3(H)
All Ref Stations (Case, Eliza, Dabob)	59	1.7	1.7(L)	0.0(L)	6.8(L)	25.4	0.0	0.0	1.7(L)	30.5(H)
All Sinclair Stations	60	3.3	20.0(H)	5.0	50.0(H)	40.0(H)	5.0	1.7	18.3(H)	71.7(H)
All Elliott Stations	19	0.0	0.0	21.1(H)	10.5	10.5(L)	0.0	0.0	0.0	31.6

TABLE 24. Incidences (%) of Idiopathic Liver Lesions in English Sole from Reference and Urban Areas by Size Class

	Size Class[1]				
	1	2	3	4	5
Number examined					
Reference	14	34	4	2	5
Elliott Bay	0	1	4	7	7
Sinclair Inlet	11	1	3	1	43
Percent neoplasms					
Reference	0.0	0.0	0.0	0.0	20.0
Elliott Bay	-	0.0	0.0	0.0	0.0
Sinclair Inlet	0.0	0.0	0.0	0.0	4.6
Percent "preneoplasms"					
Reference	0.0	0.0(L)[2]	0.0	0.0	20.0
Elliott Bay	-	0.0	0.0	0.0	0.0
Sinclair Inlet	9.1	0.0	0.0	0.0	25.6(H)[2]
Percent specific degeneration/necrosis					
Reference	0.0	0.0	0.0	0.0	0.0(L)
Elliott Bay	-	100.0	0.0	28.6	14.3
Sinclair Inlet	0.0	0.0	0.0	0.0	7.0
Percent storage disorders					
Reference	14.3	2.9	0.0	0.0	20.0
Elliott Bay	-	0.0	0.0	28.6	0.0(L)
Sinclair Inlet	0.0	100.0	0.0	0.0	51.2
Percent nonspecific necrosis					
Reference	7.1	20.6	50.0	100.0	60.0
Elliott Bay	-	100.0	0.0	14.3	0.0(L)
Sinclair Inlet	18.2	0.0	33.3	0.0	48.8
Percent vascular disorders					
Reference	0.0	0.0	0.0	0.0	0.0
Elliott Bay	-	0.0	0.0	0.0	0.0
Sinclair Inlet	0.0	0.0	0.0	0.0	7.0

[1] Size class corresponds approximately to age class as follows:

Size class	1	2	3	4	5
Age class	1+	2+	3+	4+	>5+

[2] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.

TABLE 24. Incidences (%) of Idiopathic Liver Lesions in English Sole from Reference and Urban Areas by Size Class (Continued)

	Size Class[1]				
	1	2	3	4	5
Percent proliferative disorders					
Reference	0.0	0.0	0.0	0.0	0.0
Elliott Bay	-	0.0	0.0	0.0	0.0
Sinclair Inlet	0.0	0.0	0.0	0.0	2.3
Percent inflammations					
Reference	7.1	0.0	0.0	0.0	0.0
Elliott Bay	-	0.0	0.0	0.0	0.0
Sinclair Inlet	9.1	0.0	33.3	0.0	20.9
Any idiopathic liver lesion					
Reference	21.4	23.5	50.0	100.0	60.0
Elliott Bay	-	100.0	0.0	57.1	14.3(L)[2]
Sinclair Inlet	36.4	100.0	66.7	0.0	83.7

[1] Size class corresponds approximately to age class as follows:

Size class	1	2	3	4	5
Age class	1+	2+	3+	4+	>5+

[2] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.

class of 5+ year fish, significantly lower incidences of lesions were found among fish from the Elliott Bay stations.

Kidney Lesions. Lesions were primarily observed in the excretory (as opposed to the hematopoietic) component of the kidney. Lesions were detected in the glomerular and tubular segment of the of the nephron, and were categorized as 1) depositional disorders (mesangiosclerosis of the glomeruli, and hypermembranous glomeruli), 2) necrotic lesions (mesangiolysis of the glomeruli, tubular epithelial necrosis and tubular epithelial vacuolization), and 3) proliferative lesions (mesangial hypercellularity in the glomeruli, proliferation of the visceral/parietal layers of Bowman's capsule). All three of the above categories were found in the glomerular segment, whereas necrotic lesions were almost exclusively found in the tubular segment. Depositional disorders were found only in sole from Sinclair Inlet, with a higher incidence at Station 6 than at other Sinclair stations combined (Table 25). Necrotic and proliferative lesions were widely distributed throughout the areas sampled, with only the incidence of necrotic lesions in sole from Station 6 in Sinclair Inlet being higher. A higher incidence of one or more idiopathic kidney lesions was also observed in sole from this station and in Sinclair Inlet in general.

Gill Lesions. Three principal types of gill lesions were detected: proliferative disorders, vascular disorders, and inflammation. Proliferative lesions were observed in the respiratory epithelium, filament epithelium, and mucous cells of both epithelial components. Respiratory epithelial hyperplasia (a proliferative disorder) was often associated with fusion of adjacent gill lamellae and/or filaments. Proliferation of the pillar cells forming the internal structural component of the lamellae was also found. Vascular disorders were characterized by multiple sites of intralamellar capillary dilations, microaneurysms or telangiectasis. These occurred in absence of

TABLE 25. Incidences (%) of Idiopathic Kidney and Gill Lesions in English Sole

I.D. (NODC #)	Kidney Lesions (%)					Gill Lesions (%)				
	Sample Size	Despositional Disorders	Necrotic	Proliferative Lesions	One or More Lesions	Sample Size	Proliferative Lesions	Vascular Disorders	Inflam-mation	One or More Lesions
Case 5 (12134)	30	0.0(L)[1]	6.7	20.0	26.7	30	3.3(L)	6.7	56.7	56.7
Eliza Is. (04015)	26	0.0	3.9	30.0	30.8	26	23.1	19.2	61.5	73.1
Dabob 15 (07026)	1	0.0	0.0	0.0	0.0	1	0.0	0.0	0.0	0.0
Fourmile Rock-[2] Elliott Bay 17 (10061)	5	0.0	20.0	20.0	40.0	4	0.0	25.0	50.0	75.0
Fourmile Rock-[2] Elliott Bay 23 (10063)	12	0.0	0.0	25.0	25.0	12	0.0(L)	16.7	41.7	41.7
West Pt. (10065)	2	0.0	0.0	0.0	0.0	2	0.0	0.0	50.0	50.0
Sinclair 17 & 18 (08009 & 08006)	30	6.7	13.3	23.3	40.0	28	42.8(H)	10.7	42.8	67.8

[1] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.
[2] Dump site vicinity.

TABLE 25. Incidences (%) of Idiopathic Kidney and Gill Lesions in English Sole (Continued)

I.D. (NODC #)	Kidney Lesions (%)					Gill Lesions (%)				
	Sample Size	Despositional Disorders	Necrotic	Proliferative Lesions	One or More Lesions	Sample Size	Proliferative Lesions	Vascular Disorders	Inflam-mation	One or More Lesions
Sinclair 6 (08010)	30	25.9(H)	25.9(H)	33.3	66.7(H)	30	53.3(H)	20.0	40.0	30.0
All Ref Stations (Case, Eliza, Dabob)	57	0.0	5.3	24.6	28.1	57	12.3	12.3	57.9	63.2
All Sinclair Inlet	60	15.8(H)	19.3	28.1	52.6(H)	58	48.3(H)	15.5	41.4	74.1
All Elliott Bay	19	0.0	5.3	21.1	26.3	18	0.0(L)	16.7	44.4	50.0

hemorrhage, but the dilated capillaries often contained fibrin accumulations indicative of a coagulation disorder or a tendency towards thrombosis, possibly from endothelial injury. Most commonly, however, the lesion consisted of simple capillary dilation and congestion, possibly associated with the shock response induced in capture. Inflammation generally consisted of multifocal sites of lymphocytic and histiocytic infiltration in the subepithelial regions of the gill lamellae and filaments. There was generally no accompanying necrosis, and this condition can be interpreted as a nonspecific chronic response to injury.

Proliferative lesions of the gill were most prevalent in sole from Sinclair Inlet and near Eliza Island - Bellingham Bay; the incidence of these lesions was highest at stations in Sinclair Inlet (Table 24). This condition was not detected in sole from any Elliott Bay site and was found in only one sole from Case Inlet. Vascular disorders and inflammatory conditions were found in sole from most of the sampling stations at generally similar incidences.

3.4.1.2 Dover Sole

Liver Lesions. Liver abnormalities observed in Dover sole were similar to those described for English sole, except no hepatic neoplasms were detected in Dover sole. Preneoplastic liver lesions were detected only in Dover sole from Station 17 in Fourmile Rock - Elliott Bay (6.7%, 2 of 30 fish) (Table 26). Specific hepatocellular degenerative lesions were found only in sole (10%, 3 of 30 fish) from the station near West Point in Elliott Bay. The incidences of storage disorders, nonspecific degenerative lesions, vascular disorders, and proliferative lesions in sole were generally similar at all of the Elliott Bay stations. The incidence of inflammatory lesions in Dover sole was significantly higher at Station 17 (23.3%, 7 of 30 fish), although this condition was also found at the West Point Station (6.7%, 2 of 30 fish).

TABLE 26. Incidences (%) of Idiopathic Liver Lesions in Dover Sole from Elliott Bay

Lesion Type	Lesion Incidence (%) at Each Station				
	Fourmile Rock-[1] Elliott Bay 17 10061	Fourmile Rock-[1] Elliott Bay 23 10063	West Pt. 10065	Duwamish Head 10064	All Elliott Bay
N=	30	20	30	3	83
Neoplasms	0.0	0.0	0.0	0.0	0.0
Preneoplasms	6.7(H)[2]	0.0	0.0	0.0	2.4
Specific degenerative	0.0	0.0	10.0	0.0	3.6
Storage disorders	13.3	15.0	10.0	0.0	12.1
Nonspecific degenerative	30.0	5.0	13.3	33.3	18.1
Vascular disorders	3.3	0.0	6.7	0.0	3.6
Proliferative	3.3	5.0	0.0	0.0	2.4
Inflammation	23.3(H)	0.0(L)	6.7	0.0	10.8
Any lesion	53.3	25.0	33.3	33.3	38.6

[1] Dump site vicinity.
[2] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p<0.05$.

3.4.1.2.1 Kidney Lesions. Very low incidences (3.3%) of the kidney lesions previously described for English sole were detected in Dover sole (Table 26). Only Dover sole from the West Point Station had detectable kidney lesions.

Gill Lesions. Gill lesions previously described for English sole were also observed in Dover sole. Gill necrosis and proliferation were found only in Dover sole from the West Point Station, 3.5% (1 of 28 fish) and 21.4% (6 of 28 fish), respectively (Table 27). The incidences of vascular disorders and inflammatory lesions in Dover sole were generally similar at all of the Elliott Bay Stations.

3.4.2 Shellfish Pathology

A total of 148 *Cancer magister*, 73 *Cancer gracilis*, 55 *Pandalus platyceros*, and 53 *Pandalopsis dispar* were collected and examined from 14 stations. Histopathological conditions were placed into several general categories, as follows.

1. vascular disorders: congestion and microaneurysms
2. inflammatory conditions: hemocytic responses, melanized lesions, and unmelanized granulomata
3. degenerative disorders: cellular degenerative changes, nuclear atypia, and hydropic degeneration with membrane lyis
4. proliferative conditions as reflected by increased mitotic activity and
5. parasitic/infectious disorders.

Vascular disorders were typically confined to the gill, and congestion of the gill filaments by hemocytes and microaneurysms (marked dilation of gill filaments caused by hemolymph accumulation in the hemal space) was the most common condition.

TABLE 27. Incidences (%) of Idiopathic Kidney and Gill Lesions in Dover Sole from Elliott Bay

Lesion Type	Lesion Incidence (%) at Each Station				
	Fourmile Rock-[1] Elliott Bay 17 10061	Fourmile Rock-[1] Elliott Bay 23 10063	West Pt. 10065	Duwamish Head 10064	All Elliott Bay
Lesion Type					
Kidney					
N=	29	19	30	3	81
Deposition	0.0	0.0	0.0	0.0	0.0
Necrotic lesions	0.0	0.0	3.3	0.0	3.3
Proliferative	0.0	0.0	0.0	0.0	0.0
Inflammation	0.0	0.0	0.0	0.0	0.0
One or more lesions	0.0	0.0	3.3	0.0	3.3
Gill					
N=	29	20	28	3	80
Necrotic lesions	0.0	0.0	3.6	0.0	1.3
Proliferative	0.0	0.0	21.4	0.0	7.5
Vascular disorders	31.0	35.0	42.9	0.0	35.0
Inflammation	65.5	75.0	78.6	33.3	71.3
One or more lesions	72.4	85.0	85.7	33.3	78.8

[1] Dump site vicinity.

Inflammatory conditions included hemocytic responses, which are thought to be analogous to the short-term inflammatory response in vertebrates. These responses typically were represented by hemocyte infiltration into supportive connective tissues or epithelial layers, hyalinocytic encapsulation and/or hyalinocytic phagocytosis. Lesions that probably represent an analog to the chronic inflammatory response in vertebrates were melanized lesions and unmelanized granulomata, which are general, nonspecific responses to injury or parasitic/infectious agents (Bang 1983; Fontaine and Lightner 1975; Sparks 1980).

Lesions within the degenerative category were represented by a broad spectrum of conditions characteristic of degenerative or dying cells. These lesions included pyknotic nuclei, nuclear enlargement and other nuclear atypia, crystalline inclusions, hyalinization, vacuolar degeneration, and hydropic degeneration in the cytoplasm. Two more specific lesion types within the degenerative category included a nuclear change characterized by a clear halo around the nucleus just inside the nuclear membrane, and hydropic degeneration with membrane lysis, which was present only in the digestive epithelium of the hepatopancreas. The latter lesion was characterized by cytoplasmic cloudy swelling, dissolution of the plasma membrane, decreased clarity of cellular contents, and epithelial exfoliation from the underlying basement membrane.

Proliferative conditions were characterized by increased numbers of mitotic figures per microscopic field in various tissues, particularly in the hemopoietic tissue. This increased mitotic activity is probably a compensatory response to cell injury in these tissues or a host response to an unspecified endogenous or exogenous mitogen. In no case was there any evidence of neoplastic transformation of the tissues involved.

Parasitic/infectious conditions included nematode and trematode helminth infections, microsporidian and other unspecified protozoan infections, fungal infections, suspected bacterial infections, and nuclear/cytoplasmic inclusion bodies suggestive of viral infections.

3.4.2.1 Cancer magister

Cancer magister were caught in three locations: Bellingham Bay, Samish Bay and Dungeness Spit (Table 2). No microscopic lesions were detected in the cardiac and pyloric stomachs, or the gonads of C. magister. Two main categories of inflammatory conditions appeared consistently in most of the organs of C. magister: melanized lesions and unmelanized granulomas. The incidences of these two lesion types varied considerably among the different collecting sites and from station to station within each sampling area, revealing no clear distributional pattern. Incidences of melanized lesions in some tissues (hindgut, eye and bladder, Table 28; see Table 34 for codes) were found to be somewhat higher (11% to 40%) in animals from Samish Bay and Dungeness Spit compared to Bellingham Bay (0% to 7.7%). These same lesions also occurred less frequently in the hindgut of crabs from Stations 11 and 12 and Stations 23 and 24 in Bellingham Bay. Unmelanized granulomas in the antennal gland were detected only in animals from Samish Bay and Dungeness Spit. Unmelanized granulomas in the bladder were not detected in crabs from Dungeness Spit, but were detected at low incidences at the other two areas.

A variety of distribution patterns were observed with the other histopathological conditions. One type of degenerative disorder, hydropic degeneration/membrane lysis of digestive epithelial cells of the hepatopancreas, was found only in animals from Bellingham and Samish Bays (Table 29). This lesion was found to occur at significantly greater frequencies in crab from Stations 4 and 5 in Bellingham Bay and significantly lower frequencies in crab from Dungeness Spit. The incidence of nuclear atypia in digestive epithelial cells of the hepatopancreas fluctuated broadly among the crab from different areas. The incidence of proliferative conditions in the hemopoeitic tissue of C. magister was significantly lower in Samish Bay and higher at Stations 11 and 12 in Bellingham Bay. The incidences of all inflammatory conditions (Table 29) were either similar or varied too broadly to determine clear distributional patterns among the sampling areas. For

TABLE 28. Incidences (%) of Individual Histopathological Conditions in *Cancer magister*

Tissue organ lesion type[1] (no. examined)	Bellingham Bay Station 11-12	Bellingham Bay Station 4-5	Bellingham Bay Station 23-24	Samish Bay Station 1	Samish Bay Station 20	Dungeness Spit
Ampulla (N)	(28)	(27)	(15)	(26)	(29)	(11)
Mel lesion	43	30	60	46	55	64
Unmel gran	0	0	0	0	3.4	0
ANG (N)	(28)	(26)	(13)	(22)	(24)	(10)
Mel	3.6	0	0	0	8.3	0
Unmel gran	0	0	0	4.5	4.2	10
Degen dis	7.1	7.7	0	4.5	4.2	0
Hemo infil	11	15	7.7	14	17	0
Bladder (N)	(30)	(29)	(17)	(28)	(28)	(11)
Mel lesion	6.7	3.4	0	11	21	18
Unmel gran	10	3.4	0	3.6	0	0
Viral inf	3.3	3.4	0	0	0	10
Epidermis (N)	(30)	(28)	(17)	(29)	(28)	(10)
Mel lesion	3.3	3.6	0	6.9	11	0
Viral inf	0	0	0	3.4	3.6	0
Esophagus (N)	(22)	(25)	(12)	(17)	(18)	(9)
Mel lesion	4.5	8.0	0	5.9	17	11
Hemo infil	0	12	8.3	0	0	0
Eye (N)	(28)	(27)	(13)	(25)	(25)	(10)
Mel lesion	3.6	7.4	7.7	12	24	20
Unmel gran	3.6	0	7.7	4.0	0	20
Hemo infil	18	22	15	24	0	20
Gill (N)	(28)	(23)	(17)	(27)	(28)	(10)
Mel lesion	29	26	29	56	32	40
Unmel gran	11	0	0	7.4	7.1	20
Hemo tissue (N)	(24)	(23)	(14)	(23)	(26)	(9)
Prol cond	54(H)[2]	35	29	8.7(L)	12(L)	33
HEP (N)	(30)	(29)	(17)	(28)	(28)	(10)
Mel lesion	6.7	3.4	5.9	7.1	7.1	10
Unmel gran	6.7	6.9	0	11	7.1	20
Hyd deg	33	45(H)	12	11	14	0(L)
Nuc atypia	20	24	18	50	32	20
Viral inf	0	3.4	0	0	0	0
Hindgut (N)	(30)	(29)	(16)	(25)	(29)	(10)
Mel lesion	0(L)	6.9	0(L)	32	31	40
Unmel gran	0	3.4	13	12	21	0
Hemo infil	13	0(L)	13	12	0(L)	0
Midgut(N)	(29)	(27)	(17)	(25)	(26)	(11)
Mel lesion	10	11	12	20	7.7	18
Unmel gran	0(L)	11	0(L)	25	15	36(H)

[1] Definitions of codes for tissue and lesion types in Tables 28-33 are given in Table 34.

[2] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.

TABLE 29. Incidences (%) of Major Histopathological Categories in *Cancer magister* from Bellingham Bay, Samish Bay, and Dungeness Spit

Tissue/organ[1] lesion type	Bellingham Bay Station 11-12	Bellingham Bay Station 4-5	Bellingham Bay Station 23-24	Samish Bay Station 1	Samish Bay Station 20	Dungeness Spit
Ampulla Inflam cond[2]	43	30	60	46	55	64
ANG Inflam cond[2]	18	15	7.7	14	21	10
Bladder Inflam cond[2]	17	6.7	0(L)[3]	14	21	18
Epidermis Inflam cond[2]	3.3	3.6	0	6.9	11	0
Esophagus Inflam cond[2]	4.5	12	8.3	5.9	17	11
Eye Inflam cond[2]	25	33	23	36	24	60
Gill Inflam cond[2]	39	35	29	56	36	60
HEP Inflam cond[2]	13	6.9	5.9(L)	18	14	30
Degen dis[4]	37	59	18(L)	54	36	20
Hindgut Inflam cond[2]	13	10(L)	19	48(H)	41	40
Midgut Inflam cond[2]	10	19	12	36	23	55(H)

[1] Definitions of codes for tissue and lesion types in Tables 28-33 are given in Table 34.
[2] Inflammatory conditions consisting of melanized lesions, and unmelanized granulomas and hemocytic infiltration.
[3] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.
[4] Degenerative disorders consisting of hydropic degeneration/membrane lysis and nuclear atypia.

example, the incidences of these conditions in the bladder and hepatopancreas were significantly lower in crabs from Stations 23 and 24 in Bellingham Bay, significantly lower in the hindgut of crab from Stations 4 and 5 in Bellingham Bay, but significantly higher in both the hindgut of crab from Station 1 in Samish Bay and in the midgut of crab from Dungeness Spit.

3.4.2.2 Cancer gracilis

Cancer gracilis were caught in three areas: Case Inlet, Sinclair Inlet and Bellingham Bay (Table 2). Histopathological lesion categories were the same as for C. magister, except that hydropic degenerative/ membrane lysis in the hepatopancreas was not observed in C. gracilis. Also no lesions were observed in the cardiac stomach, esophagus, gonad or midgut ampulla of C. gracilis.

The incidence of melanized lesions in all tissues but eye, bladder, and hindgut were consistently higher in crabs from Case Inlet than in those from Sinclair Inlet (Table 30). These lesions were significantly more prevalent in the antennal gland and gill of crab from Case Inlet. The incidences of unmelanized granulomas in the antennal gland, bladder, and midgut were significantly higher in crab from Case Inlet. The incidences of these lesions in epidermis and pyloric stomach were similar between the two areas; however, they were more frequently found in hemopoietic tissue and hindgut of crab from Sinclair Inlet. A distinct distributional pattern among the sampling sites was not observed for proliferative conditions in the hemopoietic tissue.

The incidence of combined inflammatory conditions of the gill (Table 31) was significantly higher in crabs from Case Inlet, and these lesions were significantly less prevalent in the antennal gland and epidermis in crab from Sinclair Inlet (Stations 17-20). Incidences of these conditions in other tissues were generally similar amongst the sampling areas. The small sample (4 individuals) of C. gracilis captured in Bellingham Bay precluded any meaningful comparisons in lesion incidences with the other areas.

TABLE 30. Incidences (%) of Individual Histopathological Conditions in *Cancer gracilis*

Tissue organ lesion type[1] (no. examined)	Case Inlet Station 11	Bellingham Bay Station 23-24	Sinclair Inlet Station 17-20	Sinclair Inlet Station 7
ANG (N)	(16)	(4)	(13)	(25)
Mel	29(H)[2]	0	7.7	4.0
Unmel gran	35(H)	0	0(L)	8.0
Hemo infil	5.9	0	7.7	8.0
Degen dis	18	0	7.7	4.0
Bladder (N)	(23)	(4)	(16)	(27)
Mel lesion	26	0	25	11
Unmel gran	26(H)	25	6.3	3.7
Viral inf	22	50	19	19
Degen dis	26	50	19	26
Epidermis (N)	(23)	(4)	(16)	(28)
Mel lesion	52	0	6.3	41
Unmel gran	8.7	0	6.3	3.6
Gill (N)	(22)	(4)	(14)	(25)
Mel lesion	41(H)	0	7.1	4.0(L)
Unmel gran	8.7	0	14	12
Hemo tissue (N)	(20)	(2)	(16)	(22)
Mel lesion	15	0	0	0
Unmel gran	0	0	0	5
Prol cond	10	0	13	4.5
Eye (N)	(22)	(4)	(12)	(25)
Mel lesion	0	0	0	12
Hemo infil	4.5	0	8.3	0
HEP (N)	(22)	(4)	(17)	(28)
Mel lesion	27	0	18	11
Unmel gran	23	25	5.6	18
Nuc atypia	32	0	29	36
Viral inf	4.5	25	12	0
Hindgut (N)	(17)	(3)	(17)	(26)
Mel lesion	5.9	33	5.9	3.8
Unmel gran	5.9	0	12	7.7
Midgut(N)	(18)	(4)	(15)	(25)
Mel lesion	17	0	6.7	12
Unmel gran	50(H)	75	20	20(L)
PYL STO (N)	(19)	(4)	(12)	(28)
Mel lesion	11	0	0	3.6
Unmel gran	5.3	0	0	3.6
Parasite Prev	40	50	29	27
Viruses	30	50	29	19
Nematodes	5	0	0	0
Trematodes	0	0	0	3.8
Microsporidans	5	0	0	3.8
Fungus	5	0	0	0
Unidentified	0	25	0	3.8

[1] Definitions of codes for tissue and lesion types in Tables 28-33 are given in Table 34.

[2] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.

TABLE 31. Incidences (%) of Major Histopathological Categories in *Cancer gracilis* from Case and Sinclair Inlets

Tissue/organ[1] lesion type	Case Inlet Station 11	Sinclair Inlet Station 17-20	Sinclair Inlet Station 7
ANG Inflam cond[2]	59	7.7(L)[3]	20
Bladder Inflam cond[2]	48	25	15
Epidermis Inflam cond[2]	52	6.3(L)	43
Gill Inflam cond[2]	15(H)	0	5
Hemo tissue Inflam cond[2]	15	0	5
Eye Inflam cond[2]	4.5	8.3	12
HEP Inflam cond[2]	32	18	32
Hindgut Inflam cond[2]	12	18	12
Midgut Inflam cond[2]	50	27	28
PYL STO Inflam cond[2]	11	0	7.1

[1] Definitions of codes for tissue and lesion types in Tables 28-33 are given in Table 34.
[2] Inflammatory conditions consisting of melanized lesions, and unmelanized granulomas and hemocytic infiltration.
[3] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.

