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CHEMICAL CONTAMINANTS AND ABNORMALITIES IN FISH AND INVERTEBRATES FROM PUGET SOUND

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PREFACE

This report was prepared for the MESA Puget Sound Project, Office of Marine Pollution Assessment, National Oceanic and Atmospheric Administration in Seattle. The overall goal of the Project is to develop an understanding of the existing levels, fates, and effects of contaminants in Puget Sound. The Project supports and coordinates many individual studies conducted by private, academic and government groups. This report was prepared by scientists of the Northwest and Alaska Fisheries Center for the Project. It summarizes information on chemical concentrations and biological indices of contaminant conditions gathered during studies performed from 1978 to 1981. The Project technical monitor for these studies was Edward Long.

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EXECUTIVE SUMMARY

Beginning in 1978 and continuing through the spring of 1981, samples of sediment and selected bottom-dwelling fish and invertebrates were collected at regular intervals from urban embayments and from nonurban (reference) areas in Puget Sound and adjacent waters. Sediment and tissue samples were analyzed for a large number of organic and inorganic chemicals, including aromatic hydrocarbons (AHs), polychlorinated biphenyls (PCBs), chlorinated pesticides, other chlorinated organic compounds, and metals. In most cases, the same animals from which tissues were taken for chemical analyses were also examined for grossly visible and microscopic abnormalities. In addition, the community characteristics (i.e., abundance and species diversity) of the sediment-associated invertebrates and fish were defined.

The purpose of this report is to summarize data collected from 1978 through 1981. A substantial portion of these data was previously reported by Malins et al. (1980, 1981), and is also available from the NOAA National Oceanographic Data Center. The present report includes major results reported previously, as well as subsequently obtained data.

Seventy-three sediment and 148 biota samples have been chemically analyzed since 1978. AHs, PCBs, and chlorinated butadienes (CBDs) were widely distributed in Puget Sound sediment; however, the concentrations varied extensively both among and within embayments. The sediments in embayments adjacent to the most populated areas, Elliott Bay and Commencement Bay, contained the highest concentrations of AHs and PCBs; CBDs were highest in Commencement Bay.

More than 500 AHs were revealed in a chromatogram of one urban-associated sediment sample (Hylebos Waterway, Commencement Bay). Mean concentrations of 27 AHs selected as target compounds, and reported as the sum of their individual concentrations, in sediments from urban areas were as much as 30 times higher (e.g., up to 63,000 parts per billion dry weight, in Elliott Bay) than the mean concentrations of these compounds in sediments from reference areas.

Scores of halogenated compounds in addition to PCBs and hexachlorobenzene (HCB) were associated with certain embayments. This was especially evident for sediments collected near Tacoma, Port Angeles, Bellingham, and Everett. Identities of these halogenated compounds have not been confirmed because reference mass spectra have not been published, and many compounds remain unidentified because the mixtures are so complex that mass spectra could not be interpreted.

Arsenic and mercury were detected only in sediment from urban areas, whereas lead was high in both urban (ca. 130,000 ppb, dry weight) and nonurban (ca. 15,000 ppb, dry weight) areas.

Worms, clams, shrimp, and crabs from urban embayments contained levels of AHs that were higher than detected levels in these animals from nonurban areas (e.g., up to 17,000 ppb, dry weight, in animals from urban areas and up to 1,500 ppb, dry weight, in animals from nonurban areas). Concentrations of selected AHs in fish livers from all areas were generally low (less than 1,000 ppb, dry weight). Concentrations of AHs from virtually all biota samples were lower than the concentrations of AHs in sediment from areas from which the animals were captured, and only a small number of the AHs found in sediment were detected in biota.

Concentrations of PCBs and other halogenated compounds were also generally higher in biota from urban than from nonurban areas. The highest PCB concentrations (up to 35,000 ppb, dry weight) were found in crab hepatopancreas and in sole liver. PCB concentrations in edible tissue (muscle) were low (70-150 ppb, wet weight) in salmon and cod from both urban and nonurban areas. Concentrations of PCBs in livers of salmon from both types of areas were also low (41-190 ppb, dry weight). The concentrations of PCBs in liver of English sole caught from urban areas were as much as 15 times higher than those from nonurban areas (24,000 ppb, wet weight, compared to 1,600 ppb, wet weight).

CBDs were found in biota from urban areas, but, with a few exceptions, were not detected in biota from the nonurban areas. Livers of sole from the Hylebos Waterway had the highest concentrations of CBDs detected (up to 9100 ppb, dry weight). Hexachlorobutadiene, a carcinogenic CBD, was detected in liver and muscle of English sole, salmon, and cod from Elliott Bay, Commencement Bay, and from a nonurban (reference) area. The highest concentrations were found in the liver and muscle of English sole from the Hylebos Waterway (120 to 570 ppb and 2 to 110 ppb, wet weight, respectively). Less than 1 ppb (wet weight) was detected in the muscle of salmon and cod from all three areas.

DDT was present in livers of English sole from urban areas at mean concentrations 6.6 times those in nonurban areas (60 vs. 9.1 ppb, wet weight). In contrast, cod livers from urban and nonurban areas had similar mean DDT levels (ca. 20 ppb, wet weight). Lower concentrations of DDT (less than 6 ppb, wet weight) were found in salmon liver and in cod, sole, and salmon muscle.

Muscle of English sole, cod, and salmon from both urban and nonurban areas contained low concentrations of lead and mercury (less than 200 ppb, wet weight). Arsenic (up to ca. 5,800 ppb, wet weight) was found in sole muscle from both urban (Duwamish and Hylebos Waterways) and nonurban (Port Susan) areas, and in cod (2,700 ppb, wet weight) and salmon muscle (700 ppb, wet weight) from Elliott Bay, Commencement Bay, and Point Jefferson.

Bottom fish, crabs, and shrimp examined during this study had a variety of pathological conditions. The most commonly observed lesions were either associated with infectious agents (parasites or micoorganisms) or they were caused by unknown factors (idiopathic lesions). Some of the idiopathic lesions were found only, or were most prevalent, in fish from the urban embayments. In English sole, the fish species most widely distributed throughout Puget Sound, these urban-associated lesions included liver neoplasms, and "preneoplastic" and necrotic liver lesions. English sole with liver neoplasms and "preneoplastic" lesions were most prevalent in Seattle's Duwamish Waterway [8% and 12%, respectively (535 fish were examined)] and in Tacoma's Waterways [4% and 9%, respectively (573 fish were examined)]. English sole with specific necrotic lesions of the liver were most prevalent in the Duwamish Waterway (18%, 535 fish were examined) and along Seattle's Waterfront (20%, 161 fish were examined). Fish with the above-mentioned lesions, as well as other types of lesions had abnormal changes in blood cell counts and in the concentrations of serum components. Some of these changes were indicative of severe organ dysfunction.

Statistical methods were used to evaluate possible relationships between the prevalence of English sole with the above-mentioned liver lesions and the chemical composition of the sediment in the areas from which the affected fish were captured. In one method, the sampling stations were arranged into eight groups on the basis of cluster group analysis. The highest prevalences of English sole with these lesions were found at stations in two cluster groups. These groups were characterized as having high concentrations of sediment-associated metals and AHs. This apparent association between the prevalence of these liver lesions and the sediment concentrations of the two classes of chemicals was supported by the results of another statistial test, the Spearman rank correlation. The prevalence of English sole with liver neoplasms and specific necrotic lesions was positively correlated (at a significance level of $p \leq 0.05$) with the relative sediment concentrations of AHs and metals, whereas the prevalence of this species with "preneoplastic" lesions was positively correlated only with aromatic hydrocarbons ($p \leq 0.01$).

The organs of shrimp and crabs most frequently found with histopathological lesions included the gill, hepatopancreas, midgut, and antennal gland. These lesions were not generally recognizable as externally visible abnormalities (except crab gills) and are considered to be idiopathic. Although shrimp and crabs with some of these lesions were commonly found in urban areas, the significance of these observations is not presently known due to the low numbers of animals captured and examined which precluded meaningful statistical analyses.

The abundance of fish, and the number of fish species were generally higher in the estuarine bays (Commencement and Elliott Bays) compared to the inlets and open bays. For sediment-dwelling invertebrates, the average highest species richness values were found in the reference areas (Port Madison 11.5 ± 2.9) and the outer portions of the estuarine

bays (West Point, 11.1±5.0). The lowest values were in sediments from the urban waterways (Hylebos Waterway, 3.0±1.8) and inner portions of the urban associated areas (Budd Inlet, 2.4±1.5).

Overall, the findings indicate that hundreds of potentially toxic chemicals are present in Puget Sound sediments from as far north as Bellingham Bay to as far south as Budd Inlet. Many of the chemicals are also found in a variety of benthic and pelagic organisms. The question of the threat they pose to these organisms or the consumer is not known at present and can only be determined through further research.

1. INTRODUCTION

Between the spring of 1978 and the spring of 1981, a study was conducted in Puget Sound, Washington, to determine the extent and nature of chemical pollution and to investigate adverse effects on fish and invertebrates. This study represents a cooperative effort between the Marine Ecosystems Analysis (MESA) Puget Sound Project, a part of the Office of Marine Pollution Assessment (OMPA), and the National Marine Fisheries Service (NMFS), both of the National Oceanic and Atmospheric Administration (NOAA). The MESA Puget Sound Project was established to focus scientific research on environmental problems relating to Puget Sound. The objective of the Project is to use an integrated program of multidisciplinary research to document the occurrence and fluxes of contaminants of special concern, the dynamic processes influencing their physical and chemical transport and fate, and their biological and ecological effects.

The scientific disciplines involved in this study included fish pathology, invertebrate pathology, fish ecology, invertebrate ecology, and analytical chemistry. Fish and invertebrate species were examined for gross and microscopic pathological conditions, and tissues from selected animals were subjected to chemical analyses. Chemical analyses were also performed on bottom sediments from each of the sampling stations.

The locations and types of sampling stations in the urban-associated and reference areas are displayed in Figures 1 and 2; the major urban-associated sampling areas were Seattle's Elliott Bay, Tacoma's Commencement Bay, Olympia's Budd Inlet, and Bremerton's Sinclair Inlet. The reference (or nonurban) areas were Case Inlet in south Puget Sound, Port Madison in central Puget Sound, Port Susan in north Puget Sound, and Discovery Bay just outside the entrance to Puget Sound. (In the interest of brevity, the terms "urban" and "nonurban" will be used to identify these areas.) The locations of the sampling stations in the urban areas were chosen, in part, on the basis of their proximity to point sources of pollutants, and from predictions of the probable distribution of biological anomalies. Chemical analyses of bottom sediments from these areas, previously performed by the NOAA National Analytical Facility (NAF), demonstrated the presence of pollutants in these areas (Malins et al. 1980). Also, previous reports of pathologic conditions of flatfish in Puget Sound identified some locations as having substantial numbers of abnormal animals (Pierce et al. 1978). Another criterion for choosing the location of sampling stations was the type of sediment, with a preference being given to depositional areas (In Puget Sound, such areas are characterized by a high mud to sand ratio.) Depositional areas were selected because many industrial chemicals readily sorb to aquatic sediments (Wildish et al. 1980), and such areas should give an indication of levels of pollutants in the environment. Therefore, the majority of the sites were selected to reflect "worse-case" situations, and proportionally fewer sites were selected in dilution zones and reference areas.

During 1980, effects studies were initiated to determine whether or not selected species of invertebrates were adversely affected by exposure to chemically-contaminated bottom sediments. These studies were conducted under semicontrolled field conditions. In one type of experiment, cages containing molluscs or crabs were placed on bottom sediments at four urban sites and one reference site. In the other type, trays of sediment were placed on sediment at these same sites and recolonization by benthic invertebrates was measured.

The results of the first two years of the field survey were previously reported by Malins et al. (1980, 1981). The results of the entire project, including the previously reported data, are summarized in this report.

Background Information

The following classes of chemical pollutants were characterized in sediment and biota: Aromatic hydrocarbons (AHs), polychlorinated biphenyls (PCBs), other chlorinated organics, and metals.

Aromatic hydrocarbons are a class of organic compounds that contain one or more benzene rings. AHs occur in fossil fuels and are also formed through combustion processes (Clark and Brown 1977; Payne et al. 1979; Dunn and Stich 1976; and Bjorseth and Dennis 1979). AHs enter the marine environment from industrial and municipal waste disposal, atmospheric fallout, runoff, and the seepage and spillage of petroleum and petroleum-derived products. Sediments are a major repository of many AHs, primarily because the AHs become associated with suspended particles which then settle to the bottom. Sorption of AHs to sediment is more pronounced in seawater than in freshwater, because of the much higher ionic strength of seawater (Callahan et al. 1979).

The AHs are lipophilic and can accumulate in fish. For example, after one week, starry flounder (*Platichthys stellatus*) exposed to water-soluble hydrocarbons from Prudhoe Bay crude oil accumulated in muscle 10^4 times the concentration of C_4- and C_5-substituted benzenes present in the water column (Roubal et al. 1978). Such accumulated hydrocarbons are converted to metabolites in the fish and thereby may escape detection by chemical analysis (Roubal et al. 1978; Varanasi et al. 1979; Varanasi and Gmur 1981).

Polychlorinated biphenyls (PCBs) have been widely used in industry as heat exchanger, dielectric, hydraulic and lubricating fluids, as plasticizers, as ingredients for caulking compounds, and as flame retardants. The PCBs have been released in the environment for many years and have become virtually ubiquitious (EPA 1980; Wasserman et al. 1979). PCBs are slightly soluble in water, highly toxic, and resistant to metabolism. They substantially bioconcentrate in food chains (EPA 1980; Wasserman et al. 1979; Fishbein 1974); and bioconcentration factors as high as 10^6 have been reported for marine organisms (Callahan et al. 1979).

The chlorinated pesticide DDT was used extensively in the United States prior to 1972, but is presently restricted. Many such chlorinated pesticides and their metabolites are highly resistant to degradation in both soil and water. As with AHs and PCBs, the chlorinated pesticides are only slightly soluble in water and tend to be concentrated in sediments. These pesticides accumulate in fatty tissues of marine organisms, are generally resistant to metabolism, and are readily bioconcentrated (EPA 1980).

Hexachlorobenzene (HCB) has been used as an agricultural fungicide, for the synthesis of pentachlorophenol and chlorinated hydrocarbons, and as a plasticizer and flame retardant. HCB has not been produced commercially in the United States since 1976; however, it is still a by-product in the industrial preparation of chlorinated hydrocarbons and in the production of chlorine gas from sodium chloride. HCB is relatively stable in the environment and is bioaccumulated in marine organisms. A variety of toxic effects in animals are associated with the ingestion of this compound (EPA 1980).

Other chlorinated compounds found include the chlorinated butadienes (CBDs). One such compound, hexachlorobutadiene (HCBD), is toxic to aquatic organisms at less than 1 ppm, and is fetotoxic, neurotoxic, nephrotoxic, and carcinogenic (EPA 1980).

Certain heavy metals in trace amounts (e.g., iron, zinc, copper, and selenium) are essential for the health of organisms. Other heavy metals, e.g., mercury, appear to serve no essential biological function. Elevated concentrations of both essential and nonessential metals can have adverse effects on growth, reproduction, behavior, survival rate, and the overall health of marine organisms (Phillips and Russo 1978). The biological effects of metals in the marine environment are influenced by their oxidation states and the nature of complexes formed with organic and inorganic compounds. Moreover, the toxicity of metals is affected by environmental factors, such as pH, concentrations of other organic and inorganic compounds, and the metal's capability of being oxidized or reduced (Phillips and Russo 1978).

Sediments are a primary sink for most heavy metals in marine environments (Renfro 1973). Marine organisms accumulate metals through the ingestion of food or particulate matter and by diffusion through external membranes (i.e., gills and skin). Metals are excreted in fecal material, urine, and through membrane diffusion.

The Food and Drug Administration (FDA) has designated mercury, lead, cadmium, arsenic, selenium, and zinc as the most hazardous toxic metals in human food (Phillips and Russo 1978). Of these elements, the FDA has established regulatory limits in food only for mercury; however, guidelines established by the EPA to protect marine organisms state that the concentrations of cadmium, copper, selenium, and silver in marine water should not exceed at any time 59, 23, 410, and 2.3 μg/L, respectively.

2. METHODS AND MATERIALS

Because of the large numbers of fish and invertebrate species found in Puget Sound, it was necessary to select certain target species to be examined for pathobiological characteristics and/or chemical analysis. Target species were defined by one or more of the following criteria: (1) wide distribution throughout Puget Sound, (2) life history stages directly or indirectly associated with sediments, and (3) previously observed abnormalities. The target species are listed in Table 1. The animals examined included selected fish, crustacea (crabs and shrimp), and sediment-dwelling invertebrates (bivalves and polychaete worms).

2.1 Logistics and General Sampling Procedures

The sampling vessels were the R.V. Malka and the R.V. Harold W. Streeter in 1979, and the R.V. Streeter in 1980 and 1981. Demersal fish and epibenthic invertebrates were collected with an otter trawl with 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 tows were made for 5 minutes and covered a distance of approximately 386 m or 0.2 nautical miles. Blackmouth salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*) and Pacific cod (*Gadus macrocephalus*) were captured by hook and line from Commencement Bay, Elliott Bay, and Point Jefferson (near Port Madison). Sediment samples and benthic infauna were collected with a modified 0.1 m^2 Van Veen sediment grab (Word 1976). Temperature, salinity, and dissolved oxygen measurements were taken at or near the bottom at each sampling station during each sampling period. All samples of tissue and sediment were coded when collected, and logs were maintained to assure proper sample identification during sample processing.

2.2 Chemical Analyses

The methods used for chemical analyses were described in detail by Malins et al. (1980) and are summarized here. Unless otherwise stated, all concentrations are reported on a dry weight basis. Selected toxic organic chemicals in Table 2 and inorganic elements in Table 3 were routinely analyzed in sediment and biota. The organic chemicals listed include aromatic hydrocarbons (AHs), polychlorinated biphenyls (PCBs), chlorinated butadienes (CBDs), and several chlorinated pesticides. Sediment and biological samples were placed in solvent-cleaned glass containers and frozen until needed for chemical analysis. For the analysis of organic compounds, samples were extracted with methanol and dichloromethane and the extract was fractionated, using both silica gel and Sephadex LH-20 column chromatography (Brown et al. 1980; Ramos and Prohaska 1981). The desired fractions were concentrated to

about 1 mL and analyzed for the target aromatic and chlorinated compounds (Table 2) by capillary column gas chromatography. Hewlett-Packard 5840 gas chromatographs, modified as described by Ramos et al. (1979), were equipped with capillary columns (ca. 30-m x 0.25-mm i.d.) coated with SE-54. Chromatography conditions included: helium carrier gas at a pressure of 12 psi; 2-μL splitless injection; split valve opened after 18 seconds. Initial oven temperature was held at 50°C for 5 minutes, then programmed at 4° per minute to 280°C. Flame ionization, electron capture, and mass spectrometer detectors were used. Target compounds were quantitated using internal standards (Table 2). The mass spectrometer system consisted of a Finnigan 3200 mass spectrometer, an INCOS data system, and a Hewlett-Packard 5840 gas chromatograph. Sediment and tissue samples were analyzed for metals (Table 3) by digesting with hot acid, followed by plasma emission spectrometry or atomic absorption spectrophotometry. Sediment samples were also analyzed for organic carbon and grain size distribution (Krumbein and Pettijohn 1938).

Quality assurance included analyses of replicate samples and blanks, interlaboratory comparisons, and the use of recovery and internal standards.

Cluster analysis was performed on sediment chemistry data using the programs of Wishart (1975). Concentrations of those chemicals listed in Tables 2 and 3 in sediments from 40 sampling stations were used in performing the cluster analysis. The data were standardized to a mean of zero and unit variance of 1. Clustering was performed using Ward's method (Everitt 1974). This method can be thought of as attempting to match stations with similar chemical profiles.

2.3 Fish Pathology

Necropsy Procedures. During each sampling period, up to 30 individuals from each of the five target fish species (Table 1) at each sampling station were necropsied. The necropsy procedure for most fish included measurement of total length and weight, determination of sex, collection of otoliths for age determination, description of the gross appearance of the animal, collection of blood for hematological tests, and excision and preservation of tissue from grossly visible lesions and major organs. Blood for hematological and blood chemistry tests was collected by cardiac puncture, or from caudal vessels with a syringe, or by excising the caudal fin. Aliquots of whole blood were used immediately after collection to determine hemoglobin concentrations and to make blood smears for differential white blood cell counts. The remaining blood was allowed to clot, and the serum was returned to the laboratory and frozen. An aliquot of the serum was used for chemical analyses (see Hematology and Blood Chemistry).

Ten adult Pacific cod, 16 blackmouth salmon, and 3 coho salmon were necropsied in a manner similar to that used for the target species, except no blood samples were taken.

Histological Procedures. For light microscopic examination, all preserved tissues were routinely embedded in paraffin and sectioned at 5 μm (Preece 1972). When necessary, tissues such as gills and bones were decalcified using a commercial decalcification solution (Scientific Products, Redmond, WA) prior to processing. Paraffin sections were routinely stained with Mayer's hematoxylin and eosin-phloxine, and for further characterization of specific lesions, additional sections were stained using standard special staining methods (Thompson 1966, Preece 1972, Armed Forces Institute of Pathology 1968).

Histopathological Procedures. All slides of stained tissues were examined using a "blind" system. Each fish was assigned a field number when necropsied; then prior to microscopic examination the fish was assigned an identification number derived from a random number table. Histopathologists examining tissue sections had information on the species, length, weight, sex, and the presence of grossly visible lesions, but no knowledge of the area from which the fish was collected.

Hematology and Blood Chemistry. Hematology and blood chemistry data described in this report are from analyses of blood from English sole collected during the winter of 1980. The hematological measurements included hematocrit and differential white blood cell counts taken on EDTA-chelated whole blood. Assessments of organ function by measurement of serum parameters were based on approved methods sanctioned by the American Association of Clinical Chemistry as outlined by Tietz (1976) and Kaplan and Szabo (1979).

Statistical Methods

The G-statistic was used to test the null hypothesis that the fish lesion frequency within a given area or at a particular station did not differ from the average lesion frequency (for the particular lesion being analyzed) for all areas sampled. In other cases the G-statistic was also employed to assess the statistical significance of lesion frequency variations between subareas and between cluster groups. Since the data consisted usually of two categories (fish with lesions vs. normal fish, or two lesion frequencies being compared), the Yates correction for continuity (Sokal and Rohlf 1969) was used. This method takes into account both the number of fish examined and the number of lesions observed.

2.4 Invertebrate Pathology

Necropsy Procedures. Crab and shrimp species were captured with the fish species in epibenthic trawls. The target species were separated from the total catch and up to 10 animals of each species were necropsied. The necropsy procedure was similar to that described for fish, with the following exceptions: for shrimp, the length of the carapace from the eye socket to the dorsal medial edge of the posterior end was measured, and crabs were measured across the widest points of the dorsal aspect of the carapace.

Shrimp were serially cut into 6 mm-thick cross sections through the entire length of the cephalothorax and the anterior two segments of the abdomen. These cross sections were immediately fixed in Davidson's fixative and later transferred to 50% ethanol (EtOH), and then to 70% EtOH.

The following organs and tissues were excised from crabs for examination: eye, brain, thoracic ganglion, cardiac stomach, pyloric stomach, midgut, anterior caecum, posterior caecum, heart, hemopoetic tissue, bladder, epidermis, mandibular organ, ovary, testis, and gill. These samples were preserved in Helly's fixative and later transferred sequentially through 50% EtOH and 70% EtOH.

Bivalves and polychaetes were sorted from sediment grab samples. Bivalves were opened and the tissues were extracted. These tissues and polychaetes, which were removed from their tubes, were fixed in Davidson's fixative. All specimens were transferred to 50% EtOH, then to 70% EtOH. Polychaetes were later identified and sorted.

Histologic Procedures. Tissues were processed for histology by standard techniques in a Tissue Tek II automatic tissue processor. All specimens were vacuum infiltrated with Paraplast for 15 minutes, then embedded in Paraplast blocks. Tissue sections, 5 to 7 μm thick, were routinely stained with hematoxylin and eosin (H&E). In some cases, where bacteria or certain protozoan parasites were suspected, Giemsa stain was employed. Fungal infections were demonstrated with the use of Grocott's Methenamine-Silver (GMS) stain.

Histopathological Procedures. Specimens were examined for the presence of parasites, secondary fungal and bacterial infections, and for lesions using a "blind" system similar to that described for fish.

2.5 Fish and Epibenthic Crustacean Ecology

Fish. At each sampling station, the catch of fish was sorted into target species (Table 1) and additional species. Up to 30 individuals of each target species were transferred into holding tanks for subsequent pathological analysis. If any additional individuals of target fish species remained, up to 20 more specimens were individually weighed, measured, and the otoliths removed for age determination. All, or a representative subsample, of any remaining target species were individually measured for length. All nontarget fish species were counted and the total weight of the species catch recorded.

Catch per unit of effort (CPUE) for the total catch was calculated using a 5-minute tow as a single unit. The CPUE for a given station or area was calculated by summing the total catch over the location involved and dividing this value by the number of tows made. Species richness was determined by counting the number of species occurring at a station or area. The diversity of species (H') was determined by using the Shannon-Weaver diversity index (Zar 1974).

$$H' = \frac{n \ln n - \sum_{i}^{k} f_1 \ln f_1}{n},$$

where n is the sample size, f_1 is the number of fish in species i, and k is the number of species.

Epibenthic Crustacea. Crabs and shrimp were also captured by otter trawl. The catch was sorted, and the numbers of individuals of each target species were enumerated.

2.6 Benthic Invertebrate Ecology

Benthic grab samples were taken using a modified Van Veen grab (Word, 1976). Two core samples each with a surface area of 100 cm^2 and a depth of 10 cm were taken from each grab for analysis of infaunal organisms. Three grab samples were taken at each station. In those areas with uniform depth, such as waterways, these samples were taken at the same depth. At other stations with a depth gradient, such as bays, samples were taken at depths which corresponded to the fish sampling program (Figures 1 and 2).

Sediment from the 1000 cm^3 core samples was wet-sieved through 1 mm-mesh stainless steel sieves and all the material remaining was preserved and analyzed. Wet weight biomass of molluscs, crustaceans, annelids, and ophiuroids was measured for winter of 1979 samples. Dry weight was then calculated using conversion factors reported by Lie (1968).

Molluscs were identified using the taxonomic keys of Kozloff (1974), Keen and Coan (1974), and Abbott (1954). Polychaetes were identified using the keys of Fauchald (1977) and of Banse and Hobson (1974). Other invertebrates were identified using the keys of Kozloff (1974). Representative specimens of each species were placed in a reference collection.

The taxonomic data were used to calculate several indices of community composition. These indices, which included the Infaunal Trophic Index (ITI) (Word 1978, Thom et al. 1979), and diversity measures, such as taxon richness (the number of taxa in a sample), and the Shannon-Weaver diversity index (Zar 1974) (Table 4) are thought to be indicative of the effects of environmental stress on benthic marine communities. For computation of the ITI, benthic marine invertebrate taxa were divided into four groups on the basis of feeding strategy (Thom et al. 1979): Group 1, suspension feeders; Group 2, suspension and surface detritus feeders; Group 3, surface detritus feeders; and Group 4, subsurface detritus feeders (see Table 5 for a list of taxa in each

group). Infaunal Index values can range from 0 to 100, with Group 1 organisms being most abundant in sediments with ITI values between 78 and 100, and Group 4 organisms being most abundant in sediments with ITI values below 24 (Word 1978).

2.7 Effects Studies

The effects studies consisted of *in situ* field exposures in which caged crabs and molluscs were placed at test sites in urban-associated areas [Duwamish (Station 10031), Hylebos (Station 9028), and City (Station 9031) Waterways; and the Seattle Waterfront (near Station 10015)] and a reference area (Port Susan, Station 9031) (Figures 1 and 2). A colonization assay was another type of *in situ* field study conducted in these areas. Using this assay it was possible to measure the ability of benthic invertebrates to recolonize sediment from urban-associated and reference sites.

2.7.1 Crab Exposures

In the first experiment, juvenile *C. gracilis* were held in cages at test sites for 60 days during July, August, and September, 1980. The crabs were initially collected by beach seine during low tide in the shallow waters of Mutiny Bay, Whidbey Island. Two cages, each containing four crabs, were placed at each test site. The crabs had carapace widths of approximately 45 mm, and were placed in cages within five days of capture. The weighted cages had dimensions of 60 x 100 cm, and were constructed of 2 cm-mesh Vexar screen. At all sites, except the reference site, the cages were raised and lowered by a line attached to a dock. At the reference site the line was attached to a mooring buoy. At the beginning of the experiment and at 2-week intervals thereafter, the crabs were fed freshly frozen English sole which had been captured at Port Susan. At each feeding, the number of crabs in each cage was determined and their gross appearance was described.

The second experiment also was performed using juvenile *C. gracilis* of the same approximate size and from the same area as the crabs used in the first experiment. The animals were put in cages and the cages were placed both in the Duwamish Waterway and near our research facilities in Mukilteo. The Mukilteo station served as a reference. The experimental procedures were similar to those used in the first experiment, except the crabs were fed and examined more frequently (at 3- to 4-day intervals). The exposure was for 56 days.

2.7.2 Mollusc Exposures

In these experiments, bivalves were collected from the intertidal sediments in Quartermaster Harbor, Vashon Island. Each cage received 23 *Macoma nasuta*, 4 *M. secta*, 2 *M. inquinata*, 3 *Tapes phillippinarum*, and 3 *Protothaca staminea*. The cages were made of Vexar mesh and consisted of an outer cylinder of 2 cm mesh, 30 cm in diameter and 30 cm high,

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and an inner cylinder of 1 cm mesh, 12 cm in diameter and 30 cm high. Varying numbers (17 to 23) of smaller (less than 4 cm long) individuals were placed in the inner chamber, and the larger individuals remaining were placed in the outer chamber. The cages were set out at a depth of 10 m by divers at the same stations used in the first crab exposures. Cage bottoms were worked into the sediment to a depth of 10 cm by rotating the cage back and forth.

After 71 days, the cages were collected by divers and agitated to remove mud or sand. The cages were then suspended about 5 m above the bottom for 18 to 24 hours to permit deposit-feeders to purge their guts of sediment. The cages were then hauled to the surface and the clams in the inner and outer compartments were placed in separate basins, examined for shell damage, and identified to species. The condition of empty shells (damaged or undamaged) was noted, and remaining live clams were dissected. Tissues from half of the number of *M. nasuta* were used for chemical analysis, and tissues from the remaining *M. nasuta* and individuals of the other species were used for histopathological examination.

2.7.3 Sediment Recolonization

Experiments were initiated in August 1980 using sediment from the five experimental sites. The sediment was collected with the modified Van Veen grab and undisturbed cores of sediment were placed in plastic freezer containers (size approximately 15 x 15 x 10 cm) with porous bottoms. The containers, frozen and thawed in order to kill most benthic infauna, were arranged as six subunits in a case. Each case contained subunits with only one sediment type and was covered with a 0.5 cm-mesh Vexar cage to limit predation. Four cases were placed at each of the urban sites; two of the cases had sediment taken from that particular site, and two of the cases had sediment collected from Port Susan, the nonurban reference site. The cases were spaced at 5 m intervals. At the Port Susan site, 10 cases of sediment were deployed; 2 of the cases contained sediment from Port Susan, and the remaining 8 cases contained contaminated sediment from each of the four urban areas, 2 cases for each area. The first sediment samples were collected 8 weeks after deployment and at 8-week intervals thereafter. The longest exposure time was for 24 weeks. The samples were collected by SCUBA divers who first sealed the undisturbed individual subunits with plastic lids, then brought the samples to the surface. The sediment samples were sieved through 1.0 mm-and 0.5 mm-mesh sieves, and the retained animals were preserved and identified by procedures described in Section 2.6.

In a second recolonization experiment, sediment was collected from the Duwamish Waterway and from Discovery Bay in May 1981, placed in polyethylene containers, and frozen overnight to kill the infauna. On May 22, 1981, four containers of sediment from each location were placed in trays of the same design as in the first experiment. The two trays were tied together and lowered to the bottom of the Duwamish Waterway

(Station 10031) from a dock near that used in the first experiment. Water depth was about 10 m. A line secured to the dock was used to retrieve the trays on August 10, 1981 (11 weeks of exposure). The sediment in the containers was sieved and the organisms preserved as before. Identification of all taxa collected has not yet been completed.

3. RESULTS

3.1 Chemical Analyses

Seventy-three sediment and 148 biota samples have been analyzed since 1978 (Table 6) and a major portion of the data was reported by Malins et al. (1980, 1981). All of the data are available from the NOAA National Oceanographic Data Center, 2001 Wisconsin Ave. N.W., Washington, D.C. 20235.

This report summarizes the major results, reported in parts per million (ppm) or parts per billion (ppb) on a dry weight basis unless noted otherwise.

3.1.1 Aromatic Hydrocarbons (AHs)

Sediment. GC profiles of AHs in sediment extracts from the urban-associated Hylebos and Duwamish Waterways and a reference area (Figure 3) show that a wide variety of AHs were present in sediment samples from both urban and reference areas. For example, more than 500 AHs were revealed in the chromatogram of the Hylebos sediment sample.

Concentrations of the target AHs (Table 2) were summed (ΣAHs), and the results show that the areas with highest ΣAHs were located in two urban-associated embayments, Elliott Bay and Commencement Bay (Table 7). Mean ΣAHs in sediments from urban areas were as much as 30 times the mean ΣAHs in Case Inlet and Port Madison (reference areas); however, ΣAHs varied considerably among stations within these embayments (e.g., values in Elliott Bay ranged from 150-63,000 ppb).

The distribution for individual AHs was similar to that shown for ΣAHs. For example, the concentrations of three carcinogenic AHs (fluoranthene, benz[a]anthracene, benzo[a]pyrene) were high in the same Elliott Bay stations (Table 8) as were the ΣAHs. Nontarget AHs were also found in some sediment samples; these may be associated with specific industrial processes. For example, retene (1-methyl-7-isopropylphenanthrene) was found in sediment collected near pulp mills (Table 9). In addition to retene, sediment from Port Gardner (Station 05210, Figure 4) also contained ca 22,000 ppb of a compound identified as ferruginol (Heller and Milne 1978).

Biota. Worms, clams, shrimp, and crabs from urban embayments contained ΣAHs significantly higher than ΣAHs in animals from reference areas (e.g., one worm sample from the Hylebos Waterway contained 85 times the ΣAHs detected in the worm from the reference area, Table 10). The

ΣAHs in fish liver samples from all areas were generally low (less than 1,400 ppb); ΣAHs from virtually all biota samples were lower than ΣAHs in sediment obtained from areas where the organisms were collected.

Chromatograms of AHs from biota (Figures 5 and 6) were much less complex than those for sediment from the same sampling stations. For example, fewer than a dozen AHs were identified in the biota (Figure 5) in comparison to as many as 500 AHs present in the sediment (Figure 3).

3.1.2 Polychlorinated Biphenyls (PCBs)

Sediment. Mean summation values for PCBs (ΣPCBs) in sediments from three urban areas: Elliott Bay, Commencement Bay and Sinclair Inlet were up to 300 times as high as ΣPCBs from the reference areas (Table 11). Generally, stations containing elevated ΣPCBs also contained elevated ΣAHs (compare Tables 7 and 11). Also, similar to the results obtained with AHs, a wide variation in values was evident for ΣPCBs at stations within an embayment (e.g., ΣPCBs for Elliott Bay ranged from "not detected" to 2,100 ppb). GC profiles of halogenated compounds in sediment from Hylebos and Duwamish Waterways and from Port Madison permit a comparison of the levels of PCBs found in the three areas (Figure 7).