3.4.2.3 Pandalopsis dispar

Pandalopsis dispar were caught in two areas: Fourmile Rock - Elliott Bay dump site vicinity and Dabob Bay (Table 2). No pathologic conditions were observed in the epidermis, pyloric stomach, or hemopoietic, heart, and nervous tissues. Some pathological conditions were detected only in shrimp from Dabob Bay, including melanized lesions of the bladder, hyalinocytic encapsulation of the eye, and gill, and gonads. Parasitic infections were also primarily found in shrimp from Dabob Bay. Melanized lesions and hyalinocytic encapsulation in the gill and hepatopancreas of shrimp from Dabob Bay were particularly severe. Congestion of the gills was also detected only in shrimp from Dabob Bay. Proliferative conditions, hemocytic infiltration and hyalinocytic encapsulation generally accompanied the melanized lesions in the hepatopancreas. These conditions were probably associated with unidentified parasitic infections. Degenerative disorders of the antennal gland were found only in shrimp from the Fourmile Rock - Elliott Bay dump site vicinity. Inflammatory conditions in all tissues (except eye) (Table 32) were more prevalent in shrimp from Dabob Bay.

3.4.2.4 Pandalus platyceros

Pandalus platyceros were also only captured in the Fourmile Rock - Elliott Bay dump site vicinity and Dabob Bay (Table 2). As with P. dispar, no pathological conditions were observed in the epidermis, hemopoietic tissue, nervous tissue, pyloric stomach, eye, or gonad of P. platyceros (Table 33). Degenerative disorders of the antennal gland and heart were detected only in shrimp from the dump site vicinity. On the other hand, this condition was detected only in the cardiac stomach of shrimp from Dabob Bay. Increased hyalinocytic phagocytosis was detected only in the gills of the dump site vicinity specimens, whereas hyalinocytic encapsulation was more prevalent in the gills and heart of Dabob Bay specimens. Melanized lesions in the hepatopancreas were also observed only in shrimp from the dump site vicinity, but the incidence of these lesions was higher in the gills and heart of shrimp from Dabob Bay.

TABLE 32. Incidences (%) of Individual Histopathological Conditions in Shrimp from Elliott and Dabob Bays

Tissue organ lesion type[1] (no. examined)	*Pandalopsis dispar* Fourmile Rock-Elliott Bay[2] Station 17	*Pandalopsis dispar* Dabob Bay Station 15	*Pandalus platyceros* Fourmile Rock-Elliott Bay[2] Station 17	*Pandalus platyceros* Dabob Bay Station 5
ANG (N)	(16)	(16)	(13)	(24)
Mel	6.3	13	0	0
Hyal encap	13	13	0	0
Para inf	0	13	0	0
Degen dis	6.7	0	15	0
Bladder (N)	(18)	(29)	(21)	(31)
Prol cond	0	6.7	4.8	3.2
Hyal encap	5.6	14	0	0
Mel lesion	0	14	0	0
Degen dis	17	10	4.8	6.5
Para inf	0	6.7	0	0
Hyal phago	0	3.4	0	0
CAS (N)	(23)	(30)	(21)	(31)
Prol cond	4.3	3.3	5.0	19
Degen dis	0	0	0	9.7
Mel lesion	0	6.7	0	0
Esophagus (N)	(22)	(28)	(20)	(30)
Prol cond	4.5	14	10	10
Eye (N)	(22)	(29)	(20)	(31)
Hyal encap	0	3.4	0	0
Mel lesion	0	3.4	0	0
Hemo infil	9.1	3.4	0	0
Gill (N)	(22)	(30)	(21)	(30)
Mel lesion	0	23(H)[3]	4.7	23
Hyal encap	0	13	0(L)	37
Congestion	0	6.7	14	33
Hyal phago	0	6.7	4.8	0
Microan	4.5	13	23	30
Para inf	0	13	0	0
Prol cond	4.5	3.3	0	3.3
Gonad (N)	(22)	(29)	(21)	(30)
Mel lesion	9.1	14	0	0
Hyal encap	0	6.9	0	0
Heart (N)	(17)	(18)	(20)	(25)
Degen dis	0	0	9.1	0
Hyal encap	0	0	0	0
Mel lesion	0	0	0	4.2
HEP (N)	(23)	(30)	(22)	(30)
Prol cond	8.7	20	15	27
Hyal encap	13	30	9.1	13
Mel lesion	13	27	9.1	0
Hyal phago	4.2	17	0	10
Hemo infil	8.3	37	4.5	10
Para inf	8.3	27	0	9.1
Viral inf	0	3.3	0	0

[1] Definitions of codes for tissue and lesion types in Tables 28-33 are given in Table 34.
[2] Dump site vicinity.
[3] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p < 0.05$.

TABLE 33. Incidences (%) of Major Histopathological Categories for Shrimp from Elliott and Dabob Bays

Tissue/organ[1] lesion type	*Pandalopsis dispar* Fourmile Rock-Elliott Bay[2] Station 17	*Pandalopsis dispar* Dabob Bay Station 15	*Pandalus platyceros* Fourmile Rock-Elliott Bay[2] Station 17	*Pandalus platyceros* Dabob Bay Station 5
ANG				
Inflam cond[3]	13	19	0	0
Bladder				
Inflam cond[3]	5.6	14	0	0
CAS				
Inflam cond[3]	0	6.7	0	0
Eye				
Inflam cond[3]	9.1	3.4	0	0
Gill				
Inflam cond[3]	0(L)[4]	23	5.0(L)	31
Vas dis[5]	4.5	20	43	63
Gonad				
Inflam cond[3]	9.1	14	0	0
Heart				
Inflam cond[3]	0	0	0	7.4
HEP				
Inflam cond[3]	17	37	9.1	18

[1] Definitions of codes for tissue and lesion types in Tables 28-33 are given in Table 34.
[2] Dump site vicinity.
[3] Inflammatory conditions including melanized lesions, unmelanized granulomas, hyolinocytic encapsulation, hyalinocytic phagocytosis and hemocytic infiltration.
[4] L indicates stations classified in the low incidence group, and H indicates stations in the high incidence group. Significance level for G-statistic was $p<0.05$. These designations also apply to Tables 3 and 4.
[5] Vascular disorders including congestion and microaneurysms.

TABLE 34. Lesion and Tissue Codes used in Tables 28-33

ANG = Antennal gland
CAS = Cardiac stomach
Degen dis = Degenerative disorders
Hemo infil = Hemocytic infiltration
H = Incidence is significantly higher at the 0.05 confidence level
Hemo tissue = Hemopoietic tissue
HEP = Hepatopancreas
Hyal encap = Hyalinocytic encapsulation
Hyal phago = Hyalinocytic phagocytosis
Inflam cond = Inflammatory conditions
L = Incidence is significantly lower at the 0.05 confidence level
Prol cond = Proliferative conditions
Mel lesion = Melanized lesions
Microan = Microaneurysms
N = Number of tissues examined
Nuc atypia = Nuclear atypia
Para inf = Parasitic infection
Parasite prev = Parasite incidence
Hyd deg = Hydropic degeneration/membrane lysis
PYL STO = Pyloric stomach
Unmel gran = Unmelanized granuloma
Viral inf = Viral infection

3.5 AMPHIPOD BIOASSAY (PERFORMED BY EPA)

3.5.1 Screening Surveys

Results of amphipod bioassays on sediment collected in screening surveys (August 2 to September 18, 1983) are presented in Figures 32 through 39. Tabular data are presented in Appendix C.

Analyses shown in Table 35 indicate that results of the bioassays for urban bays were not clearly distinct from results for the baseline bays. Case Inlet, a baseline bay, showed the lowest mean survival (14.0), followed by another baseline bay, Dabob Bay, and an urban bay, Port Gardner - Everett Harbor (both with mean survival of 15.1). Sinclair Inlet, another urban bay, also demonstrated relatively low mean survival (15.2). Samish Bay, a baseline bay, and Bellingham Bay, a third urban bay, showed intermediate mean survival (16.0 and 16.4, respectively). Sequim Bay, the remaining baseline bay, and Fourmile Rock - Elliott Bay dump site vicinity, the remaining urban area, demonstrated highest mean survival (16.9 and 17.2, respectively).

While the four urban bays showed both higher levels of metals and IR than baseline bays (Table 35), correlation analyses performed on the 207 bioassays from 180 stations only detected a weak negative correlation ($p < 0.025$) between silver and amphipod survival, and IR and amphipod survival ($p < .10$) (Tables 36 and 37). However, a significant negative correlation ($p < 0.001$) also occurred between percent fines and amphipod survival, and similarly between percent water and amphipod survival. The tendency of lower survival became more evident when the exposure sediment was fine-grained, contained a higher percent water, and contained more silver. Examining the frequency distribution of amphipod survival in Table 38 further confirmed that fewer survived in sediment with higher percent fines and percent water.

Despite the finding that lower survival occurred almost exclusively in fine-grained sediments, fine-grained sediments did not automatically mean low amphipod survival. There were 87 cases where 17 or more of 20 amphipods survived. Of these 6 or 6.9% exhibited percent fines $\geq$90% and percent water $\geq$65%.

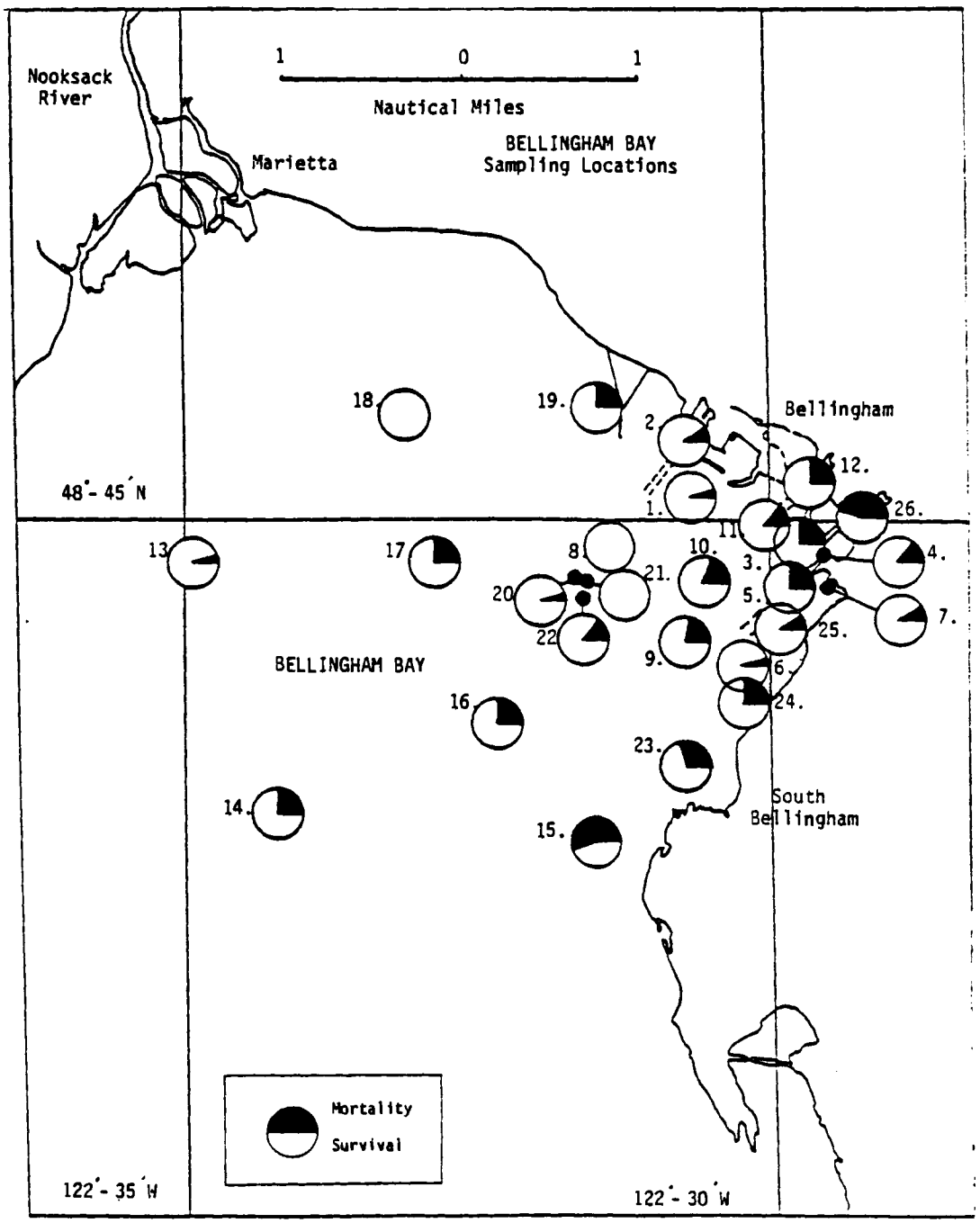


FIGURE 32. Results of Amphipod Bioassays on Sediments Collected in Bellingham bay during Screening Surveys (August 2 to September 18, 1983).

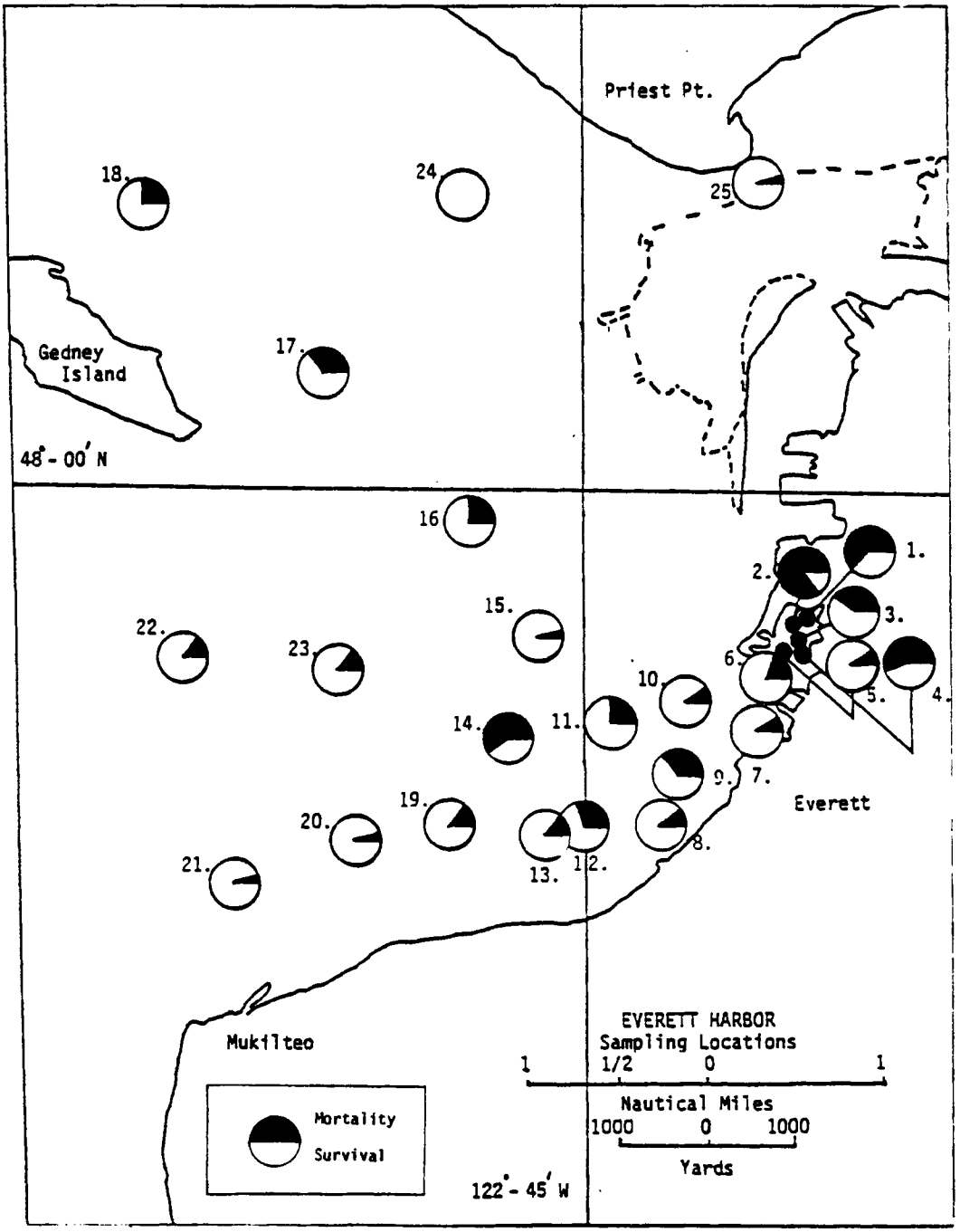


FIGURE 33. Results of Amphipod Bioassays on Sediment Collected in Port Gardner - Everett Harbor During Screening Surveys (August 2 to September 18, 1983)

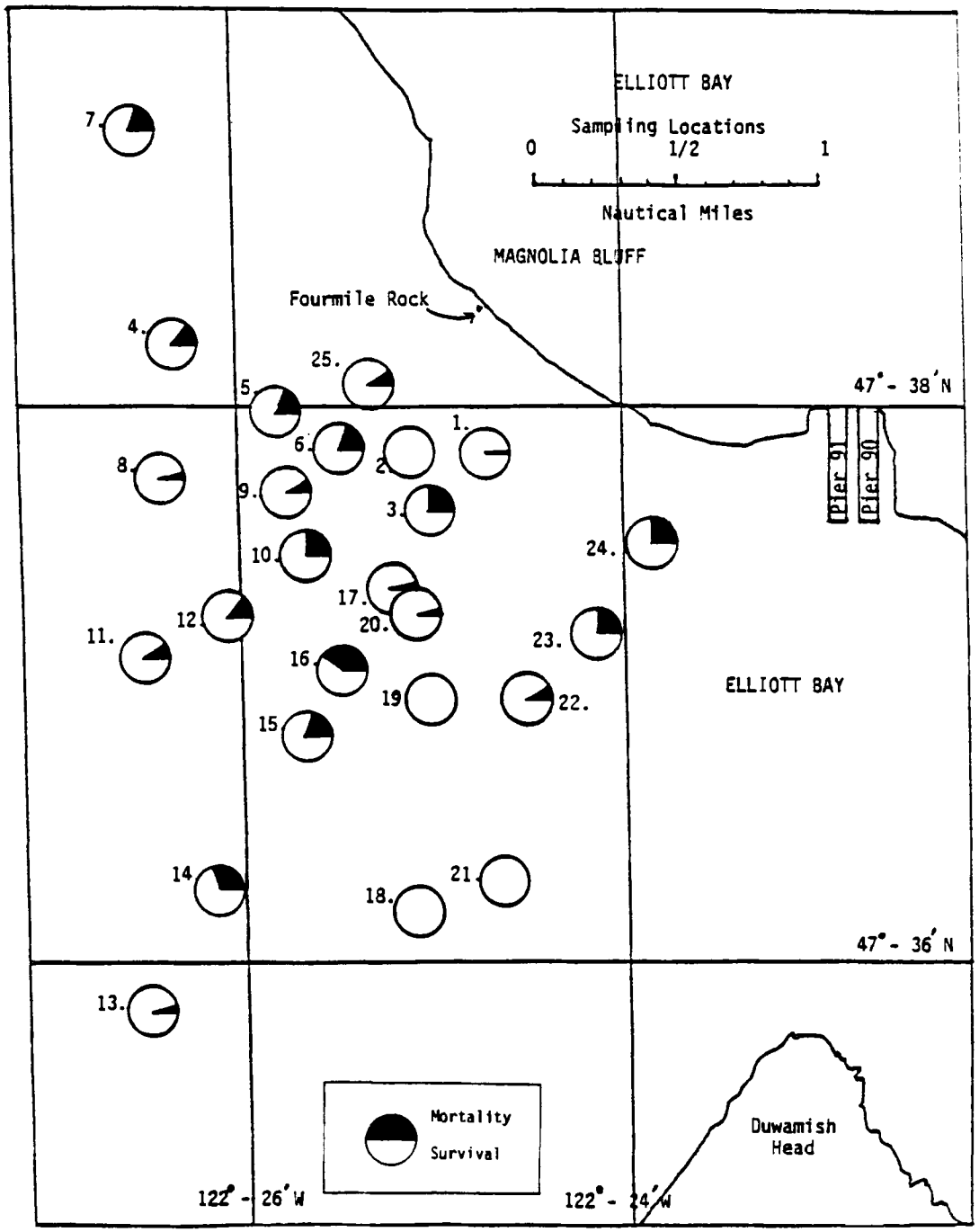


FIGURE 34. Results of Amphipod Bioassays on Sediments Collected in the Fourmile Rock - Elliott Bay Dump Site Vicinity During Screening Surveys (August 2 to September 18, 1983)

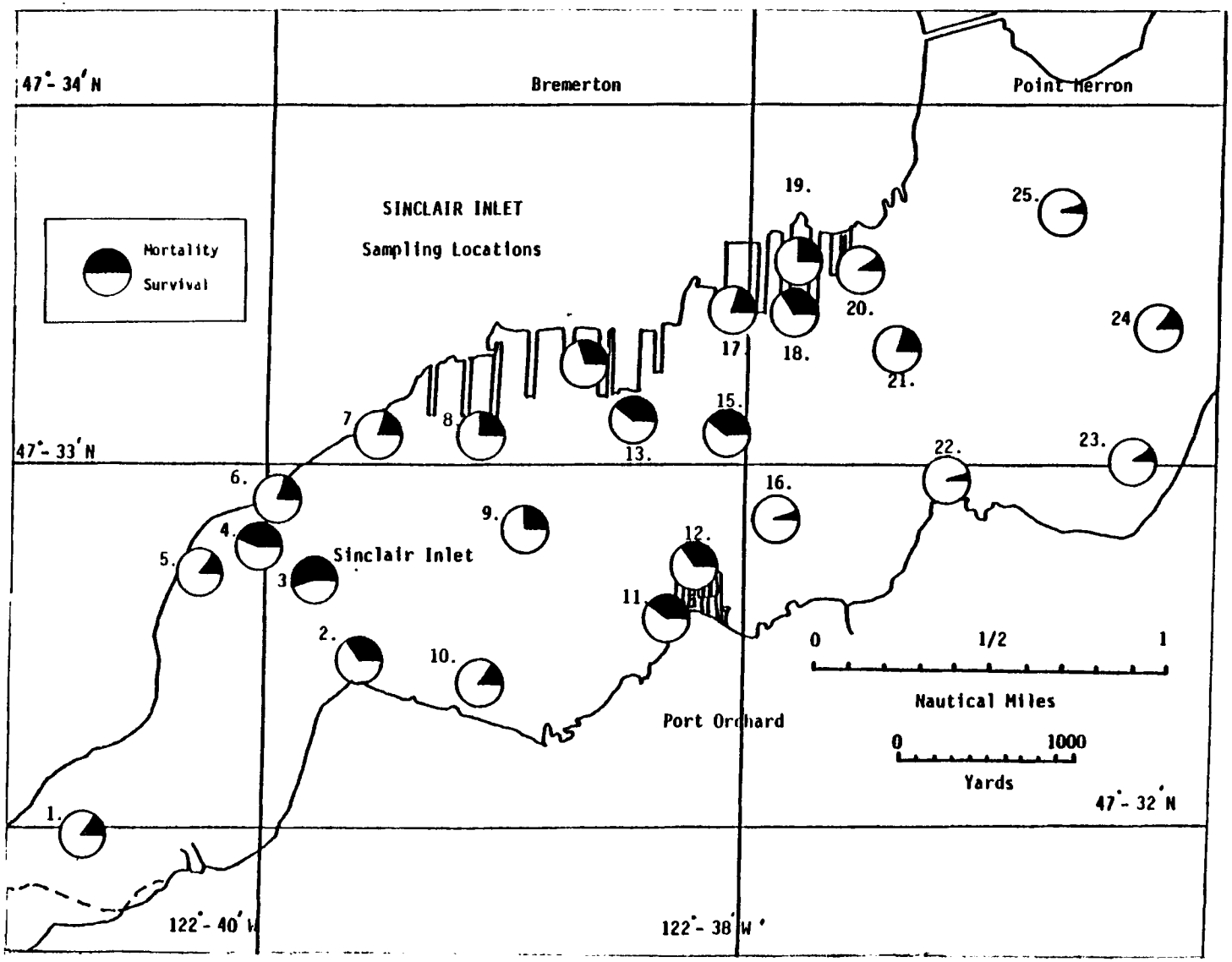


FIGURE 35. Results of Amphipod Bioassays on Sediments Collected in Sinclair Inlet during Screening Surveys (August 2 to September 18, 1983)

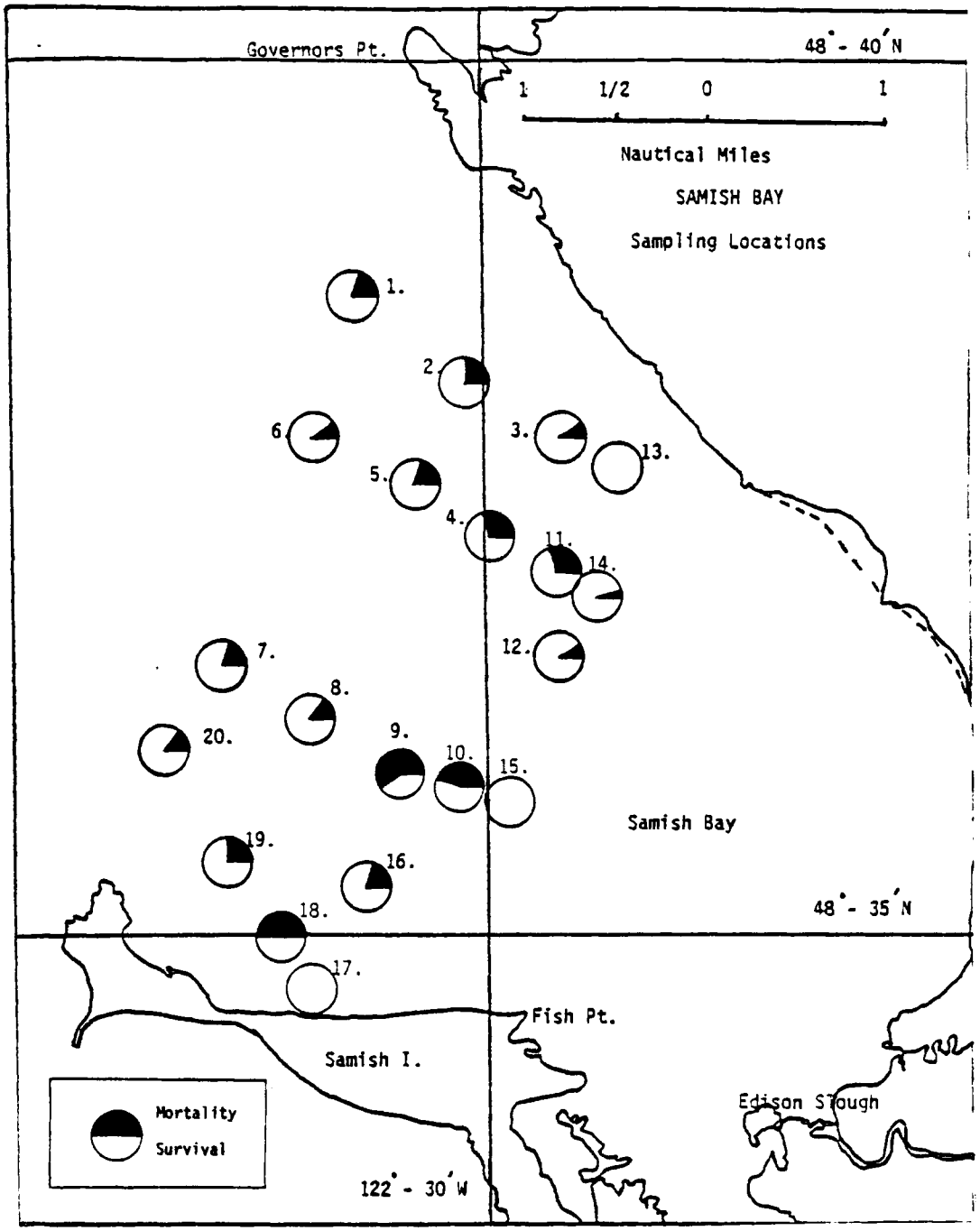


FIGURE 36. Results of Amphipod Bioassays on Sediments Collected in Samish Bay During Screening Surveys (August 2 to September 18, 1983)

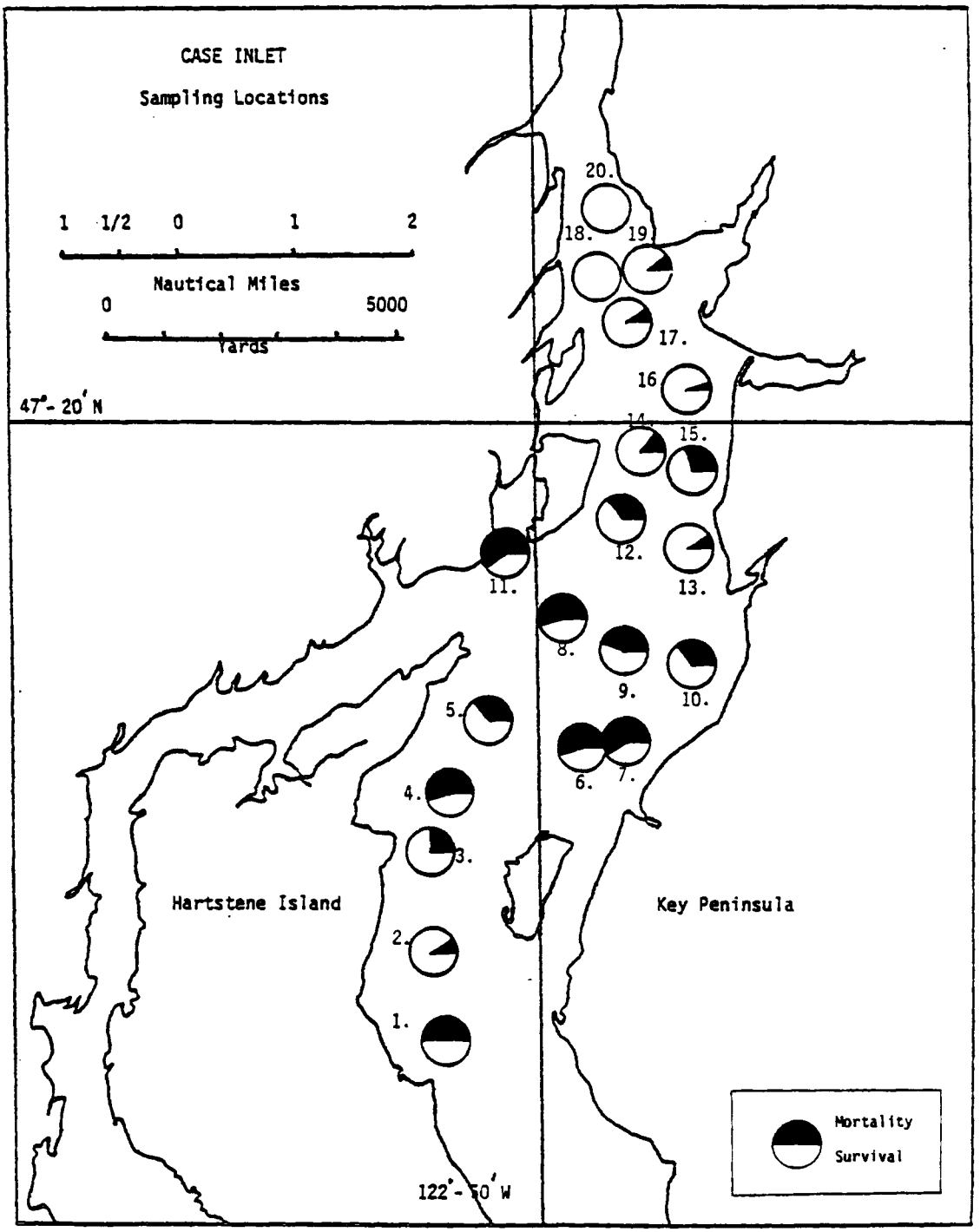


FIGURE 37. Results of Amphipod Bioassays on Sediments Collected in Case Inlet During Screening Surveys (August 2 to September 18, 1983)

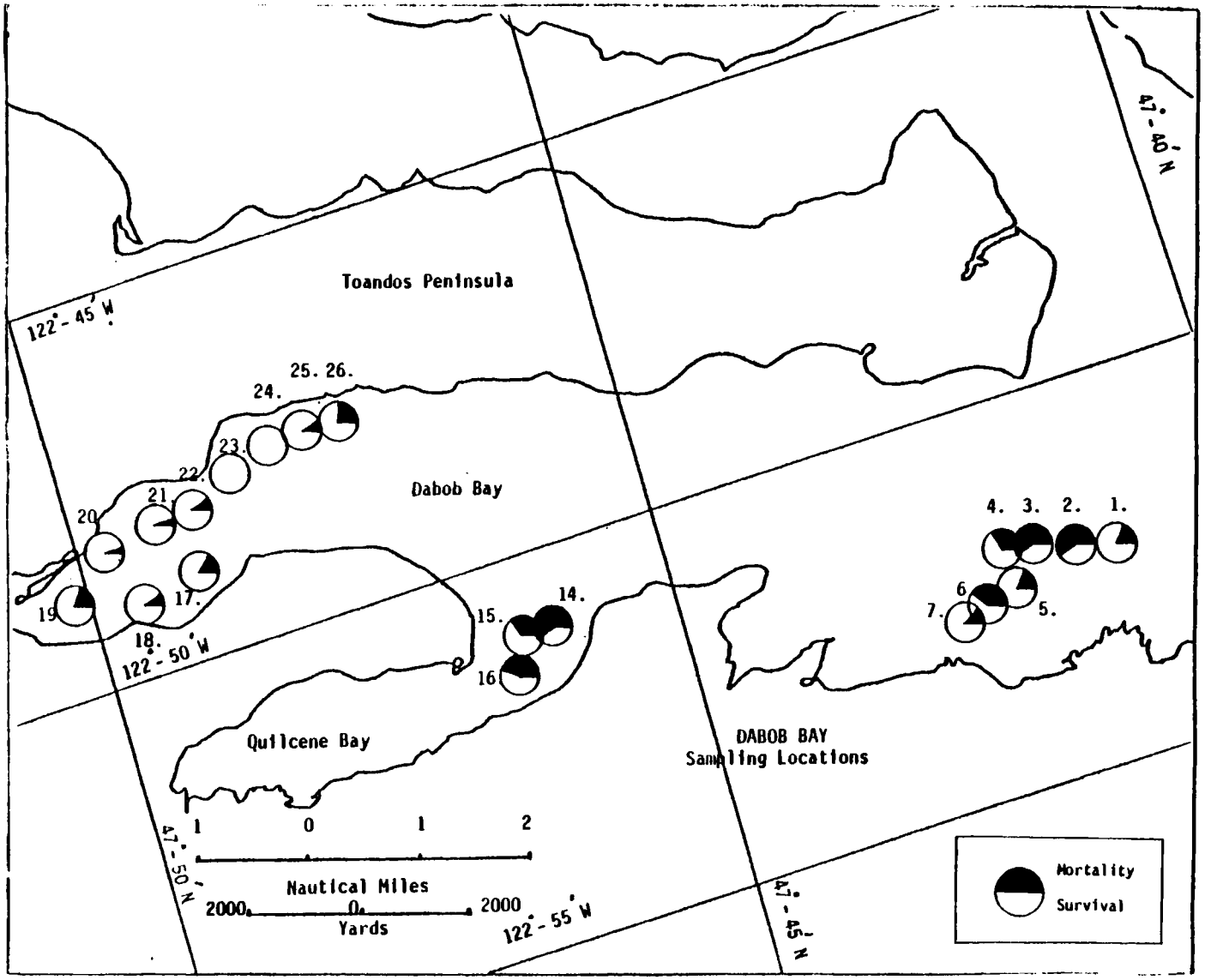


FIGURE 38. Results of Amphipod Bioassays on Sediments Collected from Dabob Bay During Screening Surveys (August 2 to September 18, 1983)

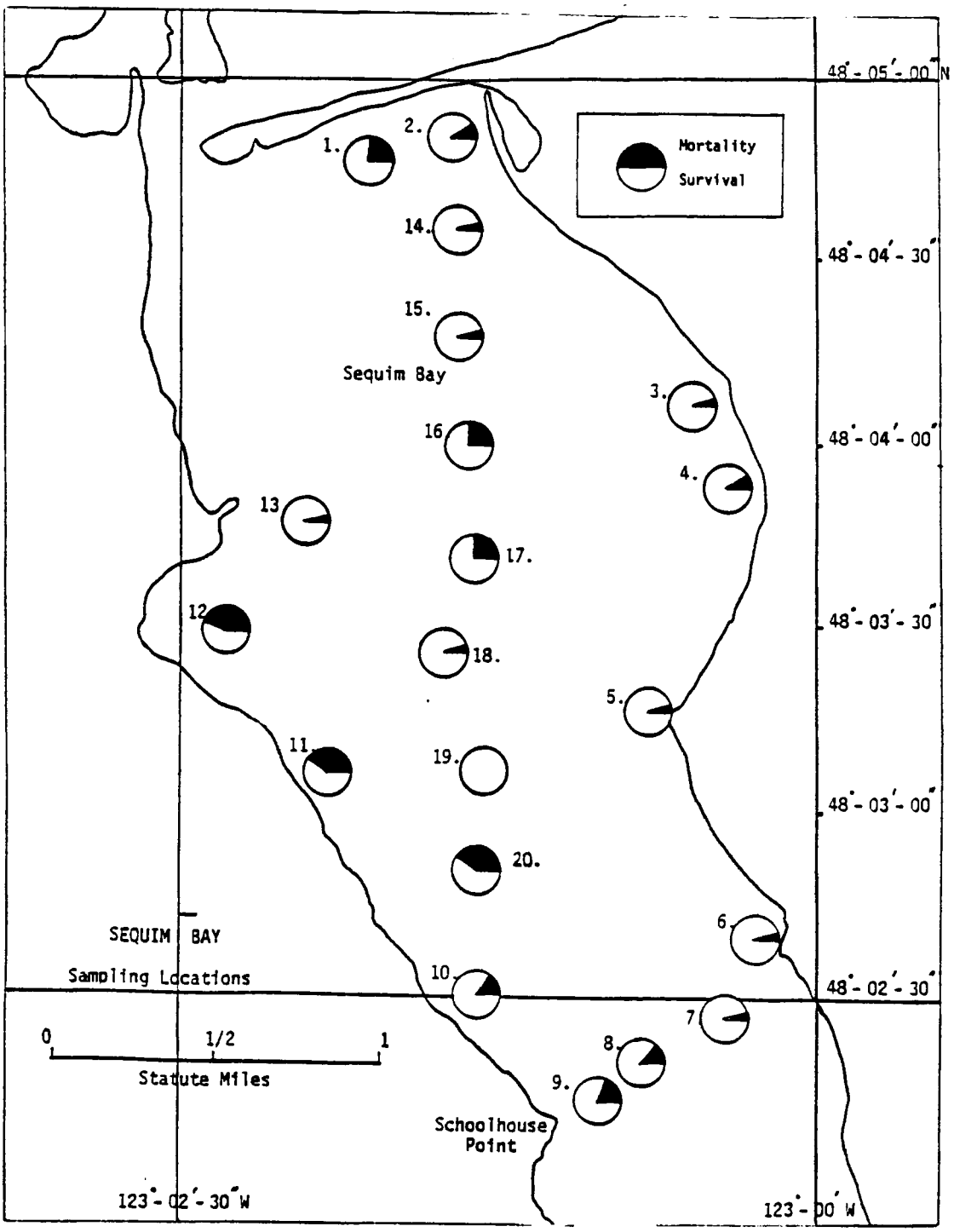


FIGURE 39. Results of Amphipod Bioassays on Sediments Collected in Sequim Bay During Screening Surveys (August 2 to September 18, 1983)

TABLE 35. Mean Values (± Standard Deviation) for 1983 Amphipod Survival and Sediment Characteristics for All Bays (180 Stations)

	No. of Survivors	No. of Bioassays with 16 or or Less Survivors/ Total No. Bioassays[1]	Percent Water in Sediment	Percent Fines in Sediment	Ag (ppm)	Hg (ppm)	Pb (ppm)	IR (ppm)
Overall	15.7 ± 3.4	92/180	50.1 ± 14.9	65.2 ± 32.5	0.371 ± 0.477	0.429 ± 0.751	40.6 ± 57.0	477.8 ± 1381.3
Case Inlet	14.0 ± 4.3	12/20	64.6 ± 9.7	89.0 ± 19.8	0.299 ± 0.098	0.160 ± 0.043	24.9 ± 6.9	154.4 ± 81.6
Dabob Bay	15.1 ± 4.0	11/20	41.8 ± 20.1	46.7 ± 40.6	0.079 ± 0.075	0.054 ± 0.042	9.5 ± 7.6	29.3 ± 14.5
Samish Bay	16.0 ± 3.4	11/20	46.2 ± 11.0	63.4 ± 37.1	0.078 ± 0.038	0.097 ± 0.052	13.2 ± 6.4	87.5 ± 83.5
Sequim Bay	16.9 ± 2.7	7/20	53.0 ± 11.0	66.8 ± 26.5	0.136 ± 0.052	0.068 ± 0.029	11.1 ± 3.6	96.7 ± 50.6
Bellingham Bay	16.4 ± 2.7	13/26	50.7 ± 11.6	79.6 ± 23.4	0.192 ± 0.108	0.675 ± 0.663	24.9 ± 28.7	566.4 ± 440.1
Fourmile Rock-Elliott Bay[2]	17.2 ± 2.2	10/24	48.4 ± 14.6	61.3 ± 31.0	0.529 ± 0.194	0.771 ± 1.078	88.3 ± 87.4	288.2 ± 218.1
Port Gardner-Everett Harbor	15.1 ± 4.3	12/25	49.7 ± 15.2	52.7 ± 26.6	0.261 ± 0.122	0.158 ± 0.127	29.0 ± 20.6	1627.3 ± 3419.3
Sinclair Inlet	15.2 ± 2.7	16/25	47.1 ± 15.3	62.6 1 35.4	1.230 ± 0.763	1.183 ± 1.176	105.6 ± 77.9	652.4 ± 568.2
West Beach (Control)	17.3 ± 6.4	4/41						

[1] There were some stations with replicate bioassays (actually test replicates). These numbers reflect only stations, not total number of replicated bioassays. Where test replicates existed, averages were used for that station. Thus, while there were 207 bioassays, these only represent 180 stations.
[2] Dump site vicinity.

TABLE 36. Correlation Matrix for Numbers of Amphipods Surviving (1983) and Sediment Characteristics from All Bays (180 Stations)

	No. Survivors	Percent H_2O	Percent Fines	Ag	Hg	Pb
Percent H_2O	-0.408					
Percent Fines	-0.331	0.827				
Ag	-0.177	0.273	0.283			
Hg	-0.059	0.095	0.128	0.574		
Pb	-0.105	0.140	0.104	0.677	0.871	
IR	-0.123	0.223	0.002	0.146	0.161	0.219

TABLE 37. Results of Regression Analyses for Numbers of Amphipods Surviving (1983) and Sediment Characteristics from all Bays (180 Stations)

No. Survivors	R	R^2	p-level
Percent H_2O	-0.408	0.166	$p < .001$
Percent Fines	-0.331	0.110	$p < .001$
Ag	-0.177	0.031	$p < .025$
Hg	-0.059	0.003	-
Pb	-0.105	0.011	-
IR	-0.123	0.015	$p < .100$

TABLE 38. Mean Percent H_2O and Percent Fines at Various Survivor Levels

No. Survivors	No. of Cases	Percent H_2O	Percent Fines
3	1	70.05	72.93
4	1	66.73	64.28
6	1	67.10	70.30
7	1	66.73	64.28
8	7	62.72	85.56
9	6	67.19	90.21
10	2	45.46	81.87
11	8	58.29	75.66
12	8	54.56	72.33
13	13	56.88	79.67
14	9	55.67	66.14
15	25	48.77	68.72
16	22	51.28	68.30
17	21	48.39	70.48
18	31	45.12	52.36
19	31	45.16	57.33
20	20	41.77	48.47

3.5.2 Detailed Surveys

Results of amphipod bioassays collected during detailed surveys (April 23 to May 29, 1984) are presented in Figures 40 through 47. Tabular data are presented in Appendix D.

Results of analyses, shown in Tables 39 through 44, demonstrated that statistically significant ($p < 0.05$) differences among mean amphipod survival and reburial occurred in all assays (1 through 6). Subsequent analyses applying Dunnett's procedure demonstrated statistically significant ($p < 0.05$) differences among the station means as summarized in Table 45. These latter analyses were performed with survival data and not reburial data. By inspection, however, reburial results parallel survival results with only minor exception.

The analyses shown in Table 46 indicated the lack of a significant batch effect: $P(\chi^2_5 \geq 2.21) = 0.81$. Each batch of amphipods responded similarly to the reference toxicant PCP, thus eliminating handling or test organism condition as a cause of the differences among treatments observed.

Based on these data, and the data presented in Table 47, the urban bays were not clearly distinct from baseline bays. Of the urban bays, Port Gardner - Everett Harbor and Sinclair Inlet exhibited the lowest mean survival (12.3 and 13.5, respectively). Intermediate survival was observed in Bellingham Bay, while the highest mean survival of amphipods in urban bay sediments occurred in the Fourmile Rock - Elliott Bay dump site vicinity. Of the baseline bays, Case Inlet and Samish Bay showed the lowest mean survival (13.0 and 13.3, respectively), followed by Dabob Bay (16.1) and Sequim Bay (17.0).

Examination of the correlation matrix in Table E.2 revealed that amphipod survival correlated with several physical and chemical parameters. The trend toward lower survival became more evident when the sediments were finer grained and contained more water and more organic contaminants as exemplified by PCB-1254, aromatic hydrocarbons, and IR.

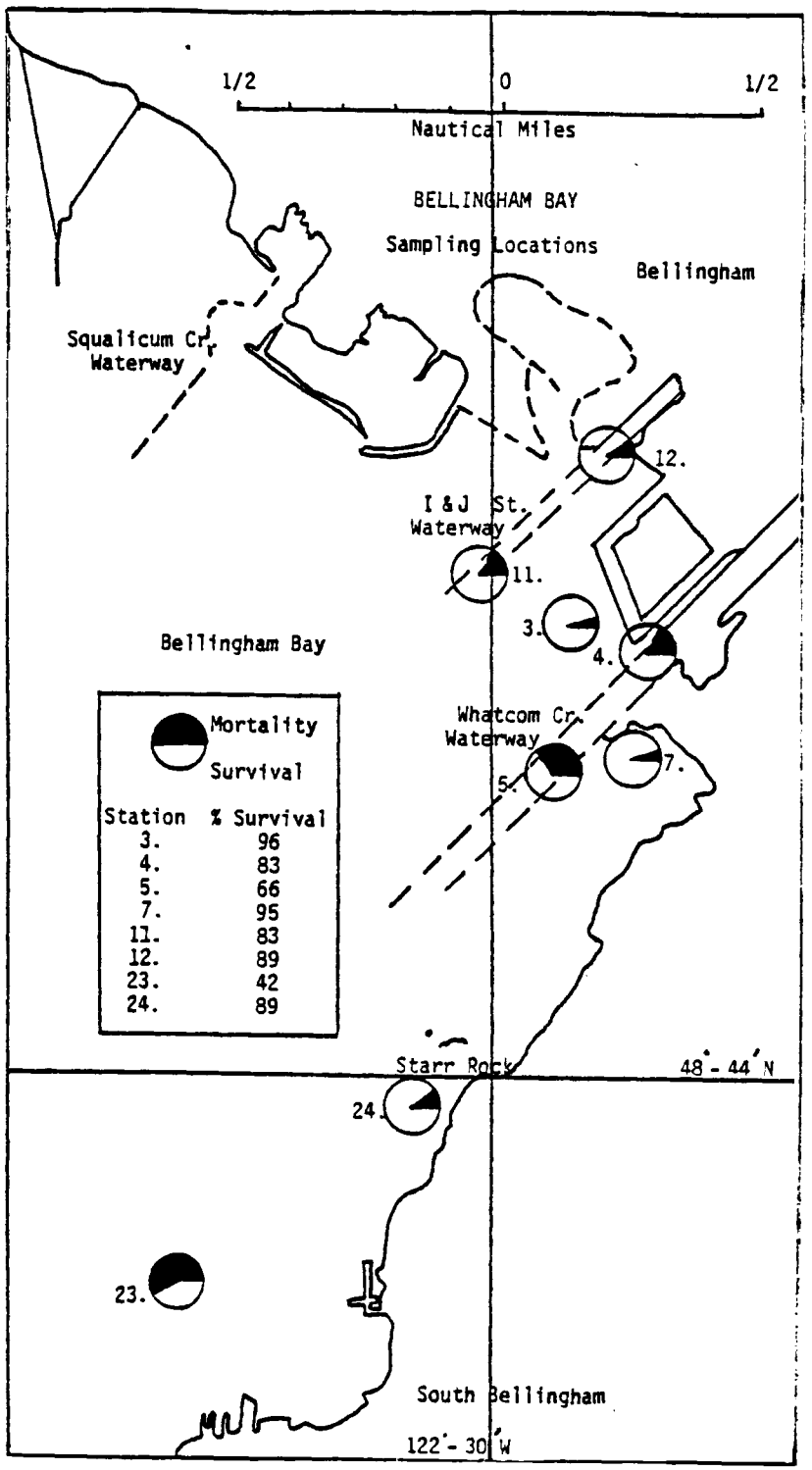


FIGURE 40. Results of Amphipod Bioassays on Sediments Collected in Bellingham Bay During Detailed Surveys (April 23 to May 29, 1984)