Biota. The ΣPCBs for all biota samples from the Duwamish and Hylebos Waterways were from 2 to 66 times higher than those found in the same organisms from reference areas (Table 12). The highest ΣPCBs (as high as 35,000 ppb) were found in crab hepatopancreas and sole liver. The ΣPCBs in biota from the Duwamish Waterway (e.g., worm, clam, shrimp) were 0.6-4 times the mean ΣPCBs in the sediment from the Duwamish Waterway (Stations 1, 2, 3 and 16; Table 11). The ΣPCBs in crab hepatopancreas and English sole liver from the Duwamish Waterway were 24-64 times greater than the concentrations in sediment from the same area.

The distribution of PCBs in liver and muscle of English sole, salmon and cod from Elliott Bay, Commencement Bay, and reference areas is given in Table 13. All samples, except English sole liver and muscle, had ΣPCB values similar to the values for such fish from the reference areas. Concentrations of ΣPCBs in English sole liver were from 1 to 15 times those from reference areas (Table 13).

3.1.3 Chlorinated Butadienes (CBDs) and Hexachlorobenzene (HCB)

Sediment. Although CBDs (Table 14) and HCB (Table 15) were found in nearly every sediment, only those from Commencement Bay had levels much higher than those from the reference areas (up to 20,000 ppb ΣCBDs and 1300 ppb HCB). The mean for ΣCBDs for Commencement Bay was as much as 800 times the mean for ΣCBDs from Port Madison (reference area). As with the ΣAHs and ΣPCBs, values for ΣCBDs and HCB varied greatly among stations (the range of ΣCBDs in Commencement Bay was <0.1 to

20,000 ppb). Of the CBDs, tetrachlorobutadiene (TCBD) was found in sediments (Figure 7) at concentrations higher than those for hexachlorobutadiene (HCBD).

Biota. CBDs (Table 16) and HCB (Table 17) were found in all biota samples from the Hylebos Waterway; however, these compounds generally were barely detected (10 ppb or less) in biota from the Duwamish Waterway or reference areas. Sole liver from the Hylebos Waterway had the highest concentrations of ΣCBDs (up to 9100 ppb) and HCB (up to 3700 ppb). Worm, clam, shrimp, and crab from the Hylebos Waterway had concentrations of 7 to 360 ppb ΣCBDs, and 10 to 370 ppb HCB (with one exception - 1800 ppb HCB in one crab sample). HCBD, a carcinogenic CBD, was generally found in the liver and muscle of English sole, salmon, and cod from Elliott Bay, Commencement Bay, and a reference area (Point Jefferson). Muscle of salmon and cod from all three areas contained less than 1 ppb of HCBD (wet weight); liver of salmon and cod contained less than 10 ppb (wet weight). However, English sole from Commencement Bay (Hylebos Waterway) contained 120-570 ppb of HCBD in liver and 2-110 ppb in muscle (wet weight).

In contrast to the sediment data, the concentration of HCBD was higher than the other CBDs in biota (Figures 8, 9, 10). The concentrations of individual CBDs in sediment and sole liver from the Hylebos Waterway (Figure 11) show that HCBD predominates in biota and other CBDs (with fewer chlorine atoms) predominate in sediment.

3.1.4 Chlorinated Hydrocarbon Pesticides

Sediments. The concentrations of chlorinated hydrocarbon pesticides (Table 2) in sediment were low. The mean concentration of DDT in sediments from Elliott and Commencement Bays was 7 ppb (dry weight); this value was 20 times the mean value in reference areas (0.35 ppb, dry weight).

Biota. Mean concentrations of DDT in English sole livers from Hylebos and Duwamish Waterways were four times the mean concentration from reference areas (180 ppb vs. 45 ppb). In contrast, cod livers from Commencement and Elliott Bays and reference areas had similar mean DDT levels (ca. 20 ppb). Low concentrations of DDT (<6 ppb) were found in salmon liver and muscle from all three areas.

3.1.5 Other Halogenated Compounds

Sediment. Sediments collected near Bellingham, Everett, Port Angeles, and Tacoma contained a variety of nontarget halogenated compounds (Figures 7, 12, and 13). Burrows et al. (1981) identified 36 compounds or groups of isomers in sediments (Table 18) from the Hylebos Waterway and Old Tacoma Stations. Although more than 300 compounds were identified in these samples (Appendix A), numerous compounds remain unidentified. Dichloro-, trichloro-, tetrachloro-, and penta-chlorobenzenes were found in sediment samples from Port Angeles, Bellingham, and Everett.

Biota. Nontarget chlorinated compounds were also found in biota from Commencement Bay, particularly from Hylebos Waterway (Figures 8 and 9). Mass spectra showed some of these compounds to be the same highly chlorinated aliphatic hydrocarbons found in some of the Commencement Bay sediments.

3.1.6 Metals

Sediment. Mercury, lead, arsenic, silver, and cadmium were generally distributed throughout Puget Sound sediments (Tables 19-23). Lead was present in the highest mean concentrations, ca. 130,000 ppb in Elliott Bay, Commencement Bay and Sinclair Inlet and 20-50% of that amount in the other urban areas (Table 20). The mean concentration of arsenic was highest in Elliott and Commencement Bays (e.g., 90,000 and 85,000 ppb, respectively). Mercury was found in urban area sediments at concentrations about equivalent to the highest concentration found in Port Madison or Case Inlet (reference areas, Table 19). Bellingham Bay samples had the highest mean mercury concentration (1,400 ppb). Both urban and reference areas generally had similar mean concentrations of silver (ca. 2,000 ppb) and cadmium (ca. 5,000 ppb) (Tables 22 and 23).

Biota. Muscle of English sole, cod, and salmon from urban and reference areas contained low concentrations of three heavy metals (cadmium <100 ppb; lead <200 ppb; and mercury <140 ppb). In contrast, arsenic concentrations were high (ca. 5,800 ppb) in English sole muscle from the Duwamish and Hylebos Waterways and a reference area. Mean arsenic levels in cod (2,700 ppb) and salmon (700 ppb) muscle from Elliott and Commencement Bays and reference area were lower than those for sole.

3.1.7 Cluster and Principal Components Analyses of Sediment Chemistry Data

The principal components analysis of the sediment chemistry data yielded four principal components axes (Table 24). The first principal components axis consisted primarily of AHs, and the second axis included primarily metals. The third axis contained a combination of certain metals plus PCBs. The fourth axis consisted primarily of chlorinated organic compounds. Almost 75% of the variability in the 28 metals and 36 hydrocrabon compounds and hydrocarbon compound classes could be accounted for by four principal component axes. The chemicals loading most strongly on these axes formed groups in which concentrations tended to vary together. This tendency of stations with similar chemicals to group together when compared statistically was utilized in the cluster analysis. The results of this analysis yielded eight cluster groups of sampling stations (Figures 14-17; Table 25). Plots of the stations and groupings on the first four principal components axes are given in Figures 15-17.

3.1.8 Analyses of Clam and Crab Samples from Caged Studies

To determine the extent to which clams and crabs bioconcentrate the target chemicals, fauna were taken from reference areas and placed in cages in urban areas. Caged clams accumulated high levels of ΣAHs (15,000 ppb) compared to reference clams (clams that were collected at the same time and from the same areas as the clams held in cages, but were frozen immediately after capture) (970 ppb); the differences in ΣAHs were small between caged crabs (390 ppb) and reference crabs (150 ppb). The chlorinated hydrocarbon concentrations (Table 26) were much higher in the caged clams and crabs than in the reference samples (Figures 18, 19). For example, the concentration of HCB was 310 and 70 times higher in caged than in reference clams and crabs, respectively.

3.2 Fish Pathology

A variety of pathologic conditions were observed in all fish species examined, some of which were species-specific, while others were common to all target species (Table 27). In this section, lesions will be broadly classified as either idiopathic or infectious. Idiopathic lesions are defined as those with an unknown etiology that are independent of any infectious agent visible at the detection limit of light microscopy. These lesions may be caused by chemical contaminants, nutritional disorders, microorganisms (not generally diagnosable at the light microscope level), genetic disorders, trauma, or as yet undefined environmental factors.

Within each broad classification, specific lesions will be arranged in standard lesion types possessing similar histopathological features. For example, idiopathic lesions of the liver are grouped as neoplasms, "preneoplastic" lesions, degenerative/necrotic lesions, intracellular storage disorders, vascular disorders, and inflammatory lesions. Table 27 presents a description of each lesion type for each major organ and the specific lesions which comprise each lesion type.

The results will consist of a brief description of the main morphologic characteristics of selected lesions, and a presentation of the prevalence and geographical distribution of affected fish. For the purpose of simplifying the presentation of the geographical distribution, the sampling stations will be organized into subareas (Table 28). The subareas will represent either an entire embayment (i.e., Sinclair Inlet), or subdivisions of a large embayment (i.e., Elliott and Commencement Bays).

Data on the prevalence of fish from each subarea with the various lesions are presented in Figures 20 to 27. The G-statistic (Sokal and Rohlf 1969) was used to test whether the prevalence of fish with a lesion type in any subarea was significantly different from the average overall prevalence of fish with that lesion at all the stations sampled. Whether differences were higher (H) or lower (L) was determined by inspection of the frequency data. Thus, statements indicating that the

prevalence of a particular lesion type was higher in fish from certain subareas are based only on statistically significant differences ($p \leq 0.05$). Because the number of fish examined varied among the sampling stations, it was important to consider the sample size in comparing lesion frequencies, particularly in those cases having a small sample size. The computational formula for the G-statistic includes this factor. Consequently, lesion prevalence in some subareas appeared much higher or lower than prevalence in other subareas, but failed to be significantly different due to the smallness of the sample. When no lesions were observed in fish from a subarea or cluster group, a value of zero was substituted for the term "(observed affected frequency) X (the natural logarithm of the observed affected frequency)" in the computation of the G-statistic. Also, in the interest of brevity, only those lesions which are identified in Table 27 as "significant" (i.e., the geographical distribution was nonuniform) will be further described.

3.2.1 Idiopathic Liver Lesions

Neoplasms. Hepatic neoplasms were primarily of hepatocellular, cholangiocellular (biliary), and mesenchymal cell origin, although neoplasms of mixed cell origin were also encountered. Adenomas and carcinomas were classified according to commonly utilized morphologic criteria employed in distinguishing benign vs. malignant hepatic neoplasms (Newberne and Butler 1978, Ward and Vlahakis 1978, Squire and Levitt 1975). In a separate study, a single case of a metastasizing cholangiocellular carcinoma was found in an English sole from the Duwamish River (McCain et al. 1982). Additional information on the characteristics of these lesions is shown in Table 27.

Fish with hepatic neoplasms were found in all the urban-associated embayments (Figure 20a). No tumor-bearing fish were found in the reference areas. Pacific staghorn sculpin with hepatic neoplasms were found only in the Hylebos Waterway (1 of 116 fish) and the Commencement Bay Waterways (1 of 98 fish), while quillback rockfish with hepatic neoplasms were found only in Sinclair Inlet (1 of 3 fish). No hepatic neoplasms were detected in Pacific tomcod. English sole with hepatic neoplasms were found in all urban embayments except Budd Inlet, with the highest prevalence occurring in the upper Duwamish Waterway (16%, 22 of 136). Similarly affected rock sole were found in all the urban embayments.

"Preneoplastic" Conditions. "Preneoplastic" lesions were included in this group strictly on the basis of histologic similarity to previously reported "preneoplastic" conditions found in mammals which generally progress to neoplasia and are thought to be irreversible (Squire and Levitt 1975; Bannasch 1978; Becker 1978, 1976; Farber 1976). In general they are proliferative and/or regenerative in nature and possess atypical cellular characteristics.

The geographical distribution of fish with "preneoplastic" conditions was similar to that observed for fish with hepatic neoplasms (Figure 20b), with the following exceptions: (a) one Pacific tomcod

from Brown's Point, Commencement Bay, had a "preneoplastic" hepatic lesion; (b) no "preneoplastic" lesions were detected in quillback rockfish; (c) rock sole with "preneoplastic" lesions were found in the reference areas, Case Inlet (7%, 2 of 28) and Port Madison (9%, 2 of 22), while English sole with these lesions were found in only one reference area, Discovery Bay (4%, 2 of 51); and (d) affected English sole were most prevalent in the Commencement Bay Waterways (12%, 33 of 276) and in the upper Duwamish Waterway (21%, 28 of 136).

Nonspecific Degenerative/Necrotic Lesions. Cases of nonspecific cellular degeneration and necrosis were included in this lesion group only if no infectious agent was found to be associated with the liver. The severity of these lesions ranged from mild cellular or subcellular perturbations, such as hydropic degeneration, to increased cytoplasmic eosinophilia or migration of nuclear chromatin, to cell necrosis (Trump and Arstila 1975) (Table 27).

Demersal fish with these nonspecific lesions were found in a variety of sampling areas; however, the highest frequencies were generally in fish from Elliott and Commencement Bays (Figure 21). One Pacific cod (1 of 4 fish) from Elliott Bay and no cod (0 of 6 fish) from Point Jefferson had these lesions.

Specific Degenerative/Necrotic Lesions. The major lesions grouped in this category are megalocytic hepatosis (MH) and nuclear pleomorphism (Table 27) (Koller and Zinkl 1973; Hinton et al. 1978; Jones and Butler 1975). Although this lesion type was identified by Malins et al. (1980) as MH, nuclear pleomorphism and MH were so frequently found to be closely associated that a new name, specific degenerative/necrotic lesions, is now used. The distribution of fish with these specific lesions was very species dependent. These lesions were detected in only one Pacific tomcod, from the lower Duwamish Waterway (1 of 65), and were detected in only one quillback rockfish from the Seattle Waterfront (1 of 93). These types of lesions were observed in English sole, rock sole, and Pacific staghorn sculpin primarily from urban areas (Figure 22).

Intracellular Storage Disorders. Intracellular storage disorders are not considered to be lesions, *per se*, but represent variability in the amounts and types of intracellular storage products (Table 27). Disruption of cellular homeostatic mechanisms can produce abnormal accumulations of both normal and abnormal intracellular products. These products include lipids (excessive accumulation of these products can result in fatty change or steatosis), as well as iron-containing storage products such as hemosiderin (excessive accumulations of this product can result in hemosiderosis) (Robbins and Cotran 1979).

English sole, rock sole, and Pacific staghorn sculpin with storage disorders were found in most of the sampling areas. Affected Pacific tomcod and quillback rockfish were primarily found in Elliott Bay and Commencement Bay; although affected Pacific tomcod were also found in Sinclair Inlet (Figure 23). One Pacific cod (1 of 4 fish) from Elliott Bay and no cod (0 of 6 fish) from Point Jefferson had fatty change.

3.2.2 Sex/Age/Length/Weight Relationships of Fish with and without Idiopathic Liver Lesions

The prevalence of males and females of each target species with any of the five above-mentioned idiopathic liver lesions was compared by computation of the G-statistic using the Yates Continuity Correction factor. No significant difference was observed between the prevalence of idiopathic liver lesions in either sex of the target fish species. Comparisons of liver lesion prevalence for each age group of English and rock sole were also compared by computation of the G-statistic. The prevalence of a lesion among all fish with age determinations was used to calculate the expected frequencies. Males and females were analyzed separately. Ages of English sole from the fall sampling periods of 1979 and 1980, and of rock sole from the fall of 1980 were determined by otolith readings. Fish smaller than 100 mm were considered to be less than 1 year old.

The prevalences of English sole with the five types of idiopathic liver lesions were significantly ($p<0.025$) nonuniform among the age groups of both sexes (Table 29). With the exception of English sole with neoplasia, young fish (up to 1 year old for females and up to 2 years old for males) tended toward prevalences significantly lower ($p\leq0.05$) than overall lesion prevalences. Neoplasia did not occur in males less than 2 years old and females less than 1 year old. In contrast, English sole older than 6 years old had a general pattern of higher than expected lesions frequencies. Among males, lesion prevalence was significantly greater ($p\leq0.05$) for the five lesion categories in either the 6-7 or 7-8 year old groups or in both age groups. The prevalence of sole with neoplasia, "preneoplasia", nonspecific degeneration/necrosis and total liver lesions was significantly higher ($p<0.025$) in the 8-15 year old males. The prevalences of females with neoplasia, "preneoplasia", storage disorders, and nonspecific degeneration/necrosis were greater than the expected value ($p\leq0.025$) in the 6-7 year old group.

In contrast to the patterns observed in English sole, rock sole showed highly uniform lesion prevalence across the age groups. No age group of male rock sole had a significantly different level of disease prevalence than expected. With the exception of hepatic neoplasia and storage disorders, this was also true for female rock sole. Three-to-four year old females had a higher prevalence of neoplasia than expected, and 1-2 year old females exhibited a lower prevalence of storage disorders than expected. However, the test for uniformity for both lesions across all ages showed that the null hypothesis could not be rejected, i.e., that lesion prevalence was the same for each age group.

The lengths and weights of English sole and rock sole of the same age, with and without each of the five idiopathic liver lesions, were compared using the T-test (Zar 1974). Data for males and females were analyzed separately. Among English sole and rock sole males and females, no significant differences were observed between the length or weight of sole with or without liver lesions.

3.2.3 Kidney Lesions

Degenerative/Necrotic Lesions. Lesions were observed in both the glomerular and the tubular systems of the kidney. Mesangiolysis (Morita et al., 1978) represents a primary glomerular lesion, whereas tubular vacuolation and necrosis (Trump et al., 1975) involves the tubular system (Table 27). English sole with these types of lesions were generally widespread throughout the sampling areas, with the highest prevalence in the Duwamish and Hylebos Waterways (Figure 24). The other target species with these lesion types were found in fewer sampling areas and the prevalence values were generally lower.

The most significant and prevalent lesion found in salmon was a primary glomerular lesion in the kidney, characterized by a massive thickening of the peripheral basal lamina in the glomerular tuft, often with irregular, subendothelial, eosinophilic depositions. The "wire loop" appearance of these glomeruli corresponded closely to the morphology seen in mammalian syndromes generally classified as immune complex glomerulonephritis. This idiopathic lesion affected 43% (3 of 7 fish) of the Elliott Bay salmon, 57% (4 of 7 fish) of the Commencement Bay salmon, and 40% (2 of 5 fish) of those from Point Jefferson.

3.2.4 Gill Lesions

Proliferative lesions were observed in respiratory epithelial cells, filament epithelial cells, and mucous cells. Respiratory epithelial hyperplasia was often associated with fusion of adjacent gill lamellae and/or filaments (Smith and Piper 1975; Walsh and Ribelin 1975). Proliferation of the pillar cells forming the internal structural component of the lamellae were found, often in association with other hyperplastic conditions in the gill (Table 27). A single depositional lesion type, characterized by a thickening of the basal lamina surrounding the lamellar capillaries, was observed. This lesion was often associated with pillar cell proliferation, and an increase in pericapillary connective tissue and/or matrix was also observed.

Fish with proliferative gill lesions were widely distributed in both urban and nonurban areas (Figure 25). The prevalence of English sole with gill lesions was significantly higher in Hylebos Waterway and Discovery Bay. The prevalence of rock sole and Pacific tomcod was significantly higher only in the Hylebos Waterway.

Respiratory epithelial hyperplasia was observed in Pacific cod from Elliott Bay (1 of 4 fish) and Point Jefferson (1 of 6 fish), and in salmon from Point Jefferson (1 of 5 fish). Respiratory epithelial hypertrophy was detected in only one salmon (1 of 7 fish) from Elliott Bay. Lamellar capillary microaneurysms were found in one Pacific cod (1 of 6 fish) from Point Jefferson and in one salmon (1 of 7 fish) from Commencement Bay.

3.2.5 Lesions of the Skin and Fin

Two types of grossly visible skin lesions were observed. The first lesion type was characterized as tumorous growths known as angioepithelial nodules (AEN) and epidermal papillomas (EP) (Wellings et al., 1976b). The second type of lesion, known as fin erosion, was often grossly recognizable as partial loss, fusion, or distortion of fin rays, generally accompanied by hemorrhage and granulation tissue. In some cases, entire fins were destroyed. Rock sole and English sole were the only target fish species observed with these lesions. The only rock sole with AENs or EPs were found in southwest Commencement Bay (9%, 2 of 23), while affected English sole were captured in a number of urban and nonurban areas. The only rock sole with fin erosion were found in the Hylebos Waterway (2.5%, 4 of 161). Fin-eroded English sole were captured in the Commencement Bay Waterways (1 of 287), and in the upper (2 of 149) and lower (2 of 418) portions of the Duwamish Waterway.

Because of interest in fin erosion in starry flounder generated by a previous study in the Duwamish Waterway (Wellings et al., 1976b), this species was routinely examined for eroded fins. Only one affected starry flounder was found in the upper Duwamish Waterway (1 of 43). In Commencement Bay, two fin-eroded starry flounder were found in the Puyallup River (2 of 14), and one was collected from the City Waterway.

3.2.6 Spleen Lesions

Both lymphoid and erythroid hypoplasia and/or depletion were observed in fish spleens. All target species had individuals with these lesion types. With the exception of English sole, all of the affected animals of the other species were from the urban embayments (Figure 26). English sole with these lesions were present in all of the sampling areas except Port Madison and West Point.

3.2.7 Lesions of the Gall Bladder

Inclusion cysts of the mucosal epithelium were found within the gallbladders of Pacific staghorn sculpin, rock sole, and English sole from the urban-associated embayments (Figure 27). The other two target species were not affected. Inclusion cysts were detected in only one Pacific cod (1 of 6 fish) from Point Jefferson, and were not detected in salmon.

3.2.8 Multiple Lesions in Fish

A statistical test (using the G-statistic) was performed that was designed to identify which subareas had target species with prevalences of multiple liver lesions significantly higher or lower than other subareas. The highest prevalences ($p \leq 0.05$) of English sole with more than one liver lesion were in the Commencement Bay Waterways and the

Duwamish Waterway. The lowest prevalences for English sole with multiple lesions were in Sinclair Inlet and at West Point. Rock sole and Pacific tomcod with multiple liver lesions were found only in the Duwamish Waterway.

3.2.9 Hematology and Blood Chemistry

The differential white blood cell counts in English sole with various pathological lesions and parasitic infestations in the liver, kidney, gills and heart are shown in Table 30. In this portion of the study, corresponding blood and tissue samples were taken from 309 English sole (15-35 cm) captured in 1980. Control levels for differential cell distribution were formulated by incorporating data from English sole which had no observable pathological condition(s) (evaluated by light microscopy) in any of the tissues examined, nor showed any major effect from the parasitic lesions documented. Seventy-one fish met the above criteria and were used to formulate normal ranges for differential leukocyte counts for English sole.

Many of the pathological lesions identified in the liver were significantly correlated ($p \leq 0.05$) with certain hematological changes in English sole. Consistent decreases in the percent of lymphocytes with a corresponding increase in the percent thrombocytes, were found in association with all the major liver lesions except in vascular disorders of the liver. An increase in the percent of PAS-positive granulocytes occurred only in a selected number of idiopathic hepatic conditions, such as neoplastic, "preneoplastic", and vascular disorders.

Of the parasitic infestations of the liver only *Myxosporidia* and *Coccidia* were associated with any marked alteration of leukocyte differential counts. Fish with all other parasitic infestations of the liver either were not found to have counts which were significantly different from normal leukocyte counts or they could not be evaluated appropriately due to insufficient samples.

Certain lesions in the kidney were also found to be associated with differential leucocyte counts that differed from normal. Both kidney depositional disorders and necrotic lesions were associated with a significant change in the lymphocyte and thrombocyte differential counts, with a corresponding decrease and increase, respectively. Additionally, depositional disorders of the kidney were associated with an increase in the percent PAS-positive granulocytes. These findings are similar to those observed with fish having proliferative lesions of the liver. No kidney parasitic infestation was found to be correlated with abnormal hematology.

English sole with certain parasitic infestations of the gill showed a marked change in differential cell counts. Interestingly, in these cases, significant changes in the percent lymphocytes were not associated with a corresponding increase in the percent thrombocytes. It is possible that this may be reflective of specific changes associated with these conditions in the gill of English sole.

Blood samples from the same 309 English sole were also used for determinations of the concentrations of 11 serum components. Preliminary results showed that fish with various lesions of the liver, kidney, and heart had significant changes in the levels of serum components relative to levels in normal fish. Control values for each of these parameters were formulated from the 71 fish selected on the bases described above.

In almost all English sole with liver disorders and in some with kidney disorders, serum albumin levels were strikingly low (Table 31). This parameter is a marker for general liver pathology in mammalian clinical sciences. Lower-than-normal levels of calcium and total serum proteins were also consistently observed in fish with liver and kidney disorders. The levels of all other serum components were either unchanged or altered only in fish with certain lesions, such as increased bilirubin concentrations associated with specific necrosis and "preneoplastic" lesions.

3.2.10 Interrelationships between Lesion Prevalence and Sediment Chemical Composition

The prevalence of fish with the above-mentioned types of lesions from stations within each of the eight chemically-similar cluster groups (Table 25) was determined. As described in Section 3.1, cluster group analysis was used to group the sampling stations according to similar composition of chemical contaminants in sediment. The G-statistic was used to examine which of the cluster group stations had significantly higher or lower ($p \leq 0.05$) prevalences of fish with the selected lesions.

The prevalence of fish (at least one of the five target species) with five of the eight types of liver lesions was significantly higher at cluster group-4 stations (Figures 28-31). The lesion types and the species affected included the following: hepatic neoplasms, specific degeneration/necrosis, storage disorders, and "preneoplasia" in rock and English sole; and hepatacellular necrosis in rock sole. Cluster group-7 stations had significantly higher prevalences of English sole with megalocytic hepatosis, storage disorders, hepatocellular necrosis, and "preneoplasia"; and of rock sole with neoplasms and megalocytic hepatosis. The prevalence of fish from cluster group-1 stations with three types of liver lesions: neoplasms, specific degeneration/necrosis, and "preneoplasms" was significantly lower, or too few fish were collected and examined to be statistically significant (Figures 28 and 29).

The prevalence of kidney necrosis was significantly higher only among English sole at cluster group-4 stations (Figure 32). The prevalence of English sole with this lesion type was significantly lower only at cluster group-1 stations.

The frequencies of three of the target fish species (the exception was rock sole) with proliferative disorders of the gill were significantly

higher at cluster group-7 stations (Figure 33). Several cluster groups had significantly lower prevalences of fish with gill lesions, but no pattern of lesion-free animals was observed.

Statistical analyses were performed using the nonparametric method of Spearman's rank correlation (Zar 1974) to evaluate the relationships between lesion prevalence for English sole and the relative concentrations of chemicals in the sediment from the stations where the affected fish were captured. The ranked prevalence values were compared with the ranked factor scores for each sampling station on each of the four axes obtained by the principal components analyses of sediment chemistry data (see Table 24). The results of these comparisons demonstrated that the frequencies of English sole with hepatic neoplasms, "preneoplasia", specific degeneration/necrosis, and storage disorders were all positively correlated with the concentrations of the chemicals in the first principal components axis and the second principal components axis (significant at $p \leq 0.038$) (Table 32).

Statistically significant ($p \leq 0.05$) differences were observed in the values of certain hematological and serum chemistry parameters for English sole between some of the cluster groups. However, because sole with significantly different values were not consistently found in any of the cluster group stations, the interpretation of these differences is presently very difficult.

Kendall's rank correlation method (Zar 1974) was used to evaluate the relationships between the hematological and blood chemistry values for English sole and the relative sediment concentrations of the chemicals on each of the four principal component axes. Those hematological parameters which were significantly correlated with any of the groups of chemicals were all negatively correlated (Table 33). The strongest correlations ($p \leq 0.001$) were between the percentage of an unclassifiable group of blood cells termed lymphocyte/thrombocytes and the third and fourth principal components axes, and between the percent granulocytes and the third principal components axis.

The serum chemistry parameters which had values positively correlated ($p \leq 0.001$) with any of the four groups of chemicals included the following: alanine aminotransferase (axis one), bilirubin (axis three), creatinine (axis two and three), and phosphate (axis one) (Table 34). Serum parameters which were negatively correlated ($p \leq 0.001$) included phosphate (axis four) and magnesium (axis three).

3.3 Invertebrate Pathology

The pathological conditions observed in crabs and shrimp are outlined in Table 35. No definitive pathological conditions were observed in polychaete worms or in molluscs. The organs of shrimp and crabs most frequently found with histopathological lesions included the gill, hepatopancreas, midgut, and antennal gland. These lesions were not generally recognizable as externally visible abnormalities, and are

considered to be idiopathic. The only grossly recognizable abnormality was discoloration (darkening) of crab gills, even in newly molted animals. A variety of parasitic infestations were also observed. Because of the great variability in sample sizes of target species from station to station and from one sampling quarter to the next meaningful statistical analyses were not possible. These data are most appropriate as descriptions of the characteristics of microscopically detectable parasites and lesions found in particular sampling environments.

3.3.1 Lesions of the Hepatopancreas

Necrosis and melanized lesions were observed in the hepatopancreas of both crabs and shrimp. Affected C. alaskensis were found only in the upper and lower Duwamish Waterway, Sinclair Inlet, outer Elliott Bay and Case Inlet. The trend was somewhat similar in pandalid shrimp except that animals with lesions were found in Commencement Bay Waterways, Hylebos Waterway, and Seattle Waterfront, but not in Case Inlet, or outer Elliott Bay. At one collection site (Station 10016, the north end of Harbor Island in the Duwamish Waterway), seven of eight C. alaskensis sampled there had necrosis of the hepatopancreas. In Cancer crabs, hepatopancreatic necrosis was most common in animals collected in Commencement Bay and Hylebos Waterway; and also was observed in animals from upper and lower Duwamish, Sinclair Inlet, and Discovery Bay (Figure 34).

3.3.2 Lesions of the Antennal Gland

Necrotic lesions, melanized nodules, abnormal concretions and granulomas were at times present in the antennal glands, part of the excretory system of of shrimp and crabs. Necrosis of the antennal gland generally was observed in C. alaskensis collected in Elliott Bay, but was also fairly frequently encountered in C. alaskensis collected from upper and lower Duwamish Waterway and Sinclair Inlet. In pandalids, necrosis of this gland occurred in animals from the Duwamish, Hylebos, and Commencement Bay Waterways (Figure 35A).

3.3.3 Lesions of the Gill

Two histopathological conditions occurred in the gills of shrimp and crabs: (1) necrosis of the connective tissue and epithelium of both the gill stem and lamellae and (2) melanized nodules, granulomas, and extensive necrosis or melanization of portions of a lamella or several lamellae. Crangon alaskensis with gill necrosis were common in the upper Duwamish Waterway, Outer Elliott Bay and Hylebos Waterway (Figure 35B). At two collection sites, Station 09033 (between Hylebos, and Blair Waterways) and Station 08005 (drydock at Sinclair Inlet) C. alaskensis had unusually high occurrences of necrotic gills, seven of eight observed and six of seven observed, respectively. One of six C. alaskensis from Discovery Bay also had gill necrosis. Melanization in the form of small melanized nodules or granulomas were found in crabs. Nodules and granulomas of the gills were observed in animals from the upper and lower Duwamish Waterway and Sinclair Inlet, Hylebos Waterway, and Commencement Bay Waterways (Figure 35C).

3.3.4 Lesions of the Midgut

Lesions of the midgut included both necrosis and/or melanized nodules and granulomas in crabs. Midgut nodules were found only in crabs from Commencement Bay, and the upper and lower Duwamish Waterway; while crabs with midgut necrosis were also found in these same areas as well as Sinclair Inlet and the Seattle Waterfront (Figure 35D,E).

3.3.5 Infectious Diseases of Miscellaneous Tissues

Microsporidian infection of the musculature was the most common infectious disease of *Crangon alaskensis*. These infections were found at all stations except Case Inlet, West Point, and Discovery Bay. They were quite common at Sinclair Inlet, with 33% (23 of 70) infected. There were trematode metacercaria encysted in the nervous system of 13% (9 of 70) of the *C. alaskensis* examined from Sinclair Inlet, as well; all infections occurred in the 1980 collections. A systemic yeast infection was also observed there only in 1980, in 4 of 70 (6%) *C. alaskensis*. (All other infection rates are based on combined catches for 1979 and 1980.) Fungal infections of the gills occurred only in pandalid shrimp collected in Hylebos Waterway and Commencement Bay Waterways. A single case of a dinoflagellate parasite of shrimp eggs of *C. alaskensis* was found in Hylebos Waterway. This is particularly interesting because it has not been reported from this area, but is considered a serious parasite of pandalid shrimp in Alaskan waters.

3.3.6 Interrelationships between Lesion Prevalence and Sediment Chemical Composition

Crabs with melanized nodules in the gills were found in stations representing every cluster group where crabs were collected except in cluster group-1 stations (Figure 36), and were more common in clusters 2 and 4. Melanized nodules were also observed in the gills of pandalid shrimp, primarily in shrimp from cluster group-7 stations (Figure 36). *Crangon alaskensis* with gill necrosis were found in all cluster group stations except cluster groups 5, 7, and 8 (Figure 36). Crabs and shrimp with lesions in other organs were either widely distributed in stations representing most of the cluster groups, or their prevalence was very low.

3.4 Fish and Epibenthic Crustacean Ecology

3.4.1 Fish Ecology

The objectives of the fish ecology study were to characterize the abundance and distribution, community characteristics, and biological characteristics of target fish species. Catch per unit effort (CPUE) was the principal means of measuring abundance. Community characteristics were defined by species diversity, richness, and composition. The biological characteristics included measurements of length, weight, and age.

As a consequence of the findings from the fish ecology portion of the investigation during 1979, modifications were made in the selection of target species and study areas used in 1980. Initially five target species were selected to represent bottom- and midwater-dwelling fishes. The results from the 1979 sampling season demonstrated, however, that English sole, rock sole, and staghorn sculpin were the most appropriate target species because of their broad distribution, high abundance, and seasonal availability. Thus, these three species were the only target fish species studied in 1980.

Analysis of results from the first year also indicated that Case Inlet and Port Madison were unsuitable as reference areas for Elliott and Commencement Bays. These latter bays were the only embayments with major rivers and thus as estuarine bays had different species occurrence patterns than the inlet and open water reference areas. One reference area used in 1980 was, therefore, Port Susan, an estuarine embayment.

Abundance and Geographical Distribution. For those areas sampled in consecutive years, the highest average CPUE values were obtained in the estuarine embayments (Figure 37). The abundance of fish in Case Inlet, Budd Inlet, Sinclair Inlet, and Port Madison was lowest during the winter and spring. The catch rates in the estuaries were highest in the fall and generally similar during the remainder of the seasons. In fact, the highest catch rate of the 2-year program in Commencement Bay occurred during the fall of 1979. Inter-year variability between the catch rates in Elliott Bay and Sinclair Inlet was generally low. The catch-rates in Port Susan and Discovery Bay were relatively high compared to the other areas sampled during 1980.

English sole and rock sole were numerous and well distributed in most of the study areas (Figure 38). Commencement Bay had the highest average English sole CPUE over the entire six sampling periods with many of the fish being captured in the waterways. The Duwamish estuary in Elliott Bay also had many English sole which appeared to follow the leading edge of the demersal saltwater wedge up the river during seasons of low runoff, and, conversely, were more abundant in the lower estuary during periods of high runoff. Rock sole were not as abundant in the waterways and estuaries as were English sole, but were most abundant in the outer stations in the estuarine bays (Figure 37). Few rock sole were collected in Case Inlet, Port Madison, Port Susan, and Discovery Bay.