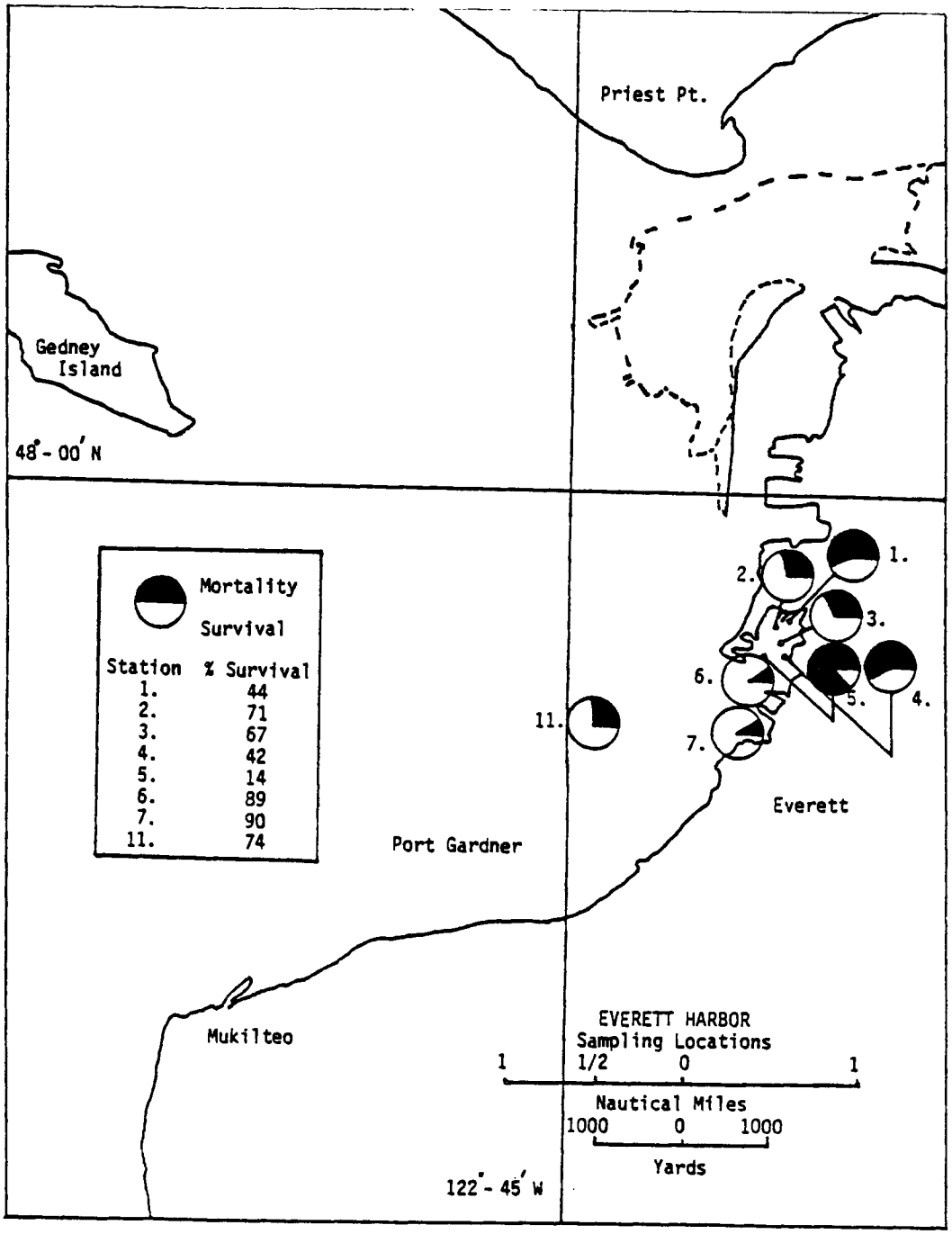
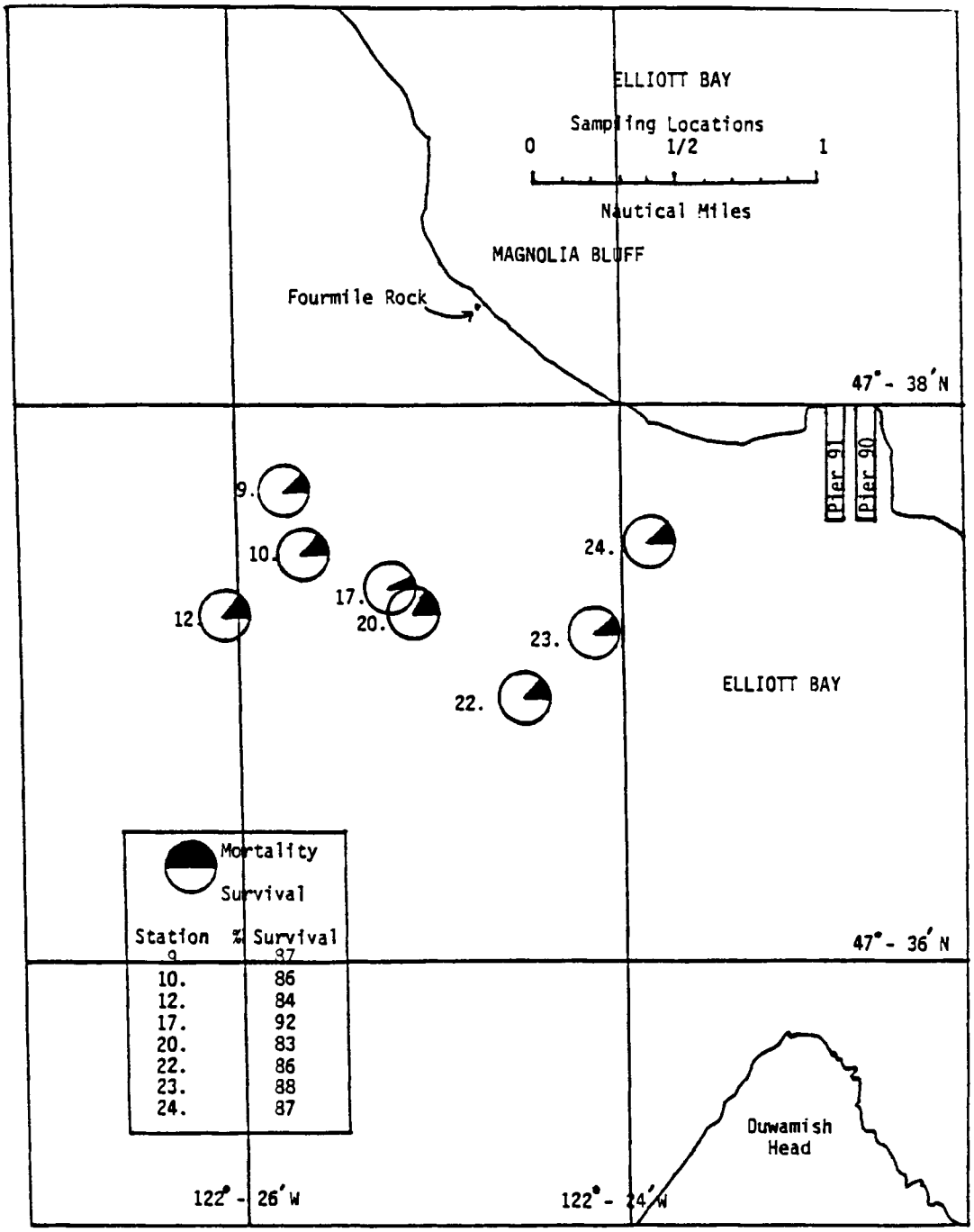


FIGURE 41. Results of Amphipod Bioassays on Sediments Collected in Port Gardner - Everett Harbor During Detailed Surveys (April 23 to May 29, 1984)



Station	% Survival
9.	87
10.	86
12.	84
17.	92
20.	83
22.	86
23.	88
24.	87

FIGURE 42. Results of Amphipod Bioassays on Sediments Collected in the Fourmile Rock - Elliott Bay Dump Site Vicinity During Detailed Surveys (April 23 to May 29, 1984)

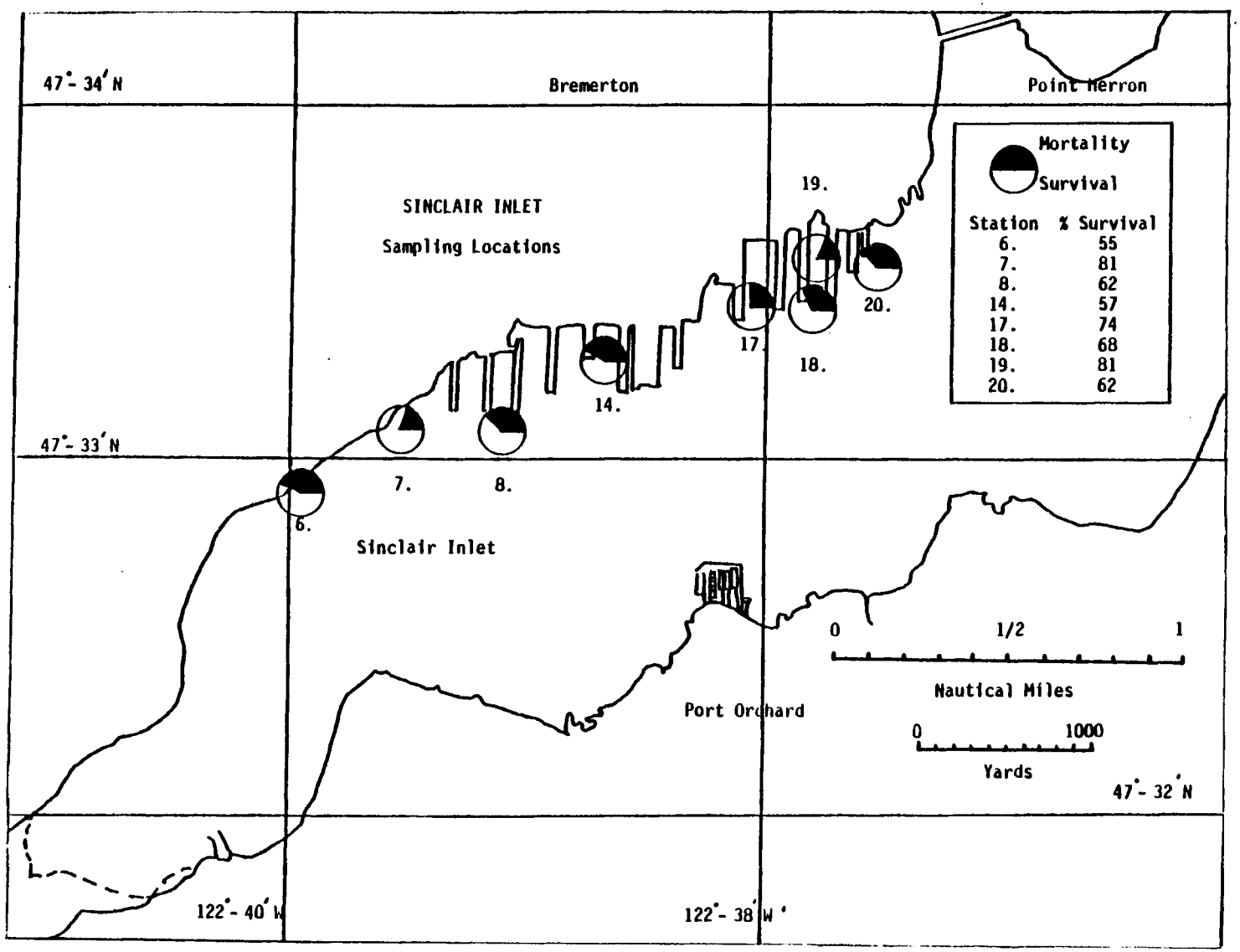


FIGURE 43. Results of Amphipod Bioassays on Sediments Collected in Sinclair Inlet During Detailed Surveys (April 23 to May 29, 1984)

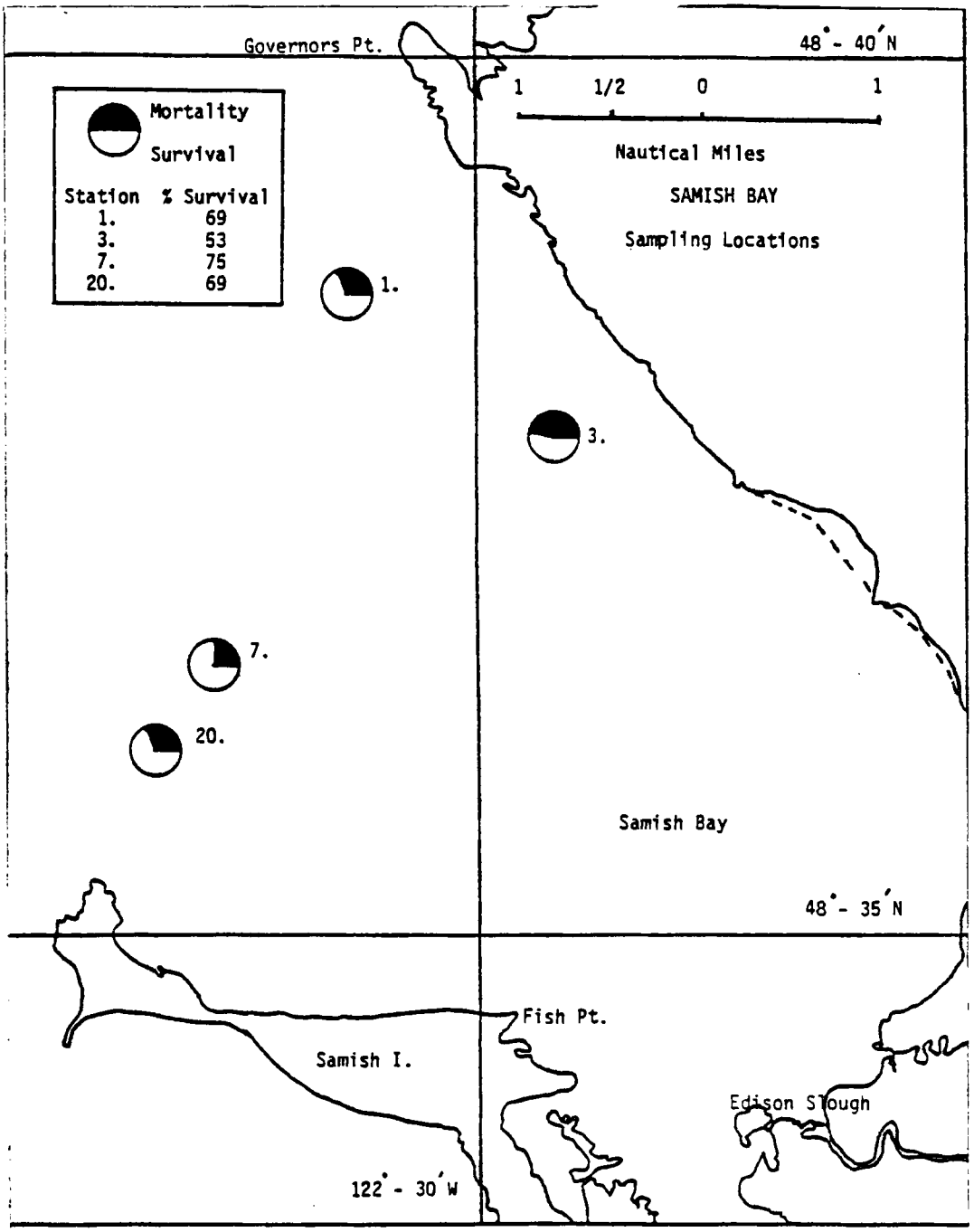


FIGURE 44. Results of Amphipod Bioassays on Sediments Collected in Samish Bay During Detailed Surveys (April 23 to May 29, 1984)

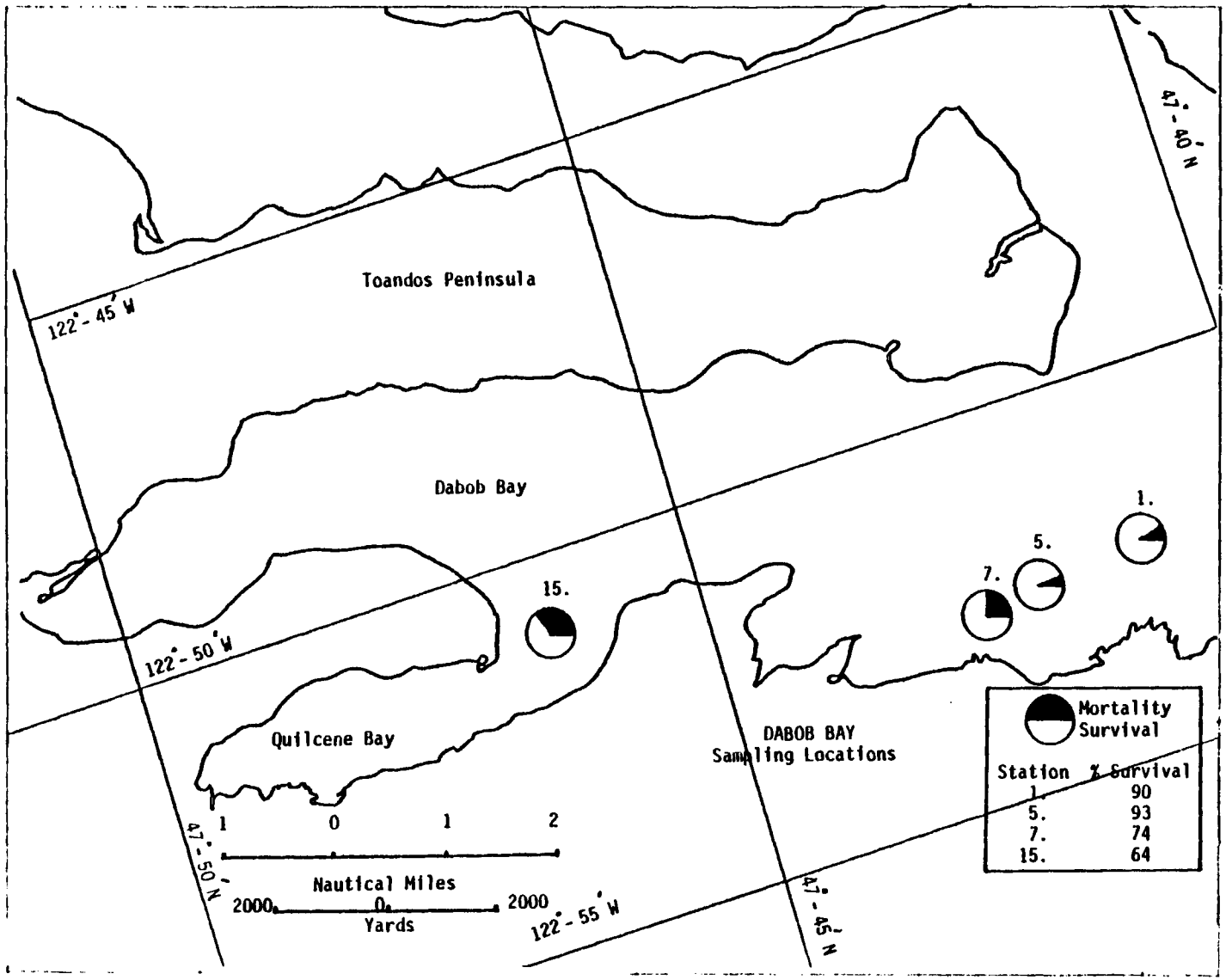


FIGURE 45. Results of Amphipod Bioassays on Sediments Collected from Dabob Bay During Detailed Surveys (April 23 to May 29, 1984)

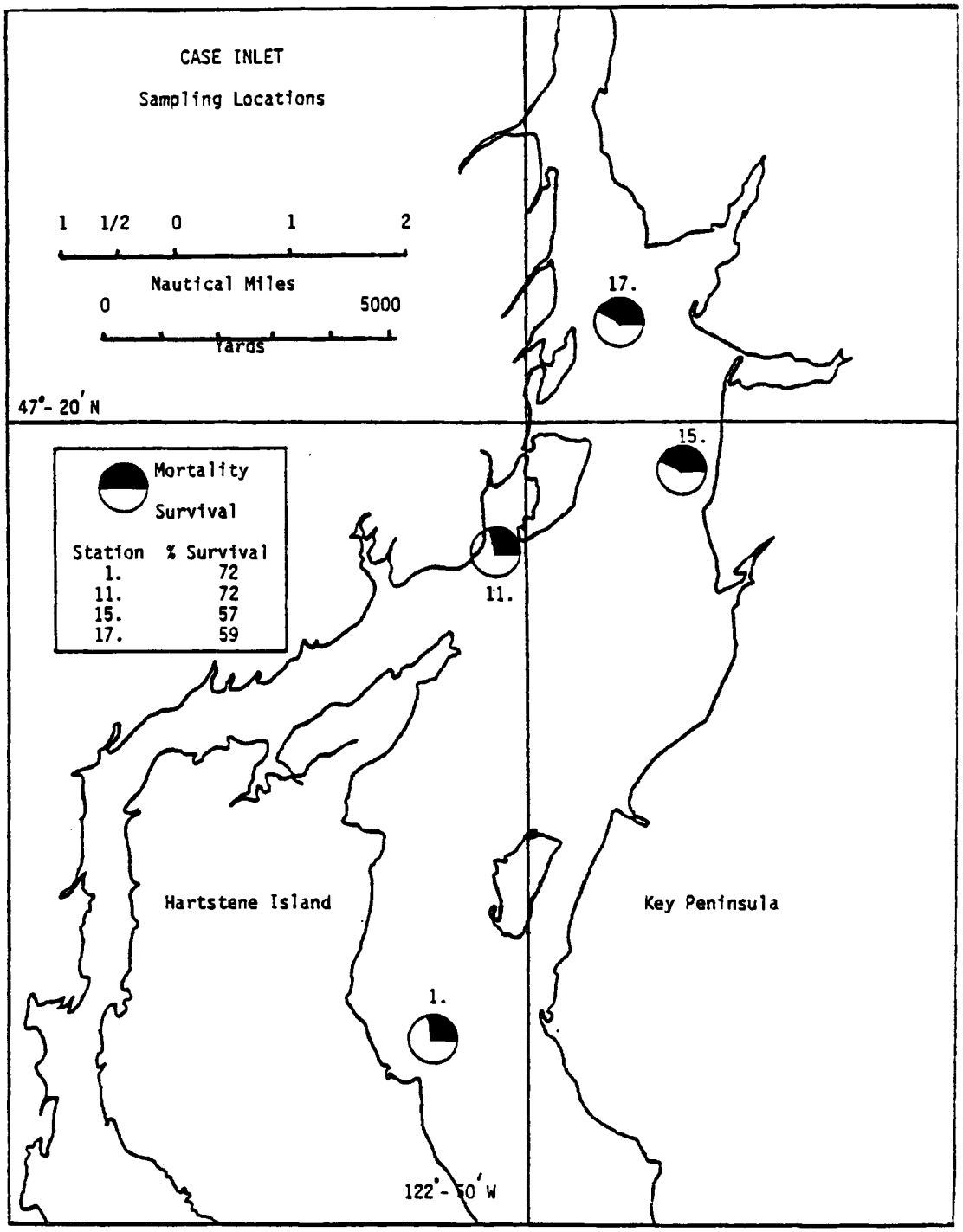


FIGURE 46. Results of Amphipod Bioassays on Sediments Collected from Case Inlet During Detailed Surveys (April 23 to May 29, 1984)

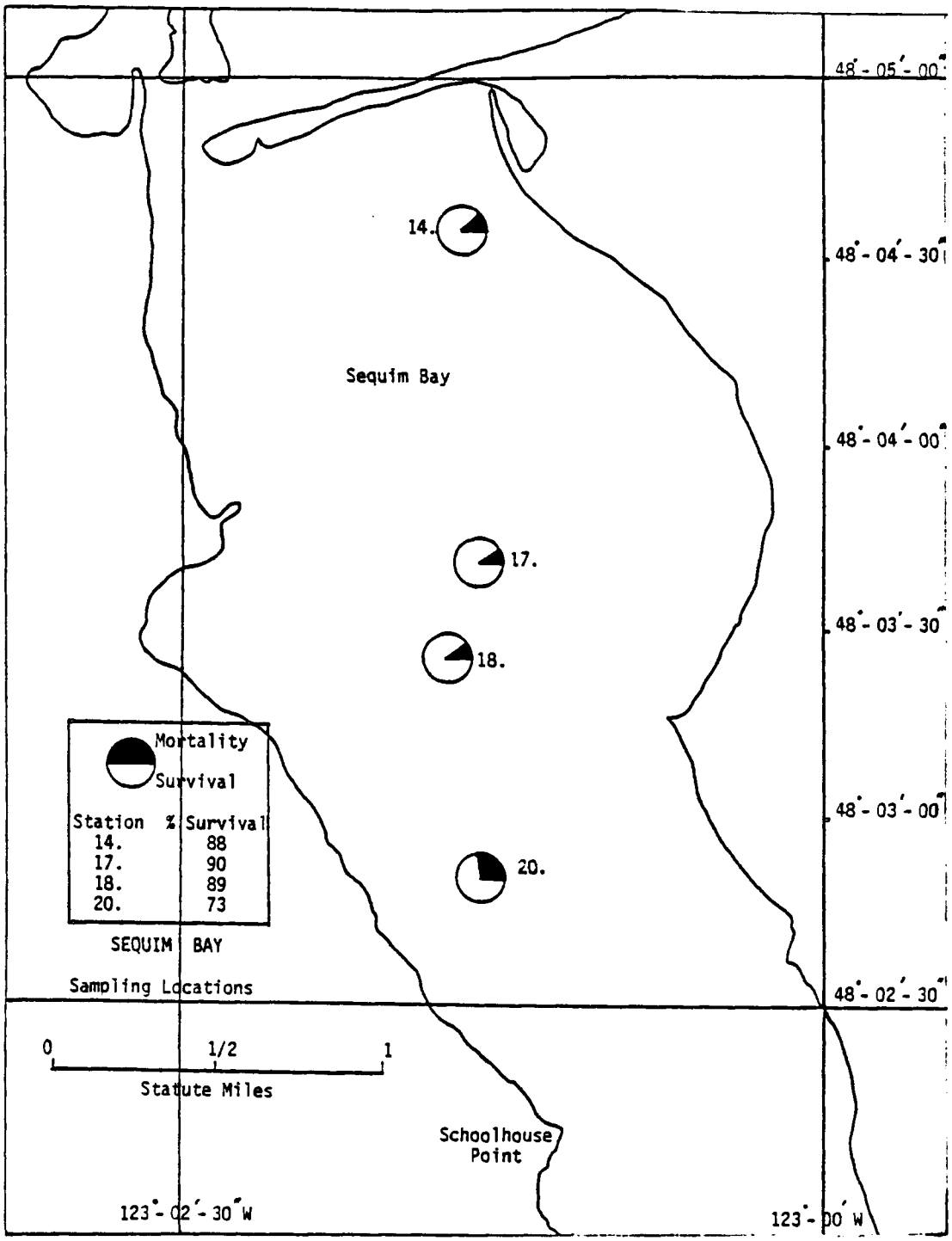


FIGURE 47. Results of Amphipod Bioassays of Sediments Collected from Sequim Bay During Detailed Surveys (April 23 to May 29, 1984)

TABLE 39. Summary of Statistical Analyses (HOMOV, ANOVA, SNK, Dunnett's Test) of Survival and Reburial Data for *Rhepoxynius abronius* Exposed to Puget Sound Sediments (Assay No. 1 - Bellingham Bay [B-1], Samish Bay [B-2])

SURVIVAL

HOMOV: F_{max} = 33.5 $F_{max.05\ (9,4)}$ = 41.1

X^2C = 16.15 $X^2_{.05(8)}$ = 15.507

C = 0.267 $C_{.05(9,4)}$ = 0.3584

ANOVA: EMS = 2.78889 $F_{.001(8,36)}$ = 3.794

F = 18.0896 $F_{.05(8,36)}$ = 2.216

SNK: B-1 = Bellingham Bay B-2 = Samish Bay

Sample	B-2 S-3	B-1 S-5	B-2 S-1	B-2 S-20	B-2 S-7	B-1 S-4	B-1 S-7	B-1 S-3	Cont.
$\bar{x}$	10.6	13.2	13.8	13.8	15.0	16.6	19.0	19.2	19.8
	6	3	5	8	7	2	4	1	9