The abundance of staghorn sculpin, Pacific tomcod, and quillback rockfish was more seasonally variable, and their distribution was more restricted. Staghorn sculpin were most common in the summer and fall in all areas, but were most abundant in the inlets. Within the estuarine bays, staghorn sculpin were most abundant in the shallow waterways. Pacific tomcod were rarely captured during the winter and spring seasons, but were abundant in Budd Inlet and Commencement Bay during the summer and fall of 1979. Quillback rockfish were found typically in low numbers in the deeper parts of central Puget Sound throughout the year.

Biological Characteristics. Only English Sole and rock sole were sufficiently widespread to permit observations of seasonal migration and recruitment. For English sole, the waterways, estuaries, and inlets were important as juvenile rearing areas with the initial settling of the very young fish beginning in the fall and continuing through the winter. The abundance of young English sole was lowest in the spring and summer, suggesting a migration to areas outside of the sampling areas during this time period (Figure 39). Rock sole young-of-the-year did not appear to have such a migration pattern, but were present in the sampling areas during most of the year.

Species Composition. English sole and rock sole were the most frequently caught species among the 10 most abundant species captured throughout the sampled regions of Puget Sound during most of the year. Sand sole (*Psettichthys melanostictus*) were present throughout the seasons, primarily in the inlets and deeper waters of the bays, although some were present in the estuaries. Shiner perch (*Cymatogaster aggregata*), flathead sole (*Hippoglossoides elassodon*), Dover sole (*Microstomus pacificus*), Pacific herring (*Clupea harengus pallasi*), and rex sole (*Glyptocephalus zachirus*) appeared to be present all year in the estuaries. Species consistently present in the inlets included starry flounder (*Platichthys stellatus*), plainfin midshipman (*Porichthys notatus*), Pacific staghorn sculpin, and speckled sand dab (*Citharichthys stigmaeus*). Roughback sculpin (*Chitonotus pugetensis*) were also present during most of the year in many areas.

Discovery Bay, the only sampling area located outside of Puget Sound, had a species composition very similar to that found in the Puget Sound sampling areas.

Species Richness. The estuarine bays (Elliott and Commencement Bays) always had significantly higher numbers ($p \leq 0.05$) of species than any other sampled area. The three inlets (Case, Budd, and Sinclair Inlets) all had typically lower species richness values throughout the seasons and years. Port Susan and Discovery Bay, however, had moderate species richness values, intermediate between the values for estuarine bays and the inlets values (Table 36).

Species Diversity. As was the case with species richness and abundance, the estuarine embayments had the highest species diversity values of all areas for each of the respective sampling periods (Table 36). The inlets had lower diversity values, although the values increased progressively from winter through fall.

3.4.2 Interrelationships between Fish Catch Rates (CPUE) and Sediment Chemical Composition

In a few cases, the CPUE values for some of the target fish species were negatively correlated ($p \leq 0.03$, using the Spearman's rank correlation method) with the relative concentrations of chemicals in the sediment at the sampling stations from where the fish were captured. The CPUE values for English sole were negatively correlated with the chemicals in

the third principal components axis; whereas the CPUE values for rock sole were negatively correlated with the first, second, and fourth principal component axes, and the catch rates for Pacific staghorn sculpin were negatively correlated with the third axis. A positive correlation ($p \leq 0.05$) was observed between the CPUE values for Pacific staghorn sculpin and English sole and the sediment concentrations of chemicals in the fourth axis.

3.4.3 Epihenthic Crustacean Ecology

Two of the target shrimp species (*Crangon alaskensis* and *Pandalus danae*) were captured in all of the sampling areas; although, very few of these species were captured in Port Madison (Figure 40). The other target shrimp species, *P. jordani*, was collected only in Commencement and Elliott Bays and Port Madison; however, this species was the most abundant of the target species.

The most abundant and widely distributed of the target crab species was *Cancer gracilis* (Figure 40). This species was collected in all areas, except Port Madison and Port Susan. *C. productus* was much less abundant, but was found in the same areas as *C. gracilis*, except in Discovery Bay. The distribution of *C. magister* differed from the other two species in that they were collected in Port Madison and Port Susan, but not in Case and Budd Inlets (Figure 40).

3.5 Benthic Infauna Invertebrate Ecology

3.5.1 Field Survey

Benthic infaunal samples were collected from 37 stations in Puget Sound (and adjacent waters) during quarterly collection periods in 1979 and in two collections during 1980. Data concerning the species composition at these sampling stations were analyzed using various statistical and indexing methods. Stepwise multiple regression with backward elimination of variables was used to evaluate which indexing method correlated most highly with the levels of toxic chemicals in sediments from which the animals were collected.

The Infaunal Trophic Index (ITI) was one indexing method used to evaluate species composition. The ITI has been proposed as a community index indicative of pollution effects on benthic marine environments (Word 1978). It has advantages over other methods because it incorporates information on feeding mode as well as taxonomic composition, and it uses an abbreviated taxonomic list which speeds processing of benthic samples. This abbreviated taxonomic list was used not only in the calculation of the ITI but also in the calculation of other indices, including taxon richness, taxon diversity, and taxon abundance. These latter methods provide estimates of species richness (S) and species abundance (A), respectively.

Species or taxa which were found to be the most abundant and widely distributed were the molluscs, Axinopsida serricata and Macoma spp., and the polychaete families, Capitellidae, Spionidae, and Cirratulidae. These organisms were present in at least 31 out of the 38 sampling stations.

The ITI values obtained from individual samples in this study ranged from a low of 13, indicating a community dominated by subsurface-deposit-feeding organisms, to a high of 95, indicating a community dominated by suspension-feeding organisms (Figure 41). Thom et al. (1979) reported that the ITI values obtained from reference stations in Puget Sound ranged from 59 to 82. Values below this range suggest infaunal communities in which subsurface deposit feeders are unusually abundant. ITI values at 14 of the 38 stations were lower than 59 during at least one season. The only stations which had ITI values under 59 for all sample collections were the City Waterway, the Puyallup River, and the Puyallup Disposal Site in Commencement Bay. Although no stations in this study had ITI values consistently higher than 82, the stations with the highest average ITI values were in Case Inlet (83) and near Magnolia Bluff in Elliott Bay (78). A high value of 82 was found at one station in Port Susan (Station 50200). Average ITI values of 71, 72, and 73 were obtained during the winter quarter of 1979 from the deepest sampling sites (135 ft to 155 ft) at West Point, Magnolia Bluff and Alki Point, respectively. These values compare closely to values of 68, 68, and 75 reported by Thom et al. (1979) for the same sites (at a depth of 150 ft) which they sampled during the winter of 1979.

Taxon richness values calculated from the taxonomic groups used in the Infaunal Index ranged from a low of zero to a high of 15 (Figure 42). The quarterly average taxon richness values were 4.3, 6.7, 7.4, and 6.3 for winter, spring, summer, and fall quarters, respectively. The winter quarter data were significantly different ($p \leq 0.05$) from the other quarter's data; however, no other significant differences were seen between spring, summer, and fall data. The highest average taxon richness values were found in sediments from Port Madison (Station 08107, 11.5 ± 2.9), West Point (Station 10023, 11.1 ± 50), and the southeast side of Duwamish Head (Station 10045, 10.6 ± 2.8); while the lowest values were from Budd Inlet (Station 12131, 2.42 ± 1.5), the East Channel of the Duwamish Waterway (Station 10039, 2.9 ± 1.4), and the turning basin in the Hylebos Waterway (Station 09027, 3.0 ± 1.8).

In 1980, additional studies were conducted in the Duwamish (Station 10031), Hylebos (Station 09028) and City (Station 09031) Waterways using 10 replicate, 1000 cm^3 cores per station to obtain an estimate of sampling variability. When the taxon richness values were analyzed using the methods of Gaufin et al. (1956) it appeared that six replicate cores (the number of cores taken at each station during the 1979 portion of this study) provided 90 to 95% of the Infaunal Index species which 10 replicate cores would provide. Thus, at least for these stations, less than one new Infaunal Index taxon would likely be added by taking four additional core samples.

The taxon richness and ITI values obtained for the benthic samples collected in 1980 (summer) were compared statistically to the values obtained for samples from the same three stations in the summer of 1979 (Table 31). No significant difference ($p \leq 0.05$) was seen in taxon richness values calculated for the three stations between the two sampling periods. ITI index values were not significantly different between the two sampling periods at the stations in City and Hylebos Waterways, but a significant difference was seen between the two sampling periods at the Duwamish River site. This difference was probably due to the presence of suspension-feeding sabellid worms which were common in summer of 1979 samples and absent in summer of 1980 samples, and to the presence of numerous surface-deposit-feeding *Macoma carlottensis* in summer of 1980 samples which were nearly absent in summer of 1979 samples taken at this site.

The abundance of fauna per 1,000 cm^3 sediment sample averaged over each quarter in 1979 was 31.42, 35.12, 46.97, and 52.00 for winter, spring, summer, and fall sampling periods, respectively. There was no significant difference between these values ($p \leq 0.05$).

Little variation was seen in water temperatures during each sampling season; however, average temperatures rose from 6.6°C to 12.6°C during the period from winter through fall. Salinity varied less than 2 ppt between stations and between seasons at most stations during the entire study. No seasonal or between-station trends in oxygen levels were observed. Sediment grain analysis showed that 9 of the 34 infaunal sampling stations had a sand-to-mud ratio greater than 1.0, indicative of sandy substrate. All other stations contained predominantely mud. Total organic carbon values ranged from 0.14 to 6.98%.

3.5.2 Interrelationships between Community Parameters and Sediment Chemical Composition

The mean values for taxon richness and ITI obtained during this study were calculated for the sampling stations in each of the cluster groups (see Table 25 for a list of the stations within each cluster group). For taxon richness, cluster group stations 3, 4, and 7 had significantly ($p \leq 0.001$) lower values than did the other cluster groups (Figure 43). The mean taxon richness value for cluster group 1 was statistically higher than any of the other cluster groups ($p \leq 0.05$). The mean ITI value for stations in cluster group 1 was significantly higher ($p \leq 0.025$) than the mean ITI values for the other six cluster groups, and 2, 3, 4, 5, 6, and 7 were indistinguishable from each other ($p < 0.05$).

These comparisons of taxon richness and ITI values demonstrate some correspondence between the two benthic community parameters. The mean values of both indices were high at stations in cluster group 1, and low in cluster group-7 stations. Little agreement between the two indices was observed in the other five groups. Therefore, at stations populated by a high proportion of suspension feeders, as indicated by a high ITI value, a large number of taxa was observed. The reverse was true in the case of stations inhabited largely by deposit feeders, i.e., a low ITI value.

The relationships between abiotic factors (chemical concentrations, sediment grain size, etc.) and several benthic invertebrate community indices (particularly taxon richness and the Infaunal Trophic Index) were investigated using stepwise multiple regression, a multivariate statistical method. The primary statistical methods used in the infaunal benthic community study were the computation of simple correlation coefficients (r), coefficients of determination (r^2), multiple correlation coefficients, and multiple coefficients of determination (Kleinbaum and Kupper, 1978). These methods were chosen because all variables were continuous or approximately continuous and because it was desirable to take variability, as well as average value, into account.

The aim of these statistical analyses was to determine the nature (positive, negative, or no correlation) and the strength (measured by r^2) of the relationship between several biological community indices and the chemical and physical characteristics of the bottom sediment. The coefficient of determination (r^2) is the percentage of variation in a dependent variable such as the number of taxa collected at particular sites which can be "explained" on the basis of the physical or chemical characteristics of those sites. All physical and chemical variables measured at each sampling station during 1979 were included in the analysis except temperature and salinity (which were relatively constant at all stations during any given season). The chemicals included in the analysis were the same as those used in the principal components analysis and cluster analysis of the station data. Only biological samples collected at the same site as the chemical samples were collected were used in the analysis.

Multiple regression analysis was performed using backward-stepwise selection to remove any variables which did not contribute significantly to the regression equation at the 0.05 significance level when entered after all other variables. The significance level of 0.05 was also chosen for the overall regression equation. To investigate relationships between the community indices and the abiotic variables, these variables were divided into eight classes (A through H) on the basis of their chemical affinities and their simple correlation coefficients with taxon richness. All correlation and multiple regression analyses were performed using the computer programs of Nie et al. (1975). Data management used the programs of Robinson et al. (1979).

All of the community indices were significantly correlated ($p<0.001$) with the concentrations of chemicals in classes A, B, and C, and with the physical variables in class H (Tables 38 and 39). The overall r^2 for all of the variables being tested was highest for taxon diversity (0.59). The ITI ranked second (overall $r^2 = 0.56$) and taxon richness ranked third (overall $r^2 = 0.48$). All of the community indices were significantly correlated with the concentrations of chemicals in classes A, B, C, and D, and with the physical variables in class H. In general, the strongest correlations with chemical variables were with those of class A. The ITI was most strongly correlated with the variables of class B, and was less strongly correlated with the aromatic hydrocarbons of class D than the other indices.

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Taxon richness was negatively correlated with all variables (see Table 38) included in the overall analysis except the chemicals of class C and several physical variables in class H, with which it was positively correlated. Taxon diversity was negatively correlated with all of the variables in classes A, B, and F, and positively correlated with the variables in class C. Total abundance was also negatively correlated with all of the variables in classes A and B. Taxon evenness was positively correlated with all of the variables in classes C, D, and E. The ITI was negatively correlated (without a significant overall correlation) with all variables in classes F and G; other classes included some variables with negative and some with positive simple correlations with this index.

Although taxon diversity was the "best" index (in terms of having the strongest overall correlation with the chemical and physical variables), taxon richness is closely related to taxon diversity from a mathematical standpoint (Table 4) and has the advantage of simplicity of interpretation (being merely the number of different taxa present in a sample). For this reason, our results are reported primarily in terms of taxon richness rather than taxon diversity in the remainder of this report. In general, results obtained using taxon richness are qualitatively similar to those based on taxon diversity.

A different statistical method, the Spearman rank correlation (a nonparametric method), was used to evaluate the relationships between infaunal benthic invertebrate community indices and the relative concentrations of chemicals in sediment. In these tests, values of the community indices were compared with the factor scores for each sampling station (from which the infauna were collected) on each of the four axes obtained by the principal components analyses of sediment chemistry data (see Table 24).

Taxon diversity and taxon richness were most strongly correlated (negatively) with the second and third principal components of the sediment chemistry data (Table 40 and Figure 44). Both indices were also correlated with the first principal component, but less strongly. Total abundance was strongly correlated only with the third principal component. The Infaunal Trophic Index was the only index correlated with the fourth principal component, and was not correlated with the first three components.

3.6 Effects Studies

The primary objectives of the effects studies, were to develop experimental procedures and design enclosures for exposing crabs and molluscs to natural environments and to determine whether crabs and molluscs at sites with high levels of chemical contaminants were adversely affected.

3.6.1 Crab Exposures

During the initial 6 weeks of the first experiment involving *in situ* exposures of caged crabs, all of the crabs in the Hylebos and Duwamish Waterways and near the Seattle Aquarium survived. One of the eight crabs in the City Waterway died between the fourth and sixth week of the experiment. At Port Susan, the reference site, two crabs died during the first week of exposure. These two crabs were replaced by new crabs from the same group initially used as a source of crabs for all the cages. By 6 weeks, one more crab at Port Susan died.

After 8 weeks of exposure, the caged-crab experiment was terminated. By this time, six of eight crabs were dead in the Duwamish Waterway, three of eight were dead in City Water, and three of ten crabs held in Port Susan had died. All animals survived in the Hylebos Waterway and at the Seattle Aquarium. The two crabs remaining in the cages (one crab per cage) in the Duwamish Waterway were each missing four or more legs. Only the carapaces of the six dead crabs remained. One dead crab and two carapaces were found in the cages from the City Waterway.

Eight-week survival at the five locations was analyzed using analysis of variance to test the null hypothesis that survival was the same at all sites. For the purposes of this analysis, all mortalities at Port Susan were assumed to have occurred among the eight crabs originally placed in the cages at that site; the two crabs added 1 week later were omitted from totals for survivors in those cages. The null hypothesis was rejected ($F=31.0$, $p<0.005$), and so mean survival at each site was compared using the Student-Neuman-Keuls multiple range test (Zar 1974). Mean survival in the Duwamish Waterway was significantly lower than that at Port Susan ($p<0.01$) (Table 48). Mean survival at Port Susan could not be distinguished from that in the City Waterway, but mean survival at both of those sites was significantly less than that at either Seattle Aquarium or Hylebos Waterway ($p<0.05$).

In general, histopathological examination of the survivors of this first experiment demonstrated cellular changes both in crabs caged in contaminated areas and in crabs caged at Port Susan (reference site); the frequencies of abnormalities were not significantly different among the groups. One change, however, that was not observed in reference crabs, involved tubular metaplasia of the hepatopancreas. This lesion was found only in crabs held near the Seattle Aquarium (25%, 2 of 8), in the Hylebos Waterway (25%, 2 of 8), and in the City Waterway (14%, 1 of 7).

Chemical analyses were performed on samples of crab hepatopancreas from caged crabs kept in the Hylebos Waterway for 8 weeks, and from crabs freshly captured in Mutiny Bay. Sufficient tissue was available only for analyses of organic compounds by GC/MS. The results of these analyses are presented in Section 3.1.7.

The second experiment involved holding C. gracilis in cages in the Duwamish Waterway (Station 10031) and near our research facility in Mukilteo for 9 weeks. The crabs were fed at 3- to 4-day intervals. After exposure for 8 weeks, six of eight crabs survived at Mukilteo (two crabs were accidentally crushed during the first week) and seven of eight crabs survived in the Duwamish Waterway (one crab was missing after 4 weeks).

3.6.2 Mollusc Exposures

The molluscs held in cages were collected after approximately 9 weeks (71 days). The number of mollusks in each cage was determined, and one half of the animals were necropsied and the tissue excised for histopathological examination. Because both cages at the Port Susan site had been separated from the bottom sediment, probably due to strong currents, the health status of these animals was not included in the experimental data. Good survival of Macoma sp. was generally observed at the sites in the Duwamish, Hylebos, and City Waterways, and near the Seattle Aquarium. The soft tissues of the remaining half of the animals were analyzed for organic compounds. The results of analyses of tissues from M. nasuta held in the Hylebos Waterway for 71 days are reported in Section 3.1.7.

3.6.3 Sediment Recolonization

Mean taxon richness values in the first recolonization experiment are given in Table 12. Significantly more taxa colonized the Port Susan sediment than the Duwamish Waterway sediment at the Duwamish Waterway location ($p<0.05$, Mann-Whitney U test). Port Susan sediment was also colonized by more taxa than the local sediment at two other locations (Hylebos Waterway and City Waterway), and by fewer taxa at the fourth location (Seattle Aquarium); however, none of these differences were statistically significant ($p\leq0.05$).

In the summer of 1981, a second, small-scale recolonization experiment used sediment taken from the Duwamish Waterway and Discovery Bay. This sediment was frozen to kill the infauna and then thawed.

Although identification and enumeration of specimens collected in the second recolonization experiment have not yet been completed, one difference between the experiments was immediately apparent: 11 pandalid shrimp (primarily Pandalus danae) were found in the Discovery Bay sediment containers (range 1 to 6 per container), while only a single ghost shrimp (Callianassa californiensis) and no pandalid shrimp were found in the Duwamish Waterway sediment containers. This difference in pandalid abundance was statistically significant ($p<0.05$, Mann-Whitney U test).

4. DISCUSSION

In addition to reviewing the most important aspects of the results of each phase of this study, comparisons between the biological data and analytical chemistry data will be discussed below. The value of comparisons between biological and chemical parameters is limited in part because not all sediment-associated chemicals were, or can presently be, identified or quantified. However, as a first step in maximally utilizing the present data for indications of causes and effects, such comparisons are considered to be justified. An additional problem of comparing sediment chemistry data with biological data, such as lesion prevalence in fish and crustacea, relates to the mobility of these animals. Even though the target species are not known to migrate great distances within Puget Sound, the exposure times of fish and crustacea at the sites of capture are not known. Also, even if one or more of the identified chemicals in the sediment directly or indirectly caused some of the observed biological anomalies, the mechanisms of action may have involved synergistic and antagonistic interactions between a variety of other chemicals (e.g., a metal acting synergistically with a chlorinated hydrocarbon).

4.1 Chemical Analyses

4.1.1. Chemicals in Sediments

Based on the initial findings of this study (Malins et al., 1980), a list of chemicals (including AHs, PCBs, CBDs, chlorinated hydrocarbon pesticides, and metals) indicative of environmental contamination was developed. These chemicals have a demonstrated toxicity in aquatic organisms (Konasewich et al. in press). The present report provides additional information on chemical analyses of sediment samples from areas not included in the previous report (e.g., near Bellingham, Everett, Port Angeles, and Shelton).

AHs, PCBs, and CBDs were widely distributed in Puget Sound, but the concentrations varied extensively both among embayments and within embayments. The sediments in embayments adjacent to the most populated areas, Elliott and Commencement Bays, contained the highest concentrations of AHs and PCBs. Scores of halogenated compounds in addition to PCBs and CBDs were associated with specific sampling sites, for example, Tacoma, Port Angeles, Bellingham, and Everett. The identities of many compounds have not been confirmed because reference mass spectra have not been published, and many compounds remain unidentified because the mixtures are so complex that proper mass spectra could not be obtained for interpretation and identification. Some of these chemicals probably are waste products from chemical manufacturing. For example, high concentrations of CBDs in sediment found in Commencement Bay are probably related to industrial activity, past or present. Wide ranges in the concentrations of CBDs (and other chlorinated compounds) were found in sediment samples collected from the Hylebos Waterway and Old Tacoma stations that suggest pockets of these chemicals. Such pockets probably exist as a result of methods of chemical waste disposal.

The aromatic hydrocarbon retene, detected in sediments collected near Bellingham, Everett, Shelton, and Port Angeles, is probably a waste product from wood pulp industries. Retene results from the dehydrogenation of abietic acid, a rosin acid (Fieser and Fieser 1949). Another compound related to rosin acids, ferruginol, was also found in the sediment sample collected near Everett.

Arsenic and mercury were present in high concentrations only in urban areas, whereas lead was high in both urban and reference areas. Arsenic and mercury are probably source-specific, while lead is ubiquitous, entering the environment from many sources, e.g., from the use of tetraethyl lead in gasoline.

4.1.2 Chemicals in Biota

Many of the chemicals identified in sediments from Elliott Bay and Commencement Bay were also present in the demersal, benthic, and pelagic organisms examined. The demersal species, such as English sole, clearly accumulated much higher concentrations of chemicals than the semi-demersal cod and pelagic salmon species. These differences may be related to the fact that the demersal species live in intimate contact with sediments that contain substantially higher concentrations of chemicals than the surrounding seawater. Significant differences in the food organisms and the chemical contamination of the food organisms between the two types of fish are also suspected of playing an important role in governing the tendency to accumulate chemicals. In general, benthic organisms from urban-associated areas contained substantially higher concentrations of chemicals in tissue than those from nonurban (reference) areas.

It is important to emphasize that the aromatic hydrocarbons are readily converted to other products in marine organisms. In fact, the finding that gas chromatograms of AHs in biota were substantially less complex then those in sediment appear to reflect the influence of hydrocarbon metabolizing enzyme systems. However, the data do not reflect concentrations of hydrocarbon conversion products, some of which are believed to initiate long-term damage (e.g., benzo[a]pyrene), including tumor formation. The findings clearly stress the need to develop techniques to analyze such compounds.

Studies with caged crabs and molluscs kept in Commencement Bay indicated that significant differences exist between the species in their tendency to accumulate AHs. Specifically, the concentration of ΣAHs in caged clams was about 40 times greater than in caged crabs. Although a number of factors may have contributed to this difference, it is likely that the more active metabolizing enzyme system of crabs, which increased AH metabolism and/or excretion, contributed to the lesser accumulation of AHs in these invertebrates.

PCBs, which are widely distributed in Puget Sound, were generally found in higher concentrations in biota from urban-associated areas. These compounds are readily bioconcentrated by marine organisms and are resistant to metabolism. Almost invariably the concentrations of PCBs

in biota were substantially greater than those in sediment. The crab hepatopancreas and English sole liver contained particularly high concentrations of PCBs and are thus particularly useful organs in appropriate species for assessing the cumulative impact of industrial chemicals in marine organisms.

Concentrations of chlorinated hydrocarbon pesticides were not markedly different in biota from urban-associated and reference areas. It is important to note that complex mixtures of nontarget chlorinated compounds were detected in biota from Commencement Bay, particularly the Hylebos Waterway. Compounds that were positively identified, such as CBDs and HCB, were also found in all biota examined from the Hylebos Waterway. The relatively high concentrations of CBDs and HCB in benthic species from the Hylebos Waterway are attributed to uptake from sediments that have acquired these compounds from industrial inputs. The low concentrations of CBDs and HCB in the muscle of salmon and cod from Commencement Bay indicate that these more pelagic fish are less reflective of the pollution condition of the Bay than are the benthic organisms. The findings support the conclusion that the nature and distribution of chlorinated organic compounds in Puget Sound biota are exceedingly complex.

Of all the metals analyzed, only arsenic was present in moderate to high concentrations in the muscle of cod (ca. 2,700 ppb) and salmon (ca. 700 ppb), and in high concentrations in English sole muscle (ca. 6,000 ppb) from the Hylebos Waterway, Duwamish Waterway, and even the reference area. Recognizing the toxic properties of arsenic (e.g., a carcinogen), it appears that the concentrations of this metal in edible tissue of Puget Sound organisms should be examined further.

Overall, it should be pointed out that no credible data presently exist to permit an assessment of how the chemicals are actually affecting the health of Puget Sound organisms or human consumers. The extreme complexity of the chemicals, together with the fact that many unidentified substances also exist, makes the task of establishing cause-and-effect relations very difficult. Additional problems in interpreting the results arise from the fact that interactions between chemicals alter the biological effects of single compounds (Malins and Collier 1981). Accordingly, solutions to the confounding problems of relating chemicals to biological change in Puget Sound will require a well-conceived intermeshing of appropriate laboratory and field investigations.

4.2 Fish Pathology

One of the principal objectives of this study was to determine if marine fish captured in polluted areas possessed unique lesions or disease conditions that occurred primarily in those areas. One approach in this determination is to compare the types and frequencies of pathological conditions in fish from urban and nonurban (reference) areas or from the most chemically-contaminated and the least-contaminated

areas. Since English sole and rock sole were the most abundant and widely distributed fish species examined in this study, they are the most appropriate for this comparison.

Liver neoplasms, found in both species, were restricted to fish from the most chemically-contaminated urban areas. This limited occurrence of hepatic neoplasms in English sole and rock sole suggests that a relationship exists between the presence of sole with these pathological conditions and the chemical contaminants in the sediments. The pattern of occurrence of two other types of hepatic lesions, "preneoplastic" and specific degenerative/necrotic lesions, is also suggestive of such a relationship. However, the distinction between the prevalence of fish with these lesions in urban and in nonurban areas is less clear-cut than with neoplasms. "Preneoplastic" lesions are predictive only in English sole. Fish with "preneoplastic" lesions were most prevalent (statistically significant at $p \leq 0.05$) at stations in the most contaminated urban areas; however, two affected English sole were found in Discovery Bay (a reference area). Similarly, English sole and rock sole with specific degenerative/necrotic lesions were most prevalent in the most contaminated urban areas, but one affected English sole was also found at a station in Port Madison.

The reasons why some fish with these apparently pollution-related lesions were restricted to certain stations in urban areas, while sole with other lesions had a slightly broader geographical distribution, are not clear. In the latter case, a sole may be exposed to the causative agent(s) in an urban area and subsequently migrate to a reference area. Also, the lesions could be caused by low concentrations of chemicals present at relatively higher levels in urban areas and at lower levels in reference areas. Alternatively, a variety of other factors (infectious organisms, physicochemical, and physiological) may cause these lesions, and while these factors are present in both urban and nonurban areas, more of these factors may be present in urban areas.

An important progression from finding several types of lesions and syndromes in Puget Sound fishes is to attempt to define possible causes of these conditions. Three basic approaches can be used to help identify cause-and-effect relationships: (1) the histopathological and physiological characteristics of affected fish can be compared with similar characteristics described in the scientific literature for laboratory animals (i.e., rodents and fish) exposed to specific toxic chemicals or infectious agents; (2) a technique commonly used in epidemiological studies can be employed in which statistical methods are used to correlate the prevalence of fish having certain types of lesions with environmental concentrations of particular classes of toxic chemicals; and (3) normal fish can be exposed to suspected causative agents (either individually or in combination) under controlled laboratory conditions, and the similarities between observed effects and lesions found in the field can be evaluated.

Histopathological conditions similar to the lesions observed in this study have been reported in other animal species exposed to toxic chemicals under laboratory conditions. The hepatocellular neoplasms resemble nodules induced by DDT, dieldrin, acetylaminofluorene, nitrosamine, and phenobarbitone in mice (Jones and Butler 1975). Similarly, trabecular carcinomas induced by aflatoxin B_1 in rainbow trout (Salmo gairdneri) displayed a similar cytologic morphology (Ashley 1967, Sinnhuber et al. 1977). Proliferation of bile duct epithelial cells has been described commonly in a variety of lesions induced by toxic chemicals. Kasza et al. (1976) reported bile duct hyperplasia in rats treated with dietary PCBs. Kimbrough et al. (1972) described adenofibrosis, the most prominent hepatic lesion in rats exposed to PCBs, as possibly cholangioproliferative. Rainbow trout, mammals, and birds exposed to dietary aflatoxin all developed bile duct hyperplasia and proliferation, with and without fibrosis (Ashley 1967, Simon et al. 1967, Newberne 1967). Arsenic, which accumulates in the liver and kidney of mammals and is excreted in the bile, has been linked with various bile duct changes (Beliles 1975).

Rats, mice, rabbits, and monkeys exposed to acute and chronic levels of commercial mixtures of PCBs have developed lesions with characteristics similar to the specific degenerative/necrotic lesions found in this study (Koller and Zinkl 1973, Nishizumi 1970). Hepatocytomegaly was also reported in channel catfish (Ictalurus punctatus) acutely and subacutely exposed to Aroclor mixtures (Hinton et al. 1978), and in rainbow trout exposed to dietary cyclopropenoids (J. Hendricks, Oregon State Univ., Pers. Comm.). In all these cases, cellular enlargement was a result of accumulation of intracellular products, such as glycogen or lipid, and proliferation of endoplasmic reticulum. Nuclear enlargement can be caused by derangements in the mitotic process, and is a common feature in cellular atypia in animals exposed to toxins (Zimmerman 1978).

Mice treated with phenobarbitone and other microsomal-enzyme-inducing drugs exhibited marked cytomegaly and cellular hypertrophy, originating in the centrilobular regions of livers (Jones and Butler 1975).

The nonspecific degenerative/necrotic liver lesions widely distributed in Puget Sound have also been described in other species exposed to toxic chemicals. Nonspecific hepatocellular necrosis is a common response to poisoning in many vertebrates (Plaa 1975), including teleosts. Couch (1975), and Walsh and Ribelin (1975) found coagulation necrosis to be a general reaction in fish exposed to a spectrum of compounds, including organophosphate, organochlorine and chlorinated cyclodiene pesticides and PCBs. A variety of metals, including arsenic, chromium, silver, indium, iron, molybdenum, selenium, tellurium, and thallium, also possess hepatotoxic properties in humans and mammals (Beliles 1975).

Suspected "preneoplastic" liver lesions have been demonstrated in dogs and rats administered dietary Aroclor (Fishbein 1974, Kimbrough et al. 1972, Kasza et al. 1976). Newberne (1967) reported that aflatoxin-exposed mammals and birds developed bizarre pleomorphic nuclei. Cutthroat trout (Salmo clarki clarki) exposed to organochlorine pesticides in both the water and diet demonstrated several toxic liver changes including cellular and nuclear pleomorphism (Eller 1971).

A variety of agents are capable of injuring the functional segments of the teleost nephron, mainly the glomerular and the proximal tubular systems (Trump et al. 1975, Hook 1980, Suzuki et al. 1963). Our study revealed examples of injury to both of these systems, with mesangiolysis representing a primary glomerular lytic lesion, whereas degeneration/necrosis involved the tubular system. Deposition of normal or abnormal products in the renal glomerulus can result from a host of etiologies (Schillings and Stekhoven 1980).

Proliferative lesions of the gill, such as respiratory epithelial hyperplasia (REH), are common, nonspecific responses to many irritants. Chronic exposure to ammonia, a pollutant frequently discharged into river systems via domestic waste waters and agricultural and urban drainage water, has been known to cause epithelial proliferation and aneurysms in the gills of salmonid fishes (Burrows 1964, Smith and Piper 1975). Gill epithelial proliferation has also been reported in fish exposed to pesticides such as mirex (Van Valin et al. 1968), parathion (Walsh and Ribelin 1975) and toxaphene (Lowe 1964); herbicides including dichlobenil (Cope et al. 1969), diuron (McCraren et al. 1969) and endothal (Eller 1969); and heavy metals such as iron salts (Ashley 1979). Additionally, REH has been associated with infectious agents such as bacteria, parasites, and fungi (Eller 1975).

Another approach in evaluating cause-and-effect relationships between the prevalence of English sole and rock sole with hepatic lesions and concentrations of chemical contaminants in their environment was presented in Section 3.2.9. A statistical method (the G-statistic) was used to test the null hypothesis that the prevalence of affected sole from a particular cluster group did not differ from the average prevalence of similarly affected sole for all of the cluster group stations. Sole with the three types of hepatic lesions mentioned above, neoplasia, "preneoplasia", and specific degeneration/necrosis, were consistently most prevalent at cluster group-4 stations (the Duwamish Waterway) and/or 7 (Sitcum and Hylebos Waterways). The relative concentrations of the four basic groups of chemicals [aromatic hydrocarbons (arenes), metals, PCBs, and other chlorinated compounds] found in sediment are best depicted in Figures 15 to 17. Cluster group-4 and cluster group-7 stations had relatively high concentrations of metals and intermediate levels of aromatic hydrocarbons (Figure 15). As can be seen in Figure 16, cluster group 4 stations had relatively higher levels of PCBs than did the cluster group 7. The concentrations of other chlorinated hydrocarbons were relatively low at cluster group-4 stations, whereas the relative concentrations of these hydrocarbons were low in some

stations in cluster group 7 and high in others (Figure 17). Therefore, the groups of chemicals which were consistently present at moderate-to-high levels at both groups of stations were the aromatic hydrocarbons and the metals.

Another statistical approach to examining the relationships between the prevalence of fish with certain types of liver lesions and the chemical composition of the sediments at sampling stations from which they were captured is presented in Section 3.2.9. This approach involves the use of the nonparametric method of Spearman rank correlation. In the cluster group analyses, the sampling stations were arranged into the cluster groups and a statistical method (the G-statistic) was used to test whether fish with certain lesions were more prevalent at certain cluster group stations. The correlation approach is a more detailed method of determining if the prevalence of fish with specific lesion types at all the sampling stations were positively or negatively correlated with the relative concentrations of the chemicals on each of the four principal components axes. The results of this latter statistical technique showed that the prevalance of English sole with liver neoplasms, specific hepatocellular degeneration/necrosis, and hepatocellular storage disorders was positively correlated with the relative sediment concentrations of those aromatic hydrocarbons in the first principal components axis and those metals in the second principal components axis. These positive correlations agree in general with the results obtained with the cluster group analysis; that is, these diseases were more prevalent at stations where concentrations of sediment-associated aromatic hydrocarbons and toxic metals were high.

The finding of statistically significant positive correlations between the prevalence of liver lesions in English sole and these two classes of sediment contaminants emphasizes the complexities of defining cause-and-effect relationships in the marine environment. First, the actual causative factors of these liver lesions could be other than the AHs and metals on the principal component axes. The causative factors may be chemicals not measurable by the analytical procedures used in this study, but whose presence and abundance are correlated with the above-mentioned AHs and metals. Secondly, the fact that two chemically different classes of sediment contaminants are correlated with the prevalence of fish with similar lesions suggests that these classes of chemicals may interact in causing the lesions.