DUNNETTS:	*	*	*	*	*	*	N.S	N.S	Cont.
	10.6	13.2	13.8	13.8	15.0	16.6	19.0	19.2	19.8

* Significant $p<0.05$

REBURIAL

HOMOV: F_{max} = 31.5

X^2C = 18.6

C = 0.269

ANOVA: EMS = 2.600

F = 20.1688 $F_{.05(8,36)}$ = 2.216

SNK:

Sample	B-2 S-3	B-1 S-5	B-2 S-1	B-2 S-20	B-2 S-7	B-1 S-4	B-1 S-7	B-1 S-3	Cont.
$\bar{X}$	10.4	13.0	13.6	13.8	15.0	16.2	19.0	19.2	19.8
	6	3	5	8	7	2	4	1	9

DUNNETTS:	*	*	*	*	*	*	N.S	N.S	Cont.
	10.4	13.0	13.6	13.8	15.0	16.2	19.0	19.2	19.8

* Significant $p<0.05$

TABLE 40. Summary of Statistical Analyses (HOMOV, ANOVA, SNK, Dunnett's Test) of Survival and Reburial Data for *Rhepoxynius abronius* Exposed to Puget Sound Sediments (Assay No. 2 - Bellingham Bay [B-1], Port Gardner - Everett Harbor [B-5])

SURVIVAL

HOMOV: $F_{max} = 12.875$ $F_{max.05(9,4)} = 41.1$

$X^2C = 9.758$ $X^2_{.05(8)} = 15.507$

ANOVA: EMS = 4.25556

F = 22.735 $F_{.001(8,36)} = 3.794$

SNK: B-1 = Bellingham Bay B-4 = Port Gardner - Everett Harbor

Sample	B-1 S-23	B-4 S-4	B-4 S-1	B-4 S-3	B-4 S-2	B-1 S-11	B-1 S-24	B-1 S-12	Cont.
$\bar{x}$	8.4	8.4	8.8	13.4	14.2	16.6	17.8	17.8	19.4
	3	8	5	7	6	1	4	2	9

DUNNETTS:	*	*	*	*	*	N.S	N.S	N.S	Cont.
	8.4	8.4	8.8	13.4	14.2	16.6	17.8	17.8	19.4

* Significant $p<0.05$

REBURIAL

HOMOV: $F_{max} = 14.125$

$X^2C = 8.95$

ANOVA: EMS = 4.2444

F = 29.2147

SNK:

Sample	B-1 S-23	B-4 S-4	B-4 S-1	B-4 S-3	B-4 S-2	B-1 S-11	B-1 S-24	B-1 S-12	Cont.
$\bar{x}$	5.6	7.8	8.4	12.6	14.0	16.4	17.4	17.8	19.4
	8	5	3	7	6	1	4	2	9

DUNNETTS:	*	*	*	*	*	N.S	N.S	N.S	Cont.
	5.6	7.8	8.4	12.6	14.0	16.4	17.4	17.8	19.4

* Significant $p<0.05$

TABLE 41. Summary of Statistical Analyses (HOMOV, ANOVA, SNK, Dunnett's Test) of Survival and Reburial Data for *Rhepoxynius abronius* Exposed to Puget Sound Sediments (Assay No. 3 - Port Gardner - Everett Harbor [B-4], Fourmile Rock - Elliott Bay Dump Site Vicinity [B-5])

SURVIVAL

HOMOV:

F_{max} = 32.33 $F_{max.05(9,4)}$ = 41.1

X^2_C = 12.7665 $X^2_{.05(8)}$ = 15.507

ANOVA: EMS = 3.0444

F = 41.8923

SNK: B-4 Port Gardner - Everett Harbor B-5 Fourmile Rock - Elliott Bay

Sample	B-4 S-5	B-4 S-11	B-5 S-12	B-5 S-10	B-5 S-9	B-4 S-6	B-4 S-7	B-5 S-17	Cont.
$\bar{x}$	2.8	14.8	16.8	17.2	17.4	17.8	18.0	18.4	19.4
	1	2	3	4	5	6	7	8	9

DUNNETTS:	*	*	N.S	N.S	N.S	N.S	N.S	N.S	Cont.
	2.8	14.8	16.8	17.2	17.4	17.8	18.0	18.4	19.4

* Significant $p<0.05$

REBURIAL

HOMOV: F_{max} = 27.667

X^2_C = 11.2541

ANOVA: EMS = 2.9556

F = 45.4812

SNK:

Sample	B-4 S-5	B-4 S-11	B-5 S-12	B-5 S-10	B-5 S-9	B-4 S-6	B-4 S-7	B-5 S-17	Cont.
$\bar{x}$	2.4	14.6	16.8	17.2	17.4	17.8	18.0	18.4	19.4
	1	4	7	6	5	2	3	8	9

DUNNETTS:	*	*	N.S	N.S	N.S	N.S	N.S	N.S	Cont.
	2.4	14.6	16.8	17.2	17.4	17.8	18.0	18.4	19.4

* Significant $p<0.05$

TABLE 42. Summary of Statistical Analyses (HOMOV, ANOVA, SNK, Dunnett's Test) of Survival and Reburial Data for *Rhepoxynius abronius* Exposed to Puget Sound Sediments (Assay No. 4 - Fourmile Rock - Elliott Bay Dump Site Vicinity [B-5], Sinclair Inlet [B-6])

SURVIVAL

HOMOV:

F_{max} = 62.5 $\qquad F_{max.05(9,4)}$ = 41.1

X^2C = 18.4098 $\qquad X^2_{.05(8)}$ = 15.507

ARCSIN $\sqrt{\chi c}$ $\qquad C_{.05(9,4)}$ = 0.3584

F_{max} = 42.0901

X^2C = 10.6197

C = 0.267

ANOVA: (on transformed data)

EMS = 66.23021

F = 8.9414 $\qquad F_{.001(8,36)}$ = 3.794

SNK: B-5 Fourmile Rock - Elliott Bay - B-6 Sinclair Inlet

Sample	B-6 S-7	B-6 S-14	B-6 S-8	B-6 S-7	B-5 S-20	B-5 S-22	B-5 S-23	B-5 S-24	Cont.
$\bar{x}$	11.0	11.4	12.4	16.2	16.6	17.2	17.6	17.4	19.0
$\bar{x}$ Arcsin $\sqrt{\chi c}$	48.0	49.1	52.2	64.9	66.1	68.1	70.4	71.7	78.6
	5	8	7	6	1	2	3	4	9
DUNNETTS:	*	*	*	N.S	N.S	N.S	N.S	N.S	Cont.
	48.0	49.1	52.2	64.9	66.1	68.1	70.4	71.7	78.6

* Significant $p<0.05$

REBURIAL

HOMOV:

F_{max} = "0" in variance

X^2C = 12.33

C = 0.296

ANOVA:

EMS = 4.6222

F = 9.4700

SNK:

Sample	B-6 S-7	B-6 S-14	B-6 S-8	B-6 S-7	B-5 S-20	B-5 S-22	B-5 S-23	B-5 S-24	Cont
	11.0	11.4	12.4	16.2	16.6	17.0	17.2	17.6	19.0
$\bar{x}$	5	8	7	6	1	2	4	3	9
DUNNETTS:	*	*	*	N.S.	N.S	N.S	N.S	N.S	Cont.
	11.0	11.4	12.4	16.2	16.6	17.0	17.2	17.6	19.0

* Significant $p<0.05$

TABLE 43. Summary of Statistical Analyses (HOMOV, ANOVA, SNK, Dunnett's Test) of Survival and Reburial Data for *Rhepoxynius abronius* Exposed to Puget Sound Sediments (Assay No. 5 - Sinclair Inlet [B-6], Dabob Bay [B-7])

SURVIVAL

HOMOV: F_{max} = "0" in variance

X^2C = 12.7223 $\qquad X^2_{.05(8)}$ = 15.507

C = 0.311 $\qquad C_{.05(9,4)}$ = 0.3584

ANOVA: EMS = 4.57778

F = 7.8871 $\qquad F_{.05(8,36)}$ = 3.794

SNK: B-6 = Sinclair Inlet $\qquad$ B-7 = Dabob Bay

Sample	B-6 S-20	B-7 S-15	B-6 S-18	B-6 S-17	B-7 S-7	B-6 S-19	B-7 S-1	B-7 S-5	Cont.
$\bar{x}$	12.4	12.8	13.6	14.8	14.8	16.2	18.0	18.6	20.0
	4	8	2	1	7	3	5	6	9

DUNNETTS:	*	*	*	*	*	*	N.S	N.S	Cont.
	12.4	12.8	13.6	14.8	14.8	16.2	18.0	18.6	20.0

* Significant $p<0.05$

REBURIAL

HOMOV: F_{max} = "0" in variance

X^2C = 12.8929

C = 0.216

ANOVA: EMS = 4.4778

F = 7.866

SNK:

Sample	B-6 S-20	B-7 S-15	B-6 S-18	B-6 S-17	B-7 S-7	B-6 S-19	B-7 S-1	B-7 S-5	Cont.
$\bar{x}$	12.4	12.8	13.6	14.8	14.8	15.4	18.0	18.4	20.0
	4	8	2	1	7	3	5	6	9

DUNNETTS:	*	*	*	*	*	*	N.S	N.S	Cont.
	12.4	12.8	13.6	14.8	14.8	15.4	18.0	18.4	20.0

* Significant $p<0.05$

TABLE 44. Summary of Statistical Analyses (HOMOV, ANOVA, SNK, Dunnett's Test) of Survival and Reburial Data for *Rhepoxynius abronius* Exposed to Puget Sound Sediments (Assay No. 6 - Sequim Bay [B-3], Case Inlet [B-8])

SURVIVAL

HOMOV: $F_{max} = 7.800$ $F_{max.05(9,4)} = 41.1$

$X^2C = 5.2853$ $X^2.05(8) = 15.507$

ANOVA: EMS = 4.48889 $F_{.001(8,36)} = 3.794$

SNK: B-8 = Case Inlet B-3 = Sequim Bay

Sample	B-8 S-15	B-8 S-17	B-8 S-1	B-8 S-11	B-3 S-20	B-3 S-14	B-3 S-18	B-3 S-17	Cont.
$\bar{x}$	11.4	11.8	14.4	14.4	14.6	17.6	17.8	18.0	19.0
	7	8	5	6	4	1	3	2	9

DUNNETTS:	*	*	*	*	*	N.S	N.S	N.S	Cont.
	11.4	11.8	14.4	14.4	14.6	17.6	17.8	18.0	19.0

* Significant $p<0.05$

REBURIAL

HOMOV: $F_{max} = 6.7$

$X^2C = 4.3814$

ANOVA: EMS = 4.4444

F = 8.8475

SNK:

Sample	B-8 S-15	B-8 S-17	B-8 S-1	B-8 S-11	B-3 S-20	B-3 S-14	B-3 S-18	B-3 S-17	Cont.
$\bar{x}$	11.2	11.8	14.4	14.4	14.4	17.6	17.8	17.8	19.0
	7	8	4	5	6	1	2	3	9

DUNNETTS:

	*	*	*	*	*	N.S	N.S	N.S	Cont.
	11.2	11.8	14.4	14.4	14.4	17.6	17.8	17.8	19.0

* Significant $p<0.05$

TABLE 45. Significance of Amphipod Survival Data by Bay and Station for Sediments Collected During Detailed Surveys April 23 to May 29, 1984

Location	Significant[1]	Not Significant[2]	Inconclusive[3]
Bellingham Bay	B-1 S-5	B-1 S-3	B-1 S-4
	B-1 S-23	B-1 S-7	B-1 S-11
		B-1 S-12	
		B-1 S-24	
Samish Bay	B-2 S-1		
	B-2 S-3		
	B-2 S-7		
	B-2 S-20		
Port Gardner-Everett Harbor	B-4 S-1	B-4 S-6	
	B-4 S-2	B-4 S-7	
	B-4 S-3		
	B-4 S-4		
	B-4 S-5		
	B-4 S-11		
Fourmile Rock-Elliott Bay[4]		B-5 S-9	B-5 S-10
		B-5 S-17	B-5 S-12
		B-5 S-23	B-5 S-20
		B-5 S-24	B-5 S-22

1 <16.2 surviving was significant ($p < 0.05$).
2 >17.2 surviving was not significant ($p > 0.05$).
3 Values between 16.2 and 17.2 are classified as inconclusive.
4 Dump site vicinity.

TABLE 45. Significance of Amphipod Survival Data by Bay and Station for Sediments Collected During Detailed Surveys April 23 to May 29, 1984 (Continued)

Location	Significant[1]	Not Significant[2]	Inconclusive[3]
Sinclair Inlet	B-6 S-6		B-6 S-7
	B-6 S-8		B-6 S-19
	B-6 S-14		
	B-6 S-17		
	B-6 S-18		
	B-6 S-20		
Dabob Bay	B-7 S-7	B-7 S-1	
	B-7 S-15	B-7 S-5	
Sequim Bay	B-3 S-20	B-3 S-14	
		B-3 S-17	
		B-3 S-18	
Case Inlet	B-8 S-1		
	B-8 S-11		
	B-8 S-15		
	B-8 S-17		

TABLE 46. Chi-Square 2 x 6 Analysis to Test for Batch Effect in Amphipod Test Populations

Batch	#1	2	3	4	5	6	Total
Alive	132	127	128	129	125	120	761
Dead	48	53	52	51	55	60	319
Total	180	180	180	180	180	180	1080

$$\chi^2_5 = 2.21$$

$$P\ (\chi^2_5 \geq 2.21) = 0.81$$

The results of the 1984 bioassay series generally reflected the results of the 1983 bioassay series. Assay of the Fourmile Rock - Elliott Bay dump site vicinity and Sequim Bay sediments resulted in the highest survival in both surveys. Port Gardner - Everett Harbor and Case Inlet consistently demonstrated the lowest survival, indicating good agreement between the independent series. Actually, the results of the 1984 bioassay series successfully reproduced the results of the 1983 series at the screening level 63% of the time.

Statistically speaking (see Table 48), with approximately 50% of the bioassays in each survey resulting in a "hit" (16 or fewer survivors), a totally random procedure would be expected to yield equal quantities in four categories: (1) both 1983 and 1984 a "hit"; (2) both 1983 and 1984 not "hits"; (3) 1983 a "hit," 1984 not; and 4) 1983 not, 1984 a "hit." In fact, the procedure yielded 30 of 48 (62.5%) correct pairs. Comparing the procedure with random chance yields a chi-square value of 7.17 with 3 degrees of freedom (df) which is significant at the 0.10 level to reject the hypothesis of no difference. If stations with survival from 15.1 to 16.9 are rejected as being inconclusive, then 35 cases remain (Table 49). The procedure then matched on 22 of the 35 (62.9%). Again, testing the procedure against random chance yielded a chi-square value of 6.03 with 3 df, which rejects the null hypothesis of no difference at the 0.25

TABLE 47. Mean Values (± Standard Deviation) for 1984 Amphipod Survival and Sediment Characteristics for All Bays (48 Stations)

	No. of Survivors	No. Bioassays with 16 or Less Survivors/Total No. Bioassays	Percent Water in Sediment	Percent Fines in Sediment	Ag (ppm)	Hg (ppm)	Pb (ppm)	IR (ppm)
Overall	14.8 ± 3.3	25/48	57.6 ± 10.1	69.5 ± 21.4	0.609 ± 0.800	0.850 ± 1.410	66.7 ± 90.8	743.1 ± 967.2
Case Inlet	13.0 ± 1.6	4/4	64.6 ± 8.7	72.0 ± 22.5	0.329 ± 0.189	0.127 ± 0.024	26.3 ± 5.0	91.2 ± 32.3
Dabob Bay	16.1 ± 2.7	2/4	47.1 ± 15.8	45.6 ± 32.0	0.093 ± 0.090	0.064 ± 0.027	9.9 ± 4.9	45.1 ± 24.8
Samish Bay	13.3 ± 1.9	4/4	54.6 ± 3.9	83.6 ± 3.1	0.114 ± 0.010	0.100 ± 0.007	14.9 ± 0.7	91.8 ± 9.1
Sequim Bay	17.0 ± 1.6	1/4	63.3 ± 2.8	77.6 ± 9.3	0.203 ± 0.027	0.770 ± 0.010	13.3 ± 1.3	88.9 ± 22.2
Bellingham Bay	16.1 ± 3.6	2/8	59.6 ± 7.5	86.7 ± 13.9	0.249 ± 0.058	1.025 ± 0.446	23.8 ± 8.6	924.9 ± 492.0
Fourmile Rock-Elliott Bay[1]	17.3 ± 3.5	0/8	49.4 ± 11.4	52.7 ± 23.9	0.552 ± 0.213	1.540 ± 2.810	134.1 ± 155.2	304.0 ± 182.8
Port Gardner-Everett Harbor	12.3 ± 5.2	6/8	61.6 ± 9.7	63.4 ± 12.4	0.299 ± 0.098	0.239 ± 0.109	40.1 ± 15.8	1852.0 ±1490.6
Sinclair Inlet	13.5 ± 2.0	6/8	60.5 ± 5.3	74.7 ± 15.8	2.186 ± 0.797	2.113 ± 1.050	170.3 ± 68.1	1219.3 ± 923.2
West Beach (Control)	19.4 ± 4.2	0/30						

[1] Dump site vicinity.

TABLE 47. Mean Values (± Standard Deviation) for 1984 Amphipod Survival and Sediment Characteristics for All Bays (48 Stations) (Continued)

	No. of Survivors	No. Bioassays with 16 or Less Survivors/Total No. Bioassays	PCB-1254 ppb	Total Aromatics ppb
Overall	14.8 ± 3.3	25/48	315.7 ± 1441.1	5387.1 ± 6882.8
Case Inlet	13.0 ± 1.6	4/4	Undetected	Undetected
Dabob Bay	16.1 ± 2.7	2/4	Undetected	Undetected
Samish Bay	13.3 ± 1.9	4/4	Undetected	1252.0 ± 1325.4
Sequim Bay	17.0 ± 1.6	1/4	Undetected	Undetected
Bellingham Bay	16.1 ± 3.6	2/8	48.8 ± 26.9	2591.9 ± 2384.5
Fourmile Rock-Elliott Bay	17.3 ± 3.5	0/8	319.0 ± 411.1	4141.2 ± 5153.4
Port Gardner Everett Harbor	12.3 ± 5.2	6/8	316.7 ± 252.9	8594.6 ± 7538.5
Sinclair Inlet	13.5 ± 2.0	6/8	578.5 ± 364.3	8288.3 ± 10279.0
West Beach (Control)	19.4 ± 4.2	0/30		

Dump site vicinity.
Mean of detectable values only.

TABLE 48. Comparison of Procedure with Random Chance, All 48 Stations

	1983-1984 both hits	1983-1984 not hits	1983-hit 1984-not	1983-not 1984-hit
EPA	19	11	12	6
Random	12	12	12	12

$X^2 = 7.17$ 3 df sig. $\alpha = 0.10$

TABLE 49. Comparison of Procedure with Random Chance, 13 Stations with Inconclusive Results (Mean Survival Between 15.1 and 16.9) Removed

	1983-1984 both hits	1983-1984 not hits	1983-hit 1984-not	1983-not 1984-hit
EPA	15	7	7	6
Random	8.75	8.75	8.75	8.75

$X^2 = 6.03$ 3 df sig. $\alpha = 0.25$

level. The procedure is especially accurate in duplicating the situation where both 1983 and 1984 results showed "hits" at a given station.

3.6 OYSTER LARVAL BIOASSAY (PERFORMED BY MRL)

3.6.1 Screening Surveys

Results of oyster larval bioassays in selected sediments collected during screening surveys are presented in Table 50. A summary of the experimental conditions for each bioassay is found in Appendix D.

Although rigorous statistical comparisons were not possible (nor intended), oyster larval bioassay of sediments from all urban bay stations, with the exception of Station No. 6 at the Fourmile Rock - Elliott Bay dump site vicinity, indicated clear evidence of a concentration effect. Increased abnormality occurred generally at the higher test concentrations (10 and 100 g/L). However, the Fourmile Rock - Elliott Bay dump site Station No. 6 and the only baseline bay station, Sequim Bay No. 14, showed virtually no differences among concentrations.

3.6.2 Detailed Surveys

Results of oyster larval bioassays on sediments collected in detailed surveys (April 23 to May 29, 1984) are presented in Tables 51 through 56. A summary of the experimental conditions for each bioassay is presented in Appendix D.

Analyses shown in Table 57 demonstrated that statisically significant differences ($p < 0.05$) from controls for the following stations: Stations 11, 12, 23, and 24 in Bellingham Bay; Stations 6, 7, 14, 19, and 20 in Sinclair Inlet; and Stations 1 and 7 in Dabob Bay (Tables 58 and 59).

Examination of the correlation matrix in Appendix E, shows that for all 48 oyster larval bioassays, no significant ($p < 0.05$) relationships existed between chemical variables and percentages of abnormal larvae. Examination of the correlation matrix in Appendix E also shows a negative correlation (-0.29) between percent abnormal oyster larvae and amphipod

TABLE 50. Effects of Sediment Concentration on Oyster Larval Development Measured as Percent Abnormals (Assays performed August 24 to September 24, 1983 on Fresh Puget Sound Sediments)

Sediment Concentration (g/L)	Port Gardner-Everett Harbor Station 2 8-24-83[1]	Fourmile Rock-Elliott Bay[2] Station 2 8-27-83[1]	Fourmile Rock-Elliott Bay[2] Station 6 8-27-83[1]	Sinclair Station 1 8-31-83[1]	Sinclair Station 7 8-31-83[1]	Sequim[3] Station 14 9-22-83[1]
Control	13.3	21.9	11.1	29.9	12.9	15.4
	6.3	17.1	6.8	16.6	10.9	10.0
	7.3	11.3	5.4	16.8	10.3	10.0
	$\bar{x}$ = 8.9	$\bar{x}$ = 16.6	$\bar{x}$ = 7.7	$\bar{x}$ = 21.1	$\bar{x}$ = 11.3	$\bar{x}$ = 11.8
0.01	9.5	8.9	15.1	14.5	14.5	11.6
	2.6	20.8	28.6	30.2	4.4	11.5
	8.8	27.4	6.4	13.9	6.4	7.0
	$\bar{x}$ = 6.9	$\bar{x}$ = 19.0	$\bar{x}$ = 16.7	$\bar{x}$ = 19.5	$\bar{x}$ = 8.4	$\bar{x}$ = 10.0
0.10	10.7	14.0	7.9	24.2	11.3	21.0
	0.0	24.0	16.6	30.2	10.6	0.0
	12.7	32.5	3.2	9.1	13.4	7.0
	$\bar{x}$ = 7.8	$\bar{x}$ = 23.5	$\bar{x}$ = 9.2	$\bar{x}$ = 21.1	$\bar{x}$ = 11.7	$\bar{x}$ = 9.3
1.00	9.6	17.9	8.6	31.8	17.2	29.6
	2.6	38.6	0.0	21.4	26.1	15.4
	15.3	27.1	13.3	31.0	11.5	20.4
	$\bar{x}$ = 9.1	$\bar{x}$ = 27.8	$\bar{x}$ = 7.3	$\bar{x}$ = 28.0	$\bar{x}$ = 18.2	$\bar{x}$ = 21.8
10.00	12.3	34.4	8.3	39.6	36.0	15.1
	14.6	26.7	3.8	30.9	18.8	11.9
	16.7	36.5	16.9	30.9	27.3	8.8
	$\bar{x}$ = 14.5	$\bar{x}$ = 32.5	$\bar{x}$ = 9.6	$\bar{x}$ = 33.8	$\bar{x}$ = 27.3	$\bar{x}$ = 11.9
100.00	44.0	35.3	9.4	25.0	34.7	12.8
	23.0	35.9	10.7	46.5	39.5	19.1
	8.3	22.7	7.7	58.3	31.3	8.8
	$\bar{x}$ = 25.1	$\bar{x}$ = 31.1	$\bar{x}$ = 9.2	$\bar{x}$ = 43.2	$\bar{x}$ = 35.1	$\bar{x}$ = 13.5

[1] Test date.
[2] Dump site vicinity.
[3] Sediment control.

TABLE 51. Effects of Puget Sound Sediments on Oyster Larval Development Measured as Percent Abnormals (Assay No. 1 Performed April 26 to 28, 1984)

Samish Bay

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
1	A	16	0	
	B	49	1	
	C	22	2	
	D	113	3	
	Total	200		3.0
3	A	25	0	
	B	57	1	
	C	19	0	
	D	0	0	
	Total	101	1	0.9
7	A	17	0	
	B	7	1	
	C	73	1	
	D	35	1	
	Total	132	3	2.2
20	A	42	1	
	B	120	1	
	C	40	2	
	D	13	0	
	Total	215	4	1.8
Control	A	127	8	
	B	175	3	
	C	198	17	
	D	214	6	

Bellingham Bay

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
3	A	118	2	
	B	64	0	
	C	10	1	
	D	66	0	
	Total	258	3	1.2
4	A	109	0	
	B	36	0	
	C	58	3	
	D	157	6	
	Total	360	9	2.5
5	A	34	2	
	B	41	0	
	C	109	1	
	D	34	3	
	Total	218	6	2.8
7	A	77	0	
	B	33	0	
	C	22	0	
	D	74	1	
	Total	206	1	0.5
Control	E	3	3	
	F	3	2	
	G	173	2	
	H	215	6	
	Total	1108	47	4.2[1]

[1] Percent abnormal of eight control test replicates.