Comparisons between lesion prevalence and the concentrations of xenobiotics in tissues of diseased animals would also be useful in evaluating cause-and-effect relationships. This approach is presently not appropriate for two major reasons: (1) chemical analyses were performed on too few fish; and (2) a major group of xenobiotics, AHs, are rapidly metabolized by fish and are not detectable by routine chemical analysis of fish tissues.

The third approach which can lead to a better understanding of the causes of the lesions observed in sole would involve exposing normal sole under laboratory conditions to toxic chemicals suspected of being

causative agents. The selection of these agents is facilitated by the results of the other two approaches to evaluating cause-and-effect relationships. As important as these laboratory experiments would be to evaluating the causes of these lesions, they are technically most difficult for the following reasons: (a) the long-term culture of sole under conditions mimicking the natural environment is difficult, and (b) because culture of English and rock sole from the egg to adult is presently not feasible, experimental animals must be collected from wild stocks which have a high degree of genetic heterogeneity or may have had previous contact with toxic chemicals. Another extremely important consideration in the search for cause-and-effect relationships between certain types of lesions in fish and environmental factors is the role of species specificity. Different species of fish are known to respond differently when exposed to the same xenobiotic (Roubal et al. 1978). For example, liver lesions in English sole characterized as specific degeneration/necrosis may be caused by a completely different set of factors than similar-appearing lesions in Pacific staghorn sculpin.

4.2.1 Age/Length/Weight Relationships between Fish with and without Idiopathic Hepatic Lesions

The predominant trend of homogeneity of weight and length between fish possessing and lacking liver lesions indicates that there is little or no apparent impairment of feeding and/or ability to metabolize and utilize food material in diseased animals. It is possible that differences may exist between animals of the exact same age, but this cannot be analyzed since ages are determined only to the year, not month.

The predominant pattern of low lesion incidence in English sole less than 2 years old and high lesion incidence in sole aged 6 years or older may be related to the time required for morphological expression of a disease. For example, a latent period between exposure to the etiological agent(s) and histological evidence of the disease may exist. Visibly diseased tissue may not be produced until a cumulative level of biochemical change or dysfunction is reached. No pronounced age-related pattern of lesion prevalence was observed in rock sole. The marked differences between the patterns of lesion prevalence by age of English and rock sole may have been a result of physiological and/or behavioral differences between the two species. Rock sole may respond differently to a particular disease agent than English sole. Equally, rock sole may feed, move, and interact with the environment to produce exposure times and levels different from that of English sole.

4.2.2 Comparisons with Other Field Surveys

Previous studies of abnormalities in English sole and starry flounder have been conducted in the Duwamish Waterway. In a study performed in 1974 and 1975, Wellings et al. (1976b) reported fin erosion in 8% of the starry flounder and in 0.5% of the English sole. Pierce et al. (1978 and 1980) and McCain et al. (1977) reported the results of a similar field survey conducted in 1975 and 1976 in which liver abnormalities in both English sole and starry flounder were found.

English sole were reported to have hepatomas at a prevalence of 32% (20 of 62), together with other liver abnormalities. Starry flounder had similar abnormalities, but no liver neoplasms were observed.

In a more recent investigation performed between 1978 and 1980 in the Duwamish Waterway, McCain et al. (1982) continued to characterize the prevalence, geographical distribution, and histopathology of liver abnormalities in flatfish species, as well as the chemical composition of fish tissues and bottom sediments. The results of this study differed somewhat from the previous studies. The prevalence of fin erosion was 1% in English sole and 3% in starry flounder. The prevalence of liver neoplasms in English sole was 19%; however, this reduction from the previously observed prevalence of 32% was in part due to the fact that liver neoplasms are more frequently observed in adult fish, and the early study emphasized adults, while the latter study was concerned with all age groups. Also, 1% of the starry flounder had liver neoplasms.

Liver neoplasms have also been reported in feral marine fish in other waters. Smith et al. (1979) reported hepatocellular carcinomas in Atlantic tomcod (*Microgadus tomcod*) in the Hudson River estuary in New York. Falkmer et al. (1977) found cholangiocellular carcinomas in Atlantic hagfish (*Myxine glutinosa*) near Sweden. Both of these areas were reported to be heavily contaminated with PCBs.

4.2.3 Hematology and Blood Chemistry of Fishes

The results of the hematological tests demonstrated a marked relationship between liver-associated pathological lesions and abnormal differential blood cell counts in English sole. Most fish with liver, kidney, gill, and heart lesions had lowered percentages of lymphocytes and increased percentages of thrombocytes and granulocytes. The mechanisms involved and whether or not the hematological changes are severely detrimental to the health of the organisms are not presently known.

4.3 Invertebrate Pathology

Even though shrimp and crabs with histopathological lesions of the gill and hepatopancreas were commonly found in urban areas, the significance of these observations is not presently known due to the low numbers of animals captured and examined during this study. Gill lesions (necrosis and/or melanized nodules) in *Crangon alaskensis* and *Cancer* crabs were commonly observed in areas of urban embayments with the highest levels of a variety of sediment-associated chemical contaminants. A number of heavy metals have been shown to cause lesions in the gills of crustaceans (Couch 1979). Cadmium (760 µg/liter as $CdCl_2$) exposure for 9 days or longer caused grossly observable blackening of the gills of pink shrimp (Nimmo et al. 1977) and death occurred after exposure for 30 days. Histopathological studies (Couch 1979) revealed that the blackening was caused by necrosis of the subcuticular tissues (gill epithelium) in the distal gill filaments, subsequently involving the entire filament. The formation of melanized nodules is characteristic

of the arthropods. Melanized nodule formation has been observed in insects as a response to a variety of agents including various types of particles injected into the haemocoel (Salt 1970). Melanized nodules have been observed within gill lamellae of penaeid shrimp experimentally infected with the pathogenic fungus Fusarium sp. (Solangi and Lightner 1976).

The midgut is an organ probably most vulnerable to both chemical damage and microorganism invasion because it is the only portion of digestive tract not lined with chitin. Release of substances from the hepatopancreas and anterior ceca takes place here as well as various digestive activities. In the more heavy industrialized geographic areas, crabs developed idiopathic lesions of the midgut. These lesions ranged from small melanized nodules to large granulomas in the wall. These lesions were absent in crabs from other subareas. In this organ, as in the gill, melanization is the crab's response to various sorts of injury.

The antennal gland in crustaceans is the final portion of the excretory system, probably with function similar to the proximal tubule of the vertebrate kidney. It is involved in the movement of fluid across the epithelium and reabsorption of proteins. These functions are obviously impaired when the organ becomes necrotic.

The hepatopancreas is an organ of absorption and storage; it also produces digestive enzymes. The principal microscopic abnormality observed in crab and shrimp hepatopancreas was necrosis and encapsulation of the epithelium, this being a common response to chemical injury in crustaceans (Fontaine et al. 1975).

There is considerable debate as to whether animals stressed by unfavorable environmental conditions are more susceptible to infectious diseases. In the present study, the mycotic infections in P. danae in the industrial area of Tacoma may have been secondary invasions of gills damaged by chemical injury. Other infectious diseases may or may not have been related to environmental stress. For example, microsporidan infections occurred in shrimp from a number of areas, but they were most common in Sinclair Inlet. In addition, the trematode infections and the suspected yeast infections of shrimp were found only at this location. Multiple infections occurred with two or all three of the infectious organisms present.

These studies are to be considered preliminary. However, they do show some apparent trends; that is, lesions and diseases of selected crabs and shrimp appear to be more common in industrialized areas.

4.4 Fish and Epibenthic Crustacean Ecology

4.4.1 Fish Ecology

On the basis of catch rates, species composition, species richness, and species diversity, the eight study areas fall into three categories that may be considered separate ecological habitats for the demersal and

midwater fishes sampled. These categories conform to the hydrographical areas previously mentioned: (1) the estuarine embayments (Elliott and Commencement Bays and Port Susan), (2) the open bays (Port Madison and Discovery Bay), and (3) shallow-water inlets without major contributing rivers (Case, Budd, and Sinclair Inlets). Total species seasonal catch rates were highest in the estuarine embayments. The open bays had moderate-to-high total catch rates, particularly in the fall of 1979 at Port Madison and in the fall of 1980 at Discovery Bay. During each sampling period, the total catch rates in Case, Budd, and Sinclair Inlets were generally the lowest.

The presence of major rivers in the estuaries make them unique habitat types. The presence of the longfin smelt virtually only in the estuarine embayments, i.e., in Elliott Bay, Commencement Bay and Port Susan, illustrates the uniqueness of the species assemblage in this type of habitat. Additionally, salmon and steelhead trout frequent these areas during their migrations, but were not frequently captured by the otter trawl. Juveniles of other fish species also utilize these nutrient-laden areas. English sole particularly had high proportions of young-of-the-year in the shallow waterways near the estuaries. The lower salinity of these estuaries changes the community by restricting the more stenohaline marine fish such as sand sole to deeper waters of the estuarine bays. These stenohaline species were also captured more frequently in the shallow waters of the inlets.

The open bays tend to have more midwater species than the other two habitats and the anadromous species normally inhabiting the estuaries are absent. Port Madison particularly had the schooling, midwater canary rockfish (*Sebastes pinniger*) common in the catches, and the midwater sablefish (*Anoplopoma fimbria*) were common in the catches in Discovery Bay. The inlets tended to have many marine fishes common to Puget Sound, with the open water species and euryhaline species being virtually absent.

Species richness values were greatest in the major estuarine embayments. Port Susan had a remarkably high species richness especially considering that only one major sampling effort in this area yielded 25 species of fish. The inlets typically had one half of the number of species that were collected in the estuarine bays. The open bays had moderate numbers of species.

The pattern of species diversity values was similar to that for species richness, except the inlets tended to have high diversity values. These high values were due in part to the fact that the same species of fish were consistently captured in the inlets during each collection.

The pathological and ecological inter-area comparisons of fishes assume that these fishes inhabited their respective areas all year. The recruitment patterns of the flatfishes and tomcod certainly suggest mass movement to other, presumably deeper, water and do not preclude some inter-area dispersion. However, studies by Menasveta (1958) and by Day (1976) indicated that tagged adult English sole generally remained

in the same local areas of capture. Further, English sole demonstrated some degree of homing ability as they were often caught in the area of initial capture after being released in a different area (Day 1976).

4.4.2 Crustacean Ecology

The shrimp Crangon alaskensis and the crab Cancer gracilis were the most ubiquitous of the epibenthic crustacean target species. The shrimp were much more abundant and were found in more study areas than were the crabs, making shrimp a more desirable group of animals for this type of field survey. The average CPUE for C. gracilis was seldom higher than 1, while the average CPUE for C. alaskensis was 8 (excluding the CPUE value of 195 for Discovery Bay). Also, this shrimp species was found in all the embayments sampled, compared to the crab species which was not collected in two embayments: the reference areas, Port Madison and Port Susan.

4.5 Benthic Invertebrate Ecology

The results of field surveys of benthic invertebrates can provide important information in documenting the cumulative effects of pollutants on the distribution, abundance, growth rate, food habits, and community structure of the organisms present (Armstrong et al. 1978). Various community indices and indicator species have been used to define areas impacted by pollution because they provide means of defining the zone of impact of a pollution source (Word 1978, Mearns and Green 1976, Moore 1979, Freeman and Dickie 1979, Grassle and Grassle 1974). Some authors have objected to the use of diversity indices because they feel that the variability of the natural environment, even without the influences of pollution, leads to the formation of communities which cannot be assessed by a generalized hypothesis (Hurlbert 1971).

Seasonal variations in abundance in infauna observed in this study were similar to the trends reported by Lie (1968) in Puget Sound. Lowest abundance values were observed during the winter, and higher values were observed in the summer and fall. A similar trend was seen in the number of taxa represented in the infaunal indices. Average Infaunal Trophic Index (ITI) values for each season were stable throughout the year, implying that whereas the abundance and species composition of a community may change during the year; in general, the dominant feeding strategy of those organisms present at a site does not change substantially from season to season.

Variations in the water quality parameters of temperature, salinity, and dissolved oxygen were probably not responsible for the observed variations in species richness and abundance in invertebrates. The small variation observed in these parameters in stations sampled during a single quarter was well within the range which the benthic organisms can tolerate. The seasonal water temperatures were very similar to those reported by Lie (1968) for Puget Sound.

With regard to the analyses of the relationships between several benthic invertebrate community indices and sediment chemical composition, it should be emphasized that not all variables were included in the best multiple regression equation for each class of chemical and physical variables because the stepwise selection method eliminated "redundant" variables - those highly correlated with variables already in the equation. Many of the chemical variables are highly correlated with one another (Malins et al. 1980); thus, those chemicals remaining in the equation are best considered as representatives of chemical classes (i.e., metallic elements or organic compounds).

4.6 Effects Studies

4.6.1 Cage Exposures

The results of the cage exposures of crabs must be interpreted in light of the pathology observations indicating that the crabs were underfed and, therefore, more susceptible to mortality from various sources than well-fed crabs. However, the 100% survival at Seattle Aquarium and Hylebos Waterway indicates that inadequate feeding was not in itself a source of mortality.

The strongest conclusion to be drawn from the results is that mortality in the Duwamish Waterway was higher than at any of the other sites, including the reference area. Of the sites chosen, all except Port Susan were high in hydrocarbon contaminants, but only one (Duwamish Waterway) was also high in heavy metals. The cause of mortality at Port Susan is unclear.

The exposure of molluscs to sediments in urban and nonurban areas demonstrated that during a period of 9 weeks, the *Macoma* spp. tolerated the experimental conditions with no detectable effects. In view of the high tissue levels of chemical contaminants found in *Macoma nasuta* held in at least one urban area, the Hylebos Waterway, adult members of this species appear to be able to tolerate short-term exposures to high levels of organic pollutants.

4.6.2 Sediment Recolonization

The results of the recolonization experiments corresponded to the results presented in the benthic ecology portion of this study. In the sediments placed in the Duwamish Waterway, fewer taxa colonized Duwamish sediment than Port Susan sediment. The exposure site in the Duwamish Waterway corresponds to a station in cluster group 4. This observation agrees with the finding of significantly lower taxon richness values for benthic invertebrate samples from stations in cluster group 4.

5. CONCLUSIONS

The data presented in this report constitute a substantial new body of knowledge for use in evaluating the impact of human activities on Puget Sound.

The most sophisticated analytical techniques available were used to identify xenobiotics in sediments and organisms. Even these approaches have substantial limitations, however. Thus, it should be clearly understood that possibly hundreds of potentially toxic chemicals were present in sediment and tissue samples in addition to those detected.

A number of pathological abnormalities were observed in several species of fishes and invertebrates. Although initial attempts were made to correlate pollutants in sediment with the prevalence of certain lesions, the extent to which pathological abnormalities are caused by pollutants is still not known. Gaining a better understanding of cause-and-effect relationships between chemicals in the environment and the etiology of pathological conditions in marine biota remains a foremost problem for future research.

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REFERENCES

Abbott, R.T. 1954. American Seashells. New York, xiv+541 pp., 310 figs., 40 pls. (Rev. ed. in press).

Armed Forces Institute of Pathology. 1968. Manual of Histologic Staining Methods. Third Edition (L.G. Luna, ed.) McGraw-Hill, New York, 258 p.

Armstrong, J., R. Thom, K. Chev, B. Arpke, R. Bohn, J. Glock, R. Hieronymus, E. Hurlburt, K. Johnson, B. Mayer, B. Stevens, S. Tettleback, and P. Waterstrat. 1978: The impact of the Denny Way combined sewer overflow on the adjacent flora and fauna in Elliott Bay, Puget Sound, Washington. A report prepared in cooperation with the Municipality of Metropolitan Seattle, Washington, 112 pp.

Ashley, L.M. 1967. Histopathology of rainbow trout aflatoxicosis. In: Trout Hepatoma Research Conference Papers (J.E. Halver and J.A. Mitchell, eds.), Bureau of Sport Fisheries and Wildlife (Dept. of Interior). Research Report, 70:130-144.

Ashley, L.M. 1979. Action of iron salts in solution on goldfish. Prog. Fish-Cult., 32:109.

Bannasch, P. 1978. Sequential cellular alterations during hepatocarcinogenesis. In: Rat Hepatic Neoplasia (P.M. Newberne and W.H. Butler, eds.), MIT Press, Cambridge, 58-93.

Banse, K. and K.D. Hobson. 1974. Benthic errantiate polychaetes of British Columbia and Washington. Bull. 185, Fish. Res. Bd. Canada, 111 pp.

Becker, F.F. 1976. Sequential phenotypic and biochemical alterations during chemical carcinogenesis. Cancer Research, 36:2563-2566.

Becker, F.F. 1978. Characterizations of hepatic nodules. In: Rat Hepatic Neoplasia (P.M. Newberne and W.H. Butler, eds.), MIT Press, Cambridge, 42-52.

Beliles, R.P. 1975. Metals in Toxicology, the Basic Science of Poisons. (L.J. Casarett and F. Doull, eds.). Macmillan Publishing Co., Inc., New York, 454-502.

Bjorseth, A. and A.J. Dennis (eds.). 1979. Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects. Battelle Press, Columbus, Ohio, 1097 pp.

Brown, D.W., L.S. Ramos, M.Y. Uyeda, A.J. Friedman, and W.D. MacLeod, Jr. 1980. Ambient-temperature extraction of hydrocarbons from marine sediment-comparison with boiling-solvent extractions. In: Advances in Chemistry Series No. 185, Petroleum in the Marine Environment (L. Petrakis and F.T. Weiss, eds.). American Chemical Society, 14:313-326.

Burrows, R.E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. U.S. Fish Wildlife Serv. Res. Rep., 66:1-12.

Burrows, D.G., W.D. MacLeod, Jr., L.S. Ramos, and D.W. Brown. 1981. Mass spectral identification of chlorinated organic compounds from sediments collected near an industrial area. In: 29th Annual Conference on Mass Spectrometry and Allied Topics Abstracts (W.L. Fite, ed.), The American Society for Mass Spectrometry, East Lansing, Mich., 615-616.

Callahan, M.A., M.W. Slimak, N.W. Gabel, I.P. May, C.F. Fowler, J.R. Freed, P. Jennings, R.L. Durfee, F.C. Whitmore, B. Maestri, W.R. Mabey, B.R. Holt, and C. Gould. 1979. Water-related environmental fate of 129 priority pollutants, EPA-440/4-79-029a.

Clark, R.C. and D.W. Brown. 1977. Petroleum: properties and analysis in biotic and abiotic systems. In: Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms (D.C. Malins, ed.), Academic Press, New York, Vol. 1:1-89.

Cope, O.B., J.P. McCraren, and L.L. Eller. 1969. Effects of dichlobenil on two fishpond environments. Weed Sci., 17:158-165.

Couch, J.A. 1975. Histopathological effects of pesticides and related chemicals on the livers of fishes. In: The Pathology of Fishes (W.E. Ribelin and G. Migaki, eds.), Univ. of Wisconsin Press, Madison, 559-584.

Couch, J.A. 1979. Shrimp (Arthropoda Crustacea: Panaeidae). Pollution Ecology of Estuarine Invertebrates. Chap. 7, Academic Press, New York, 235-258.

Day, D.E. 1976. Homing behavior and population stratification in central Puget Sound English sole (Parophrys vetulus). J. Fish. Res. Board Can., 33:278-282.

Dunn, B.P. and N.F. Stich. 1976. Monitoring procedures for chemical carcinogens in coastal waters. J. Fish. Res. Board Can., 33:2040-2046.

Eller, L.L. 1969. Pathology in redear sunfish exposed to Hydrothol 191. Trans. Amer. Fish. Soc., 98:52-59.

Eller, L.L. 1971. Histopathology lesions in cutthroat trout (Salmo clarki) exposed chronically to the insecticide endrin. Am. J. Pathol., 64:321.

Eller, L.L. 1975. Gill lesions in freshwater teleosts. In: The Pathology of Fishes (W.E. Ribelin and G. Migaki, eds.), Univ. of Wisconsin Press, Madison, 305-330.

EPA. 1980. Ambient Water Quality Criteria. Polynuclear Aromatic Hydrocarbons, EPA 440/5-80-069; Polychlorinated Biphenyls, EPA 440/5-80-068; Mercury EPA 440/5-80-058; Lead, EPA 440/5-80-57; Arsenic, EPA 440/5-80-021; Cadmium, EPA 440/5-80-025; Copper, EPA 440/5-80-036; Selenium, EPA 440/5-80-070; Silver, EPA 440/5-80-71; Chlorinated Benzenes, EPA 440/5-80-028; and Hexachlorobutadiene, EPA 440/5-80-053.

Everitt, B. 1974. Cluster Analysis. Heinemann Educational Books, Ltd., London, 122 pp.

Falkmer, S., S. Marklund, P.E. Mattson, and C. Rappe. 1977. Hepatomas and other neoplasms in the Atlantic hagfish (Myxine glutinosa): a histopathologic and chemical study. Annals New York Acad. Sci., 298:342-355.

Farber, E. 1976. On the pathogenesis of experimental hepatocellular carcinoma. In: Hepatocellular Carcinoma (K.O. Kuda and R. L. Peters, eds.), John Wiley and Sons, New York, 1-24.

Fauchald, K. 1977. The Polychaete Worms: Definitions and Keys to the Orders, Familities and Genera. Natural History Museum of Los Angeles County, 188 pp.

Fieser, L.F. and M. Fieser. 1949. Natural Products Related to Phenanthrene. Reinhold Publishing Corporation, New York, 48, 70.

Fishbein, L. 1974. Toxicity of chlorinated biphenyls. Ann. Rev. Pharmacol., 14:139-156.

Fontaine C.T., R.G. Bruss, I.A. Sanderson, and D.V. Lightner. 1975. Histopathological response to turpentine in the white shrimp, Penaeus setiferus. J. Invert. Pathol., 25(3):321-330.

Freeman, K.R. and L.M. Dickie. 1979. Growth and mortality of the blue mussel, Mytilus edulis, in relation to environmental indexing. J. Fish. Res. Board Can., 36:1238-1249.

Gaufin, A.R., E.K. Harris, and H.J. Walter. 1956. A statistical evaluation of stream bottom sampling data obtained from three standard samplers. Ecology, 37(4):643-648.

Grassle, J.F. and J.P. Grassle. 1974. Opportunistic life histories and genetic systems in marine benthic polychaetes. J. Mar. Res., 32:253-284.

Heller, S.R. and G.W.A. Milne. 1978. EPA/NIH Mass Spectral Data Base, Vol. 3. U.S. Department of Commerce, NSRDS-NBS 63.

Hinton, D.E., J.E. Klaunig, and M.M. Lipsky. 1978. PCB-induced alterations in teleost liver: A model for environmental disease in fish. Mar. Fish. Rev., 40(10):47-50.

Hook, J.B. 1980. Toxic responses of the kidney. In: Toxicology, The Basic Science of Poisons. 2nd edition (J. Doull, C.D. Klaasen, M.O. Amdur, eds.), Macmillan Publishing Co., New York, 232-245.

Hurlbert, S.H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology, 54(4):577-586.

Jones, G. and W.H. Butler. 1975. Morphology of spontaneous and induced neoplasia. In: Mouse Hepatic Neoplasia (W.H. Butler and P.M. Newberne, eds.), Elsevier Scientific Publishing Co., Amsterdam, 21-60.

Kaplan, A. and L.L. Szabo. 1979. Clinical Chemistry: Interpretation and Techniques. Lea and Febiger, Philadelphia, 440 pp.

Kasza, L., M.A. Weinberger, C. Carter, D.E. Hinton, B.F. Trump, and E.A. Brouwer. 1976. Acute, subacute, and residual effects of PCB in rats. II Pathology and electron microscopy of liver and serum enzyme study. J. Tox. Environ. Health, 1:689-703.

Keen, M.A. and E. Coan. 1974. Marine Molluscan Genera of Western North America: An Illustrated Key. Stanford University Press, Stanford, Calif., 208 pp.

Kimbrough, R.D., R.E. Linder, and T.B. Gaines. 1972. Morphological changes in livers of rats fed polychlorinated biphenyls. Arch. Environ. Health, 25:354-364.

Kleinbaum, D.G. and L.L. Kupper. 1978. Applied Regression Analysis and Other Multivariable Methods. Duxbury Press, North Scituate, Mass., 556 pp.

Koller, L.D. and J.G. Zinkl. 1973. Pathology of polychlorinated biphenyls in rabbits. Am. J. Path., 70(3):363-377.

Konasewich, D.E., P.M. Chapman, G. Vigers, E. Gerencher, and N. Treloar. In press. Effects, pathways, processes and transformation of Puget Sound contaminants of concern (Toxicant literature review), NOAA OMPA Technical Memorandum.

Kozloff, E.N. 1974. Keys to the Marine Invertebrates of Puget Sound, the San Juan Archipelago and Adjacent Regions. University of Washington Press, Seattle, 226 pp.

Krumbein, W.C. and F.J. Pettijohn. 1938. Manual of Sedimentary Petrography. The Twentieth Century Earth Science Series (Kirtley F. Mather, ed.), Appleton-Century-Crofts, Inc., New York, 549 pp.

Lie, U. 1968. A quantitative study of benthic infauna in Puget Sound, Washington, U.S.A., in 1963-1964. Fisk Dir. Skr. (Ser. Havunders), 14:229-556.

Lowe, J.I. 1964. Chronic exposure of the spot, Leiostomus xanthurus, to sublethal concentrations of toxaphene in seawater. Trans. Amer. Fish. Soc., 93:396-399.

Malins, D.C., S-L. Chan, B.B. McCain, D.W. Brown, A.K. Sparks, and H.O. Hodgins. 1981. Puget Sound pollution and its effects on marine biota, submitted as a progress report to Marine Ecosystems Analysis Puget Sound Project, Office of Marine Pollution Assessment, Seattle, Washington.

Malins, D.C., and T.K. Collier. 1981. Xenobiotic interactions in aquatic organisms: effects on biological systems aquatic toxicology. Aquat. Toxicol., 1:257-268.

Malins, D.C., M.M. Krahn, D.W. Brown, W.D. MacLeod, Jr., and T.K. Collier. 1980. The analysis of petroleum products in marine environments. Helgolander Wissenschaftliche Meeresuntersuchungen, 33:257-271.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, and H.O. Hodgins. 1980. Chemical contaminants and biological abnormalities in Central and Southern Puget Sound. NOAA Technical Memorandum OPMA-2. 295 pp.

McCain, B.B., M.S. Myers, U. Varanasi, D.W. Brown, L.D. Rhodes, W.D. Gronlund, D.G. Elliott, W.A. Palsson, H.O. Hodgins, and D.C. Malins. 1982. Pathology of two species of flatfish from urban estuaries in Puget Sound. DOC (EPA) Interagency Energy/Environment, Research and Development Program Report, EPA-600/7-82-001 (in press).

McCain, B.B., K.V. Pierce, S.R. Wellings, and B.S. Miller. 1977. Hepatomas in marine fish from urban estuary. Bull. Environ. Contam. and Toxicol., 18:1-2.

McCraren J. P., O.B. Cope, and L.L. Eller. 1969. Some chronic effects of diuron on bluegills. Weed Sci., 17:497-504.

Mearns, A.J. and C.S. Green. 1976. Comparison of the benthos at several wastewater discharge sites. In: Southern California Coastal Water Research Project, El Segundo, California. Annual Report, 211-216.

Menasveta, D. 1958. Migration and fishing mortality of English sole (Parophrys vetulus) in Saratoga Passage, and adjacent waters. Master's Thesis, University of Washington, 84 pp.

Moore, J.W. 1979. Diversity and indicator species as measures of water pollution in a subarctic lake. Hydrobiologia, 66:73-80.

Morita, T., I. Kihara, T. Oite, T. Yamamoto, and Y. Suzuki. 1978. Mesangiolysis: Sequential ultrastructural study of Habu venom-induced glomerular lesions. Laboratory Investigation, 38(1):94-102.

Newberne, P.M. 1967. Biological activity of the aflatoxins in domestic and laboratory animals. In: Trout Hepatoma Research Conf. Papers (J.E. Halver and J.A. Mitchell, eds.), Bureau of Fisheries and Wildlife, Dept. of Interior, Research Report, 70:130-144.

Newberne, P.M. and W.H. Butler, eds. 1978. In: Rat Hepatic Neoplasia. MIT Press, Cambridge, pp. 288.

Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner, and D.H. Bent. 1975. Statistical Package for the Social Sciences. 2nd edition, McGraw-Hill Book Co., New York, 675 pp.

Nimmo, D.R., D.V. Lightner, and L.H. Bahner. 1977. Effects of cadmium on the shrimps Penaelus duorarum, Palaeomonetes pugio, and Paleomonetes vulgaris. In: Physiological Responses of Marine Biota to Pollutants (F.J. Vernberg et al., eds.), Academic Press, New York, 131 pp.

Nishizumi, M. 1970. Light and electron microscope study of chlorobiphenyl poisoning in mouse and monkey liver. Archives of Environmental Health, 21:620-632.

Payne, J.F., R. Maloney, and A. Rahimtula. 1979. Are petroleum hydrocarbons an important source of mutagens in the marine environment? In: Proceedings, 1979 Oil Spill Conference, March 19-22, 1979, Los Angeles, 533-536.

Phillips, G.R. and R.C. Russo. 1978. Metal bioaccumulation in fishes and aquatic invertebrates: A literature review, EPA-600/3-78-103.

Pierce, K.V., B.B. McCain,and S.K. Wellings. 1978. The pathology of hepatomas and other liver abnormalities in English sole (Parophrys vetulus) from the Duwamish River Estuary, Seattle, Washington, J. Natl. Cancer Inst., 6D:1445-53.

Pierce, K.V., B.B. McCain, and S.R. Wellings. 1980. Histopathology of abnormal livers and other organs of starry flounder Platichthys stellatus from the estuary of the Duwamish River, Seattle, Washington, U.S.A. J. of Fish Diseases, 3:81-91.

Plaa, G.L. 1975. Toxicology of the liver. In: Toxicology: The Basic Science of Poisons (L.J. Casarett and J. Voull, eds.), Macmillian Publishing Co., Inc., New York, 170-189.

Preece, A. 1972. A Manual for Histologic Technicians. 3rd edition. Little, Brown and Co., Boston, 428 pp.

Ramos, L.S., D.W. Brown, R.G. Jenkins, and W.D. MacLeod, Jr. 1979. Modification of conventional gas chromatographic inlets for the use of glass capillary columns. In: Trace Organic Analysis: a New Frontier in Analytical Chemistry (H.S. Hertz and S.N. Chesler, eds.), National Bureau of Standards Special Publication 519, Washington, D.C., 713-718.

Ramos, L.S. and P.G. Prohaska. 1981. Sephadex LH-20 chromatography of extracts of marine sediment and biological samples for the isolation of polynuclear aromatic hydrocarbons. J. Chromatography, 211:284-289.

Renfro, W.C. 1973. Transfer of Zn^{65} from sediments by marine polychaete worms. Mar. Biol., 21:305-316.

Robbins, S.L. and R.S. Cotran. 1979. Pathologic Basis of Disease. 2nd ed. W.B. Saunders Company, Philadelphia, 1598 pp.

Robinson, B.N., G.D. Anderson, E. Cohen, and W.F. Gazdzik. 1979. Scientific Information Retrieval User's Manual (second edition). Northwestern University, Evanston, Illinois, 458 pp.

Roubal, W.T., S.I. Stranahan, and D.C. Malins. 1978. The accumulation of low molecular weight aromatic hydrocarbons of crude oil by coho salmon (Oncorhynchus kisutch) and starry flounder (Platichthys stellatus). Arch. Environ. Contam. Toxicol., 7:237-249.

Salt, G. 1970. The cellular defense reactions of insects. Cambridge monographs in experimental biology. No. 16. Cambridge University Press, Cambridge, 118 pp.

Schillings, P.H.M. and J.H.S. Stekhoven. 1980. Atlas of Glomerular Histopathology. Skarger, Basel, 143 pp.

Simon, R.C., A.M. Dollar, and E.A. Smuckler. 1967. Descriptive classification on normal and altered histology of trout livers. In: Trout Hepatoma Research Conf. Papers, Bureau of Sport Fisheries and Wildlife (Dept. of Interior), Research Report, 70:130-144.

Sinnhuber, R.O., J.D. Hendricks, J.H. Wales, and G.B. Putman. 1977. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. Annals New York Acad. Sci., 298:389-408.

Smith, C.E. and R.G. Piper. 1975. Lesions associated with chronic exposure to ammonia. In: The Pathology of Fishes (W.E. Ribelin and G. Migaki, eds.), Univ. of Wisconsin Press, Madison, 497-512.

Smith, C.E., T.H. Peck, R.J. Klauda, and J.B. McLaren. 1979. Hepatomas in Atlantic tomcod Microgadus tomcod (Walbaum) collected in the Hudson River estuary in New York. J. Fish Diseases, 2:313-319.

Sokal, R. and F. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco, 776 pp.

Solangi, M.A. and D.V. Lightner. 1976. Cellular inflammatory response of Penaeus aztecus and Penaeus sitiferus to the pathogenic fungus, Fusarium sp. isolated from the California Brown Shrimp, Penaeus californiensis. J. Invert. Path., 27:77-86.

Squire, R.A. and M.H. Levitt. 1975. Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Research, 35:3214-3223.

Suzuki, Y., J. Churg, E. Grishman, W. Martner, and S. Dacha. 1963. The mesangium of the renal glomerulus-electronmicroscopic studies of pathologic alterations. Am. J. of Pathology, 43(4):555-578.

Thom, R.M., K.K. Chew, and J.Q. Word. 1979. Abundance biomass and trophic structure of the subtidal infaunal communities of the eastern side of central Puget Sound. A report to the Municipality of Metropolitan Seattle, 43 pp.

Thompson, S.W. 1966. Selected Histochemical and Histopathological Methods. Charles C. Thomas, Springfield, Illinois, 1639 pp.

Tietz, N.W. 1976. Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadelphia, 1263 pp.

Trump, B.F., R.T. Jones, and S. Sahaphong. 1975. Cellular effects of mercury on fish kidney tubules. In: Pathology of Fishes (W.E. Ribelin and G. Migalki, eds.), Univ. of Wisconsin Press, Madison, 585-612.

Trump, B.F. and A.V. Arstila. 1975. Cellular reaction to injury, pp. a-96. In: Principles of Pathobiology (second edition), M.F. LaVia and R.B. Hill, eds.), Oxford University Press, New York, 295 p.

Van Valin, C.C., A.K. Andrews, and L.L. Eller. 1968. Some effects of mirex on two warm-water fishes. Trans Amer. Fish Soc., 97:185-196.

Varanasi, U., D.J. Gmur, and P.A. Treseler. 1979. Influence of time and mode of exposure on biotransformation of naphthalene by juvenile starry flounder (Platichthys stellatus) and rock sole (Lepidopsetta bilineata). Arch. Env. Contam. Toxicol., 8:673-692.

Varanasi U. and D.J. Gmur. 1981. Hydrocarbons and metabolites in English sole (Parophrys vetulus) exposed simultaneously to [^{3}H]benzo[a]pyrene and [^{14}C] naphthalene in oil-contaminated sediment. Aquat. Toxicol., 1:49-67.

Walsh, A.H. and W.E. Ribelin. 1975. The pathology of pesticide poisoning. In: The Pathology of Fishes (W.E. Ribelin and G. Migaki, eds.), Univ. of Wisconsin Press, Madison, 515-558.

Ward, J.M. and G. Vlahakis. 1978. Evaluation of hepatocellular neoplasms in mice, J. Natl. Cancer Inst., 61:807:811.

Wasserman, M., D. Wasserman, S. Cucos, and H. Miller. 1979. World PCB's map: storage and effects in man and his biologic environment in the 1970's. In: Health Effects of Halogenated Aromatic Hydrocarbons (W.J. Nicholson and J.A. Moore, eds.), Annals of the New York Acad. Sci., 320:69-124.

Wellings, S.R., B.B. McCain, and B.S. Miller. 1976a. Epidermal papillomas in Pleuronectidae of Puget Sound, Wash. Prog. Exp. Tumor Res., 20:55-74.

Wellings, S.R., C.E. Alpers, B.B. McCain, and B.S. Miller. 1976b. Fin erosion disease of starry flounder (Platichthys stellatus) and English sole (Parophrys vetulus) in the estuary of the Duwamish River, Seattle, Washington. J. Fish. Res. B. Can., 33:2577-2586.

Wildish, D.J., C.D. Metcalf, H.M. Akagi, and D.W. McLeese. 1980. Flux of Aroclor 1254 between estuarine sediments and water. Bull. Environ. Contam. Toxicol., 24:20-26.

Wishart, D. 1975. Clustan 1C User's Manual. University College London, London, 124 pp.

Word, Jack Q. 1976. Biological comparison of grab sampling devices. Annual Report, Southern California Coastal Water Research Project, Long Beach, Calif., 189-194.

Word, Jack Q. 1978. The infaunal trophic index. Coastal Water Research Project (W. Bascom, ed.), Annual Report, 19-39.

Zar, J.H. 1974. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, 620 p.

Zimmerman, H.J. 1978. Hepatoxicity. Appleton-Century-Crofts, New York, 47-90.

Table 1. List of target fish and invertebrate species.

FISH

English sole (*Parophrys vetulus*)

Rock sole (*Lepidopsetta bilineata*)

Pacific staghorn sculpin (*Leptocottus armatus*)

Pacific tomcod (*Microgadus proximus*)[1]

Quillback rockfish (*Sebastes maliger*)[1]

INVERTEBRATES

Crabs (*Cancer magister*, *C. gracilis*, and *C. productus*)

Shrimp (*Pandalus danae*, *P. jordani*, and *Crangon alaskensis*)

Clams (*Macoma carlottensis* and *Acila castrensis*)[1]

Worms (*Capitella capitata*, *Glycera capitata*, and *Prionospio pinnata*)[1]

[1] Species collected only in 1979

Table 2. Target organic compounds analyzed in sediment and biota from Puget Sound, plus internal and recovery standards.

Aromatic Hydrocarbons (AHs):

1. Isopropylbenzene
2. *n*-Propylbenzene
3. Indan
4. 1,2,3,4-Tetramethylbenzene
5. ✓ Naphthalene
6. Benzothiophene [1]
7. 2-Methylnaphthalene
8. 1-Methylnaphthalene
9. Biphenyl
10. 2,6-Dimethylnaphthalene
11. ✓ Acenaphthene
12. 2,3,5-Trimethylnaphthalene
13. ✓ Fluorene
14. Dibenzothiophene [1]
15. ✓ Phenanthrene
16. ✓ Anthracene
17. 1-Methylphenanthrene
18. 3,6-Dimethylphenanthrene
19. ✓ Fluoranthene
20. ✓ Pyrene
21. ✓ Benz[a]anthracene
22. ✓ Chrysene
23. Benzo[e]pyrene
24. ✓ Benzo[a]pyrene
25. Perylene
26. ✓ Dibenzanthracene
27. ✓ Benzofluoranthene

Chlorinated Pesticides:

Hexachlorobenzene (HCB)
Lindane (γ- BHC)
Heptachlor
Aldrin
o,p'-DDE
α-Chlordane
trans-Nonachlor
p,p'-DDE
o,p'-DDD
m,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT

Dichlorobiphenyls)
Trichlorobiphenyls)
Tetrachlorobiphenyls)
Pentachlorobiphenyls) PCBs
Hexachlorobiphenyls)
Heptachlorobiphenyls)
Ochtachlorobiphenyls)
Nonachlorobiphenyls)

Dichlorobutadienes
Trichlorobutadienes (3CBD))
Tetrachlorobutadienes (TCBD)) CBDs
Pentachlorobutadienes (PCBD))
Hexachlorobutadienes (HCBD))

Internal and Recovery Standards

D8-Naphthalene (D8N)
D10-Acenaphthene (D10A)
D12-Perylene (D12P)
D4-1,4-Dichlorobenzene (D4D)
Triisopropylbenzene (TPB)
Hexamethylbenzene (HMB)
n-Decylcyclohexane (DCH)
Technazene (TEC)

1 A heteroaromatic compound (aromatic ring contains a nonpolar sulfur moiety)

Table 3. Metals analyzed by either inductively coupled argon plasma emission spectroscopy (Group A) or atomic absorption spectroscopy (Group B).

GROUP A

Aluminum[1]	Al	Mercury[2]	Hg
Antimony	Sb	Molybdenum	Mo
Arsenic	As	Nickel[1,2]	Ni
Barium	Ba	Phosphorus[1]	P
Beryllium	Be	Potassium	K
Bismuth	Bi	Scandium	Sc
Boron[1]	B	Selenium	Se
Cadmium[1]	Cd	Silicon	Si
Calcium	Ca	Silver[2]	Ag
Chromium[1]	Cr	Sodium	Na
Cobalt	Co	Strontium[1]	Sr
Copper[1]	Cu	Tin	Sn
Gallium	Ga	Titanium	Ti
Germanium	Ge	Tungsten	W
Iron[1]	Fe	Vanadium[1]	Va
Lead[1]	Pb	Yttrium	Y
Lithium[1]	Li	Zinc[1,2]	Zn
Magnesium[1]	Mg	Zirconium	Zr
Manganese[1]	Mn		

GROUP B

Aluminum	Lead[1,2]
Arsenic[1,2]	Manganese
Cadmium[1,2]	Mercury[1,2]
Chromium[1,2]	Selenium[1,2]
Copper[1]	Zinc[1,2]
Iron	

[1] Determined in tissue
[2] An EPA Priority Pollutant

Table 4. Computational formulae for infaunal community indices.

Infaunal Trophic Index (ITI) =

$$100 - \left[33.3 \left(\frac{0N_1+1N_2+2N_3+3N_4}{N_1+N_2+N_3+N_4} \right) \right]$$

where $N_1 = n_i$ for taxa in Group 1

$N_2 = n_i$ for taxa in Group 2

$N_3 = n_i$ for taxa in Group 3

$N_4 = n_i$ for taxa in Group 4

and n_i = number of individuals in the ith taxon present

Total abundance (TA) = $N_1 + N_2 + N_3 + N_4$

Taxon richness (S) = k, the number of taxa present

Taxon diversity (H') = $- \sum_{i=1}^{k} (p_i \log p_i)$, where $p_i = \frac{p_i}{TA}$

Taxon evenness (J) = $\frac{H'}{\log k}$

Table 5. Groups of benthic invertebrates used to calculate the Infaunal Trophic Index (from Thom et al. 1979).

ORGANISMS			
Group 1	Group 2	Group 3	Group 4
Ampharetidae	Capitellidae	Chaetopteridae	*Ammotrypane*
Maldanidae	Cirratulidae	Nereidae	*Armandia*
Onuphidae	Goniadidae	*Travisia*	*Capitella*
Owenia	Magelonidae	*Bittium*	*Solemya*
Sabellidae	*Myriochele*	*Macoma*	Stenothoidae
Serpulidae	Nephtidae	*Nucula*	oligochaete
Terebellidae	Orbiniidae	*Nuculana*	
Rhomboidella	Spionidae	*Parvilucina*	
Nemocardium	Syllidae	*Yoldia*	
Ampelisca	*Axinopsida*		
Byblis	*Mysella*		
Caprellidae	cumaceans		
Phoxocephalidae	ostracods		
Amphipolis	*Photis*		
Amphiodia	tanaids		
Cucumaria	*Golfingia*		
	Pectinaria		

Table 6. Total number of samples chemically analyzed since the initiation of the project in 1978.

TYPES OF SAMPLES	TOTAL NUMBER OF SAMPLES ANALYZED
SEDIMENT	77
FISH SPECIES	
English sole (*Parophrys vetulus*)	
Liver samples	32
Skeletal muscle samples	10
Salmon (*Oncorhynchus tshawytscha*, *O. kisutch*)	
Liver samples	12
Skeletal muscle samples	12
Cod (*Gadus macrocephalus*)	
Liver samples	8
Skeletal muscle samples	8
Rock Sole (*Lepidopsetta bilineata*)	
Liver samples	17
Pacific staghorn sculpin (*Leptocottus armatus*)	
Liver samples	9
Pacific Tom Cod (*Microgadus proximus*)	
Liver samples	1
Quillback Rockfish (*Sebastes maliger*)	
Liver samples	6
INVERTEBRATES	
Marine Worms (*Capitella* spp.)	
Whole tissue homogenates	8
Clams (*Macoma* spp.)	
Whole tissue homogenates	9
Shrimp (Mixtures of *Pandalus danae*, *P. jordani*, *Crangon alaskensis*)	
Whole tissue homogenates	8
Crabs (*Cancer* spp.)	
Hepatopancreas	8

Table 7. The ΣAHs (ppb, dry weight) in sediment at sampling stations given in Figures 1 and 2. Multiple concentrations are different samples obtained at different times.

Area	Mean Conc. (ppb)	Sampling stations 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	13,000	9,600 11,000	30,000	4,800	18,000	63,000 36,000	2,700	3,900	4,800	1,200	5,700	150	49,000	230 4,700	2,500	6,300	1,500
2. Commencement Bay	9,700	14,000 9,500 21,000	50,000 7,900 5,500	7,000 3,400	2,700 5,300	57,000 18,000 11,000	410 220	890	2,900 1,400	280	630	740	15,000 2,200	17,000 2,700	1,900 2,600		
3. Sinclair Inlet	6,400	1,500	3,300	2,600	18,000												
4. Budd Inlet	940	1,100	620	1,100													
5. Case Inlet	350	650	60														
6. Port Madison	480	720	240														
7. Port Susan	250	240	260														
8. Everett	2,700	2,900	5,100	32													
9. Bellingham	4,000	3,300	4,600														
10. Shelton		310															
11. Port Angeles		970															
12. Liberty Bay		860															
13. Ebey Slough		26															
14. Discovery Bay		49															

Table 8. The geographic distribution of fluoranthene, benz[a]anthracene, and benzo[a]pyrene, in Elliott Bay sediment (ppb, dry weight). The locations of the sampling stations are given in Figure 2. Multiple concentrations are from different samples obtained at different times.

	Mean Conc. (ppb)	Sampling Station															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Fluoranthene	1,800	1,700 2,800	3,700	770	3,200	7,600 3,600	240	450	490	130	700	20	6,100	30 770	330	730	160
Benz[a] anthracene	1,600	1,100 1,900	2,700	510	1,400	6,800 8,400	180	290	310	80	670	10	4,800	30 260	140	660	100
Benzo[a]pyrene	740	440 440	1,500	240	960	2,200 2,600	220	180	260	40	170	6	4,000	10 140	80	440	40
ΣAHs	13,000	11,000 9,600	30,000	4,800	18,000	63,000 36,000	2,700	3,900	4,800	1,200	5,700	150	49,000	230 4,700	2,500	6,300	1,500

Table 9. The concentrations of retene in sediments (ppb, dry weight) from stations near pulp mills.

STATIONS	RETENE, ppb
Port Angeles Harbor	78
Whatcom Waterway, Bellingham, Station 2	7,500
Port Gardner, Everett, Station 1	2,900
Port Gardner, Everett, Station 2	250
Hammersely Inlet, Shelton	19

Table 10. The ΣAHs (ppb, dry weight) found in various biota (Table 6) captured in Duwamish Waterway (Elliott Bay), Hylebos Waterway (Commencement Bay) and reference areas (Port Madison and Case Inlet). N designates that AHs were not present at measurable concentrations.

Area	Worm	Clam	Shrimp	Crab Hepato-pancreas	English Sole Livers (individual)	English Sole Livers (composite)
DUWAMISH RIVER ELLIOTT BAY	1) 7,400	1) 5,300 2) 1,600	1) 720	1) 2,000	1) N 2) N	1) 1,200 2) 600 3) 300
HYLEBOS WATERWAY COMMENCEMENT BAY	1) 17,000 2) 1,200	1) 5,100 2) 1,300	1) 1,300	1) 1,900	1) 420 2) 590 3) 820 4) 1,400	1) N 2) 70
REFERENCE AREAS	1) 200	1) 160 2) N	1) 150 2) 1,500	1) 15		1) N 2) 50

Table 11. The concentrations of PCBs (ppb, dry weight) in sediment at indicated sampling stations. The location of sampling stations are given in Figures 1 and 2. Multiple concentrations are from different samples obtained at different times. N designates that PCBs were not present at measurable concentrations.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	380	530 490	670	340	170	490 2,100	310	140	160	35	99	N	61	N	240 65	170	450
2. Commencement Bay	270	1,200 980 [1]	27 [1] [1]	35 54	61 100	380 670 290	15 .7	[1]	406 180	N	.1	1.8	280 130	990 100	22 59		
3. Sinclair Inlet	130	180	220	77	28												
4. Budd Inlet	13	19	15	5													
5. Case Inlet	3	4	1														
6. Port Madison	6	9	3														
7. Port Susan	10	7.8	13														
8. Everett	41	80	[1]	1.2													
9. Bellingham		[1]	100														
10. Shelton		2.3															
11. Port Angeles		9.4															
12. Liberty Bay		32															
13. Ebey Slough		.88															
14. Discovery Bay		1.4															

$\bar{x} = 297$

[1] PCBs were not determined for sample due to interferences by other compounds

Table 12. The ΣPCBs (ppb, dry weight) in various biota (Table 6) captured in Duwamish Waterway (Elliott Bay), Hylebos Waterway (Commencement Bay), and reference areas (Port Madison and Case Inlet).

Sampling Area	Worm	Clam	Shrimp	Crab Hepato-pancreas	English Sole Livers (individual)	English Sole Livers (composite)
DUWAMISH RIVER ELLIOTT BAY	1) 1,800	1) 312 2) 1,300	1) 2,100	1) 32,000	1) 12,000 2) 13,000	1) 35,000 2) 9,200 3) 32,000
HYLEBOS WATERWAY COMMENCEMENT BAY	1) 1,220 2) 380	1) 874 2) 340	1) 3,800	1) 28,000	1) 16,000 2) 16,000 3) 10,000 4) 14,000	1) 6,100 2) 20,000
REFERENCE AREAS	1) 190	1) 24 2) 160	1) 300 2) 134	1) 480		1) 3,000 2) 1,600

Table 13. Comparison of the concentration of PCBs (ppb, wet weight) in liver and muscle of sole, salmon, and cod (Table 6) from Elliott Bay, Commencement Bay, and reference areas (Port Madison and Case Inlet for sole, or Point Jefferson for salmon and cod).

SAMPLING AREA	ENGLISH SOLE Liver/Muscle	SALMON Liver/Muscle	COD Liver/Muscle
ELLIOTT BAY	1) 9,700/360	1) 99/140[1]	1) 3,300/38[2]
	2) 2,100/270	2) 160/150[2]	2) 4,200/14
	3) 12,000/2100		
	4) 16,000/1100		
	5) 6,000/1300		
COMMENCEMENT BAY	1) 4,400/700	1) 63/57	1) 2,700/46
	2) 1,500/160	2) 71/22	2) 2,700/31
	3) 24,000/610	3) 190/43	3) 2,200/14
	4) 3,100/640	4) 63/41	
	5) 3,900/850	5) 48/42	
REFERENCE AREA	1) 3,000/[3]	1) 88/35	1) 700/10
	2) 1,600/[3]	2) 41/120	2) 2,000/7.0
		3) 120/29	3) 1,700/14
		4) 150/130	
		5) 60/100	
		Σ 96/96	

[1] Composite of 3 fish
[2] Composite of 2 fish
[3] Not analyzed

Table 14. The ΣCBDs in sediment (ppb, dry weight). The locations of sampling stations, are given in Figures 1 and 2. N designates that CBDs were not present at measurable concentrations. Multiple concentrations are from samples obtained at different times.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	12	1.2 4.2	7.2	3.1	10	18 53	15	42	7.4	3.8	6.7	2.8	2.9	N 5.2	7.6	11	N
2. Commencement Bay	1600	540 600 1,000	70 6,500 20,000	40 51	23 26	52 69 120	4.8 N	260	1,000 190	17	30	36	300 250	9,000 260	18 31		
3. Sinclair Inlet	57	19	68	90	51												
4. Budd Inlet	4	2.5	5.3	2.7													
5. Case Inlet	7.5	7.5	N														
6. Port Madison	2	1.9	2.8														
7. Port Susan	6	2.7	10														
8. Everett	21	21	[1]	N	N												
9. Bellingham		[1]	N														
10. Shelton		.79															
11. Port Angeles		.51															
12. Liberty Bay		12															
13. Ebey Slough		N															
14. Discovery Bay		N															

[1] Sample was not analyzed for CBDs

Table 15. The concentrations of HCB in sediment (ppb, dry weight). The locations of sampling stations, are given in Figures 1 and 2. N designates that HCB was not detected at measurable concentrations. Multiple concentrations are from samples obtained at different times.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	0.24	0.2 .72	0.4	0.2	0.1	0.3 .78	0.2	0.2	0.3	0.07	0.07	0.02	0.03	N 0.11	0.1	0.5	0.1
2. Commencement Bay	74	20 30 50	0.6 150 1300	3 2.8	2 2.9	3 3.2 6.2	1 .14	10	60 16	0.1	0.2	1	50 33	250 2.7	2 2.1		
3. Sinclair Inlet	0.25	0.2	0.4	0.2	0.2												
4. Budd Inlet	0.07	0.1	0.1	0.01													
5. Case Inlet	0.03	0.04	0.01														
6. Port Madison	0.07	0.1	0.03														
7. Port Susan	.30	0.29	0.31														
8. Everett	.2	.38	[1]	.01	.01												
9. Bellingham		[1]	13														
10. Shelton		0.03															
11. Port Angeles		1.9															
12. Liberty Bay		.13															
13. Ebey Slough		.01															
14. Discovery Bay		.03															

[1] Sample was not analyzed for HCB

Table 16. The ΣCBDs (ppb, dry weight) in various biota (Table 6) captured in Duwamish Waterway (Elliott Bay), Hylebos Waterway (Commencement Bay), and reference areas (Port Madison and Case Inlet). N designates that CBDs were not present at measurable concentrations.

Areas	Worm	Clam	Shrimp	Crab Hepato-pancreas	English Sole Livers (individual)	English Sole Livers (composite)
DUWAMISH WATERWAY ELLIOTT BAY	1) N	1) N 2) N	1) N	1) N	1) 3 2) N	1) 3 2) 10
HYLEBOS WATERWAY COMMENCEMENT BAY	1) 360 2) 40	1) 7 2) 62	1) 150	1) 70	1) 820 2) 1,600 3) 9,100 4) 1,900	1) 270 2) 2,900
REFERENCE AREAS	1) N	1) N 2) N	1) 10 2) 5	1) N		1) 4 2) 2

Table 17. The concentrations of HCB (ppb, dry weight) in various biota (Table 6) captured in Duwamish Waterway (Elliott Bay), Hylebos Waterway (Commencement Bay), and reference areas (Port Madison and Port Susan). N designates that HCB was not present at measurable concentrations.

Area	Worm	Clam	Shrimp	Crab Hepato-pancreas	English Sole Livers (individual)	English Sole Livers (composite)
DUWAMISH RIVER ELLIOTT BAY	1) 2	1) 0.4 2) N	1) 2	1) 30	1) 10 2) 10	1) 20 2) 50
HYLEBOS WATERWAY COMMENCEMENT BAY	1) 140 2) 370	1) 10 2) 130	1) 80	1) 1,800	1) 1,100 2) 1,300 3) 3,700 4) 840	1) 270 2) 2,300
REFERENCE AREAS	1) N	1) N 2) N	1) 1 2) N	1) 2		1) 10 2) 10

Table 18. Chlorinated compounds identified in two sediment samples from Commencement Bay. If more than one isomer of a compound was present, their concentrations were summed.

COMPOUNDS	CONCENTRATIONS (ppb, dry wt.) SEDIMENT Hylebos	SEDIMENT Old Tacoma
Dichlorobutadiene	10	6
Trichlorobutadiene	4000	90
Tetrachlorobutadiene	3000	300
Pentachlorobutadiene	1000	90
Hexachlorobutadiene	1000	90
Dichlorocyclohexadiene*	0.03	
Trichlorocyclohexadiene*	10	
Tetrachlorocyclohexadiene*	20	
Pentachlorocyclohexadiene*	40	
Hexachlorocyclohexadiene*	50	3
Heptachlorocyclohexadiene*	6	
Octachlorocyclohexadiene*	30	
Trichlorostyrene	1	
Tetrachlorostyrene*	10	
Pentachlorostyrene*	50	
Hexachlorostyrene*	40	
Heptachlorostyrene*	10	
Octachlorostyrene	20	
Trichlorocyclopentene*	0.04	
Tetrachlorocyclopentene*	100	60
Pentachlorocyclopentene*	20	
Hexachlorocyclopentene*	60	20
Heptachlorocyclopentene*	10	
Chlorofluoranthene/pyrene†	0.8	80
Dichlorofluoranthene/pyrene*	0.7	50
Trichlorofluoranthene/pyrene*		6
Tetrachlorofluoranthene/pyrene*	0.2	30
Pentachlorofluoranthene/pyrene*		30
Bromochlorofluoranthene/pyrene*	1.	20
Bromofluoranthene/pyrene*	60	50
Dibromofluoranthene/pyrene*	30	200
Chlorophenanthrene/anthracene	0.4	30
Dichlorophenanthrene/anthracene	0.3	40
Trichlorophenanthrene/anthracene		10
Tetrachlorophenanthrene/anthracene*		2
Bromophenanthrene/anthracene*		0.6

*Not confirmed by standards
†Slash (/) means either chlorofluoranthene or chloropyrene

Table 19. The concentrations of mercury in sediment (ppb, dry weight). Locations of sampling stations are given in Figures 1 and 2. Multiple concentrations are from samples obtained at different times. N designates that mercury was not present at measurable concentrations.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	460	380 100	800	350	1,400	1,200 620	630	450	1,100	100	150	30	100	30 280	420	360	250
2. Commencement Bay	360	790 220 1,200	430 280 320	130 260	490 260	1,000 970 620	65 23	110	170 N	100	260	63	200 310	340 210	160 N		
3. Sinclair Inlet	910	1,100	1,000	1,200	320												
4. Budd Inlet	240	330	120	280													
5. Case Inlet	70	120	20														
6. Port Madison	75	110	40														
7. Port Susan	470	580	360														
8. Everett	160	190	260	27													
9. Bellingham	1,400	1,900	870														
10. Shelton		26															
11. Port Angeles		75															
12. Liberty Bay		290															
13. Ebey Slough		48															
14. Discovery Bay		30															

Table 20. The concentrations of lead in sediment (ppb, dry weight). Locations of sampling stations are given in Figures 1 and 2. Multiple concentrations are from samples obtained at different times. N designates that lead was not present at measurable concentrations.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	130,000	270,000 250,000	630,000	160,000	61,000	110,000 280,000	89,000	78,000	74,000	37,000	110,000	13,000	16,000	8,200 45,000	65,000	73,000	40,000
2. Commencement Bay	130,000	164,000 150,000 170,000	110,000 77,000 130,000	43,000 49,000	790,000 340,000	270,000 270,000 170,000	14,000 N	28,000	40,000 44,000	29,000	65,000	18,000	50,000 45,000	170,000 160,000	49,000 64,000		
3. Sinclair Inlet	100,000	98,000	140,000	130,000	44,000												
4. Budd Inlet	44,000	60,000	23,000	49,000													
5. Case Inlet	16,000	24,000	7,900														
6. Port Madison	15,000	20,000	10,000														
7. Port Susan	22,000	22,000	21,000														
8. Everett	23,000	30,000	36,000	3,300													
9. Bellingham	65,000	34,000	95,000														
10. Shelton	1,350	3,600															
11. Port Angeles		8,200															
12. Liberty Bay		32,000															
13. Ebey Slough		2,300															
14. Discovery Bay		N															

Table 21. The concentrations of arsenic in sediment (ppb, dry weight). Locations of sampling stations are given in Figures 1 and 2. Multiple concentrations are from samples obtained at different times. N designated that arsenic was not detected at measurable concentrations. Only real values were used to calculate means.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	90,000	95,000 44,000	84,000	280,000	N	N 28,000	N	N	N	N	N	N	N	N 8,800	N	N	N
2. Commencement Bay	85,000	N 170,000 110,000	N 31,000 50,000	N 60,000	470,000 89,000	N 38,000 55,000	N 1,600	N	N 18,000	N	N	N	N 16,000	N 31,000	N 47,000		
3. Sinclair Inlet		N	N	N	N												
4. Budd Inlet	99,000	140,000	N	57,000													
5. Case Inlet		N	N														
6. Port Madison		N	N														
7. Port Susan	15,000	15,000	14,000														
8. Everett	15,000	19,000	19,000	6,000													
9. Bellingham	16,000	20,000	11,000														
10. Shelton		4,100															
11. Port Angeles		4,000															
12. Liberty Bay		12,000															
13. Ebey Slough		5,700															
14. Discovery Bay		2,600															

Table 22. The concentrations of silver in sediment (ppb, dry weight). Locations of sampling stations are given in Figures 1 and 2. Multiple concentrations are from samples obtained at different times.

Areas	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	2,500	3,000	2,600	3,300	1,900	3,900	3,500	2,700	3,800	1,300	2,000	1,300	1,500	[1] 1,600	2,200	3,200	2,100
2. Commencement Bay	3,300	2,900 [1]	2,400 [1]	2,100 [1]	11,000 6,000	5,000 3,000	1,900 [1]	2,200	2,400	1,900	2,200	2,000	2,500	2,500	2,200		
3. Sinclair Inlet	3,300	4,800	3,400	2,900	2,000												
4. Budd Inlet	3,100	3,700	2,700	3,000													
5. Case Inlet	2,100	2,300	1,800														
6. Port Madison	1,800	2,000	1,500														
7. Port Susan		[1]	[1]														
8. Everett		[1]	[1]	[1]													
9. Bellingham		[1]	[1]														
10. Shelton		[1]															
11. Port Angeles		[1]															
12. Liberty Bay		[1]															
13. Ebey Slough		[1]															
14. Discovery Bay		[1]															

[1] Sample was not analyzed for silver

Table 23. The concentrations of cadmium in sediment (ppb, dry weight). Locations of sampling stations are given in Figures 1 and 2. Multiple concentrations are from different samples obtained at different times. N designates that cadmium was not present at measurable concentrations.

Area	Mean Conc. (ppb)	Sampling Stations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Elliott Bay	7,100	12,000 1,900	11,000	18,000	5,700	8,600 2,200	7,000	8,200	6,500	4,500	7,300	3,800	4,700	4,600 N	6,500	7,400	8,300
2. Commencement Bay	4,500	9,600 1,200 3,000	6,800 390 1,500	5,400 N	16,000 1,800	9,100 3,200 500	5,200 N	5,600	6,000 920	4,200	5,800	4,700	6,100 1,300	5,600 1,100	6,000 670		
3. Sinclair Inlet	7,000	8,100	7,700	7,100	5,200												
4. Budd Inlet	9,600	11,000	8,200	9,500													
5. Case Inlet	5,400	7,600	3,200														
6. Port Madison	4,700	6,300	3,100														
7. Port Susan	880	907	860														
8. Everett	1,500	1,300	1,700	N													
9. Bellingham	1,100	930	1,300														
10. Shelton		N															
11. Port Angeles		N															
12. Liberty Bay		940															
13. Ebey Slough		N															
14. Discovery Bay		390															

Table 24. The major eigenvector loading values for the first four principal components axes. The principal component analyses were performed on the concentrations of 28 metals and 36 organic compounds in sediment samples collected from 40 sampling stations in 1979.

AXIS 1		AXIS 2		AXIS 3		AXIS 4	
Chemical variable	Eigenvector loading	Chemical variable	Eigenvector loading	Chemical variable	Eigenvector loading	Chemical variable	Eigenvector loading
benzo(e)pyrene	0.199	Fe	0.241	Ti	0.219	o,p'-DDE	0.294
chrysene	0.197	Cd	0.232	Sc	0.216	CBDs	0.282
fluorene	0.196	W	0.231	Y	0.201	p,p'-DDE	0.269
fluoranthene	0.196	Sn	0.227	Li	0.194	o,p'-DDD	0.258
pyrene	0.196	Se	0.213	Be	0.181	p,p'-DDT	0.257
benz(a)anthracene	0.195	Sc	0.203	Ba	0.156	hexachloro-benzene	0.240
anthracene	0.188	Y	0.195	PCBs	0.155	trans-nonachlor	0.235
phenanthrene	0.188	Co	0.195	V	0.153	o,p'-DDT	0.228
biphenyl	0.187	V	0.187	Mn	0.147	m,p'-DDD	0.219
2-methylnaphthalene	0.187	Be	0.187	Se	0.136	Boron	0.212
1-methylnaphthaline	0.187	Ti	0.184	Cd	-0.195	α-chlordane	0.201
acenaphthene	0.182	Li	0.182	Pb	-0.204		
2,3,5 trimethyl-naphthalene	0.181	Mo	0.181	As	-0.227		
2-6 dimethyl-naphthalene	0.179	As	0.180	Mn	-0.264		
ideno (1,2,3-cd)-pyrene	0.177	P	0.167	Ag	-0.275		
benzofluoranthenes	0.173	Sb	0.162	Sb	-0.294		
benzo(a)pyrene	0.173	Cr	0.160	Zn	-0.321		
acenaphthalene	0.171	Zr	0.160	Cu	-0.335		
perylene	0.169	Pb	0.148				
indan	0.162	Ag	0.140				
dibenzothiophene	0.157	Zn	0.135				
benzothiophene	0.155	Cu	0.126				
naphthalene	0.146	Ni	0.113				
n-propylbenzene	0.131						
Eigenvalue	21.5		14.0		5.9		5.57
Percentage of variance	33.6		21.9		9.2		8.71
Cumulative variance	33.6		55.5		64.7		73.4

Table 25. Groupings of MESA stations by cluster analysis based on similarity of concentrations of selected chemicals in sediments from 49 sampling stations. The chemicals selected included 28 metals and 36 organic compounds for stations sampled in 1979, and 7 metals and 36 organic compounds for stations sampled during 1980. Chemical data for sediment analyzed in 1981 are not included in this table.

CLUSTER GROUP	STATION NUMBER	LOCATION	DESCRIPTION
1	8107	Port Madison 2	Indianola SW
	9035	Commencement Bay 9	Sewage plant
	9037	" " " 11	Brown's Point
	9048	" " " 20	South of Brown's Point
	10014	Elliott Bay 9	Magnolia Bluff
	10028	" " 11	Alki Point
	12063	Case Inlet 2	Stretch Island
	10046	Elliott Bay 13	Pier 42
2	8007	Sinclair Inlet 4	Point Herron
	8106	Port Madison 1	Midway
	9029	Commencement Bay 3	Blair Waterway
	9032	" " 6	Puyallup disposal site
	9033	" " 7	Between Hylebos & Blair
	9036	" " 10	Tacoma Yacht Club
	9040	" " 14	Blair turning basin
	9041	" " 21	Near Old Tacoma
	9042	" " 22	Near Old Tacoma
	9044	" " 16	Head, Milwaukee Waterway
	9046	" " 18	Head, St. Paul Waterway
	10016	Elliott Bay 4	Harbor Island N
	10045	" " 10	Duwamish Head
	12062	Case Inlet 1	Reach Island
	12131	Budd Inlet 2	Priest Point
	50200	Port Susan 1	Camano Country Club
	50225	Port Susan 2	Kayak Point
3	8004	Sinclair Inlet 1	Southwest end
	8005	" " 2	Drydock area
	8006	" " 3	Point Turner
	10036	Elliott Bay 16	Duwamish 14th St. Bridge
	10040	" " 6	Pier 70
	10041	" " 8	North of Pier 71
	10042	" " 15	Pier 86
	10043	" " 14	Corps dump site
	10044	" " 7	Midway
	12130	Budd Inlet 1	Entrance channel
	12132	" " 3	Olympia Shoal

Table 25 continued

CLUSTER GROUP	STATION NUMBER	LOCATION	DESCRIPTION
4	10031	Elliott Bay 1	Duwamish Waterway
	10030	" " 2	Duwamish Waterway W
	10039	" " 3	Duwamish Waterway E
	10032	" " 17	Duwamish Waterway[1]
	10034	" " 18	Duwamish Waterway[1]
5	9028	Commencement Bay 2	Hylebos 11th St. Bridge
	9031	" " 5	City Waterway
	10015	Elliott Bay 5	Pier 54
	10023	" " 12	West Point
6	9034	Commencement Bay 8	Brown's Point S
	9038	" " 12	Outside Hylebos Waterway
	9047	" " 19	Head of Middle Waterway
7	9027	Commencement Bay 1	Hylebos turning basin
	9030	" " 4	Sitcum Waterway
	9043	" " 15	Head of Sitcum Waterway
8	9039	Commencement Bay 13	Old Tacoma
9	12133	Budd Inlet	Dofflemyer Pt.
	9045	Commencement Bay	Puyallup River
Unassigned	2041	Discovery Bay	Near Mill Pt.

[1] These two stations were placed in this cluster group on the basis of sediment chemistry data obtained in a previous study (McCain et al. 1982).

Table 26. The concentrations of ΣAHs, HCB, ΣPCBs, and ΣCBDs (ppb, dry weight) in clams (*Macoma nasuta*) and crabs (*Cancer gracilis*) from the caged studies.

SAMPLE	ΣAHs	ΣPCBs	ΣCBDs	HCB
Clam - Quartermaster Harbor	970	62	<2.5	0.88
Clam-after being caged in the Hylebos Waterway 71 days	15,000	1800	678	270
Crab - Mutiny Bay	150	99	<2.2	1.7
Crab-after being caged in the Hylebos Waterway 60 days	390	910	29	120

Table 27. Histopathological conditions observed in target fish species.

ORGAN	CONDITION	SPECIES AFFECTED[1]	SIGNIFICANCE[2]
LIVER	A. Idiopathic		
	1. Neoplasms		
	a. hepatocellular (minimum deviation basophilic nodules, liver cell adenoma, hepatocellular carcinoma)	ES,RS,PSS	NU
	b. biliary (cholangioma, cholangiocellular carcinoma)	ES,RS,PSS	
	c. mesenchymal (hemangioma, fibroma)	ES	
	2. "Preneoplastic" conditions a. nodular eosinophilic hypertrophy, b. nodular hepatocellular hyperplasia, c. hyperbasophilic foci d. hyperplastic regenerative islands	ES,RS,PTC	NU
	3. Nonspecific degenerative/necrotic lesions a. hepatocellular coagulation necrosis, b. hepatocellular hyalinization	All species	NU
	4. Specific degenerative/necrotic lesions a. megalocytic hepatosis, b. nuclear pleomorphism, c. nonneoplastic cytologic atypia	All species	NU
	5. Intracellular storage disorders a. fatty change, b. hemosiderosis, c. hepatocellular vacuolization	All species	NU
	6. Vascular disorders a. sinusoidal congestion b. intrahepatic blood cysts, c. thrombi d. hemorrhage	All species	U

1 ES, English sole; RS, rock sole; PSS, Pacific staghorn sculpin; PTC, Pacific tomcod; and QB, quillback rockfish

2 The significance of each lesion group is designated as either "nonuniform" (NU) throughout the subareas as determined by the G-statistic ($p \leq 0.05$), or affected animals were found only in urban areas; or as "uniform" (U), meaning that affected animals were evenly distributed throughout the subareas (G-statistic, $p \leq 0.05$); or as "not testable" (NT), meaning insufficient affected animals were found to be used in a statistical test.