TABLE 52. Effects of Puget Sound Sediments on Oyster Larval Development Measured as Percent Abnormals (Assay No. 2 Performed May 10 to 12, 1984)

Bellingham Bay

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
11	A	49	4	
	B	19	4	
	C	7	1	
	D	42	2	
	Total	117	11	9.4
12	A	19	7	
	B	45	9	
	C	8	0	
	D	59	4	
	Total	131	20	15.2
23	A	1	1	
	B	0[1]	0	
	C	0[1]	0	
	D	0[1]	0	
	Total	1	1	100.0
24	A	165	35	
	B	0	0	
	C	199	1	
	D	12	3	
	Total	376	39	10.4
Control	A	114	2	
	B	178	2	
	C	156	0	
	D	154	2	

Port Gardner - Everett Harbor

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
1	A	23	1	
	B	22	0	
	C	33	1	
	D	25	1	
	Total	103	3	2.9
2	A	7	0	
	B	3	0	
	C	10	0	
	D	0	0	
	Total	20	0	0.0
3	A	12	1	
	B	7	0	
	C	26	1	
	D	80	0	
	Total	125	2	1.6
4	A	6	0	
	B	27	2	
	C	85	0	
	D	6	0	
	Total	124	2	1.6
Control	E	276	2	
	F	235	1	
	G	141	1	
	H	128	0	
	Total	1382	10	0.7[2]

[1] Total mortality.
[2] Percent abnormal of eight control test replicates.

TABLE 53. Effects of Puget Sound Sediments on Oyster Larval Development Measured as Percent Abnormals (Assay No. 3 Performed May 10 to 12, 1984)

Port Gardner - Everett Harbor

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
5	A	46	22	
	B	54	20	
	C	54	6	
	D	55	6	
	Total	209	54	25.8
6	A	211	16	
	B	31	1	
	C	129	4	
	D	247	13	
	Total	618	34	5.5
7	A	155	16	
	B	36	5	
	C	124	5	
	D	166	5	
	Total	481	31	6.4
11	A	35	7	
	B	292	8	
	C	126	4	
	D	87	5	
	Total	540	24	4.4
Control	A	430	24	
	B	984	103	
	C	3160	60	
	D	576	69	

Fourmile Rock - Elliott Bay[1]

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
9	A	362	5	
	B	68	1	
	C	69	10	
	D	213	38	
	Total	712	54	7.6
10	A	5	0	
	B	0	0	
	C	185	8	
	D	66	3	
	Total	256	11	4.3
12	A	437	18	
	B	217	5	
	C	313	150	
	D	47	4	
	Total	1014	177	17.5
17	A	42	3	
	B	517	19	
	C	465	327	
	D	213	4	
	Total	1237	353	28.5
Control	E	625	23	
	F	1154	37	
	G	676	18	
	H	614	14	
	Total	8219	348	4.2[2]

[1] Dump site vicinity.
[2] Percent abnormal of 8 control test replicates.

TABLE 54. Effects of Puget Sound Sediments on Oyster Larval Development Measured as Percent Abnormals (Assay No. 4 Performed May 17 to 19, 1984)

Fourmile Rock - Elliott Bay[1]				
Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
20	A	110	2	
	B	295	3	
	C	269	5	
	D	262	2	
	Total	936	12	1.3
22	A	162	0	
	B	184	3	
	C	285	0	
	D	393	5	
	Total	1024	8	0.8
23	A	68	4	
	B	221	6	
	C	25	2	
	D	243	5	
	Total	557	17	3.1
24	A	139	1	
	B	118	6	
	C	167	1	
	D	239	4	
	Total	663	12	1.8
Control	A	836	4	
	B	753	10	
	C	764	9	
	D	1422	35	

Sinclair Inlet				
Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
6	A	58	2	
	B	114	5	
	C	82	8	
	D	29	3	
	Total	283	18	6.4
7	A	217	14	
	B	106	8	
	C	97	10	
	D	203	10	
	Total	623	42	6.7
8	A	219	7	
	B	216	4	
	C	170	6	
	D	219	12	
	Total	824	29	3.5
14	A	74	8	
	B	62	8	
	C	218	13	
	D	243	11	
	Total	597	40	6.7
Control	E	430	4	
	F	381	6	
	G	395	6	
	H	325	4	
	Total	5306	78	1.5[2]

[1] Dump site vicinity.
[2] Percent abnormal of eight control test replicates.

TABLE 55. Effects of Puget Sound Sediments on Oyster Larval Development Measured as Percent Abnormals (Assay No. 5 Performed May 24 to 26, 1984)

Sinclair Inlet

Station	Rep.	No. Scored[1]	No. Abnormal	Percent Abnormal
17	A	200	23	
	B	200	24	
	C	200	6	
	D	200	16	
	Total	800	69	8.6
18	A	200	23	
	B	200	8	
	C	200	8	
	D	200	16	
	Total	800	55	6.9
19	A	200	51	
	B	200	57	
	C	200	23	
	D	200	47	
	Total	800	178	22.3
20	A	200	30	
	B	200	28	
	C	200	18	
	D	200	19	
	Total	800	95	11.9
Control	A	200	6	
	B	200	0	
	C	200	3	
	D	200	5	

Dabob Bay

Station	Rep.	No. Scored[1]	No. Abnormal	Percent Abnormal
1	A	200	22	
	B	200	36	
	C	200	12	
	D	200	11	
	Total	800	81	10.1
5	A	61	11	
	B	200	9	
	C	200	9	
	D	200	2	
	Total	661	31	4.7
7	A	200	24	
	B	200	40	
	C	200	17	
	D	200	7	
	Total	800	88	11.0
15	A	200	23	
	B	200	15	
	C	200	6	
	D	200	19	
	Total	800	63	7.9
Control	E	200	3	
	F	200	4	
	G	200	6	
	H	200	4	
	Total	1600	31	1.9[2]

[1] Rescored, see page 179.
[2] Percent abnormal of eight control test replicates.

TABLE 56. Effects of Puget Sound Sediments on Oyster Larval Development Measured as Percent Abnormals (Assay No. 6 Performed June 4 to 6, 1984)

Sequim Bay

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
14[1]	A	51	9	
	B	50	0	
	C	124	3	
	D	31	0	
	Total	256	12	4.7
17	A	43	1	
	B	49	8	
	C	10	1	
	D	89	7	
	Total	191	17	8.9
18	A	87	3	
	B	45	12	
	C	59	10	
	D	55	13	
	Total	246	38	15.4
20	A	32	2	
	B	63	6	
	C	67	5	
	D	53	9	
	Total	215	22	10.2
Control	A	255	12	
	B	268	31	
	C	241	13	
	D	251	13	

Case Inlet

Station	Rep.	No. Scored	No. Abnormal	Percent Abnormal
1	A	57	6	
	B	73	8	
	C	71	16	
	D	119	25	
	Total	320	55	17.2
11	A	10	1	
	B	11	1	
	C	56	8	
	D	52	7	
	Total	129	17	13.2
15	A	61	5	
	B	93	13	
	C	83	4	
	D	80	7	
	Total	317	29	9.1
17	A	60	4	
	B	62	5	
	C	28	2	
	D	51	2	
	Total	201	13	6.5
Control	E	261	15	
	F	289	29	
	G	282	37	
	H	245	20	
	Total	2092	170	8.1[2]

[1] Sediment control.
[2] Percent abnormal of 8 control test replicates.

TABLE 57. Summary Analysis of Variance for Percent Abnormality (Arcsin $\sqrt{X}$) in Each Oyster Larval Bioassay

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
		Assay No. 1		
Mean	1	1.323		
Stations	8	0.817	0.102	1.33[1]
Error	30	2.299	0.077	
Total (corr)	38	3.116		
		Assay No. 2		
Mean	1	1.538		
Stations	8	2.469	0.309	14.21[3]
Error	26	0.565	0.022	
Total (corr)	34	3.034		
		Assay No. 3		
Mean	1	3.304		
Stations	8	0.417	0.052	1.40[1]
Error	30	1.118	0.037	
Total (corr)	38	0.536		
		Assay No. 4		
Mean	1	1.226		
Stations	8	0.231	0.029	9.89[3]
Error	31	0.091	0.003	
Total (corr)	39	3.321		

[1] Not significant.
[2] Significant ($p < 0.05$).
[3] Significant ($p < 0.01$).

TABLE 57. Summary Analysis of Variance for Percent Abnormality (Arcsin $\sqrt{X}$) in Each Oyster Larval Bioassay (Continued)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
		Assay No. 5		
Mean	1	3.144		
Stations	8	0.392	0.049	6.45[3]
Error	31	0.235	0.008	
Total (corr)	39	0.627		
		Assay No. 6		
Mean	1	3.717		
Stations	8	0.215	0.027	2.56[2]
Error	31	0.325	0.010	
Total (corr)	39	0.540		

TABLE 58. Comparison of Treatment and Control Abnormality (Arcsin $\sqrt{X}$) Using Dunnett's Test

Assay No. 1

Bay	Samish				Bellingham				
Station	1	3	7	20	3	4	5	7	Control
Mean	.153	.044	.169	.118	.113	.107	.161	.029	.457

Assay No. 2

Bay	Bellingham				Port Gardner - Everett Harbor				
Station	11	12	23	24	1	2	3	4	Control
Mean	.344[1]	.345[1]	1.57[1]	.358[1]	.147	.000	.123	.069	.074

Assay No. 3

Bay	Port Gardner - Everett Harbor				Fourmile Rock- Elliott Bay[2]				
Station	5	6	7	11	9	10	12	17	Control
Mean	.524	.217	.271	.263	.266	.141	.354	.399	.219

Assay No. 4

Bay	Fourmile Rock- Elliott Bay[2]				Sinclair				
Station	20	22	23	24	6	7	8	14	Control
Mean	.115	.060	.210	.130	.261[1]	.271[1]	.185	.291[1]	.114

Assay No. 5

Bay	Sinclair				Dabob				
Station	17	18	19	20	1	5	7	15	Control
Mean	.290[1]	.259	.486[1]	.350[1]	.315[1]	.242	.325[1]	.278	.130

Assay No. 6

Bay	Sequim				Case				
Station	14	17	18	20	8	11	15	17	Control
Mean	.147	.294	.415	.317	.410	.348	.299	.255	.282

[1] Significant ($p < 0.05$).
[2] Dump site vicinity.

TABLE 59. Significance of Oyster Larval Percent Abnormality Data by Bay and Station for Sediments Collected During Detailed Surveys April 23 to May 29, 1984

Location	Significant[1]	Not Significant
Samish		1, 3, 7, 20
Dabob	1, 7	5, 15
Sequim		14, 17, 18, 20
Case		8, 11, 15, 17
Bellingham	11, 12, 23, 24	3, 4, 5, 7
Fourmile Rock-Elliott Bay[2]		9, 10, 12, 17 20, 22, 23, 24
Port Gardner-Everett Harbor		1, 2, 3, 4 5, 6, 7, 11
Sinclair	6, 7, 14 17, 19, 20	8, 18

[1] Differed significantly from Control ($p < 0.05$).
[2] Dump site vicinity.

survival. The trend to higher percentages of abnormal oyster larvae becomes more evident as amphipod survival decreases.

Salinity, pH, and dissolved oxygen values remained at acceptable levels in all cultures during the assays. Control cultures also generally showed low percentages of abnormal larvae: Assay No. 1, 4.2%; Assay No. 2, 0.7%; Assay No. 3, 4.2%; Assay No. 4, 1.5%; Assay No. 5, 1.9%; and Assay No. 6, 8.1%. Although the percentages of abnormal larvae exceeded the 3% abnormal rate suggested by Woelke (1972) as acceptable for controls without correction in only three of the assays, we chose to correct all of the data (Appendix E). The ASTM (1980) suggests that if abnormality exceeds 2% in control larvae, treatment abnormality should be corrected using Abbott's equation (Finney 1980). It should also be noted that ASTM (1980) allows use of control abnormalities of up to 10% provided the data are corrected and weighted mean control abnormality values are reported.

Survival data were not routinely reported. We focused on abnormal shell development because it is typically more sensitive (ASTM 1980). It is also a less variable parameter and, hence, is a more reliable endpoint for use in bioassays.

Assay No. 5 was rescored by again subsampling the remaining screened larvae from each test container. In this case, the first 200 larvae were evaluated. This procedure was necessary because many of the subsamples used for initial scoring contained too few larvae for meaningful statistical comparisons. It was likely that the larvae were not uniformly distributed within the test cultures at the time of subsampling. Either the cultures were not thoroughly mixed, or larvae were "bound" to particulates that rapidly settled to the bottom of test containers. The problem was also encountered in a few test containers in Assay Nos. 1, 2, 3, and 6, but was decidedly more pronounced in Assay No. 5.

The inclusion of six oyster larval bioassays on screening survey sediments served to calibrate our methods and, in particular, to test various modicications of ASTM E 724-80 (ASTM 1980) to adapt the technique to sediment testing. First, these data suggested that the test sediments be added at least to the 10 or 100 g/L concentration level. Various other

investigators (Schink et al. 1974; Cummins et al. 1976) have used sediment concentrations from 0.5 to 10 g/L, and different methods for exposure including rotating the embryos together with test sediments (Cardwell et al. 1977). Although the method described here used higher sediment concentrations than others have reported, there appeared to be little or no physical effect using our method of exposure. Second, these data suggested that perhaps rigorous use of a sediment control was not required. The addition of a clean sediment from Sequim Bay (Station 14) over a range of sediment concentrations of 0.01 to 100 g/L failed to evoke a response different from the seawater controls. Accordingly, while we did not elect to include a sediment control in each batch of bioassays performed during the detailed surveys (see Section 3.5.2), we did provide for Sequim Bay Station No. 14 to be retested as part of the detailed surveys. It also seemed inappropriate to use other types of sediment controls such as a No. 4 washed mortar sand, or a manufactured sand specifying grain size, percent water, and certain chemical factors. Although Chapman and Morgan (1983) used a sediment control, they did not specify source or physical or chemical constituency.

3.7 SUMMARY STATISTICS

Summary scores for chemical, biological, and physical variables used in the statistical exercise to determine which bays were showing signs of degraded sediment quality are presented in Table 60.

The mean of the summary metals scores from the 16 baseline stations was 0.0516 with a standard deviation of 0.0150. The tolerance factor, K, for this distribution to give a probability of 0.99 that 99% of the distribution lies within $\bar{x} \pm Ks$ is K = 4.492. The upper limit for this tolerance interval is 0.1191. A score of 0.120 or greater was considered unlikely to be attained in an unimpacted environment.

The mean of the summary organics scores from the baseline bays was 0.0126 with a standard deviation of 0.0028. The tolerance factor, K, is the same, 4.492. The upper tolerance limit of the distribution is 0.0250.

A score of 0.026 or greater was considered indicative of an impacted environment.

The critical level of 0.20 for proportion of dead amphipods was established in Section 3.5. The stations that were found to be different from controls in the oyster larvae bioassay were identified in Section 3.6. All summary scores exceeding their appropriate critical value are indicated by ([1]). The column designated "signal" in Table 60 denoted whether a chemical, or biological variable, or both variables exceeded the critical value.

On the basis of the data presented in Table 60, each of the 48 stations can be classified as exhibiting "signals" in one of four categories:

1. no biological or chemical signal
2. a biological, but no chemical signal
3. a chemical, but no biological signal
4. both a chemical and a biological signal.

All stations from the urban bays provided at least a chemical signal. The Fourmile Rock - Elliott Bay dump site vicinity showed only chemical signals at all stations. The other three urban bays indicated both chemical and biological signals at all or almost all of their stations. Port Gardner - Everett Harbor and Sinclair Inlet demonstrated the most consistent and strongest biological and chemical signals.

Among the baseline bays, all stations in Case Inlet and Samish Bay showed biological signals (lowered amphipod survival) in the absence of chemical signals. Sequim Bay provided the greatest proportion of stations without any signal.

TABLE 60. Summary Scores for Chemical, Biological, and Physical Variables Used to Determine Signs of Degraded Sediment Quality

Bay	Station	Metals	Organics	Amphipods	Oyster Larvae	Capitella	Fines	Signal[3]
Samish	1	0.041	0.016	0.310[1]	0.000	0	0.812	bio
	3	0.052	0.016	0.470[1]	0.000	0	0.809	bio
	7	0.042	0.014	0.250[1]	0.000	0	0.849	bio
	20	0.047	0.014	0.310[1]	0.000	0	0.873	bio
Dabob	1	0.026	0.008	0.100	0.084[1]	0	0.203	bio
	5	0.029	0.008	0.070	0.029	1	0.237	none
	7	0.041	0.009	0.260[1]	0.093[1]	0	0.490	bio
	15	0.062	0.012	0.360[1]	0.061	0	0.897	bio
Sequim	14	0.047	0.012	0.120	0.000	0	0.639	none
	17	0.058	0.012	0.100	0.009	0	0.831	none
	18	0.057	0.016	0.110	0.079	0	0.794	none
	20	0.054	0.012	0.270[1]	0.023	0	0.839	bio
Case	1	0.084	0.017	0.280[1]	0.099	0	0.894	bio
	11	0.049	0.011	0.280[1]	0.055	0	0.390	bio
	15	0.072	0.014	0.430[1]	0.011	0	0.813	bio
	17	0.065	0.012	0.410[1]	0.000	0	0.781	bio
Bellingham	3	0.171[1]	0.186[1]	0.040	0.000	0	0.669	chem
	4	0.121[1]	0.207[1]	0.170	0.000	9[1]	0.836	bio-chem
	5	0.096	0.076[1]	0.340[1]	0.000	0	0.965	bio-chem
	7	0.102	0.152[1]	0.050	0.000	0	0.917	chem
	11	0.089	0.066[1]	0.170	0.088[1]	0	0.981	bio-chem
	12	0.072	0.072[1]	0.110	0.147[1]	0	0.642	bio-chem
	23	0.090	0.082[1]	0.580[1]	1.000[1]	1	0.954	bio-chem
	24	0.083	0.075[1]	0.110	0.098[1]	0	0.974	bio-chem
Fourmile Rock-Elliott Bay[2]	9	0.250[1]	0.146[1]	0.130	0.035	0	0.557	chem
	10	0.531[1]	0.285[1]	0.140	0.009	1	0.689	chem
	12	0.107	0.040[1]	0.160	0.139	0	0.886	chem
	17	0.162[1]	0.162[1]	0.080	0.254	0	0.559	chem
	20	0.263[1]	0.269[1]	0.170	0.000	0	0.523	chem
	22	0.117	0.209[1]	0.140	0.000	0	0.624	chem
	23	0.053	0.068[1]	0.120	0.016	0	0.245	chem
	24	0.056	0.058[1]	0.130	0.003	1	0.137	chem

[1] Higher than critical value.
[2] Dump site vicinity.
[3] Signs of degraded sediment quality based on chemical, biological, or both a chemical and biological variable exceeding critical value.

TABLE 60. Summary Scores for Chemical, Biological, and Physical Variables Used to Determine Signs of Degraded Sediment Quality (Continued)

Bay	Station	Metals	Organics	Amphipods	Oyster Larvae	Capitella	Fines	Signal[3]
Port Gardner-Everett Harbor	1	0.134[1]	0.603[1]	0.560[1]	0.022	16[1]	0.629	bio-chem
	2	0.087	0.203[1]	0.290[1]	0.007	17[1]	0.831	bio-chem
	3	0.130[1]	0.446[1]	0.330[1]	0.009	11[1]	0.635	bio-chem
	4	0.270[1]	0.768[1]	0.580[1]	0.009	43[1]	0.538	bio-chem
	5	0.118	0.294[1]	0.860[1]	0.225	50[1]	0.673	bio-chem
	6	0.060	0.108[1]	0.110	0.014	6[1]	0.402	bio-chem
	7	0.088	0.160[1]	0.100	0.023	1	0.675	chem
	11	0.071	0.061[1]	0.260[1]	0.002	0	0.691	bio-chem
Sinclair	6	0.373[1]	0.387[1]	0.450[1]	0.050[1]	0	0.868	bio-chem
	7	0.670[1]	0.296[1]	0.190	0.053[1]	0	0.555	bio-chem
	8	0.309[1]	0.202[1]	0.380[1]	0.020	0	0.899	bio-chem
	14	0.334[1]	0.458[1]	0.430[1]	0.053[1]	0	0.916	bio-chem
	17	0.326[1]	0.236[1]	0.260[1]	0.068[1]	0	0.782	bio-chem
	18	0.244[1]	0.176[1]	0.320[1]	0.051	0	0.778	bio-chem
	19	0.434[1]	0.265[1]	0.190	0.208[1]	0	0.490	bio-chem
	20	0.344[1]	0.734[1]	0.380[1]	0.102[1]	0	0.686	bio-chem

[1] Higher than critical value.
[2] Dump site vicinity.
[3] Signs of degraded sediment quality based on chemical, biological, or both a chemical and biological variable exceeding critical value.

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4.0 DISCUSSION

4.1 CONTAMINANT LEVELS

4.1.1 Relationship to Physical Properties of Sediments

Examination of the correlation matrix in Table E.2 revealed that average phi was strongly correlated ($p < 0.001$) with water content. Also, percent volatile solids correlated ($p < 0.001$) with water content. An unexpected finding was the lack of significant correlations between average phi and contaminants, either metals or organics. Previous geochemical studies in baseline bays and in the main basin of Puget Sound reported strong correlations between grain size and contaminant concentration (Crecelius et al. 1975; Dexter et al. 1981; Romberg et al. 1984). The apparent reason for the lack of correlation in our study was the finding that some coarse-grain sediments from the shallow urban bays were heavily contaminated with both metals and organics. Coarse-grained sediments are not usually highly contaminated unless they occur near a point source of pollution as in the case of some stations in urban bays.

A strong correlation ($p < 0.05$) among many heavy metals in sediments was observed. In urban bays, heavy metals often have common sources and are associated with the same suspended particles. Therefore, sediments are usually contaminated by a group of heavy metals.

The organic chemicals and organic properties of the sediment were fairly strongly correlated. The correlation between TOC and percent volatile solids was very high ($p < 0.001$) as was expected because most of the volatile matter in sediment is organic matter. PCB-1254 was also highly correlated ($p < 0.01$) with aromatic hydrocarbons. PCBs were highly correlated ($p < 0.001$) with Ag perhaps because the major source of both contaminants is sewage. Aromatic hydrocarbons were highly correlated ($p < 0.001$) with IR, as were PCBs ($p < 0.01$).

4.1.2 Comparison of Contaminants in Urban and Baseline Bays

In this study, concentrations of metals, PCB-1254, and aromatic hydrocarbons in urban bays were clearly different from those in baseline bays. Urban bay sediments were generally clearly contaminated by Ag, As, Cu, Cd, Hg, Pb, and Zn. Other metals (Sb, Be, Cr, Ni, Se, Ti) were found at about the same concentration in all bays, however, a few urban bay sediments were enriched in Sb, Cr, and Ni. Baseline bays generally contained lower concentrations of metals.

The only organics that were consistently quantified in urban bays were the aromatic hydrocarbons and PCB-1254. Other compounds occasionally quantified in urban bays included phthalates and PCB-1260. By comparison, baseline bay sediments generally contained less organic contamination. No PCBs were detected, and only a few stations in one baseline bay, Samish Bay, were found to contain detectable amounts of phthalates and aromatic hydrocarbons.

It is tempting to define baseline or "reference" conditions on the basis of chemical variables. Sequim Bay and Dabob Bay, with relatively low metals burdens and no detectable organic compounds, appear to be excellent choices for reference bays. Application of this concept is potentially useful in a regulatory sense to determine where and when remedial action is required.

The fact that all bays in Puget Sound interconnect and that circulation transports contamination from one bay to another, however, tends to detract from the concept of "reference" bays. Circulation models developed for Puget Sound predict that gradients of both dissolved and particulate materials exist between regions of higher and lower contamination (Ebbesmeyer et al. 1984).

4.1.3 Comparisons to Other Studies

The range of contaminant concentrations in surface sediments of the urban and baseline bays were compared with mean data from other sediments in Puget Sound (Table 61), including pre-1900 sediments. The urban central basin sediments (Area BB in the METRO TPPS Report [Romberg et al.

TABLE 61. Comparisons of Concentrations of Contaminants in Puget Sound Sediments

	This Study					Previous Studies of Puget Sound										
	Urban Bays				Baseline Bays	Malins et al. 1980 & 1982						EPA				
	Bellingham Bay	Fourmile Rock-Elliott Bay	Port Gardner-Everett Harbor	Sinclair Inlet	Range	Bellingham Bay	Fourmile Rock-Elliott Bay	Port Gardner-Everett Harbor	Sinclair Inlet	Commencement Bay	Baseline	Port Gardner-Everett Harbor East Waterway[3]	Puget Sound Main Basin Surface Sediment[1]	Fourmile Rock-Elliott Bay Criteria	Baseline So. Puget Sound[2]	Puget Sound Pre-1900 Sediment[2]
Pb (ppm)	24	134	40	170	13-26	64	130	23	100	130	16-22	57	38	126	10-44	5.6
Hg (ppm)	1.0	1.5	0.24	2.1	0.06-0.13	1.4	0.46	0.16	0.91	0.36	0.07-0.47	0.33	0.14	1.1	0.06 0.23	0.04
Cu (ppm)	110	98	83	305	37-49	ND[4]	68	ND[4]	127	199	10-45	97	36	92	21-76	23
AH (ppm)	15.9	4.2	4.4	5.4	<0.1-0.15	4.0	13	2.7	6.4	9.7	0.25-0.48	54	2.2	11	0.1-0.8	0.24
PCB (ppb)	4.2	319	317	579	<20	<100	380	41	130	270	3-10	400	125	160	<30	9.1

[1] Romberg et al. 1984.
[2] Riley et al. 1983.
[3] Anderson and Crecelius 1985.
[4] Not determined.

1984]) are representative of fine-grain sediments now accumulating in the main basin of Puget Sound. The Fourmile Rock - Elliott Bay data have been used by U.S. EPA Region 10 to set Interim Decision Criteria for open water disposal at Fourmile Rock in Puget Sound.

Several elements in baseline bays, including Sb, As, Be, Cd, Cr, Ni, Se, Tl, and Zn, were found at approximately the same concentrations as in the pre-1900 sediments. This similarity indicated that anthropogenic activities have not contaminated the baseline bays with these nine elements. Current baseline concentrations of Sb, Be, Se, and Tl were less than those measured in pre-1900 sediments probably because analytical techniques and detection limits were different. Several elements, including Be, Se, and Tl were not elevated even in urban sediments.

The elements As, Cd, Cr, Ni, and Zn were generally elevated by not more than a factor of 10 in the urban bays compared to the baseline bays and baseline sediments deposited in the 1800s (Table 62).

In some urban sediments, metal concentrations were more than 50 times greater than concentrations in baseline sediments. These metals included Cu, Pb, Hg, and Ag. The concentrations of these metals were also enriched in many of the baseline bay sediments. Possible sources of metals entering baseline bays include transport of contaminants from the main basin of Puget Sound, local point and nonpoint sources, and atmospheric fallout. For example, Case Inlet, which was the most contaminated baseline bay, has been shown to act as a sediment trap of contaminants transported from the Tacoma area into the southern Sound (Riley et al. 1983).

We selected three metals (Pb, Hg, and Cu) and two classes of organic compounds (aromatic hydrocompounds and PCBs) to compare contaminants in sediments of the urban and baseline bays with other bays or regions of Puget Sound. These five contaminants were chosen because they are frequently quantified in sediments and are almost always elevated in sediments from urban areas. Also, these contaminants are on the Fourmile Rock Site Interim Decision Criteria list. Table 61 lists the mean concentrations of the five contaminants in the four urban bays and

As already noted, disturbed stations with major shifts in dominant infauna only occurred in urban bays (Bellingham Bay and Port Gardner - Everett Harbor). These stations accounted for the most dramatic differences between urban and baseline bays.

After omitting the stations with high TOC levels and comparing the averages for urban and baseline bays within each sediment category (Table 22), other differences in infaunal patterns between urban and baseline bays became evident. The number of taxa (richness) in silty-mud of the urban bays was half that in the same sediment type of baseline bays. For stations with sandy-silt, richness was lower in the urban bays when compared to baseline bays. For the sandy stations, richness was about the same in both urban and baseline bays. Also, in silty-mud, the average abundance was the same for urban and baseline bays. In contrast, for sandy-silt, the average abundance in urban bays was 40% of that in baseline bays. However, this latter difference was confounded by differences in depth. The Fourmile Rock - Elliott Bay dump site vicinity had five deep stations in the sandy-silt group; and these deep sandy-silt stations showed low abundance. The sandy-silt stations in Sinclair Inlet were shallower than those in baseline bays and demonstrated intermediate to high abundance in the same range as those in Sequim Bay, the most comparable baseline bay. Setting aside the influence of high TOC levels, distinct species compositions for urban bays were not distinguished. For stations with silty-mud, the most abundant species in both urban and baseline bays were the Cirratulid polychaetes, primarily *Tharyx spp.* For stations with sandy-silt, *Axinopsida serricata* became the most abundant species in some urban bays. However, this shift to *Axinopsida serricata* occurred at the deeper stations with sand contents above 30% and was probably because of habitat differences rather than being from an urban bay. For silty-sand and sand, the shift from *Axinopsida serricata* to Cirratulid polychaetes at one urban station was probably related more to the shallow depth of the bay rather than to the type of bay.

4.2.3 Comparisons With Other Studies

Overall, the results of the benthic infauna analyses confirm the general findings of other studies of Puget Sound infauna. Lie (1968) had already shown that substrate type was a dominant factor in determining the character of benthic infauna in Puget Sound. Our finding of a change in species composition from mud to sand reinforces Lie's general conclusion. Similarly, Nichols' (1970) general conclusion that clay content of the sediment controls polychaete distribution is consistent with our finding that high clay content was associated with sparse infaunas. The general conclusions of these two studies strengthen our belief that the development of sediment quality criteria must entail examination of the effects of contaminants for different sediment types.

Most infaunal studies in Puget Sound have focused on sewage outfalls. Consequently, there is little overlap between our stations and those of other studies. Consistent with our observations is the observation by others of large abundances of enrichment opportunistic species in areas of high organic content in Puget Sound. For example, Armstrong et al. (1980) reports a shift to high abundance of *Capitella capitata* around a combined sewer overflow at 9 m depth in Elliott Bay. The Fourmile Rock - Elliott Bay dump site vicinity stations sampled in our study are much deeper (over 100 m) than those of Armstrong et al. (1980).

The recent study of Swartz et al. (1985) along a gradient of stations in relation to a California marine sewage outfall provides two major findings that are similar to those in this study. First, the three stations closest to the sewage outfall had the highest organic content (TOC >4.0%) and revealed infaunas dominated by *Capitella capitata*. Second, the same three stations where *Capitella capitata* dominated also had significantly lower amphipod survival. In addition, these impacted stations showed lower species richness and abundance. These findings of Swartz et al. (1985) compare well with those of this study and strengthen the general expectation that organic enrichment of sediment leads to a shift toward infauna dominated by particular opportunistic species and to sediment toxicity for amphipods.

In another study, Chapman et al. (1984a, b) applied several types of multivariate analyses to chemical and biological data from 23 stations in four urban Puget Sound bodies of water, Elliott Bay (5 stations), the Duwamish Waterway (3 stations), Commencement Bay (10 stations), and Sinclair Inlet (2 stations), as well as two nonurban bays, Case Inlet (1 station) and Samish Bay (2 stations). Their Elliott Bay stations were shallower (less than 40 m) than ours and located closer to shore in proximity to sewage outfalls. Factor and cluster analyses applied to benthic infauna data alone and to infaunal and chemical data combined showed that Case Inlet and Samish Bay were distinct from the urban bays. As in our study, the Samish Bay infauna revealed an important echinoderm component virtually absent from the urban bays and demonstrated a species composition distinct from the other bays. Based on our results, the finding that two nonurban bays (Sequim Bay, Dabob Bay) showed infaunas of the same general species composition as that of the urban bays (Sinclair Inlet, Bellingham Bay, some stations in Port Gardner - Everett Harbor) raises the question of whether just two stations from Samish Bay and one from Case Inlet constituted an adequate and appropriate control or baseline for multivariate analyses.

Earlier studies (1964-1966) in Bellingham Bay and Everett Harbor have reported stations with extremely impoverished infauna where sludge from pulp mill effluent had accumulated (Jones and Stokes Associates 1983). More recent studies in Everett Harbor found some recovery but the infauna near the pulp mill outfalls was still dominated by Capitella capitata (Jones and Stokes Associates 1983). Our results indicate that areas with organically enriched sediment still exist in Everett Harbor and to a lesser extent, in Bellingham Bay, and that such areas are still dominated by organic enrichment opportunists.

4.3 FISH AND SHELLFISH PATHOLOGY

4.3.1 Relationships Between Lesion Incidence and Sediment Chemical Composition

4.3.1.1 Fish

Hepatic Lesions. Two major lines of evidence suggest that three of the hepatic lesions in fish (neoplasms, preneoplasms, and specific degeneration) are causally related to exposures of environmental contaminants. First, in studies previously conducted in Puget Sound (Malins et al. 1982, 1984; McCain et al. 1982), these types of hepatic lesions were found only, or were significantly more prevalent, in flatfish from chemically contaminated urban embayments. The urban areas with the highest lesion incidences were the Duwamish Waterway and the downtown waterfront in Seattle, the waterways of Commencement Bay, and Everett Harbor. Second, these types of lesions have been reported to occur in laboratory animals exposed to toxic and/or carcinogenic chemicals. Lesions morphologically similar to specific degeneration have been induced by PCBs in mammals and fish (Koller and Zinkl 1973; Hinton et al. 1978). Hepatic neoplasms and preneoplasia are inducible in mammals by many chemicals, including carbazole and derivatives (Tsuda et al. 1982), DDT, dieldrin, N-acetyl-2-aminofluorene, nitrosamines and phenabarbitone (Jones and Butler 1975). A variety of elements, including arsenic, chromium, silver, indium, iron, molybdenum, selenium, tellurium and thallium also possess hepatotoxic properties in humans and mammals (Beliles 1975). In fish, heptocellular and cholangiocellular neoplasms similar to those seen in our study have been produced by exposure to N-methyl-N'-nigrosoguanidine (Hendricks et al. 1980), and 7,12-dimethylbenz[a]anthracene (Schultz and Schultz 1982).

The other types of hepatic lesions, storage disorders and nonspecific degenerative lesions, were found in both urban and baseline bays, although highest incidences were generally found in fish from urban bays (Malins et al. 1982, 1984). The remaining three types of hepatic lesions--

vascular, proliferative, and inflammatory disorders--were evenly distributed in both urban and baseline areas. All five types of these lesions tend to be nonspecific responses to a variety of factors, including exposure to natural and synthetic toxins and physiological changes related to reproduction or diet.

Significance of Kidney and Gill Lesions. Relationships between kidney and gill lesions in fish and chemical contaminants are not as strong as those observed for hepatic lesions. Necrosis and certain types of depositional disorders of the kidney have been found in English sole from both urban and nonurban areas of Puget Sound. However, the highest incidence of the kidney necrosis was found in the Hylebos and Duwamish Waterways (Malins et al. 1982), and the depositional disorders were most prevalent in the Duwamish Waterway and the estuary of the Lake Washington Ship Canal in Seattle (McCain et al. 1982).

A similar distribution pattern in both urban and nonurban areas was observed for the proliferative gill conditions in English sole. Malins et al. (1982) found the highest incidence in the Hylebos Waterway and Discovery Bay, and McCain et al. (1982) found such conditions only in sole from the Duwamish Waterway, Lake Washington Ship Canal, and at the mouth of the Snohomish River in Everett. Proliferative gill lesions have been found in sole widely distributed throughout Puget Sound, and can be caused by external parasitism as well as exposure to chemical irritants. Consequently, their significance in relation to sediment pollutants is questionable.

4.3.1.2 Shellfish

Vascular Disorders. These conditions were found only in the gills of the shrimp species, and were more prevalent in shrimp from the baseline bay, Dabob Bay. In several instances, congestion of the gill filaments was associated with hyalinocytic phagocytosis and encapsulation. The gill has a prime function in the elimination of foreign and necrotic material

(Fontaine and Lightner 1975). The congestion, particularly in association with phagocytosis and encapsulation, may represent efforts by the affected shrimp to remove parasites, cellular debris, or foreign materials from the animal, and, thus, does appear to be a serious condition. The cause of gill microaneurysms is largely unknown, but may be an artifactual finding related to pressure changes involved in capturing the animals at depth and then bringing them too quickly to the surface. These lesions were not associated with any observable parasite, and they occurred in all sampling areas.

Inflammatory Conditions. Hyalinocytic infiltration, encapsulation, and phagocytosis were also detected only in shrimp species, and except for encapsulation in the antennal gland and phagocytosis in the gill, were more prevalent in shrimp from Dabob Bay. The function of these processes in shrimp is generally to isolate and/or remove foreign and necrotic material, whether caused by parasites, physical debris, or chemical destruction of cells (Couch 1978; Doughtie and Rao 1983; Fontaine and Lightner 1975; Johnson 1980). Much of the encapsulation in the hepatopancreas was associated with a parasitic infection in *P. dispar*, which surrounded melanized lesions in the connective tissue between the digestive tubules. Hemocytic (hyalinocytic) infiltration accompanied these occasionally severe infestations, and hyalinocytes were observed phagocytosing small dense particles. Even in severe cases, the digestive tubules appeared quite normal, except that no B-cells (Blasenzellen) were present in the digestive epithelium. No discernible parasites could be detected in association with inflammatory conditions in the hepatopancreas of *P. platyceros* from Dabob Bay.

The incidence of hemocytic infiltration in the antennal gland was highest in *C. magister* from Bellingham Bay; this condition in the eye was equally prevalent in all three sampling areas. Hemocytic infiltration of the epithelium of the esophagus and ommatidium of the eye were minor and did not appear to represent a serious impairment. Infiltration of the coelomoduct of the antennal gland represents a potentially more serious disorder. However, the infiltration was minor in all affected animals,

and the disorder was found in crab (both C. magister and C. gracilis) from all areas except Dungeness Spit.

Melanized lesions and unmelanized granulomas can develop for a variety of reasons. Injury and disease cause the majority of these responses (Bang 1983; Fontaine and Lightner 1975; Lightner and Redman 1977). Exposure to a variety of chemicals can also cause melanization reactions (Couch 1977; Doughtie and Rao 1983, 1984; Nimmo et al. 1977). In addition, dietary deficiencies can also cause these lesions (Lightner et al. 1977; Smith and Taylor 1968). Melanized lesions and unmelanized granulomas in crab, and melanized lesions in shrimp all tended to be more prevalent in tissues of animals from baseline bays than in urban bays. This evidence would seem to preclude environmental pollutants in urban bays as a probable cause for these lesions. Parasitic infections tended to be more prevalent, at least in P. dispar and C. gracilis, in baseline bays. The presence of undetectable (at the light microscope level) parasites in the other crab and shrimp species may also have been associated with the higher incidence of melanized lesions and unmelanized granulomas in reference areas.

Degenerative Disorders. Although the incidence of degenerative disorders in both crab and shrimp was low, these lesions in the antennal gland of P. dispar and P. platyceros, and in the heart of P. platyceros, were the only ones that appeared to be exclusively associated with the Fourmile Rock - Elliott Bay dump site vicinity. In the heart of P. platyceros, the degenerative changes were associated with a suspected parasitic infection.

Hydropic degeneration/membrane lysis occurred only in the hepatopancreas of C. magister while nuclear atypia occurred in the hepatopancreas of both crab species. The high concentration of digestive enzymes present in the epithelial cells of the hepatopancreas can result in rapid dissolution of the plasma membrane and cause autolytic changes in the epithelial cells when the crab are subjected to the stress of handling and necropsy. The fact that hydropic degeneration/membrane lysis occurred only in C. magister probably represents a more delicate nature of the

digestive epithelium of this species. This condition was also much higher in C. magister from the inner waterways of Bellingham Bay (Stations 11 and 12 and Stations 4 and 5), and was not observed in crab from Dungeness Spit. The digestive epithelium of other hepatopancreatic tubules did not appear to be affected by this condition. The gradual decrease in incidence of this condition from inner areas to outer areas of Bellingham Bay (Stations 23 and 24) and Samish Bay, and the total absence of this condition in crab from Dungeness Spit, suggest that perhaps subtle degenerative changes may affect membrane stability occurring in the hepatopancreatic epithelial cells of crab from the inner areas of Bellingham Bay. These changes may predispose the hepatopancreas to membrane lysis when the animals are exposed to stress.

Nuclear atypia may also be the result of autolytic changes resulting from stress of capture, handling, or necropsy and appeared in both crab species. However, the condition was present relatively rarely within the hepatopancreas and was observed in crab from all sampling areas.

Proliferative Conditions. Proliferative conditions as represented by an increase in mitotic activity in various tissues can result from a number of factors: the stage of the molting cycle, feeding activity, parasitic infection, and environmental or physical stress (Johnson 1980). No distinct pattern of the incidence of proliferative conditions was detected in C. gracilis. The significantly higher incidence of these conditions in the hemopoietic tissue in C. magister from Stations 11 and 12 in Bellingham Bay, compared to the significantly lower incidence of this lesion in crab from Samish Bay, may represent a reaction to chemical injury at this site. However, the possible relationship to chemically contaminated environments is unclear because the incidence of this lesion in crab from Dungeness Spit was similar to that of two sites in Bellingham Bay.

4.3.2 Comparison of Fish and Shellfish Pathology in Urban and Baseline Bays

Although the original design of this study called for collecting flatfish from eight embayments in or near Puget Sound, flatfish were not collected from four of these areas (Bellingham Bay, Sequim Bay, Samish Bay, and Dabob Bay) because adult English and Dover sole (fish >150 mm in length) were scarce or absent. Three of these baseline bays (Samish Bay, Dabob Bay, and Sequim Bay) were intended to serve as "reference" areas. To partially compensate for this problem, an additional baseline station near Eliza Island (west of Bellingham Bay, Station No. 04015) was sampled, and sufficient numbers of adult English sole were collected. Malins et al. (1982) demonstrated that English sole less than two years old had significantly ($p \leq 0.05$) lower incidences of idiopathic liver diseases than fish over two years old. For example, liver neoplasms were not detected in males less than two years old or in females less than one year old. No appropriate baseline station was found for Dover sole. To correct for this situation, stations outside of, but adjacent to, the Fourmile Rock - Elliott Bay dump site vicinity were sampled. Of these two stations (one southeast of West Point [Station No. 10065] and the other near Duwamish Head [Station No. 10064]), sufficient numbers of adult Dover sole were captured only at the West Point Station.

This difficulty did not exist for shellfish as suitable numbers of crab and shrimp were collected from all sampling areas except Sequim Bay. *Cancer magister* collected from Dungeness Bay were substituted for Sequim Bay crab.

4.3.2.1 Urban Bays

Fish. For English sole, Sinclair Inlet had the highest incidences of liver, kidney, and gill lesions of all the urban bays sampled. Within the Inlet, the incidence of lesions tended to be higher near the northwestern portion. For Dover sole, relatively low percentages of occurrence of serious liver lesions were detected in the Fourmile Rock - Elliott Bay dump site vicinity near West Point. Although these observations with

Dover sole provide only suggestive information about possible effects of environmental contaminants in these two areas, they do demonstrate that Dover sole may serve as a very useful indicator fish species for assessment of environmental stresses in the deeper portions of Puget Sound.

Shellfish. A number of pathological conditions in shellfish were also identified in the urban bays. The relationships of many of these conditions to urban-associated contamination are poorly understood at this time. Nevertheless, two types of lesions were found only in animals from urban areas, suggesting that they were caused by urban-associated factors (e.g., chemical contamination). Hydropic degeneration/membrane lysis in the hepatopancreas of *C. magister* and degenerative disorders in the antennal gland of both shrimp species were observed in Bellingham Bay and in the Fourmile Rock - Elliott Bay dump site vicinity, respectively.

4.3.2.2 Baseline Bays

Fish. Of the four baseline bays selected for this study, only Case Inlet had sufficient numbers of adult sole to serve as a baseline bay. The Eliza Island baseline station near Bellingham Bay was added to partially correct for this situation. Although the incidence of serious liver, kidney, and gill lesions in English sole from Case Inlet was low compared to incidences previously detected in sole from highly contaminated urban areas (e.g., the Duwamish Waterway), these lesions were not detected in sole from the Eliza Island station. It is currently not known whether the lesions observed in sole from Case Inlet were induced in situ or were in fish that migrated there from a contaminated area, such as Commencement Bay.

Shellfish. Most identified pathological conditions were found to be more prevalent in the baseline bays for both crab and shrimp species. These conditions are believed to have been the result of existing or previous parasitic infections.

In both shrimp species, proliferative conditions occurred in several tissues and in general were more prevalent in animals from Dabob Bay. The most probable cause of this condition would be parasitic infection. For example, in the hepatopancreas of P. dispar the higher incidence of proliferative conditions was accompanied by an identifiable parasitic infection. However, from the evidence available, proliferative conditions could not be attributed to any particular causative agent. In the case of shrimp, the general trend toward greater incidence of proliferative conditions in baseline areas would seem to rule out pollution in the etiology of this condition.

Parasitic/Infectious Disorders. Parasitic infections in crab and shrimp species were detected in both urban and baseline areas. The higher incidence of parasitic infections in crab and shrimp from baseline areas does not permit any conclusions as to whether crab and shrimp in urban area environments are more susceptible to parasitic infections.

4.3.3 Comparisons with Other Studies

4.3.3.1 Fish

Neoplastic and preneoplastic liver lesions in English sole were found for the first time in Case Inlet. Although the incidence of these lesions was low (both were 3.3%), previous studies conducted in this embayment during 1979 and 1980 (a total of 34 fish were examined) did not detect such lesions. It is not known whether finding these lesions during the present study reflects a change in the health of English sole in Case Inlet, or whether a small number of diseased sole were previously present, but not detected because of the relatively small sample size examined in past studies (Malins et al. 1982, 1984).

The absence of detectable serious liver lesions (e.g., neoplasms) in English sole captured near the mouth of Sinclair Inlet (Stations 17 and 18) is an observation that agrees with the results of previous sampling surveys conducted at that location. During eight separate fish collections performed between 1979 and 1983, no hepatic neoplasms or

specific degenerative lesions were detected in the 138 English sole that were examined (Rhodes et al. 1983). A low incidence (1.4%) of hepatic preneoplasms was found. However, at sampling stations near the upper end of Sinclair Inlet, in the general area of Station 6, a low incidence of hepatic neoplasms (0.8%, 1 of 122 fish) and preneoplasms (2.5%, 3 of 122 fish), but no specific degenerative lesions (Malins et al. 1982) were found. In the present study, all three types of lesions were found in English sole from Station 6 at incidences ranging from 6.7% (neoplasms) to 30% (preneoplasms). The results of this study and our previous work suggest that the incidence of serious hepatic lesions in English sole may be higher in the upper portion of Sinclair Inlet than the entrance.

Although efforts were made in the field to collect samples of comparable size, hence age, attaining this goal was not always possible. Of the English sole collected from the reference sites, 81% were of size class 1 or 2, which corresponds approximately to the 1+ or 2+ year classes. This situation was caused primarily by the sole collected at the Eliza Island site, which displayed little size variation in spite of an intensive fishing effort. On the other hand, 73% of the English sole collected in Sinclair Inlet belonged to size class 5, which corresponds approximately to the 5+ year class. Because the incidence of some neoplasia has been found to increase with age, care was taken to only conduct statistical comparisons within individual size (age) classes.

One type of kidney lesion, and depositional disorders, were found exclusively in Sinclair Inlet. Previous studies in Sinclair Inlet have identified both these lesions in English sole, but the incidences were not significantly higher than those at other Puget Sound stations (Malins et al. 1982).

Both English sole and Dover sole were examined at the three principal sampling stations at Fourmile Rock - Elliott Bay dump site vicinity. Even though only a total of 19 English sole were captured in the bay, a sufficient number (12) of fish were captured at Station 23 to compare with previous studies. Two sets of sampling stations near Station 23 have previously been sampled, one station near Magnolia Bluff (NODC No. 10014,

47° 37' 07" N X 122° 24' 04" W, 15 to 60 m in depth), and a set of stations referred to as the "Seattle waterfront" stations (Malins et al. 1984). Station 23 resembled the Magnolia Bluff station in terms of hepatic neoplasms (none were detected at either station) and preneoplasms (0% and 1.1%, respectively), but Station 23 was more similar to the Seattle waterfront in terms of specific degenerative lesions (25.0% and 19.9%, respectively).

Hepatic neoplasms were not detected in Dover sole from Elliott Bay; however, preneoplastic lesions and specific degenerative lesions of the liver were found. Preneoplastic lesions were found only in Dover sole (7.7%, 2 of 30 fish) from Station 17 near the Fourmile Rock - Elliott Bay dump site, whereas the degenerative lesions were found only in sole (10%, 3 of 30 fish) from the West Point stations. In addition, two lesions of the gill, necrotic and proliferative conditions, were found only in Dover sole from this latter station.

Finding important hepatic and gill lesions, which have been shown in English sole to be pollution-related (Malins et al. 1984), in Dover sole from selected stations at the Fourmile Rock - Elliott Bay dump site vicinity suggests that this species may be adversely affected by environmental contaminants. However, the low incidence of some of these lesions, the lack of reference Dover sole, and the lack of information on the geographical mobility of Dover sole in the sampling area, limit our ability to further interpret these data.

4.3.3.2 Shellfish

Only two conditions detected in the present study appeared to be associated with urban bays: hydropic degeneration/membrane lysis in the hepatopancreas of *C. magister* and degenerative disorders in the antennal gland of both shrimp species. These conditions were not associated with detectable parasitic infections. The incidences of hydropic degenerative/membrane lysis were highest (33% to 45%) in crab from the inner waterways of Bellingham Bay, considerably lower (11% to 14%) in Samish Bay, and not detected at Dungeness Spit.

The incidence of degenerative disorders in the antennal gland of shrimp was low, and these disorders were detected only in shrimp from the Fourmile Rock - Elliott Bay dump site vicinity. These lesions appeared to be relatively minor, and they would not be expected to seriously impair function in the affected shrimp.

Pathological conditions have been studied in a variety of Puget Sound shellfish including crab (Cancer spp.) and shrimp (Crangon alaskensis and Pandalus spp.) (Malins et al. 1982). A variety of histopathological lesions and parasitic infections were identified; however, the abnormalities were considered to be idiopathic. Necrotic and nodular lesions had relatively high incidences (in some cases 30% to 80%) in shrimp and crab collected from urban areas (e.g., Commencement Bay Waterways, Duwamish Waterway, and the Fourmile Rock - Elliott Bay dump site vicinity). The incidence of these lesions was generally lower in organisms collected from nonindustrialized areas.

4.4 AMPHIPOD BIOASSAY

4.4.1 Relationship to Physical and Chemical Properties of Sediments

Correlation analyses illustrated significant relationships between the amphipod bioassay and various physical and chemical properties of sediments. Of particular importance were the significant correlations ($p < 0.05$) of amphipod survival to sediment grain size, percent water, organic content, and burden of organic compounds.

Amphipod survival was positively correlated with sand content but negatively correlated with silt and clay content (percent fines) and organic content (percent volatiles and TOC). Of all the physical and chemical factors, amphipod survival was the most strongly correlated with water content of the sediment. Amphipod survival was not correlated with metals content but was negatively correlated with PCB and AH burdens. Why sediment burdens of metals (Ag, Hg, Pb) did not correlate with amphipod survival is not easily explained. Perhaps, the metals existed in physical-chemical forms that were not generally bioavailable to the amphipod.

Although we would like to conclude that lowered amphipod survival always correlated with high organic loads in the urban bays, we cannot. It is true that the urban bays demonstrated a higher sediment burden of organic contaminants, but this did not always result in lowered amphipod survival (e.g., Fourmile Rock - Elliott Bay dump site vicinity). It is also true that the baseline bays were found to be relatively clean of most organics, but this also did not result in higher amphipod survival (e.g., Case Inlet, Dabob Bay).

Although direct cause-and-effect relationships between survival and concentrations of organic compounds cannot be postulated, these data add credence to the potential for such relationships. Unfortunately, the state of the art of bioassay development does not allow cause-and-effect relationships between survival and sediment burden to be rigorously defined. However, the importance of physical exposure to contaminated sediments and the potential for ingestion of contaminants associated with sediments, in particular, appear to be excellent future research opportunities to elucidate mechanisms of toxic action.