Table 27 continued

ORGAN	CONDITION	SPECIES AFFECTED	SIGNIFICANCE
LIVER (cont.)	7. Inflammatory lesions	All species	U
	B. Parasitic/Infectious		
	1. Fungus (*Ichthyophonus hoferi*)	PSS	U
	2. Protozoa		
	a. Myxosporidea (including *Myxidium* sp.)		
	b. Eucoccida	ES,RS,PSS	U
	3. Metazoa		
	a. Nematoda	ES,RS,OB,PTC	U
	b. Acanthocephala (larvae)	ES,RS,	
	c. Trematoda (Digenea)	PTC,QB,ES	
KIDNEY	A. Idiopathic		
	1. Degenerative/necrotic lesions	All species	NU
	a. mesangiolysis (G)[3]		
	b. tubular degeneration/necrosis (T)		
	c. tubular vacuolation (T)		
	d. urolithiasis (T)		
	e. tubular protein casts (T)		
	2. Depositional disorders	All species	U
	a. mesangiosclerosis (G)		
	b. hypermembranous glomeruli (G)		
	3. Proliferative conditions (glomerular hypercellularity) (G)	All species	U
	4. Inflammatory lesions	All species	U
	a. glomerulonephritis (G)		
	b. hemopoietic inflammations (H)		
	B. Parasitic/Infectious		
	1. Fungus (*Ichthyophonus hoferi*)	PSS	NT
	2. Protozoa		
	a. Myxosporidea	ES,RS,PSS	U
	b. suspected Microsporidea	PSS,ES,ES,PTC	
	3. Metazoa (Nematoda)	ES,RS,PTC	U

[3] G, glomerular; T, tubular; H, hemopoietic

Table 27 continued

ORGAN	CONDITION	SPECIES AFFECTED	SIGNIFICANCE
GILL	A. Idiopathic		
	1. Degenerative/necrotic lesions a. respiratory epithelial degeneration necrosis b. respiratory epithelial hypertrophy, hydropic degeneration c. mucous cell hypertrophy	All species	U
	2. Proliferative conditions a. respiratory epithelial hyperplasia b. filament epithelial hyperplasia c. pillar cell proliferation d. mucous cell hyperplasia	All species	NU
	3. Vascular disorders a. microaneurysms, acute and chronic b. thrombi, thromboemboli	All species	U
	4. Inflammatory lesions a. lymphoid inflammation (filament edema) b. chronic inflammation	All species	U
	5. Depositional disorders (thickening of the lamellar basement membrane/matrix)		U
	B. Parasitic/Infectious		
	1. Possible Chlamydial or Rickettsial (epitheliocystis)	All species	U
	2. Protozoa a. Microsporidea (endoparasitic) b. *Trichodina* sp. (ectoparasitic)	 PTC, rarely ES) ES,RS,PSS,PTC (rarely QB)	 U
	3. Metazoa a. Trematoda Monogenea (Monopisthocotylea and Polyopisthocotylea, ectoparasitic) Digenea (possible Sanguinicolidae metacercariae, endoparasitic) b. Nematoda (larvae, including *Philometra* sp.) c. Leeches (Hirudinoidea, ectoparasitic) d. Crustacea (primarily copepods, ectoparasitic)	 ES,RS, (less frequently QB, ES,PTC ES QB,ES,PTC,PSS	U
	4. Presumptive infectious disease of uncertain etiology (megalocytic cells in subrespiratory epithelium)	PSS (rarely PTC, RS)	NT

Table 27 continued

ORGAN	CONDITION	SPECIES AFFECTED	SIGNIFICANCE
SKIN and FIN	A. Idiopathic		
	1. Degenerative/necrotic lesions a. epidermal necrosis(with ulcerations) b. epidermal spongiosus c. subcutaneous (sub-q) necrosis	All species	NT
	2. Proliferative conditions (epidermal hyperplasia)	All species	NT
	3. Vascular disorders a. hypervascularization (sub-q) telangiectasia b. hemorrhage (sub-q) c. congestion (sub-q)	All species	NT
	4. Inflammatory lesions a. sub-q fibrogranulation b. sub-q inflammation c. epidermal inflammations d. sub-q fibrosis/fibroplasia	All species	NT
	5. Miscellaneous a. epidermal inclusion cysts	All species	NT
	b. angioepithelial nodules/ epithelial papillomas	RS,ES	
	c. fin erosion	RS,ES and Starry Flounder	NT
	B. Parasitic/Infections		
	1. Viral(lymphocystis)	ES	NT
	2. Protozoan (includes suspected Microsporidea, Myxosporidea, and Coccidia)	ES,RS,PTC	NT
	3. Metazoa a. Trematoda	ES,RS,PTC	NT
	b. Nematoda (*Philometra* sp.)	ES,RS	
HEART	A. Idiopathic		
	1. Degenerative/necrotic lesions a. myocardial degeneration/necrosis (V)[4] b. myocardial fatty change (V),(BA) c. endothelial degeneration/necrosis (BA)	All species	U
	2. Vascular disorders (thromboemboli [V,A,BA])	ES,RS,PTC	U

[4] A, atrium; BA, bulbous arteriosus; V, ventricle

Table 27 continued

ORGAN	CONDITION	SPECIES AFFECTED	SIGNIFICANCE
HEART (cont.)	3. Inflammatory lesions (V,A,BA) a. endocarditis b. myocarditis c. epicarditis d. granulomata e. epicardial edema	All species	U
	B. Parasitic/Infectious		
	1. Fungus (*Ichthyophonus hoferi*)	PSS	U
	2. Protoza a. Microsporidea b. Coccidia	 ES,RS,PSS ES	 U
	3. Metazoa a. Trematoda (Digenea) b. Nematoda (larvae, including *Philometra* sp.)	 ES,RS (rarely PSS,QB) ES,RS (rarely PTC)	 U U
GASTRO-INTESTINAL TRACT	A. Idiopathic		
	1. Degenerative/necrotic lesions a. mucosal degeneration/necrosis b. hydropic degeneration		NT
	2. Vascular disorders a. congestion b. hemorrhage		U
	3. Inflammatory lesions a. granulomata b. macrophage infiltrates c. edema		U
	B. Parasitic/Infectious		U
	1. Protozoa a. Microsporidea b. Coccidia c. flagellates (order Diplomoradida)	 ES ES,RS,PSS, QB (rarely PTC)	
	2. Metazoa a. Cestoda b. Trematoda c. Nematoda (adults and larvae) d. Acanthocephala (adults and larvae)	 ES,RS,PSS,PTC All species All species All species	U

Table 27 continued

ORGAN	CONDITION	SPECIES AFFECTED	SIGNIFICANCE
SPLEEN	A. Idiopathic		
	1. Degenerative/necrotic lesions of hemopoietic tissue	All species	U
	2. Hypocellular/hypoplastic/atrophic conditions a. lymphoid b. erythroid c. hemopoietic (both lymphatic and erythrocytic)	All species	NU
	3. Reactive hyperplastic conditions (lymphoid)	All species	U
	4. Vascular disorder a. congestion b. hemorrhage	All species	U
	5. Depositional disorders (hemosiderin in ellipsoids)	All species	U
	6. Inflammatory lesions a. inflammations b. granulomata	ES,RS,PSS,PTC	U
	B. Parasitic/Infectious		
	1. Fungus (*Ichthyophonus hoferi*)	PSS	NT
	2. Metazoa (Nematoda)	ES,RS,PSS,PTC	U
GONAD	A. Idiopathic		
	1. Degenerative/necrotic lesions of the parenchyma	All species	NT
	2. Vascular disorders a. hemorrhage b. congestion	All species	NT
	3. Inflammatory lesions a. stromal inflammations b. parenchymal inflammations	All species	NT
	4. Miscellaneous (hermaphroditism)	All species	NT
	B. Parasitic/Infectious		
	1. Metazoa		NT
	a. Nematoda	All species	
	b. Acanthocephala (larvae)	All species	

Table 27 continued

ORGAN	CONDITION	SPECIES AFFECTED	SIGNIFICANCE
GALL BLADDER	A. Idiopathic		
	1. Degenerative/necrotic lesions (epithelial vacuolization)	All species	U
	2. Inflammatory lesions (edema)	All species	U
	3. Miscellaneous (inclusion cysts of epithelium)	ES,PSS,RS	NU
	B. Parasitic/Infectious		
	1. Protozoa (Myxosporidea)	ES,RS,PSS,QB (rarely PTC)	U
	2. Metazoa (Cestoda)	ES,PSS,RS,PTC	U

Table 28. Description of the sampling stations within each geographical subarea.

NAME OF SUBAREA	SUBAREA NUMBER	STATIONS WITHIN SUBAREA UNIQUE MESA NUMBER	LOCATION DESCRIPTION
Budd Inlet	1	12130	Entrance channel, south end
		12131	Priest Point
		12132	Olympia Shoal
Case Inlet	2	12062	Reach Island
		12063	Stretch Island
Southwest Commencement Bay	3	09035	Creek at sewage plant
		09036	Tacoma Yacht Club
Old Tacoma Commencement Bay	4	09039	Near Old Tacoma
		09041	Near Old Tacoma
		09042	Near Old Tacoma
Commencement Bay Waterways	5	09029	Blair Waterway, south side
		09030	Sitcum Waterway
		09031	City Waterway
		09032	Puyallup disposal site
		09045	Puyallup Waterway
Hylebos Waterway	6	09027	Hylebos Waterway, lower turning basin
		09028	Hylebos Waterway, E. 11th St. Bridge
		09033	Between Hylebos and Blair
		09034	Brown's Point, south side
Brown's Point Commencement Bay	7	09037	North of Brown's Point
Sinclair Inlet	8	08004	Southwest end
		08005	Drydock area
		08006	Point Turner, southwest side
Upper Duwamish Waterway	9	10032	Duwamish Waterway, S. of Kellogg Is.
		10034	Duwamish Waterway, 1st Ave. S. Bridge
		10036	14th Street Bridge

Table 28 continued

NAME OF SUBAREA	SUBAREA NUMBER	STATIONS WITHIN SUBAREA UNIQUE MESA NUMBER	LOCATION DESCRIPTION
Lower Duwamish Waterway (Elliott Bay)	10	10031	Duwamish Waterway, South of Harbor Is.
		10030	Duwamish Waterway, west channel
		10039	Duwamish Waterway, east channel
		10016	Harbor Island, north end
		10045	Duwamish Head, southeast side
Seattle Waterfront (Elliott Bay)	11	10015	Pier 54
		10040	Pier 70
		10041	North of Pier 71
Outer Elliott Bay	12	10044	Midway from Pier 91 to Duwamish Head
		10014	Magnolia bluff
		10028	Alki Point, south side
West Point (Elliott Bay)	13	10023	West Point, north side
Port Madison	14	08106	Midway from Pt. Monroe to Pt. Jefferson
		08107	Indianola, southwest
Port Susan	15	50200	Camano Country Club
		50225	Kayak Pt.
Discovery Bay	16	02041	Southeast of Port Discovery

Table 29. Mean, standard deviation, range, and number of individuals used in calculations for lengths, weights, and ages of rock sole and English sole exhibiting idiopathic liver lesions.

	Hepatic nonspecific degeneration/ necrosis	Hepatic specific degeneration/ necrosis	Hepatic neoplasia	Hepatic "preneoplasia"	Hepatic storage disorders	Hepatic prolif- erative lesions	Hepatic vascular disorders	Hepatic inflam- mations	Total idiopathic hepatic lesions	Total healthy livers
ROCK SOLE										
Length (mm)										
mean	200.15	178.35	236.59	229.59	211.49	228.43	214.40	154.59	188.46	185.66
stand.dev.	63.86	59.05	65.44	88.32	57.38	51.62	70.05	72.16	72.32	69.80
range	68-365	18-348	110-350	18-392	95-352	138-327	86-350	18-352	18-392	12-398
n	170	111	17	44	119	14	25	116	382	981
Weight (grams)										
mean	125.49	91.11	182.24	212.32	132.42	153.07	150.04	73.61	115.13	103.19
stand.dev.	120.88	99.05	121.47	195.35	108.19	112.23	137.63	97.00	126.24	107.80
range	3-700	3-750	17-465	5-750	6-566	25-370	10-465	1-566	1-750	1-700
n	169	111	17	44	119	14	25	114	379	975
Age (years)										
mean	3.28	2.18	3.33	5.91	3.35	4.75	4.00	1.29	2.95	3.31
stand.dev.	2.55	1.76	2.58	3.36	2.54	1.91	2.36	1.80	2.78	2.76
range	1-10	0-8	1-8	1-10	1-11	2-8	1-8	0-7	0-11	0-14
n	65	28	6	11	55	8	10	34	121	309
ENGLISH SOLE										
Length (mm)										
mean	257.90	254.30	298.42	283.18	278.19	296.18	253.30	229.70	255.74	229.41
stand.dev.	68.04	60.80	50.42	52.27	59.32	45.16	72.76	66.94	69.21	80.61
range	63-452	101-405	200-408	115-390	63-452	185-375	105-419	67-412	63-452	50-490
n	267	191	72	142	226	22	80	115	592	1528
Weight (grams)										
mean	183.12	162.57	242.74	211.96	214.35	224.64	166.76	124.63	173.97	141.55
stand.dev.	129.35	106.79	120.94	109.49	137.99	92.49	131.19	122.75	130.87	135.44
range	2-800	8-600	60-622	8-525	2-912	50-388	8-620	1-912	1-912	1-850
n	267	192	72	142	226	22	80	115	593	1521
Age (years)										
mean	4.39	3.76	6.06	5.41	4.96	6.25	4.26	2.52	3.93	2.72
stand.dev.	2.70	2.03	2.54	2.59	2.80	1.76	3.13	3.19	2.83	2.40
range	0-14	0-9	1-14	0-14	0-14	4-9	0-14	0-14	0-14	0-12
n	142	85	36	76	117	12	35	42	258	682

Table 30. Differential leukocyte counts in English sole (*Parophrys vetulus*) with various liver and kidney lesions.

Lesion Type of Parasitic Infection	Lymphocytes	Thrombocytes	Granulocytes	N[a]
LIVER				
a) Nonspecific Necrosis	L[b]	H[c]	NS[d]	67
b) Specific Necrosis	L	H	NS	51
c) Neoplasm	L	H	H	24
d) "Preneoplasia"	L	H	H	56
e) Storage Disorders	L	H	NS	63
f) Vascular Disorders	NS	NS	H	13
g) Proliferative	L	H	H	12
h) *Myxosporidia*	L	H	NS	162
i) *Coccidia*	NS	NS	H	18
j) *Nematode*	NS	NS	NS	41
k) Acanthocephalan	NS	NS	NS	16
KIDNEY				
a) Deposition Disorders	L	H	H	85
b) Necrosis	L	H	NS	36
c) *Myxosporidia*	NS	NS	NS	7
d) *Nematode*	NS	NS	NS	7
GILL				
Vascular Disorders	NS	NS	NS	34
Inflammatory Lesions	NS	NS	NS	20
a) Epitheliocystis	L	NS	H	38
b) *Trichodina*	L	NS	NS	74
c) *Trematode*	NS	NS	L	122

a) Sample size
b) L signifies a significantly lower value (% of cell type) ($p \leq 0.05$) relative to the control (normal) group as determined by a student's T-TEST. (Control group % differentials; N=71; % lymphocytes 73.1±8.5 % thrombocytes 24.1±8.1; % PAS positive granulocytes 1.7±1.1)
c) H signifies a significant increase ($p \leq 0.05$) in % of cell type relative to control fish
d) NS signifies no significant difference

Table 31. Association of various liver, kidney, and heart lesions with changes in serum components classically used to reflect organ dysfunction.

Lesions	N[a]	SERUM COMPONENTS										
		Hematocrit	ASAT[d]	ALAT[e]	Glucose	Bilirubin	Albumin	Total Protein	Phosphate	Calcium	Magnesium	Creatinine	Urea-nitrogen
LIVER													
Nonspecific Necrosis	67						L[b]						
Specific necrosis	50				L	H[c]	L						
Neoplasm	23	L					L	L	L	L	L		
"Pre-neoplasms"	55					H	L						
Storage Disorders	68						L			L			
Vascular Disorders	13	L	H			H	L	L	L	L	L		
Proliferative Disorders	12	L		H	L		L	L	L	L			
Inflammation	9	L					L	L		L			
KIDNEY													
Necrosis	36	L					L			L			
HEART													
inflammation	6			H		H							

[a] Sample size, [b] Significant difference (decrease) from control values, $P \leq 0.05$. Evaluated by students T-Test
Blank indicates no significant difference from control values
[c] Significant difference (increase) from control values, $p \leq 0.05$. Evaluated by students T-Test
[d] Aspartate aminotransferase
[e] Alanine aminotransferase

Table 32. Idiopathic liver lesions of English sole which correlated significantly with principal components axes scores. Analyses were performed using the nonparametric method of Spearman's rank correlation with the first 4 principal components axes. Coefficient values can range from -1 (perfect negative correlation) to +1 (perfect positive correlation).

Liver Lesion	Principal Components Axis	Spearman Correlation Coefficient	Significance of Correlation
Storage disorders	1	0.4995	p=0.002
Storage disorders	2	0.3227	p=0.036
Neoplasms	1	0.4966	p=0.002
	2	0.3632	p=0.021
Specific degeneration/ necrosis	1	0.4722	p=0.003
	2	0.3180	p=0.038
"Preneoplastic" conditions	1	0.4175	p=0.009
Nonspecific necrosis	2	0.3674	p=0.019
Inflammations	1	-0.3414	p=0.028

Table 33. Hematological indices of English sole (*Parophrys vetulus*) which correlated significantly with principal components axes scores. Analyses were performed using Kendall's rank correlation with the first 4 principal components axes. Values represent Kendall's rank correlation coefficient.

	PRINCIPAL COMPONENTS AXIS			
	1	2	3	4
PARAMETER				
Hematocrits	NS	NS	-0.11[b]	NS
Lymphocytes	NS	NS	NS	NS
Thrombocytes	NS	NS	NS	NS
Lymphocyte/ Thrombocyte	NS	-0.12[b]	-0.15[c]	0.17[c]
Granulocyte	NS	-0.09[a]	-0.21[c]	NS

NS - not significant
[a] - signficant at $p \leq 0.05$
[b] - significant at $p \leq 0.01$
[c] - significant at $p \leq 0.001$

Table 34. Serum chemistry values of English sole (*Parophrys vetulus*) which correlated significantly with principal components axes scores. Analyses were performed using Kendall's rank correlation with the first 4 principal components axes. Values represent Kendall's rank correlation coefficient.

	PRINCIPAL COMPONENTS AXIS			
	1	2	3	4
SERUM CHEMISTRY				
ASAT	NS	NS	NS	NS
ALAT	0.14[c]	0.08[a]	NS	0.09[a]
Glucose	0.09[a]	NS	-0.12[b]	-0.10[a]
Bilirubin	NS	NS	0.22[c]	NS
Albumin	NS	NS	NS	NS
Total Protein	NS	NS	NS	NS
Creatinine	NS	0.23[c]	0.24[c]	NS
Phosphate	0.17[c]	NS	NS	-0.15[c]
Calcium	NS	NS	0.13[b]	NS
Magnesium	NS	NS	-0.21[c]	NS

NS - not significant
[a] - significant at $p<0.05$
[b] - significant at $p<0.01$
[c] - significant at $p<0.001$

Table 35. Histopathological lesions observed in target invertebrate species.

ORGAN OR TISSUE TYPE		CONDITION	SPECIES AFFECTED[1]
GILL	A.	Necrosis	CA,CC,PS
	B.	Melanized nodules and granulomas	CC CA,PS
HEPATOPANCREAS	A.	Epithelial necrosis and/or encapsulation	CC,PS CA
	B.	Epithelial metaplasia	CC
	C.	Phagocytic activation	CC
ANTENNAL GLAND	A.	Necrosis	CA,CC,PS
BLADDER	A.	Necrosis	CC,CA,PS
MIDGUT	A.	Melanized nodules and granulomas	CC
	B.	Hemocytic infiltration	CC
MUSCLE	A.	Microsporidan infestation	CA
NERVOUS SYSTEM	A.	Trematode infestation	CA
SYSTEMIC	A.	Yeast infestation	CA

1 Species affected were *Crangon alaskensis* (CA); *Cancer* crabs (CC), *C. magister*, *C. gracilis*, and *C. productus* (CC); and pandalid shrimp (PS), *Pandalus danae* and and *P. jordani* (PS)

Table 36. The species richness (SR) and species diversity (SD) values for the target fish species and the number of hauls (N) taken in the study areas.

SEASON		1979 W	1979 Sp	1979 Su	1979 F	1980 Su	1980 F
STUDY AREA							
Budd Inlet	N	9	8	3	4	-- [a]	--
	SR	9	11	19	22	--	--
	SD	1.12	1.88	1.73	2.37	--	--
Case Inlet	N	6	6	3	6	--	--
	SR	8	11	16	15	--	--
	SD	1.77	1.52	1.95	1.82	--	--
Commencement Bay	N	28	18	14	16	21	20
	SR	39	36	36	39	43	42
	SD	2.44	2.09	2.48	1.96	2.58	2.49
Elliott Bay	N	30	24	25	21	29	22
	SR	44	37	38	36	37	39
	SD	2.36	2.35	2.72	2.80	2.53	2.92
Sinclair Inlet	N	8	7	3	4	5	5
	SR	10	16	16	19	18	13
	SD	1.61	1.73	2.05	2.10	1.68	1.53
Port Madison	N	3	5	3	2	--	--
	SR	17	11	17	24	--	--
	SD	2.42	1.77	1.77	2.07	--	--
Port Susan	N	--	--	--	--	6	--
	SR	--	--	--	--	25	--
	SD	--	--	--	--	2.09	--
Discovery Bay	N	--	--	--	--	--	5
	SR	--	--	--	--	--	30
	SD	--	--	--	--	--	1.92

[a] "--" signifies that trawls were not performed in that area during the sampling period

Table 37. Mean (and standard deviation) of infaunal data for stations sampled in 1979 and 1980 (underbar indicates no significant difference at $p \leq 0.05$)

	Taxon Richness		Infaunal Trophic Index	
	Summer 1979 (N=6)[1]	Summer 1980 (N=10)	Summer 1979 (N=6)	Summer 1980 (N=10)
CITY WATERWAY	5.3 (2.5)	6.5 (2.4)	57.9 (4.7)	61.5 (3.3)
HYLEBOS WATERWAY	7.0 (1.0)	8.1 (1.6)	52.9 (5.4)	56.3 (5.77)
DUWAMISH WATERWAY	4.7 (1.8)	5.0 (1.2)	72.0 (4.0)	64.9 (1.4)

1 N, number of replicate sediment samples

pier 54 - one
Pt Susan - two

Table 38. The classes of variables used to perform the stepwise multiple regression analysis of the benthic infaunal community indices described in Table 39.

CLASS	VARIABLES
A	Ag, As, B, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Sr, Zn, PCBs (negatively correlated with taxon richness)
B	Be, Co, Fe, Li, P, Sc, Se, Su, Ti, V, W, Y, Zr (negatively correlated with taxon richness)
C	Mn, 1,[a] 2, 5, 6, Acenaphthylene, 14, 16, 20, 24, 25, indeno-(1,2,3-CD)-pyrene, o,p'-DDD (positively correlated with taxon richness)
D	3,9,10,11,13,15,22,23,27 (negatively correlated with taxon richness)
E	7,8,12,21 (negatively correlated with taxon richness)
F	o,p'-DDE; p,p'-DDE; m,p'-DDD; o,p'-DDT;p,p'-DDT (negatively correlated with taxon richness)
G	hexachlorobenzene, -chlordane, trans-Nonachlor, CBDs (negatively correlated with taxon richness)
H	Depth, organic carbon, proportions of 6 sediment grain size fractions (most variables positively correlated with taxon richness)

[a] See Table 2 for the aromatic hydrocarbons designated by numbers

Table 39. Results of stepwise multiple regression analysis of benthic invertebrate community indices (See Table 4 for a description of these indices) and chemical and physical data for 1979 MESA sampling stations. Values are multiple coefficients of determination (r^2). Numbers of positive (+) and negative (-) simple correlations with individual variables are given in parentheses.

Class of variables	Number of variables	S	H'	J	TA	ITI
A	15	0.25***[a/] (15-)	0.34*** (15-)	0.23*** (4+,11-)	0.25*** (15-)	0.21*** (6+,9-)
B	13	0.21*** (13-)	0.32*** (13-)	0.13*** (1+,12-)	0.17*** (13-)	0.31*** (3+,10-)
C	12	0.19*** (12+)	0.21*** (10+,2-)	0.17*** (12+)	0.19*** (9+,3-)	0.08*** (6+,6-)
D	9	0.12*** (9-)	0.12*** (7+,2-)	0.13*** (9+)	0.19*** (1+,8-)	0.04* (3+,6-)
E	5	ns (5-)	ns (5+)	0.02* (5+)	ns (1+,4-)	ns (2+,3-)
F	5	0.05*** (5-)	0.02* (5-)	0.04* (3+,2-)	ns (5-)	ns (5-)
G	4	ns (4-)	ns (4-)	0.02* (4-)	0.08*** (4+)	ns (4-)
H	8	0.26*** (5+,3-)	0.38*** (5+,3-)	0.09** (5+,3-)	0.11*** (2+,6-)	0.11*** (6+,2-)
Overall	71	0.48*** (17+,54-)	0.59*** (27+,44-)	0.33*** (39+,32-)	0.36*** (17+,54-)	0.56*** (26+,45-)
No. of samples for analyses A to G		248	228	214	248	228
No. of samples for H overall analysis		238	219	205	238	219

[a]
ns - Not significant
* Significant at $p<0.05$
** Significant at $p<0.01$
*** Significant at $p<0.001$

Table 40. Spearman rank correlation coefficients for infaunal benthic invertebrate community indices and principal components analysis (See a description of the principal components axes in Table 24) factor scores at 40 benthic sampling stations. Index values are 1979 means for the chemistry-sample site at each station.

	PRINCIPAL COMPONENTS AXIS			
INDEX	1	2	3[a]	4
Taxon Richness (S)	-0.34*	-0.52***	-0.45**	ns
Taxon Diversity (H')	-0.33*	-0.58***	-0.41*	ns
Taxon Evenness (J)	ns	ns	ns	ns
Total Abundance	ns	ns	-0.39	ns
Infaunal Trophic Index	ns	ns	ns	-0.34*

[a] Station 9030 excluded
ns - Not significant
* Significant at <0.05
** Significant at <0.01
*** Significant at <0.001

Table 41. Survival data for crabs exposed to bottom sediments for 60 days (underbar indicates means which cannot be distinguished at the .05 significance level using the Student-Newman-Keuls multiple range test).

Location	Duwamish Waterway	Port Susan	City Waterway	Seattle Aquarium	Hylebos Waterway
Cluster group	4	2	5	5	5
Mean no. surviving	1.0	2.5	3.0	4.0	4.0
Standard deviation	0.0	0.7	0.0	0.0	0.0

Table 42. Mean and standard deviation (in parentheses) taxon richness values in first recolonization experiment (n=4 for each mean).

LOCATION OF EXPERIMENT	SOURCE OF SEDIMENT Same location		Port Susan		Difference
Duwamish Waterway	5.0	(0)	6.5	(1.3)	+1.5*
Hylebos Waterway	3.75	(3.2)	5.5	(0.6)	+1.75
City Waterway	3.5	(0.6)	4.75	(2.2)	+1.25
Seattle Aquarium	5.0	(1.8)	3.25	(1.0)	-1.75

*Significant at $p \leq 0.5$

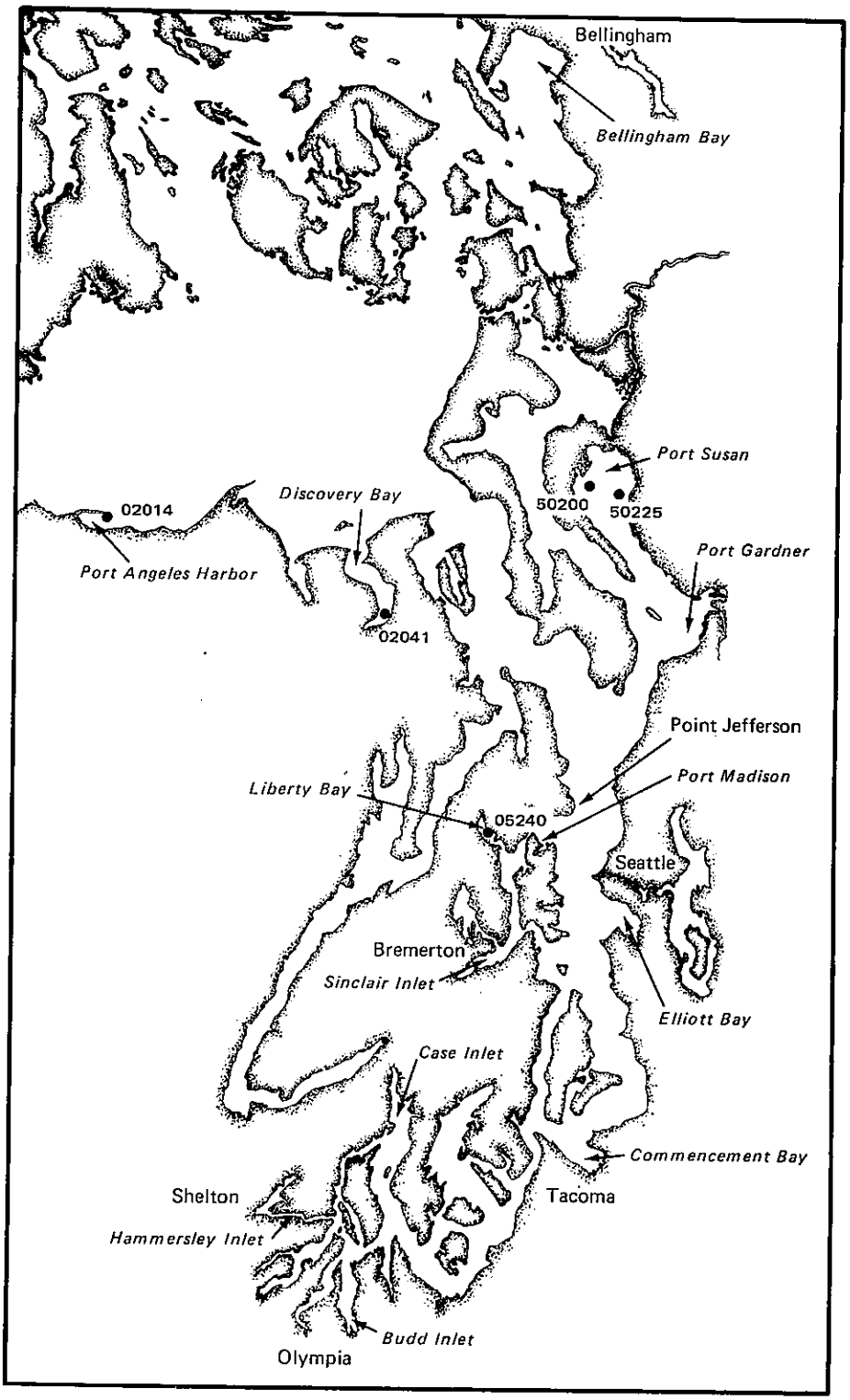
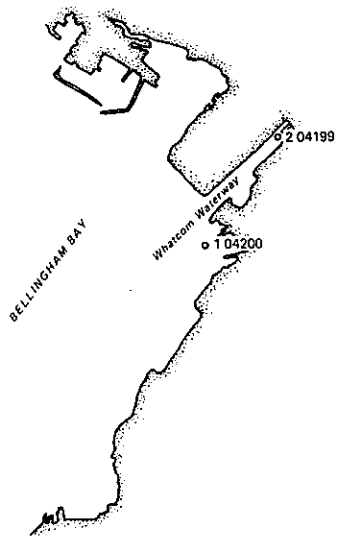


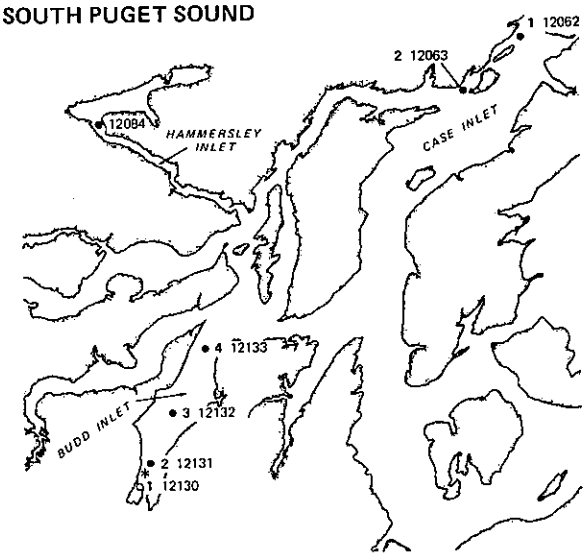
Figure 1. Locations of embayments studied. With the exception of Port Angeles Harbor (Station 02014, depth 13 m), Liberty Bay (Station 05240, depth 13 m), Discovery Bay (Station 02041, depth 32 m), and Port Susan (Station 50200, depth 14 m; and Station 50225, depth 25 m), the sampling areas are displayed in greater detail in Figure 2.

BELLINGHAM BAY

1 Between chlorine dock and flasher (8m)
2 Whatcom Waterway, inner reach (8m)



SOUTH PUGET SOUND



Case Inlet:

1 Reach Island (10–20m)
2 Stretch Island (24–30m)

Budd Inlet:

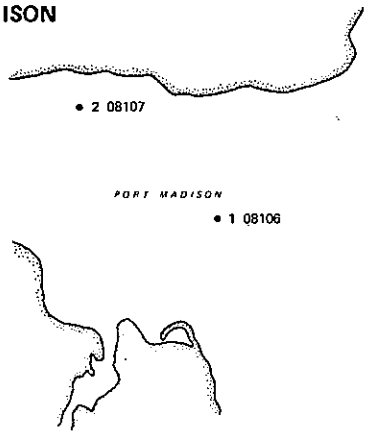
1 Entrance channel, south end (5–6m)
2 Priest Point (15–25m)
3 Olympia Shoal (15–25m)

Hammersley Inlet:

Munson Point (3m)

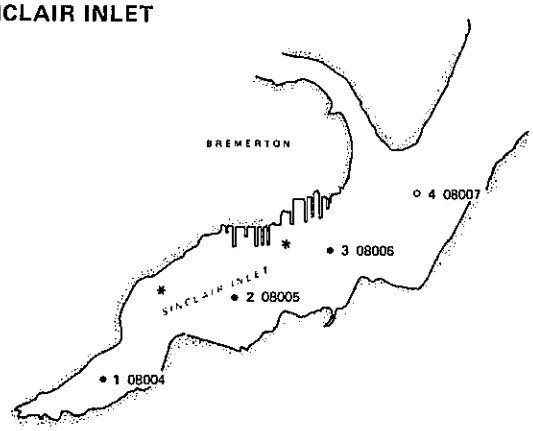
Figure 2. Locations of sampling stations. Bellingham Bay and south Puget Sound. The number of each station is the project identification number. Closed circles (•) designate stations where samples were collected for biological and chemical examination while open circles (o) designate stations where sediment samples were taken only for chemical analysis, and * designate stations samples in 1978. The depth or depth ranges sampled are given in ( ). The chemistry data for Commencement Bay stations (No. 15-22) are reported elsewhere (Malins et al. 1981).

PORT MADISON



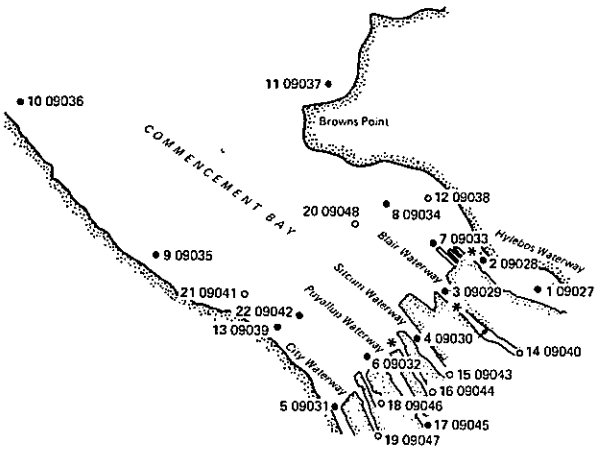
1 Midway from Pt. Monroe to Pt. Jefferson (40–100m)
2 Indianola, southwest (15–35m)

SINCLAIR INLET



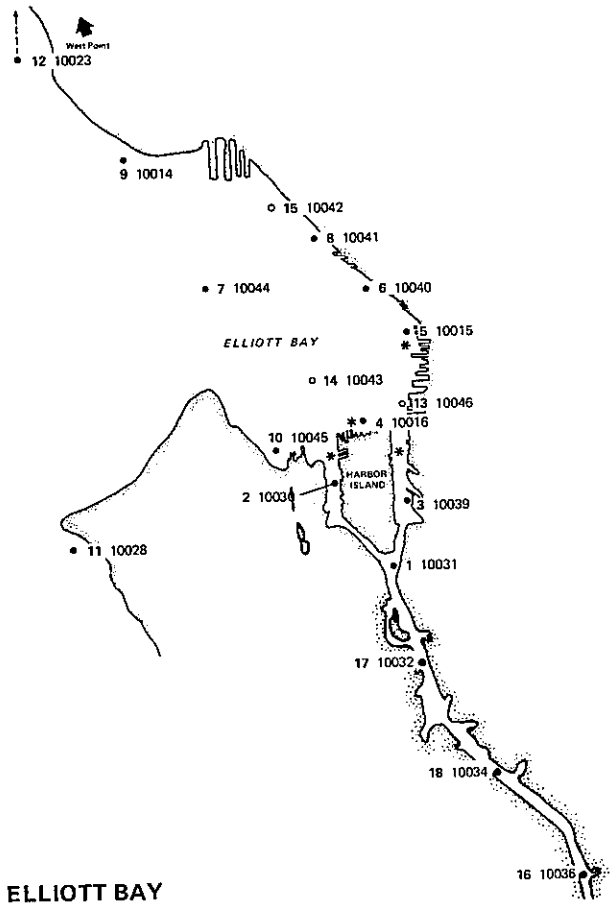
1 Southwest end (8–10m)
2 Drydock area (10–12m)
3 Point Turner, southwest side (13–15m)
4 Point Herron, south side (15–30m)

COMMENCEMENT BAY



1 Hylebos Waterway, lower turning basin (10–12m)
2 Hylebos Waterway, E. 11th St. Bridge (10–12m)
3 Blair Waterway, E. 11th St. Bridge (10–12m)
4 Sitcum Waterway (10–15m)
5 City Waterway (7–10m)
6 Puyallup disposal site (20–60m)
7 Between Hylebos and Blair (20–50m)
8 Brown's Point, south side (30–50m)
9 Creek at sewage plant (20–80m)
10 Tacoma Yacht Club (20–60m)
11 Brown's Point (20–60m)
12 Hylebos Waterway, outside, to NW (20–60m)
13 Old Tacoma (20–80m)
14 Blair Waterway, turning basin (10–12m)
15 Head of Sitcum Waterway (10m)
16 Head of Milwaukee Waterway (13m)
17 Puyallup Waterway (1–2m)
18 Head of St. Paul Waterway (9m)
19 Head of Middle Waterway (8m)
20 South of Brown's Point (45m)
21 Near Old Tacoma (100m)
22 Near Old Tacoma (100m)

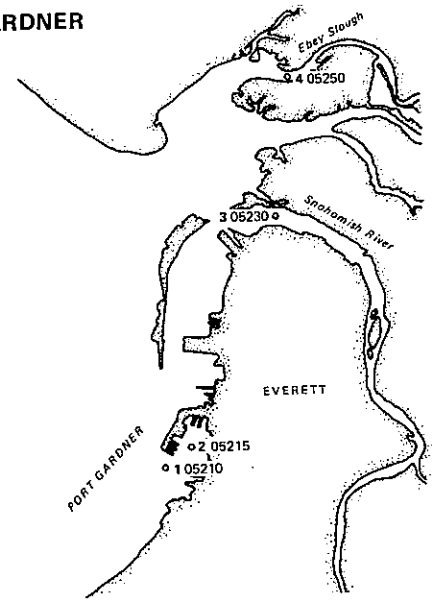
Figure 2. (continued) Port Madison, Sinclair Inlet, and Commencement Bay.



ELLIOTT BAY

1 Duwamish Waterway, near lumber mill (10–20m)
2 Duwamish Waterway, west channel (15–20m)
3 Duwamish Waterway, east channel (12–16m)
4 Harbor Island, north end (12–50m)
5 Pier 54 (20–60m)
6 Pier 70 (20–60m)
7 Midway from Pier 91 to Duwamish Head (120–170m)
8 North of Pier 71 (15–65m)
9 Magnolia Bluff (15–60m)
10 Duwamish Head, southeast side (12–50m)
11 Alki Point, south side (15–60m)
12 West Point, north side (10–50m)
13 Pier 42 (20–60m)
14 Corps dump site (70–85m)
15 Pier 86 (30–50m)
16 Duwamish Waterway, 14th Ave. bridge (10m)
17 Duwamish Waterway, south of Kellogg Is. (12m)
18 Duwamish Waterway, south of 1st. Ave. S. bridge (10m)

PORT GARDNER



1 Near Pier 1, mid-channel (13m)
2 Near Scott Plant, mid-channel, due west of Scott silver bldg. (10m)
3 Snohomish River, below green bridges, near Preston Point (5m)
4 Ebey Slough (4m)

Figure 2. (continued) Elliott Bay and Port Gardner.

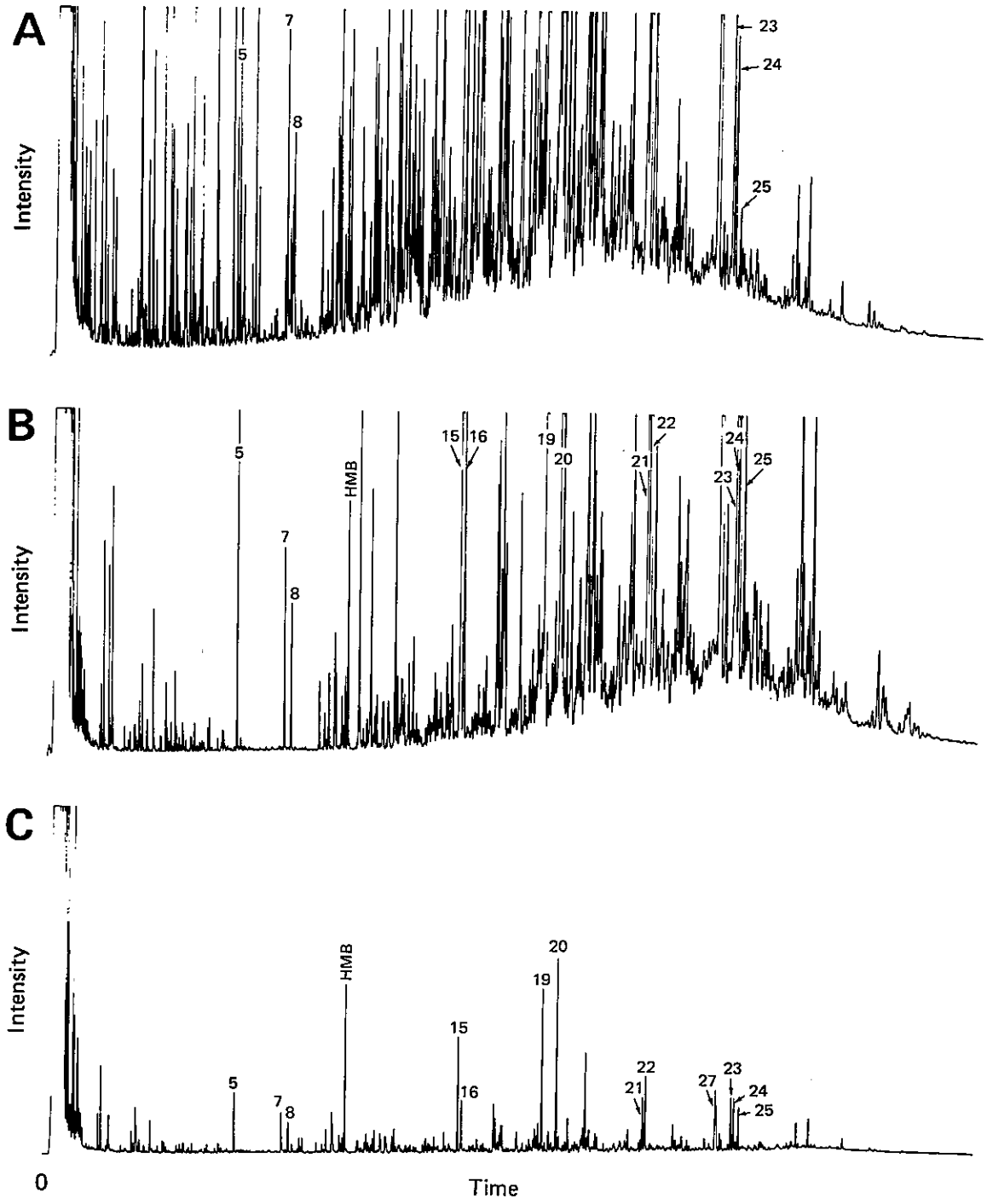


Figure 3. Gas chromatograms of AHs in extracts of similar weights of sediment from: (A) Hylebos Waterway, (B) Duwamish Waterway, and (C) Port Madison. Flame ionization detection. Labeled peaks are identified in Table 2.

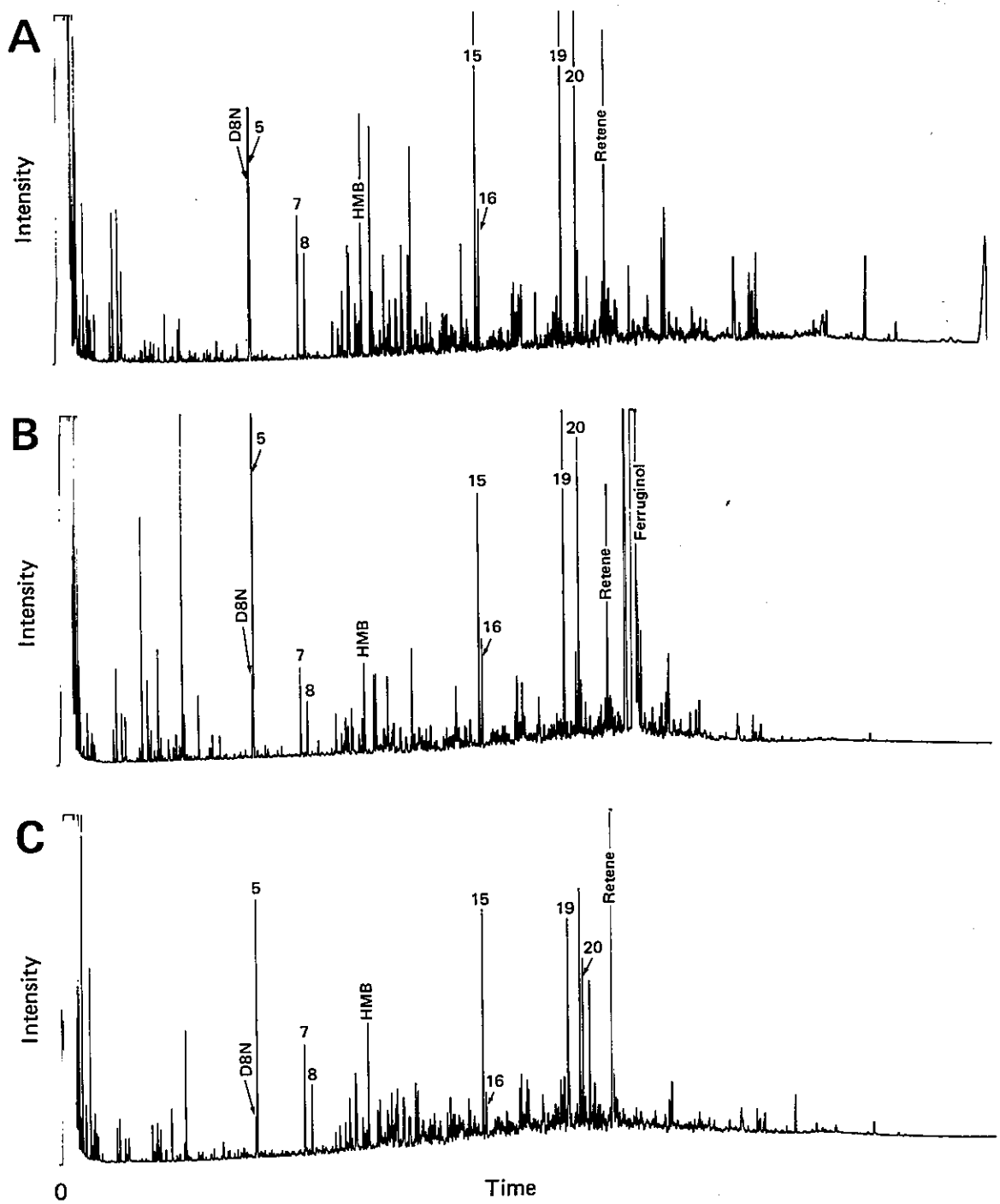


Figure 4. Gas chromatograms of AHs in extracts of sediment from: (A) Port Angeles Harbor, 100 g, (B) Port Gardner (near Everett), 33g, and (C) Whatcom Waterway (near Bellingham) 20g. Flame ionization detection. Labeled peaks are identified in Table 2.

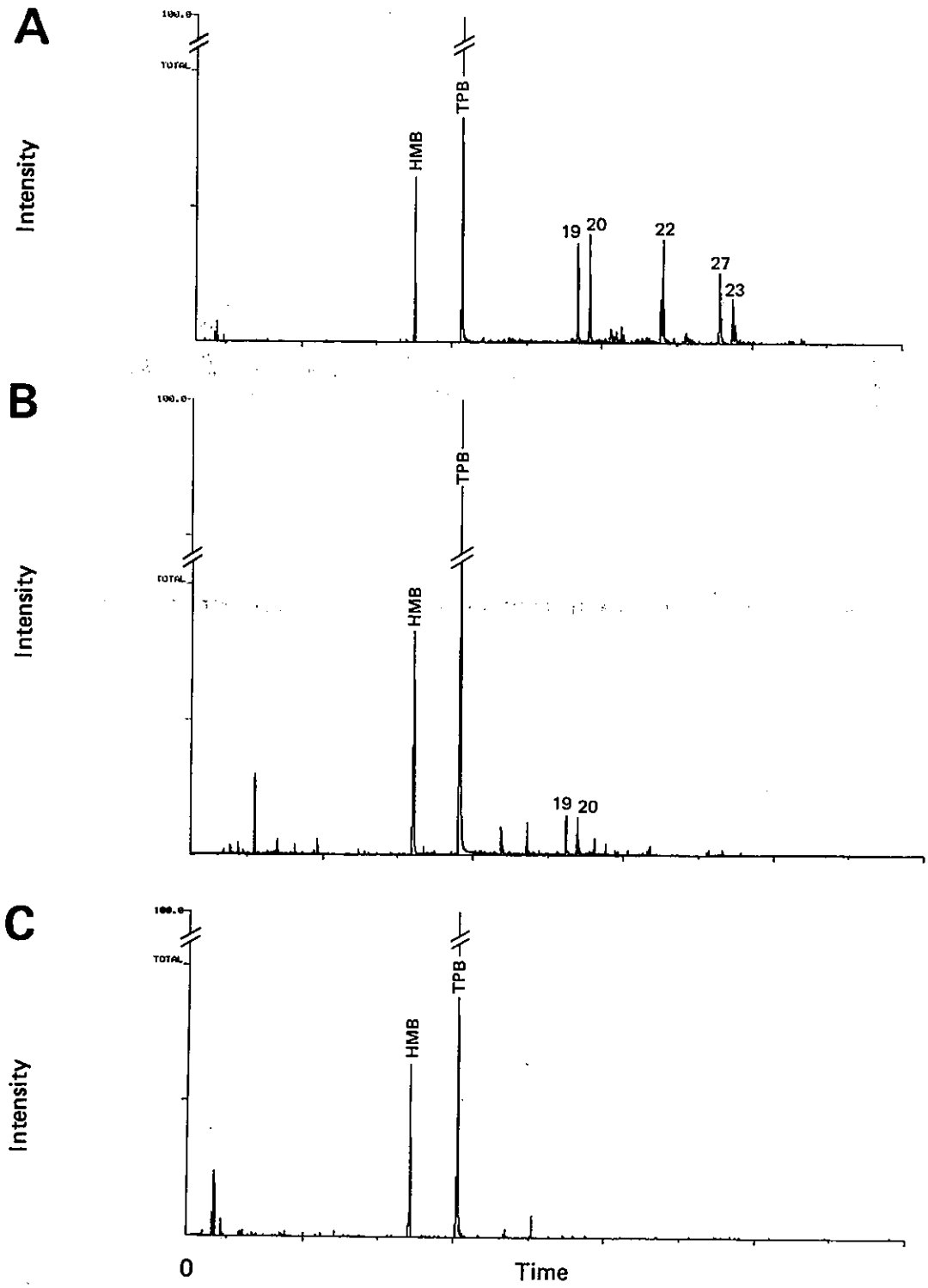


Figure 5. Gas chromatograms of AHs in extracts of: (A) worm (4.7 grams, wet weight), (B) shrimp (10.1 grams, wet weight), and (C) clams (7 grams wet weight). All samples were collected from the Hylebos Waterway. Mass spectrometer detection. Labeled peaks are identified in Table 2.

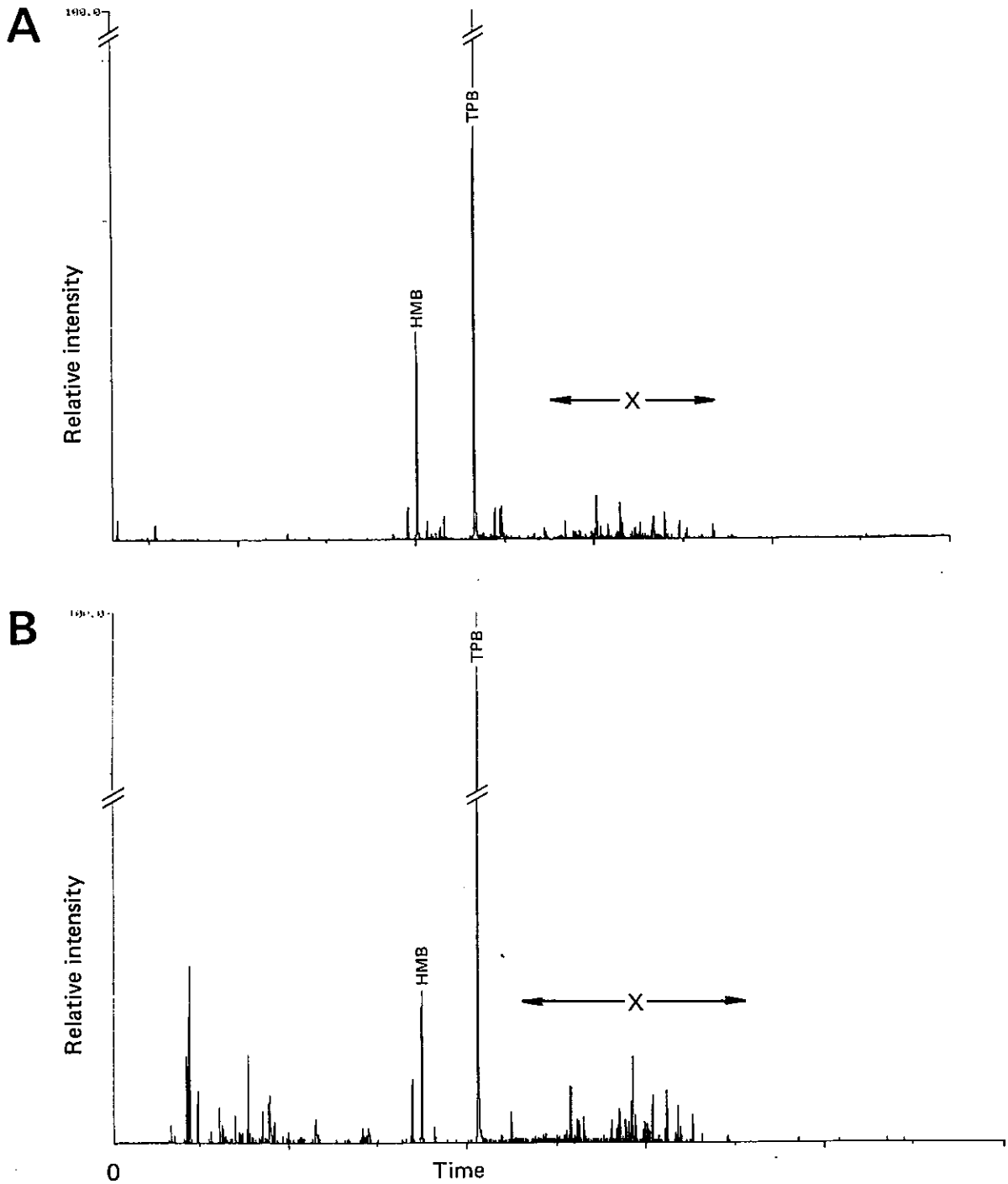


Figure 6. Gas chromatograms of AHs in extracts of: (A) English sole liver (5.7 grams, wet weight) and (B) crab hepatopancreas, Cancer spp. (9.4 grams, wet weight). Both samples were collected from the Hylebos Waterway. Mass spectrometer detection. Labeled peaks are identified in Table 2. The peaks in the area labeled X are not hydrocarbons but are PCBs and other chlorinated hydrocarbons present at high enough concentrations to show in these chromatograms.

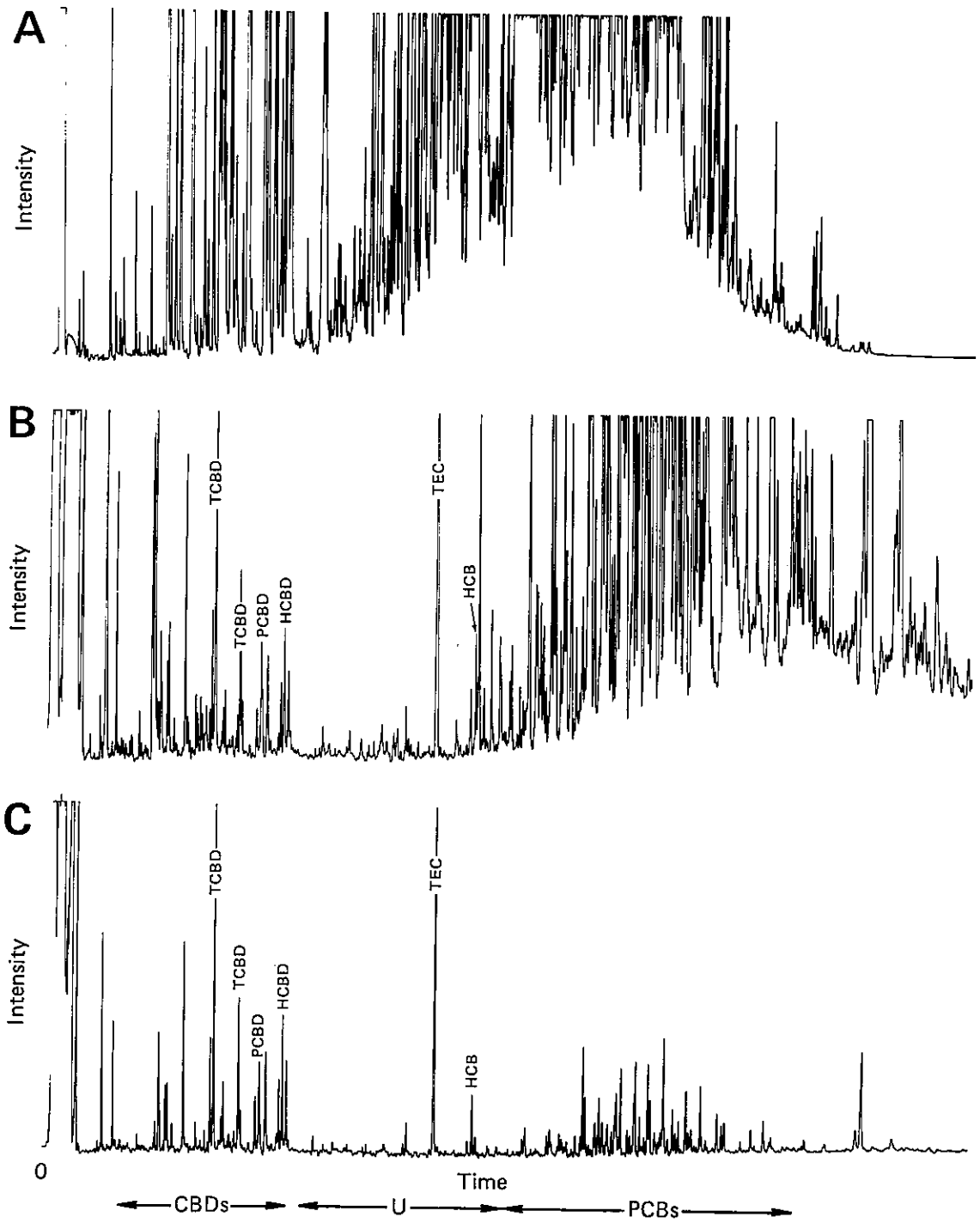


Figure 7. Gas chromatograms of chlorinated compounds in extracts of sediment from: (A) Hylebos Waterway (2 grams of sample), (B) Duwamish Waterway (100 grams), and (C) Port Madison (100 grams). Electron capture detection. Labeled peaks are identified in Table 2. The areas where CBDs, PCBs, and other chlorinated compounds (U) elute are noted.

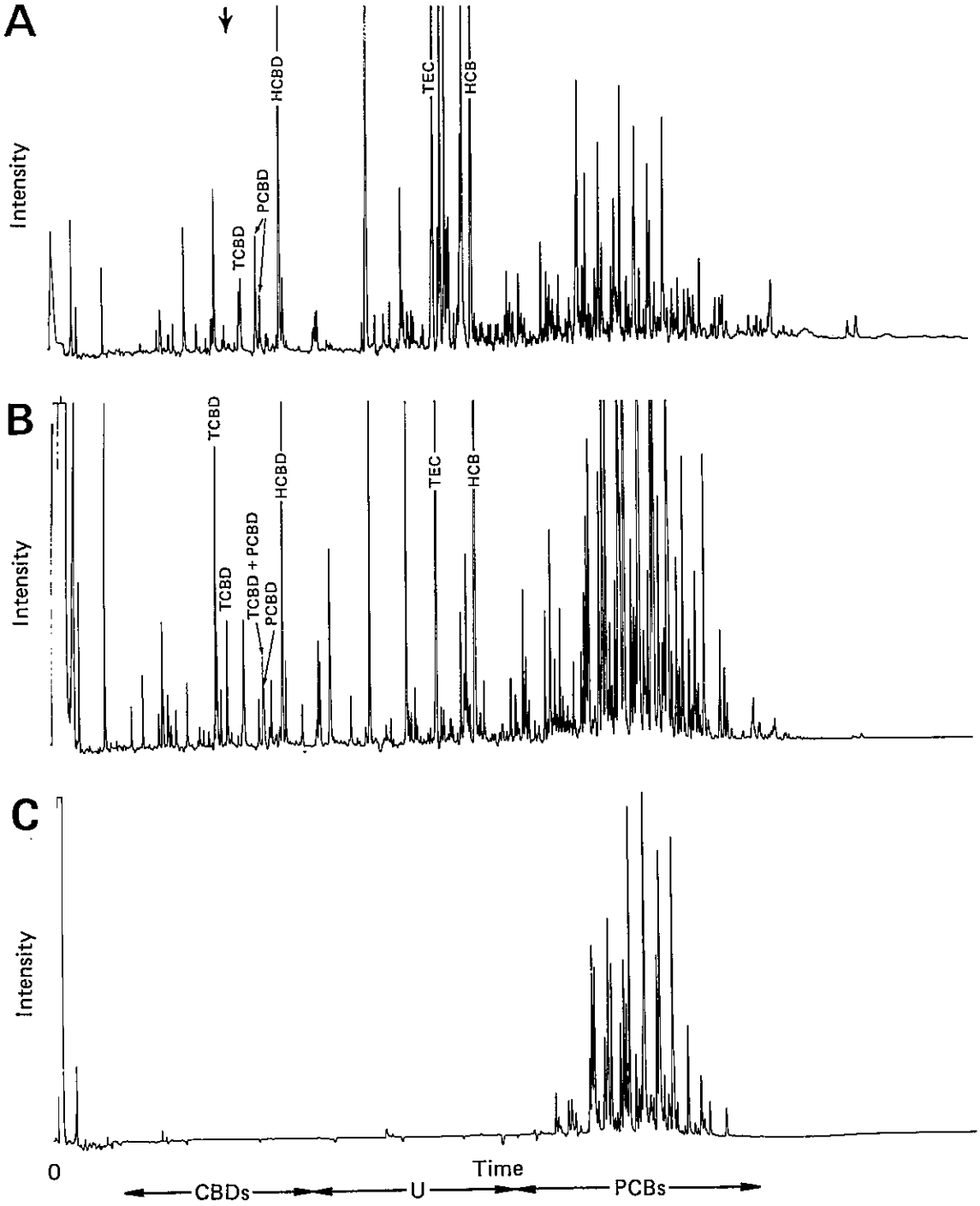


Figure 8. Gas chromatograms of chlorinated compounds in extracts of samples collected from the Hylebos Waterway of: (A) worms (4.6 grams, wet weight), (B) shrimp (10.1 grams, wet weight), and (C) PCBs (Aroclor 1254 standard). Electron capture detection. Labeled peaks are identified in Table 2. The areas where CBDs, PCBs, and other chlorinated compounds (U) elute are noted.

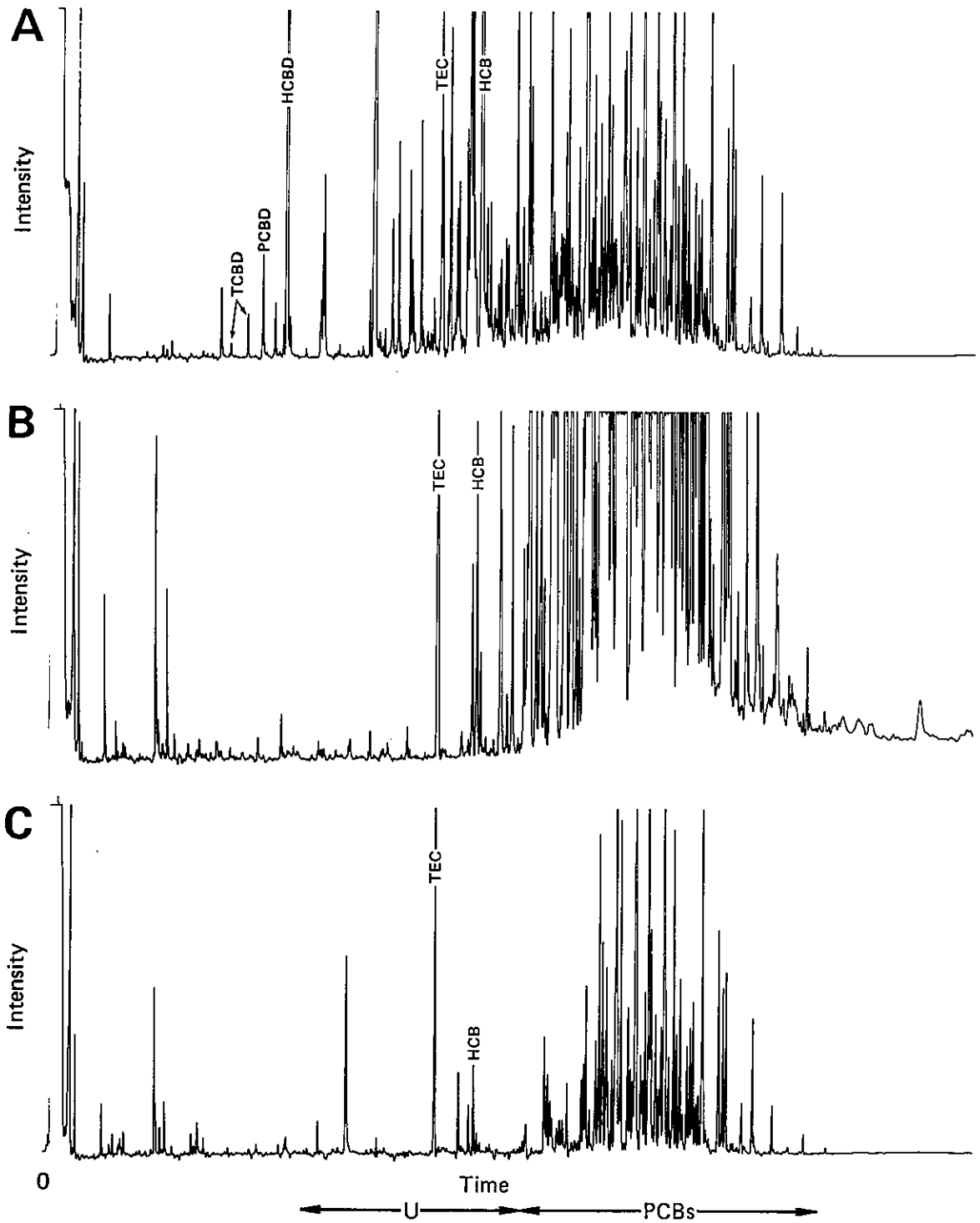


Figure 9. Gas chromatograms of chlorinated compounds in extracts of samples of English sole liver from (A) Hylebos Waterway (1.1 grams, wet weight), (B) Duwamish Waterway (10.6 grams, wet weight), and (C) Port Madison (9.8 grams, wet weight).