4.4.2 Comparison of Amphipod Survival in Urban and Baseline Bays

Based on amphipod survival data, the urban bays were not always distinguishable from the baseline bays. While Port Gardner - Everett Harbor, an urban bay, exhibited the lowest mean survival of 12.3, the Fourmile Rock - Elliott Bay dump site vicinity, another urban area, exhibited the highest mean survival of 17.3. Similarly, while Sequim Bay, a baseline bay, exhibited a mean survival of 17.0, Case Inlet, another baseline bay, exhibited the very low mean survival of 13.0. Accordingly, these data would suggest that the baseline or "reference" bay concept is not as clear-cut as hoped.

The low level of amphipod survival found in Case Inlet deserves further discussion. Although unexpected, several findings indicate that the apparent toxicity of Case Inlet sediment is real. First, low mean survival was detected in two separate surveys ($\bar{x}$ = 13.6 in the 1983 and 13.0 in 1984). Second, the bioassays were performed by a competent

laboratory, and no significant "batch" effects were observed. Third, the control survival for Case Inlet was 19.0, definitely an acceptable level. Fourth, the physical sediment characteristics in Case Inlet samples were well within the tolerance limits of the test species, Rhepoxynius abronius, as determined by Swartz et al. (1984) for percent gravel, percent sand, percent silt, percent clay, total volatile solids, and interstitial water salinity. Some low Eh readings were recorded in the bioassay beakers, but anaerobic conditions probably did not contribute to the low survival because negative Eh beakers had a higher mean survival (16.0) than beakers with positive Eh (10.8). Fifth, low amphipod survival corresponded to low infaunal species richness and abundance in Case Inlet.

However, lowered amphipod survival in Case Inlet may still be the result of a purely physical response; in this instance, exposure to a sediment with > 90% silt and clay content. Swartz et al. (1984) presented particle size limits for individual particle size categories (gravel, sand, silt, clay). Perhaps the total percentage of fine-grained sediment (percent silt and clay) should be considered. Applying this convention, the finest sediment tested by Swartz et al. (1984) was 90% silt-clay. Many of the Case Inlet stations (4-10) displaying lower survival during screening surveys had silt-clay percentages ranging from 97 to 99. In Samish Bay during screening surveys, lowered amphipod survival may also have resulted from exposure to fine-grained sediments. The three stations (9, 10, 18) showing the lowest amphipod survival all had silt-clay contents exceeding 90%. As well, this relationship may explain the finding of lowered amphipod survival at two stations in Dabob Bay.

It may also be that lowered amphipod survival in both Case Inlet and Samish Bay resulted from multiple factors: high silt and clay content, high water content, and slightly elevated metals and organics when compared to the other baseline bays. Although we have already discussed the relationship of amphipod survival to percent fines, germane to this discussion is the observation that 29 of 37 baseline bay stations showing significantly lowered amphipod survival had sediment water contents

exceeding 50%. Also, based again on the screening surveys, both Case Inlet and Samish Bay showed generally higher metals levels and organic (IR) burdens (Appendix B) when compared with the other baseline bays. The interpretation is that the level of contamination needed to produce toxicity in amphipods is somehow less in fine-grained sediments with higher water contents than that needed to produce the same level of toxicity in coarse-grained sediments holding less water. Obviously, more research on how sediment types influence the toxicity of contaminants to amphipods appears in order and would help address the question of whether or not sediment quality criteria need to be established for different sediment types.

4.4.3 Comparisons to Other Studies

The amphipod assay has been applied sparingly in Puget Sound and in other water bodies. Accordingly, few studies exist which can be rigorously compared with present findings.

Swartz et al. (1979) conducted sediment bioassays on five different test organisms including the amphipod, *Rhepoxynius abronius*, at sites in the Duwamish Waterway and adjacent sites in Elliott Bay and Puget Sound. Although *R. abronius* experienced significantly higher mortalities at three of five sediment exposures relative to controls, none of these locations corresponded to those used in the present studies. Swartz et al. (1982) also conducted 175 bioassays on Commencement Bay sediments using *R. abronius*. They determined that while sediments from central Commencement Bay, including the two designated disposal sites, were not acutely toxic, areas of both high and low toxicity were detected in the waterways and other nearshore areas of Commencement Bay. Chapman et al. (1982), under NOAA sponsorship, conducted a comprehensive sediment bioassay program at 97 sites in Elliott Bay, Commencement Bay, Sinclair Inlet, Port Madison, and Birch Bay. While *R. abronius* among other test organisms was used, the test procedures called for exposures to water and sediment slurries. In contrast to Swartz et al. (1982), and to the present studies, Chapman et al. (1982) showed essentially no acutely

lethal conditions at the sites surveyed. The only exception was when sediments from a site near the Denny Way (Elliott Bay) combined sewer outflow were assayed. Ott[1] also conducted amphipod bioassays of Puget Sound sediments. Both R. abronius and Eohaustorius washingtonianus were exposed directly for 240 h to 17 test sediments in a recirculating seawater system. Although there also occurred a spatial heterogeneity in the toxic response within localized areas, sediments from four sites (one from Elliott Bay, two from the Duwamish Waterway and one from Sinclair Inlet) resulted in significantly lower survival than the control sediment stations. More recently, Chapman et al. (1984b) surveyed sediments from 22 stations in Everett Harbor, Bellingham Bay, and Samish Bay using the amphipod bioassay. Two stations (one each in Bellingham Bay and Everett Harbor) demonstrated significant acute lethal effects, while the two Samish Bay reference stations showed no significant effects.

It should also be noted that in the present study, all sediments were assayed fresh (unfrozen). Some of the previous studies (Chapman et al., 1982, 1984a) were conducted with frozen sediments which may have altered both bioavailability and toxicity. Contaminants may have been partitioned and toxicity seriously underestimated. This problem has been discussed recently by Lee and Jones (1982) in relation to dredged material evaluations. This and other evidence (Stober et al. 1983) suggest that there is no substitute for performing either chemical or biological analyses on fresh sediment samples.

4.5 OYSTER LARVAL BIOASSAY

4.5.1 Relationship to Physical and Chemical Properties of Sediments

Although we would like to state that a higher percentage of abnormal larvae always correlated with high metals IR, TOC, or specific organics in the urban bays, we cannot. The urban bays did demonstrate a higher sediment burden of metals and organic chemicals, but this burden did not always result in a higher percentage of abnormal larvae. The baseline

[1] Personal communication, 1982. Unpublished information from a study with P.D. Plesha, R.D. Bates, C. Smith, and B.B. McCain, "An Evaluation of an Amphipod Sediment Bioassay Using Sediment from Puget Sound."

bays were found to be relatively clean of metals and chemicals, but did not always have a lower percentage of abnormal larvae. Obviously, both fine-grained and high-water-content sediments are somehow involved. However, we cannot deduce that fine-grained and high-water-content sediments per se produce increased percentages of abnormal larvae. Rather, several factors likely account for the degree of variance measured, and grain size and percent water are but two of the factors. This situation does not rule out the involvement of sediment chemistry in some way. However, because bays were sometimes tested in different assays and at different times, more rigorous comparisons are confounded by batch and time effects.

4.5.2 Comparison of Oyster Larval Abnormalities in Urban and Baseline Bays

Based on percent abnormality in oyster larvae, the urban bays were not always distinct from the baseline bays. Although a majority of stations in Bellingham Bay and Sinclair Inlet exhibited significantly higher mean percentages of abnormal larvae when compared with controls, the Fourmile Rock - Elliott Bay dump site vicinity, another urbanized area, exhibited no such differences. Similarly, although Sequim Bay, Samish Bay, and Case Inlet, all baseline bays, exhibited no differences among mean percentages of abnormal larvae when compared to controls, Dabob Bay, another baseline bay, did exhibit a higher mean percentage of abnormal oyster larvae when compared to controls. Accordingly, these data also suggest that the baseline or "reference" bay concept may not always be as helpful as first believed.

4.5.3 Comparisons to Other Studies

The oyster larval bioassay has not been routinely applied to the assessment of sediment toxicity. Accordingly, as in the case of the amphipod bioassay, relatively few studies exist that can be compared with the present findings.

Chapman and Morgan (1983) tested sediments collected from 22 stations in Puget Sound, including five stations in Elliott Bay and two stations in Sinclair Inlet. Although the station locations at Fourmile Rock - Elliott Bay and Sinclair Inlet in the two surveys did not match exactly, they were, nevertheless, in the same general vicinity. The two surveys were in general agreement that Sinclair Inlet stations are more toxic than Fourmile Rock - Elliott Bay stations. More recently, Chapman et al. (1984b) conducted oyster larval bioassays on sediments from 22 stations in Everett Harbor, Bellingham Bay, and Samish Bay. A total of 19 stations in the two urban-industrialized bays demonstrated significant abnormalities and mortalities, while the two reference stations in Samish Bay showed no significant effects. Again while station locations in the two surveys did not exactly match, they were, however, in the same general vicinity. Accordingly, we would expect that the two surveys would be in general agreement that both Bellingham Bay and Everett Harbor stations were more toxic than the reference stations in Samish Bay. The fact that we failed to detect toxicity at Port Gardner - Everett Harbor, particularly in the East Waterway, is not easily explained. One possible explanation is that relatively high variability and poor performance in control cultures in Assay No. 3 did not allow for detection of statistical differences among treatments.

4.6 SUMMARY STATISTICS

The scope of the present study did not provide for recommendations as to which remedial actions were required in the most impacted bays, but it did provide for the identification of those bays that now show signs of degraded sediment quality (Table 60). This exercise could help determine which bay to concentrate further research, mitigation, and/or clean-up efforts. These data, when taken in concert, demonstrated that the urban bays were significantly more impacted than the baseline bays. These data also indicated that the greatest impacts were found in Port Gardner - Everett Harbor, Sinclair Inlet, and Bellingham Bay. The Fourmile Rock - Elliot Bay dump site vicinity appeared to be the least impacted of the

urban-industrialized sampling areas. As well, and not surprisingly, Sequim Bay was the least impacted of baseline bays. However, it was easily seen from these data that Case Inlet and Samish Bay showed some indication of degrading sediment quality. All stations in each bay resulted in lower than mean control amphipod survival. The data for Dabob Bay also suggest some degree of impact.

Inspection of the data in Table 60 shows that the most adversely impacted stations in Port Gardner - Everett Harbor were located in the East Waterway. For Sinclair Inlet, they were close to the Puget Sound Naval Shipyard. In Bellingham Bay, they were associated with the Whatcom Creek and I and J Street waterways.

Although the fish and shellfish pathology data were not used in this summary statistical exercise, (see Section 2.8.5) they would serve to corroborate our results: English sole caught in Sinclair Inlet demonstrated the highest incidences of liver, kidney, and gill lesions. Also, two types of lesions were found only in animals from urban areas. Hydropic degeneration/membrane lysis in the hepatopancreas of Dungeness crab and degenerative disorders in the antennal gland of both shrimp species were observed in Bellingham Bay and in the Fourmile Rock - Elliott Bay dump site vicinity.

Finding a biological signal (significantly lowered amphipod survival) in Case Inlet and Samish Bay is not easily explained. The results of sediment chemistry analyses are not as clear-cut as those for Sinclair Inlet and Port Gardner - Everett Harbor or Bellingham Bay. The slightly elevated silver concentrations in both Screening and Detailed Surveys in Case Inlet could indicate the presence of sewage contamination. Particulate contaminants could enter Case Inlet through transport processes from the central basin of Puget Sound, or runoff from septic tank drain fields, and animal pastures. Certain base-neutral organic compounds found in Samish Bay during the detailed surveys perhaps suggest transport of industrial wastes from Bellingham Bay. More disturbing is that specific locations sampled in Case Inlet and Samish Bay were selected as generally the cleaner of the stations. Accordingly, the problem in

these embayments could be much worse. Whatever the explanation, much additional research must be conducted.

4.7 RELATIONSHIP OF URBAN TO BASELINE (REFERENCE) BAY CONCEPT

It would seem easy to describe baseline or "reference bay" conditions based on chemical variables. In this study, clear differences occurred in urban versus baseline bays as to concentrations of metals, PCB-1254, and aromatic hydrocarbons. Sequim Bay and Dabob Bay, with relatively low metals and no detectable organic compounds, appear to be excellent choices for reference bays.

However, because all marine waters of Puget Sound and the Strait of Juan de Fuca interconnect, contamination from one bay will result in some degree of contamination to all bays. This fact and the finding of low levels of chemical contaminants in some of the baseline bays (Samish Bay, Case Inlet) in this study would suggest that the concept of "reference bay" is not as clear-cut as first believed, and that perhaps what we have observed is evidence of a continuum or gradient of contaminants extending from the urban bays.

On the basis of biological variables, the concept of baseline or "reference bay" is also less clear, and perhaps is better described as representing a continuum of responses consistent with a gradient of contaminants existing between regions of higher and lower contamination in Puget Sound. The results of both the amphipod and oyster larval bioassays tend to best support this interpretation. Although Port Gardner-Everett Harbor, Bellingham Bay, and Sinclair Inlet, all urban bays, exhibited generally lowered amphipod survival and/or higher oyster larval abnormality, the Fourmile Rock-Elliott Bay dump site vicinity, another urban area, exhibited the highest mean amphipod survival and no difference

in percentage of oyster larval abnormality when compared with controls. Similarly, Case Inlet, Samish Bay, and Dabob Bay, all baseline bays, showed either a relatively low amphipod survival or a higher than control percentage of abnormal oyster larvae at no less than half of the stations tested. The data could be interpreted as meaning: 1. Port Gardner - Everett Harbor, Bellingham Bay and Sinclair Inlet are all typically urban bays that in Puget Sound are sources of chemical contamination. They occupy one end of the continuum. 2. Sequim Bay is typically a baseline bay located at the opposite end of the continuum far from urban sources of contamination, 3. Fourmile Rock - Elliott Bay is intermediate on the continuum located relatively close to an urban source of contamination - Elliott Bay. However, clearly the dump site is unique because not one of the eight stations in the present study indicated degraded sediment quality when evaluated by either the amphipod or oyster larval bioassay. 4. Case Inlet and Samish Bay are also located intermediate on the continuum but relatively far from urban sources of contamination and closer to Sequim Bay conditions. However, both are close enough to Commencement Bay and Bellingham Bay respectively to still show some degraded sediments. 5) Dabob Bay is also located intermediate on the continuum but closest to the Sequim Bay positon, that is, relatively far from most urban sources of contamination. The finding of some suggestion of degraded sediment quality in Dabob Bay is perhaps attributed to circulation of contaminants from the main basin of Puget Sound.

The infaunal data also suggest that the concept of baseline or reference bay is less clear-cut. In particular, the differences in physical characteristics (depth, grain size) among urban and baseline bays greatly influenced indigenous infaunal communities (species richness and abundance), and necessarily led to comparisons within sediment type. Actually, the bays differed in their depth, sediment type, etc., with as much variation within the urban and baseline categories as among all bays.

It was also evident that the influence of sediment chemistry on infauna was difficult to discern. Against the variation in infaunal indices attributed to physical factors, the influence of only one chemical variable, TOC, was consistently observed. In all sediment types, high TOC

4.0 to 16% was associated with a disturbed infauna dominated by organic enrichment opportunist species. This shift to enrichment opportunists occurred at almost all stations in Port Gardner - Everett Harbor and at several stations in Bellingham Bay. The extent of the shift was less in sandy sediments when compared with silty and muddy sediments. In addition, stations with the most impoverished infauna occurred both in urban and baseline bays and were associated with greater depth and higher clay content rather than with higher concentrations of contaminants.

5.0 CONCLUSIONS

5.1 BATHYMETRY AND SEDIMENT CHARACTERISTICS

The eight bays demonstrated different depth and sediment characteristics. The Fourmile Rock - Elliott Bay dump site vicinity and Dabob Bay were distinctly deeper (>100 m) and contained sediments which were, on the average, sandier and had lower total organic carbon (TOC) contents than all the rest. Samish and Bellingham Bays were the shallowest and the muddiest of the eight bays. Bellingham Bay showed the second highest TOC level. Case Inlet, Sinclair Inlet, and Sequim Bay were of intermediate depth, and had intermediate values for percentage of silt, clays, and TOC. These three bays exhibited sediment types ranging from sandy-silt to silty-mud. Port Gardner - Everett Harbor was distinct from the other bays in its significantly higher mean TOC level.

5.2 CONTAMINANT LEVELS

Urban bay sediments, particularly in Sinclair Inlet and at the Fourmile Rock - Elliott Bay dump site vicinity, were found to be substantially contaminated by Ag, As, Cu, Cd, Hg, Pb, and Zn. Other metals (Sb, Be, Cr, Ni, Se, and Ti) were found at about the same concentration in all bays; however, a few urban sediments in Bellingham Bay and Sinclair Inlet were enriched in Sb, Cr, and Ni. Baseline bays generally contained lower concentrations of metals, many of which were found at approximately the same concentrations as in pre-1900 sediments.

Urban bay sediments, particularly Sinclair Inlet and Port Gardner - Everett Harbor, also contained significant quantities of aromatic hydrocarbons and PCB-1254. Other organic compounds occasionally found in urban bays included phthalates and PCB-1260. However, no PCBs were detected in baseline bays, and only a few stations in Samish Bay contained detectable quantities of phthalates and aromatic hydrocarbons.

5.3 BENTHIC INFAUNA

It was determined that in all sediment types, TOC levels between 4 and 16% were correlated with a disturbed infauna dominated by organic enrichment opportunists. This shift to enrichment opportunists, primarily nematodes and *Capitella capitata*, occurred at almost all stations in Port Gardner - Everett Harbor and at several stations in Bellingham Bay. The extent of this shift was less in sandy sediments when compared to silty or mud sediments.

After omitting stations with high values of TOC, the numerically dominant infauna were shown to vary more with sediment type than by bay, except that Case Inlet and Samish Bay demonstrated distinctly different infaunas from all other bays. Also, omitting these same stations did not reveal a distinctly different set of numerically dominant species in urban bays when compared with baseline bays.

5.4 FISH AND SHELLFISH PATHOLOGY

Because flatfish species were not collected in four of the eight bays (Bellingham Bay, Samish Bay, Dabob Bay, Sequim Bay) because of seasonal scarcity, the resulting pathology data were not used in the summary statistical exercise (Section 2.8.5) to determine signs of degraded sediment quality. However, these data did serve to corroborate the results of the approaches used in the summary exercise (chemistry, infaunal analyses, amphipod and oyster larval bioassay), indicating that urban bays were generally more impacted than baseline bays.

English sole caught in Sinclair Inlet demonstrated the highest incidence of liver, kidney, and gill lesions. Also, two types of lesions in shellfish were only found in animals from urban bays. Hydropic degeneration/membrane lysis in the hepatopancreas of Dungeness crab and degenerative disorders in the antennal gland of two shrimp species were observed in Bellingham Bay and at the Fourmile Rock - Elliott Bay dump

site vicinity. And, although the incidence of serious liver, kidney, and gill lesions in English sole from Case Inlet were low compared to the incidence detected in sole from other urban bays (Duwamish Waterway), these lesions were not detected in sole from Eliza Island.

5.5 AMPHIPOD SURVIVAL

Based on the amphipod survival data alone, the urban bays were not always distinguishable from baseline bays. The Fourmile Rock - Elliott Bay dump site vicinity, selected as representative of urban bay conditions, and Sequim Bay, a baseline bay, demonstrated the highest and second highest mean survivals of 17.3 and 17.0 respectively. Similarly, Case Inlet, a baseline bay, and Port Gardner - Everett Harbor, an urban bay, demonstrated the second lowest and lowest mean survivals of only 13.0 and 12.3, respectively.

Correlation analyses illustrated significant relationships between the amphipod bioassay and various physical and chemical properties of sediments. Of particular importance were how amphipod survival related to sediment grain size, percent water, and burden of organic compounds (PCB-1254, aromatic hydrocarbons, and IR). All such properties were interpreted as accounting for lower amphipod survival. These analyses also revealed that lowered amphipod survival occurred in sediments of Port Gardner - Everett Harbor where an increased abundance of organic enrichment infaunal species was encountered.

5.6 OYSTER LARVAL BIOASSAY

Results of the oyster larval bioassay generally paralleled those of amphipod bioassays. The analyses, when taken alone, indicated that the urban bays were not always distinguished from those of baseline bays. Although a majority of stations in Bellingham Bay and Sinclair Inlet, both urban bays, exhibited significantly higher mean percentages of abnormal larvae when compared with controls, the Fourmile Rock - Elliott Bay dump site vicinity (also considered representative of urban bay conditions)

exhibited no differences when compared with controls. Similarly, although Sequim Bay, Samish Bay, and Case Inlet, all baseline bays, exhibited no differences among mean percentages of abnormal larvae when compared with controls, Dabob Bay, another baseline bay, exhibited higher mean percentages of abnormal larvae at two of its four stations.

Correlation analyses showed a significant relationship between percent abnormal oyster larvae and amphipod survival. The trend to higher percentages of abnormal oyster larvae becomes more evident as amphipod survival decreased.

5.7 SUMMARY STATISTICS

On the basis of the summary exercise which evaluated all data (chemical and biological), urban bays were significantly more impacted than baseline bays. The greatest impacts were found in Port Gardner - Everett Harbor, Sinclair Inlet, and Bellingham Bay. The Fourmile Rock - Elliott Bay dump site vicinity appeared to be the least impacted of the urban-industrialized sampling areas. Not surprisingly, Sequim Bay was the least impacted of the baseline bays. However, Case Inlet and Samish Bay, both baseline bays, showed some indication of degraded sediment quality. All sediments in both bays resulted in lower than control amphipod survival. The amphipod and oyster larvae bioassay data from Dabob Bay also suggested some degree of impact.

It was also observed that the most adversely impacted stations in Port Gardner - Everett Harbor were located in the East Waterway. For Sinclair Inlet, they were located near the Puget Sound Naval Shipyard. In Bellingham Bay, they were associated with the inner harbor. Case Inlet demonstrated the second lowest species richness, the lowest infaunal abundance, and the lowest amphipod survival of all eight bays. Samish Bay was found to contain phthalates and aromatic hydrocarbons and demonstrated the third lowest amphipod survival of all eight bays.

Clearly, the strength of this exercise was in the performance of several analyses, both chemical and biological, on the same sediment

sample. The development of a broad based (summary) index of degraded sediment quality also facilitated detection of a wider range of sediment toxicity that would not have been possible if a single analysis or bioassay had been applied.

5.8 RELATIONSHIP OF URBAN TO BASELINE (REFERENCE) BAY CONCEPT

On the basis of chemical contaminants alone, the baseline or "reference bay" concept appears to be valid. Sequim Bay and Dabob Bay, with relatively low metals burdens and no detectable organic compounds, seem to be excellent choices for reference bays. Application of this concept is potentially useful in Puget Sound in a regulatory sense to determine where and when remedial action is required.

Biological variables, however, do not always clearly distinguish between what we hypothesized at the outset of the study to be urban bays and baseline bays. Rather, the biological findings better describe a continuum of responses consistent with a gradient of contaminants existing between regions of higher and lower contamination in Puget Sound.

This continuum is perhaps best described as follows: 1) Port Gardner - Everett Harbor, Bellingham Bay, and Sinclair Inlet occupy one end of the continuum representing water bodies now showing the most evidence of degraded sediment quality. 2) Sequim Bay is a baseline bay located at the opposite end of the continuum. It currently shows the least indication of degraded sediment quality of all the bays sampled. 3) The Fourmile Rock - Elliott Bay dump site vicinity is located intermediate on the continuum, but closer to the position occupied by the urban bays. However, the dump site is somewhat anomalous because not one of the eight stations sampled during the detailed surveys showed any signs of degraded sediment quality when evaluated by either the amphipod or oyster larval bioassay. 4) Case Inlet and Samish Bay are also located intermediate on the continuum but closer to the baseline bay position occupied by Sequim Bay.

Both bays, however, are close enough to Commencement Bay and Bellingham Bay, respectively, to show some signs of degraded sediments. 5) Dabob Bay is also located intermediate on the continuum, but closest to the baseline bay position occupied by Sequim Bay. The finding of degraded sediment quality based again on the bioassay results is perhaps attributed to circulation of contaminants from the main basin of Puget Sound.

In summary, the results of our study support the "reference bay" concept; however, our results also suggest that the process to select reference bays needs refinement. It is evident that selection should be based on chemical, biological, and physical data. Also, the 1983 screening survey was particulary useful in providing relevant and recent data on which to base the selection process. Ideally, a reference bay should contain little or no chemical contamination, demonstrate few or no impacts when evaluated by infaunal analyses and sediment bioassays, but should also possess physical characteristics (depth and grain size) that closely approximate the urban bay(s) to be examined. Our results perhaps suggest that "reference stations" may be a more appropriate concept than "reference bays", particularly when time and funds limit the scope of studies performed in support of regulatory action.

There is a need to bring together a forum of scientists and regulators to refine this concept and its intended use. Also, our strategy of selecting equal numbers of the most contaminated stations within each urban bay does not address issues concerning the full range of contaminated sediments within each bay. Clearly, the present study would have benefitted from the addition of stations both in the urban and baseline bays. Consequently, our results apply only to the sediments (stations) sampled in each bay and do not likely represent all similiar depositional sediments in each bay.

5.9 USEFULNESS OF THE RECONNAISSANCE SURVEY CONCEPT

The 1983 screening survey of 181 stations from eight bays was particularly useful in appropriately focusing the 1984 detailed survey. The strategy of selecting the most contaminated stations from the urban bays and the least contaminated stations from the baseline bays permitted limited resources to be directed toward identifying those stations most likely to reveal degraded sediment quality. Moreover, the screening survey data allowed the station selection process for the detailed surveys to be based on data that was recently acquired rather than on historical data that was collected in support of other goals. The variability encountered in biological indices subsequently revealed the wisdom of the selection strategy applied. Another selection strategy would have likely provided less clear-cut biological findings. Consequently, we have learned that sampling strategies must be developed specifically for each study; and if faced with another large sediment survey, we would again recommend that a preliminary or reconnaissance level effort be undertaken to determine which sampling strategy best addressed the study goals within available resources.

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