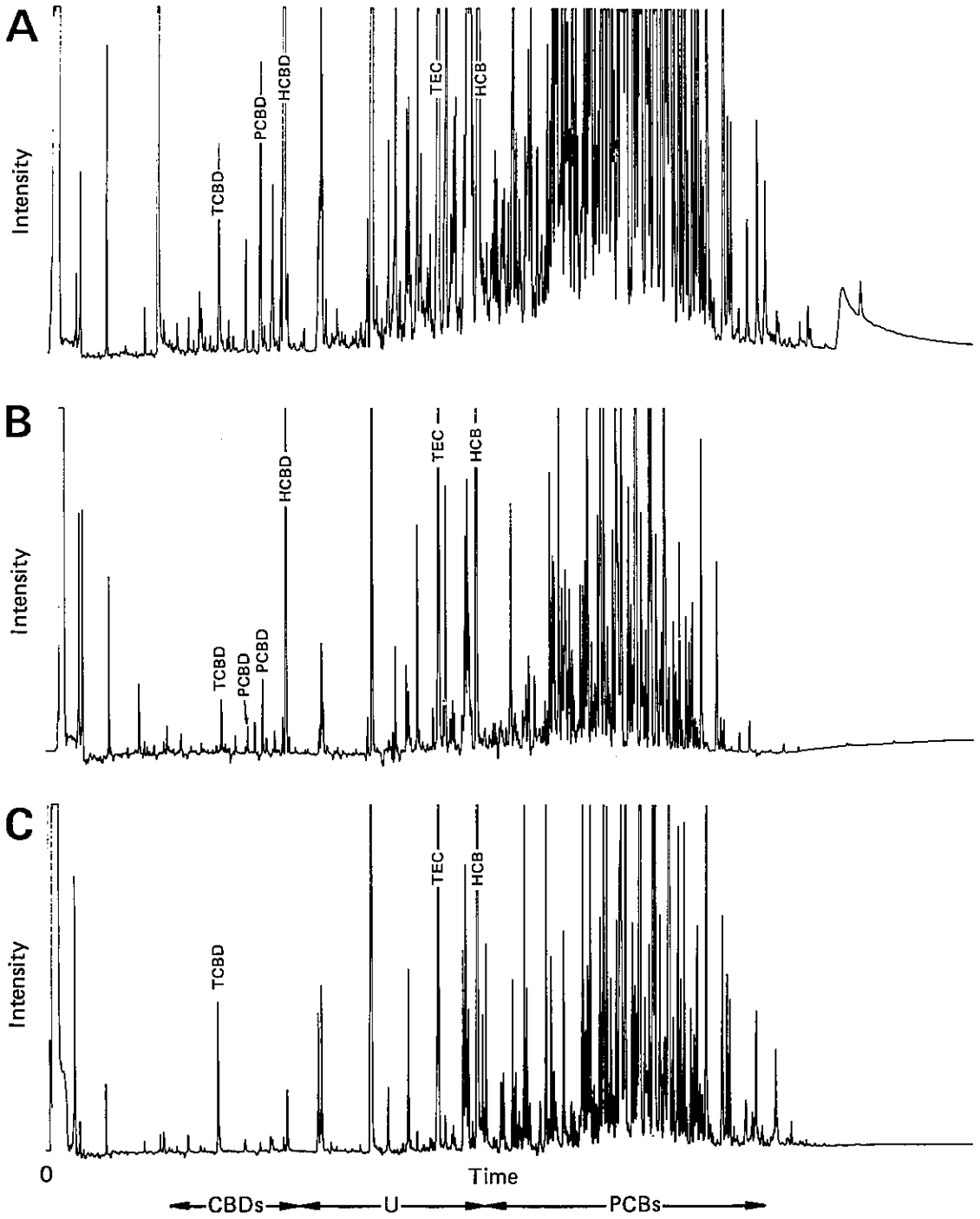


Figure 10. Gas chromatograms of chlorinated hydrocarbons in extracts of samples of: (A) English sole liver (7 grams, wet weight), (B) corresponding sole skeletal muscle (10.1 grams, wet weight), and (C) crab hepatopancreas (9.4 grams, wet weight). All samples were collected from the Hylebos Waterway. Electron capture detection. Labeled peaks are identified in Table 2. The area where CBDs, PCBs, and other chlorinated compounds (U) elute are noted.

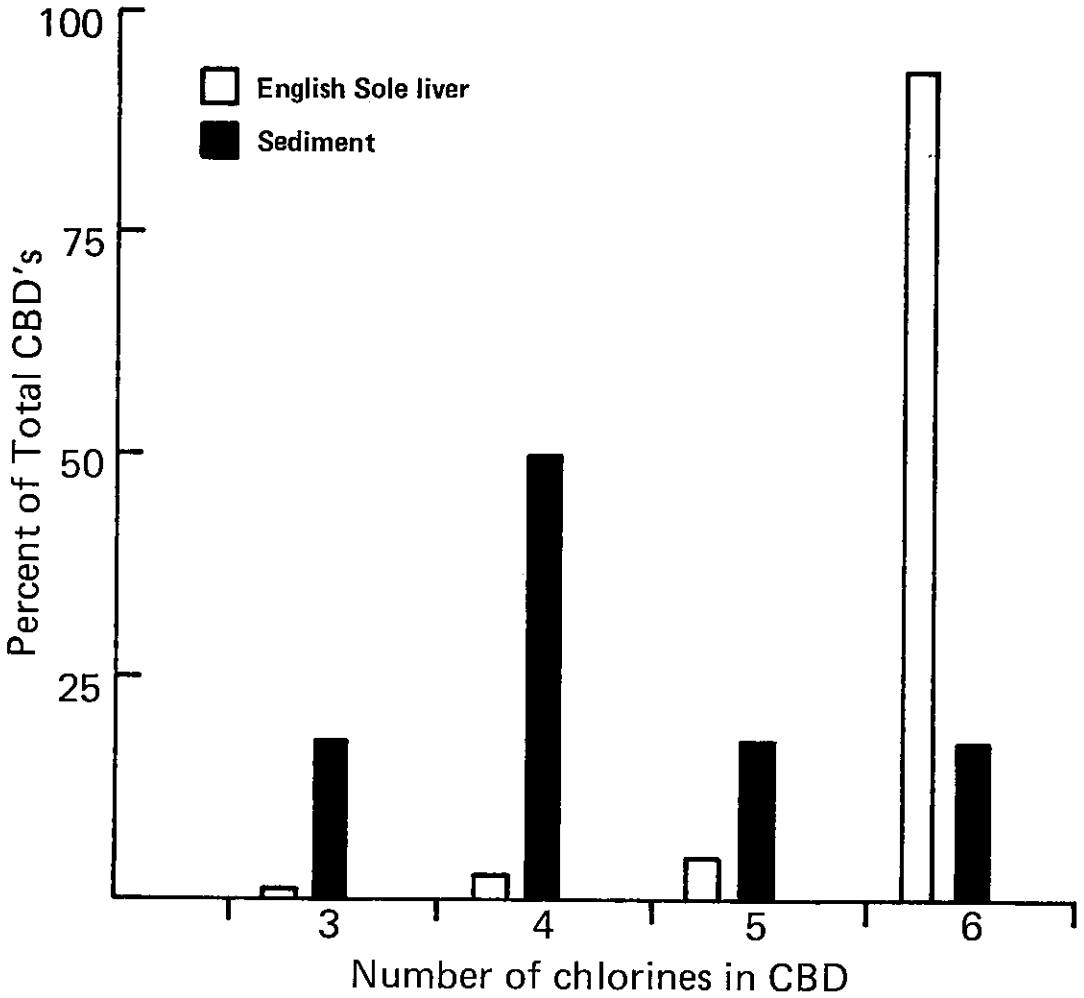


Figure 11. Tri-, tetra-, penta-, and hexachlorobutadienes in samples of sediment (average of 2 samples) and English sole liver (average of 6 samples) from the Hylebos Waterway, Commencement Bay.

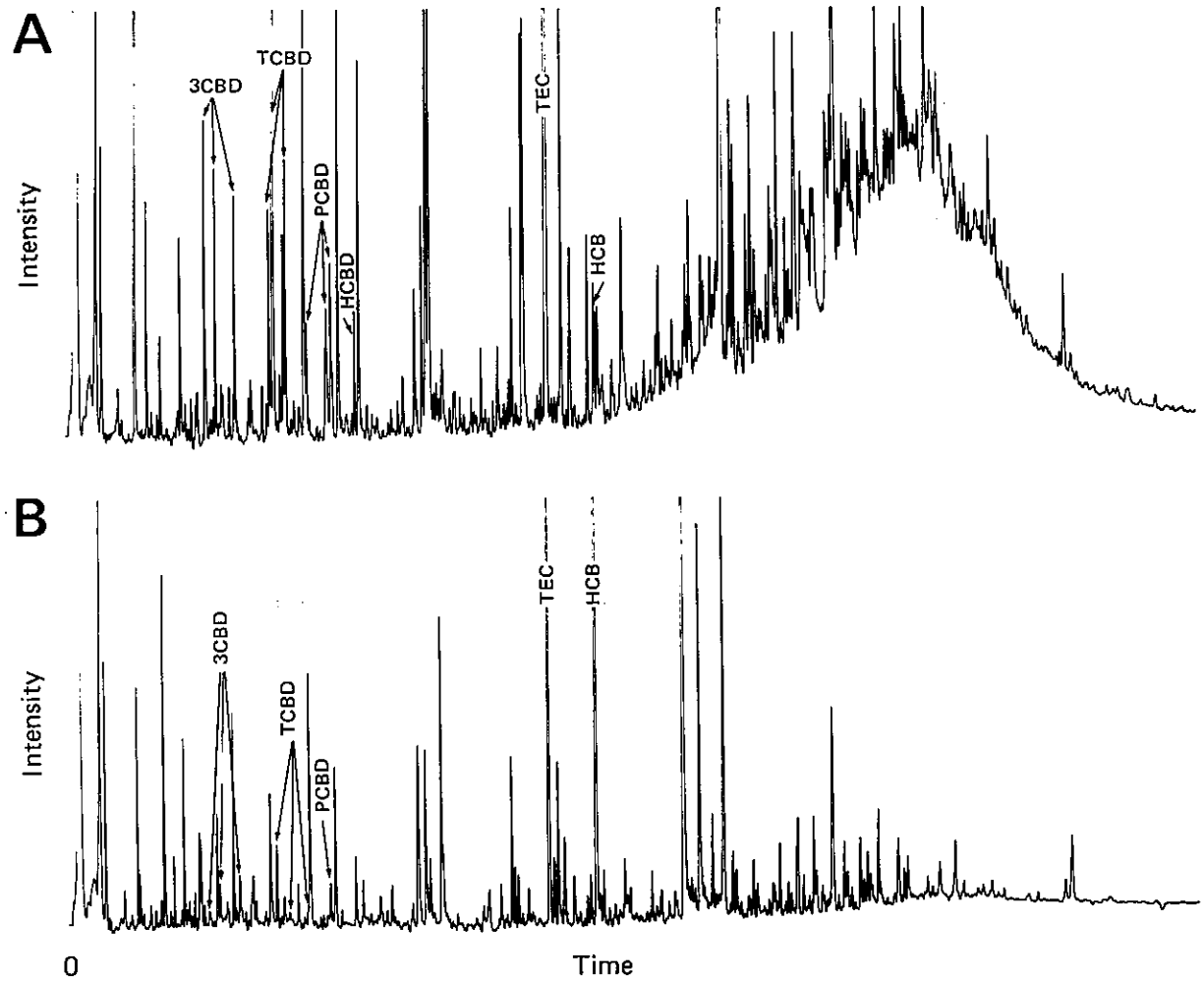


Figure 12. Gas chromatograms of halogenated compounds in extracts of 100g samples of sediment from: (A) Everett (near Pier 1) and (B) Port Angeles Harbor. Electron capture detection. Labeled peaks are identified in Table 2.

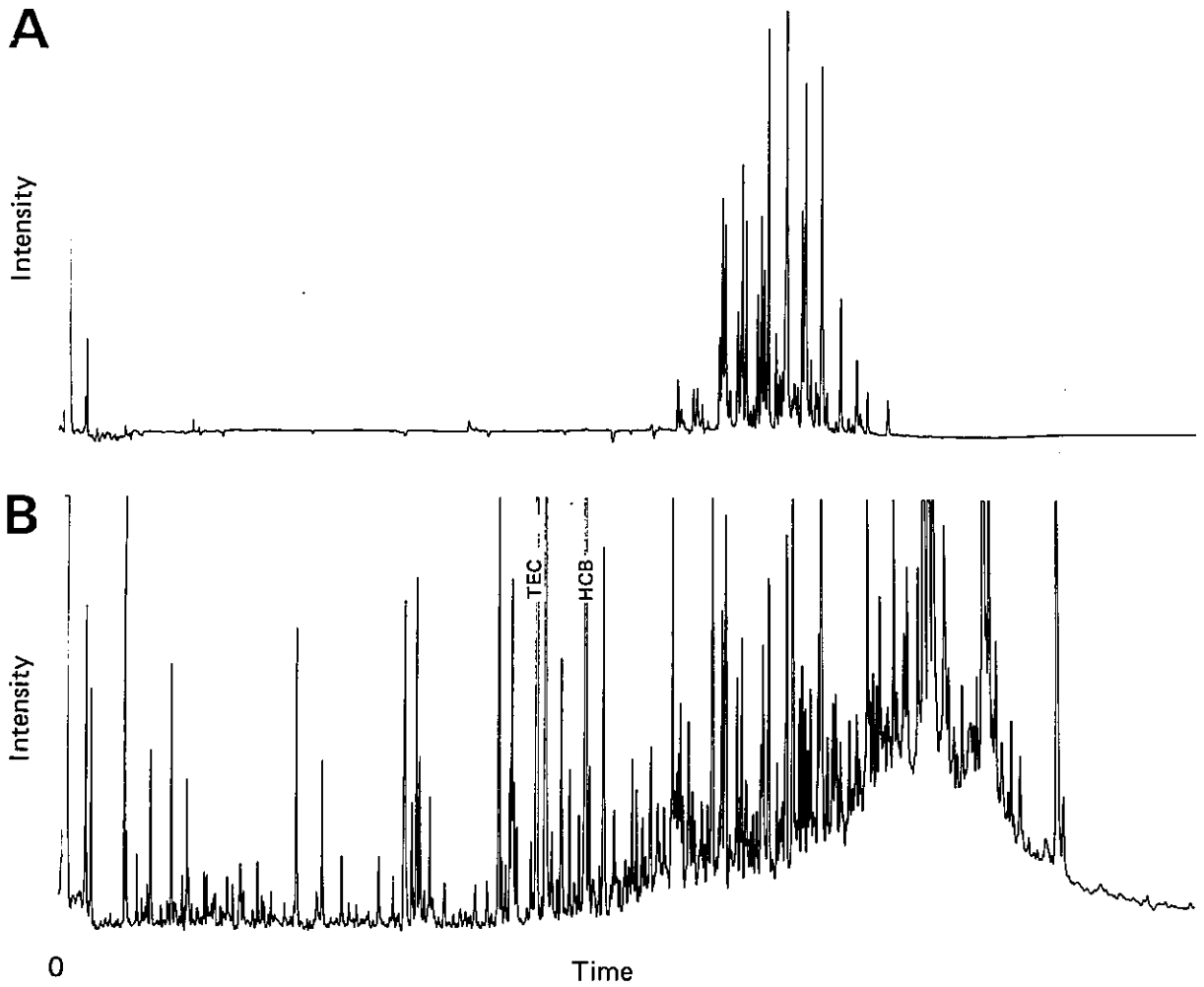


Figure 13. Gas chromatogram of halogenated compounds in: (A) Aroclor 1254 and (B) an extract from 100g of sediment collected near Whatcom Waterway, Bellingham. Electron capture detection. Labeled peaks are identified in Table 2.

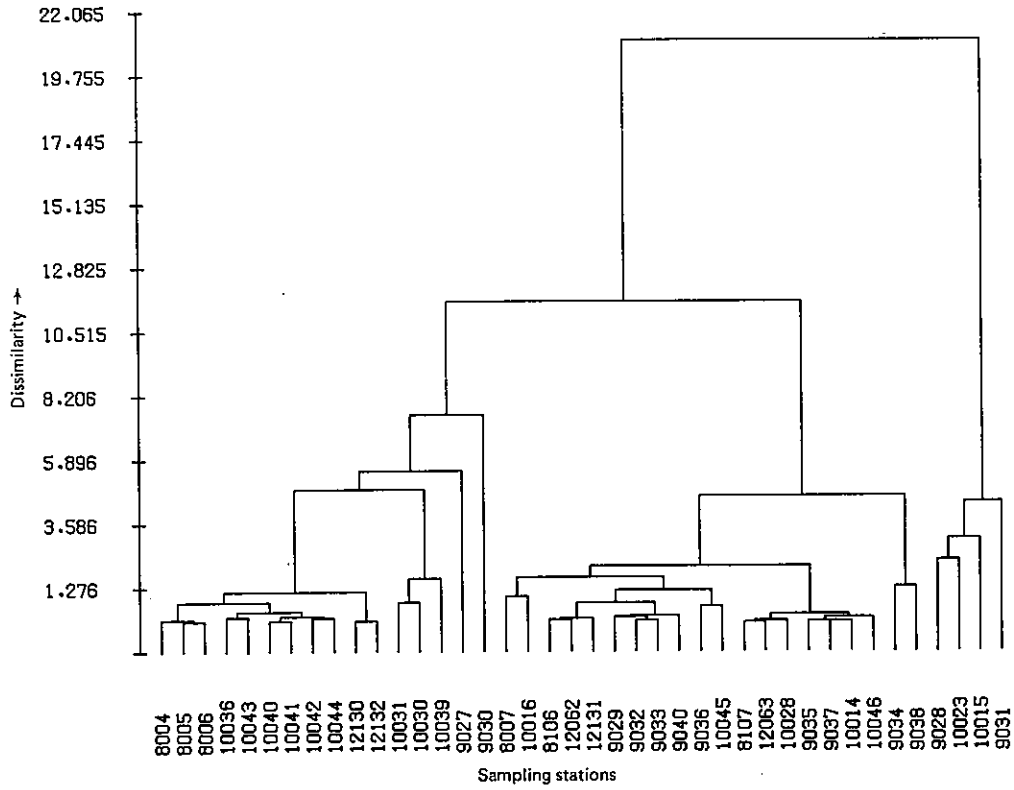


Figure 14. Dendrogram of 40 1979 MESA stations grouped by similarity of sediment chemical concentrations using cluster analysis. Ordinate is threshold distance between clusters.

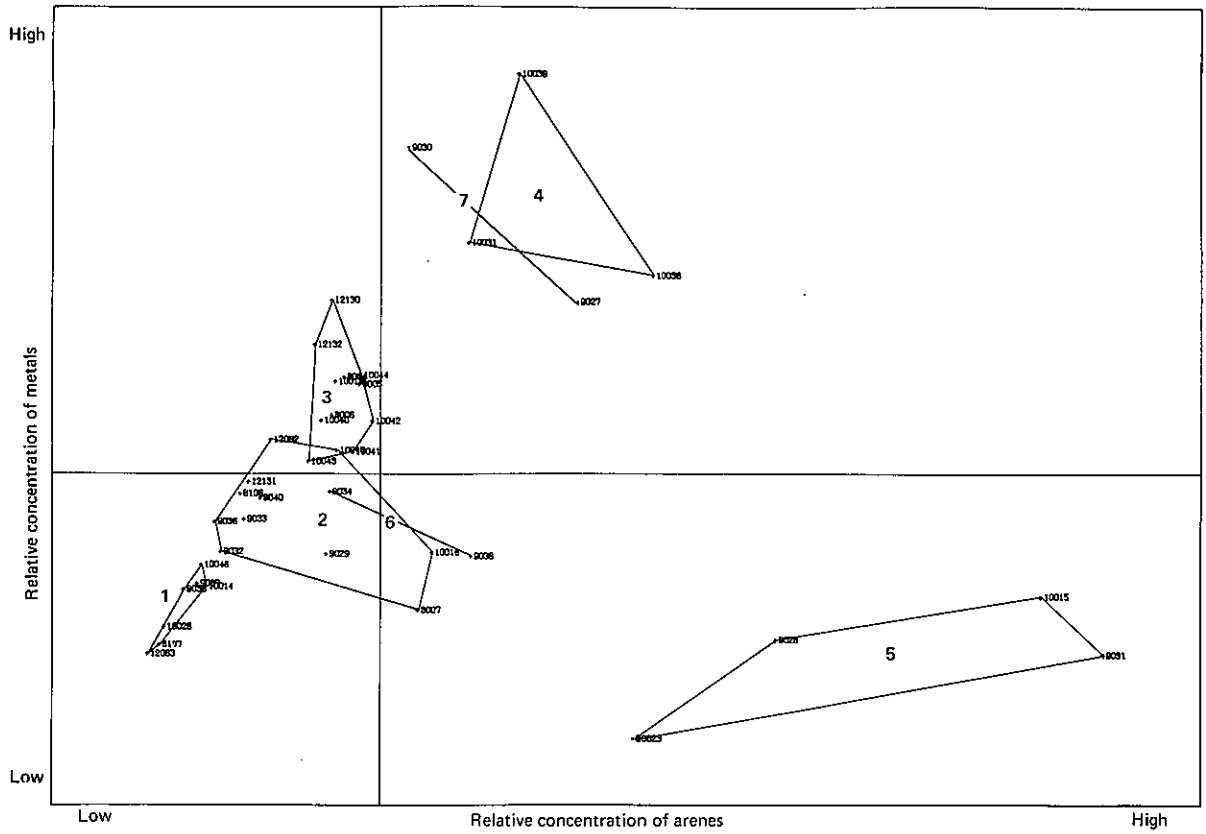


Figure 15. Groupings of 40 1979 MESA stations obtained by cluster analysis plotted on the first and second principal component axes.

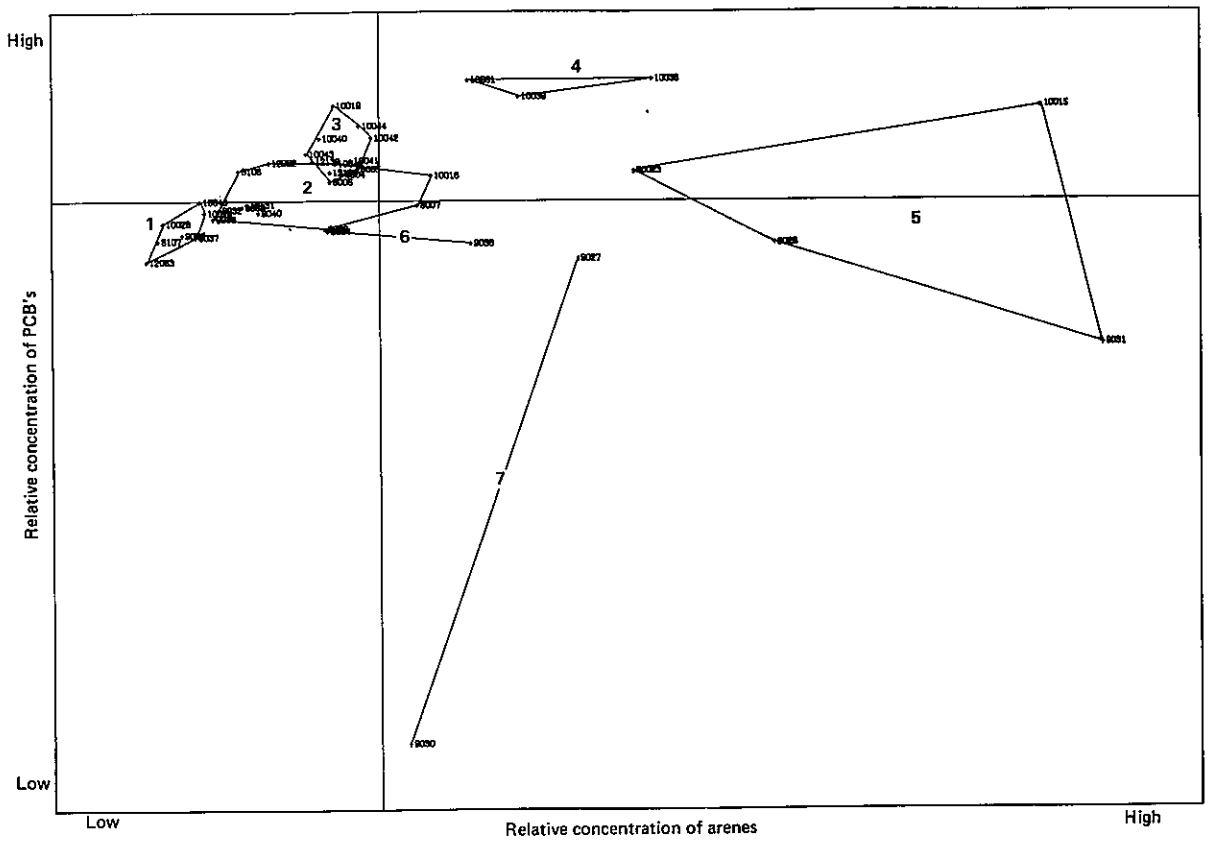


Figure 16. Groupings of 40 1979 MESA stations obtained by cluster analysis plotted on the first and third principal component axes.

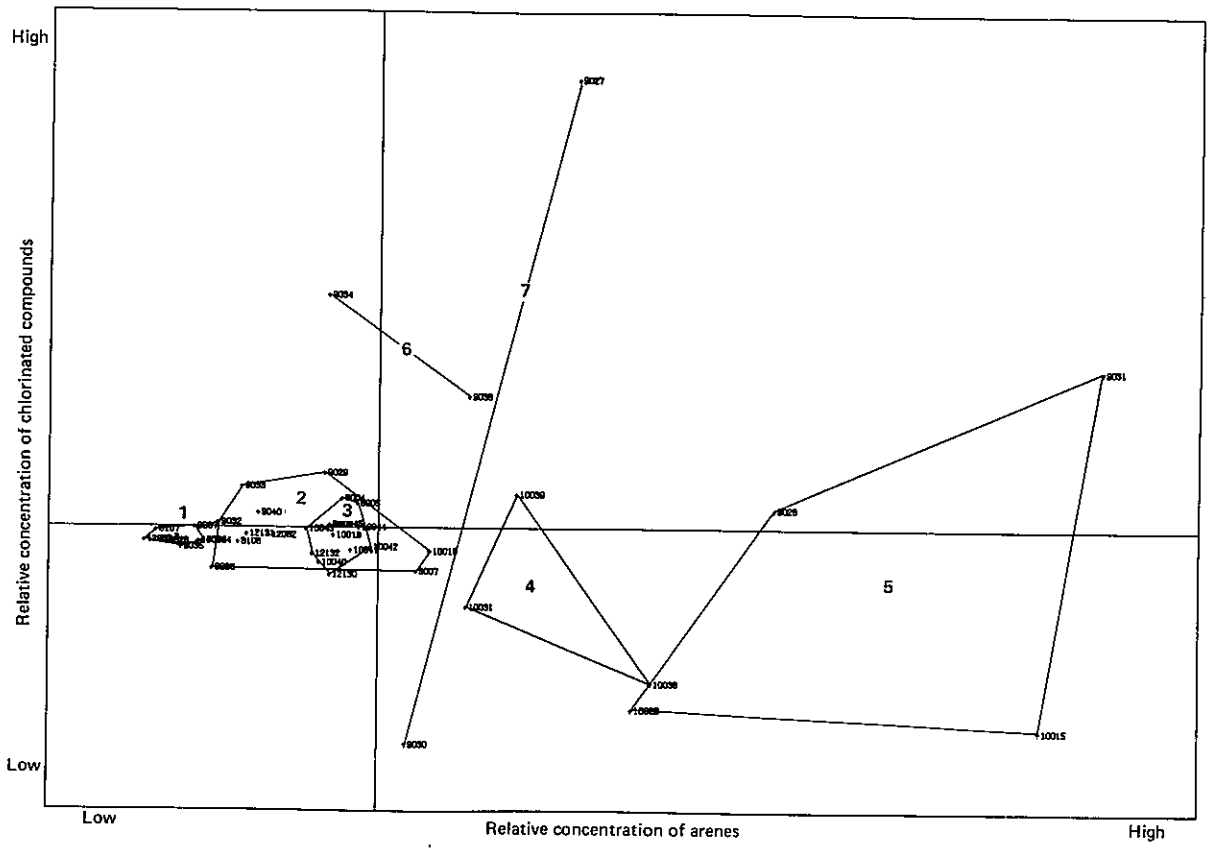


Figure 17. Groupings of 40 1979 MESA stations obtained by cluster analysis plotted on the first and fourth principal component axes.

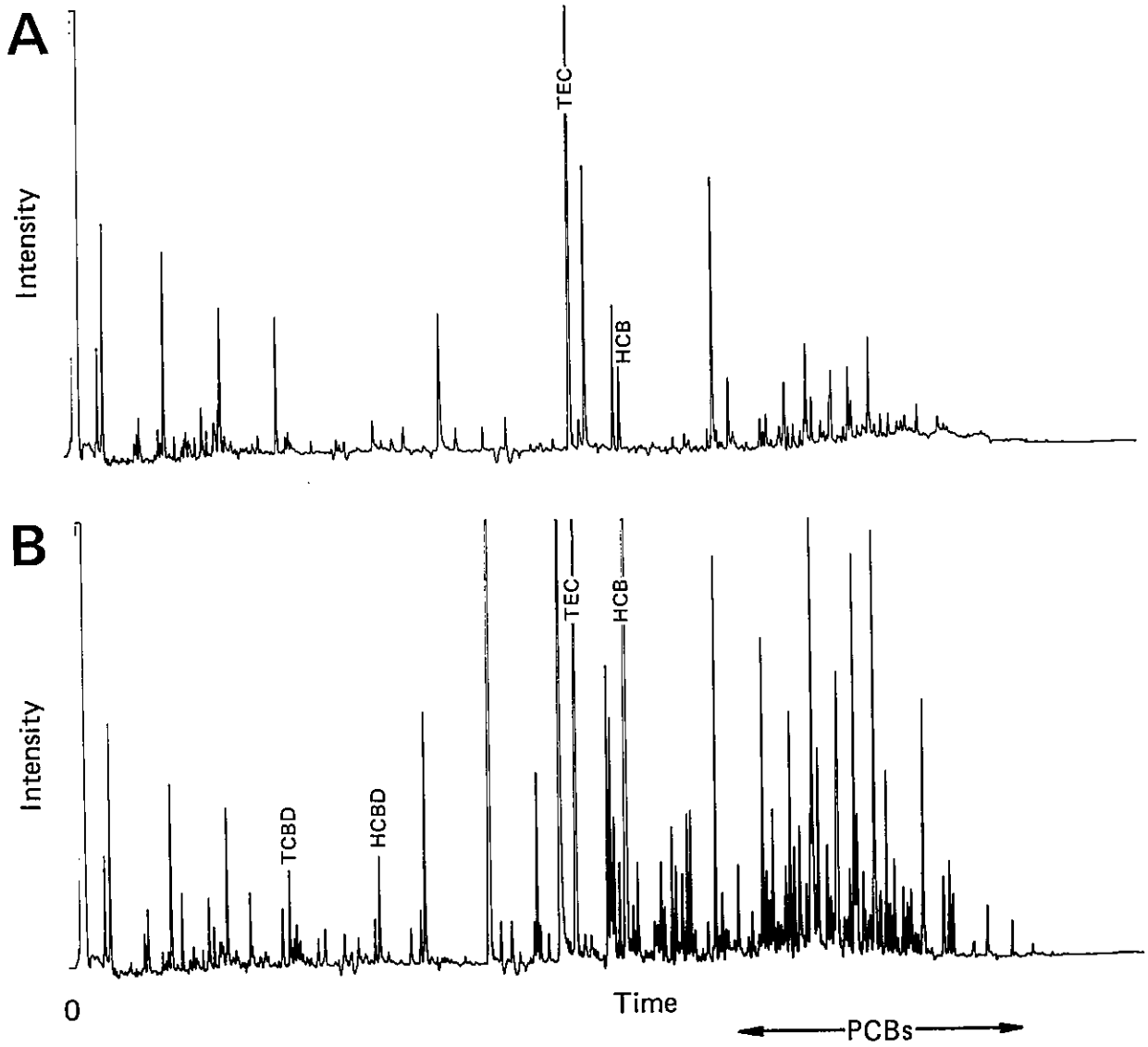


Figure 18. Gas chromatograms of chlorinated compounds in extracts of crab hepatopancreas (Cancer gracilis) from caged study experiments. (A) Crabs from Mutiny Bay (10g) and (B) crabs after being caged in the Hylebos Waterway for 60 days (6.7g). Electron capture detection. Labeled peaks are identified in Table 2.

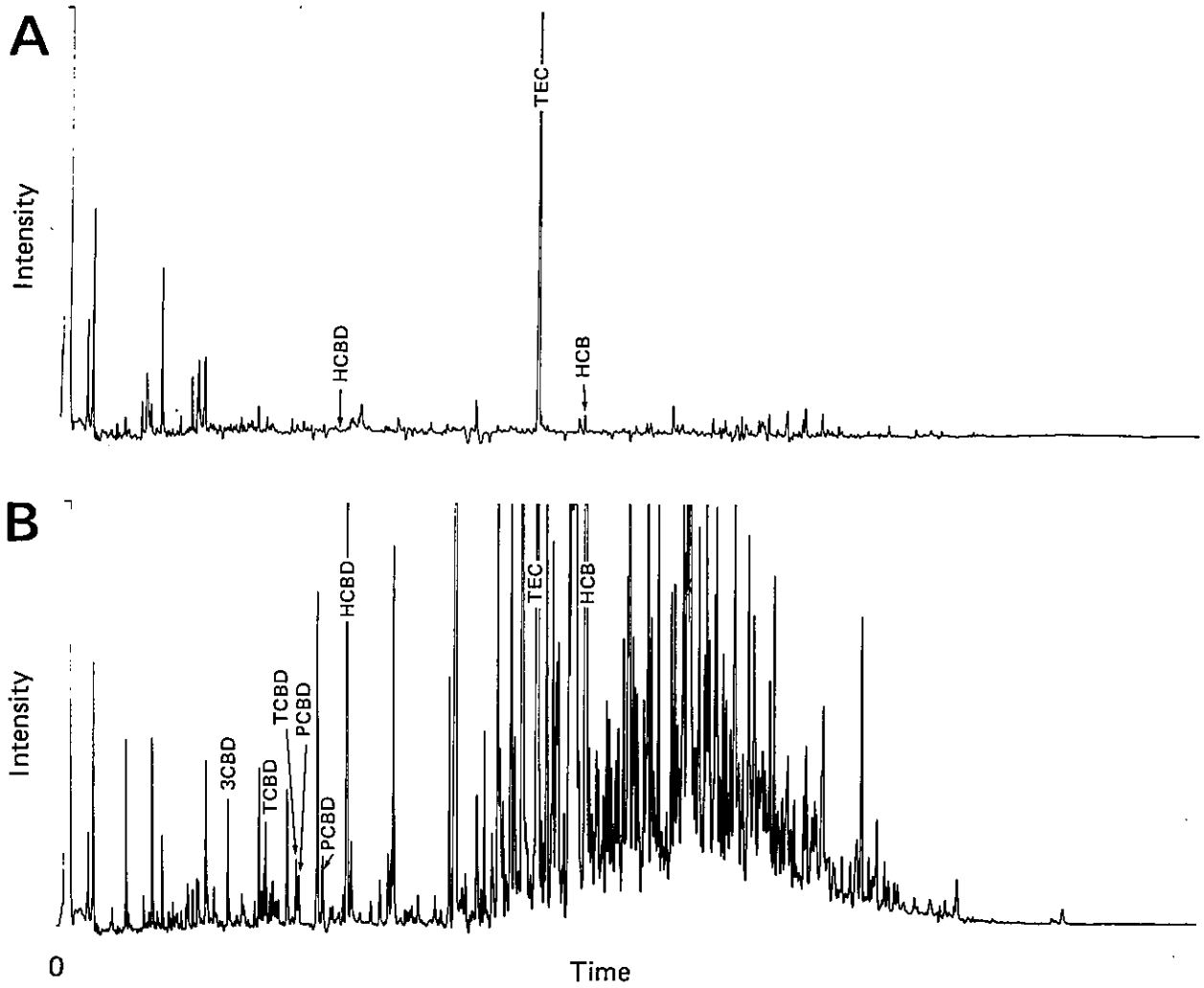
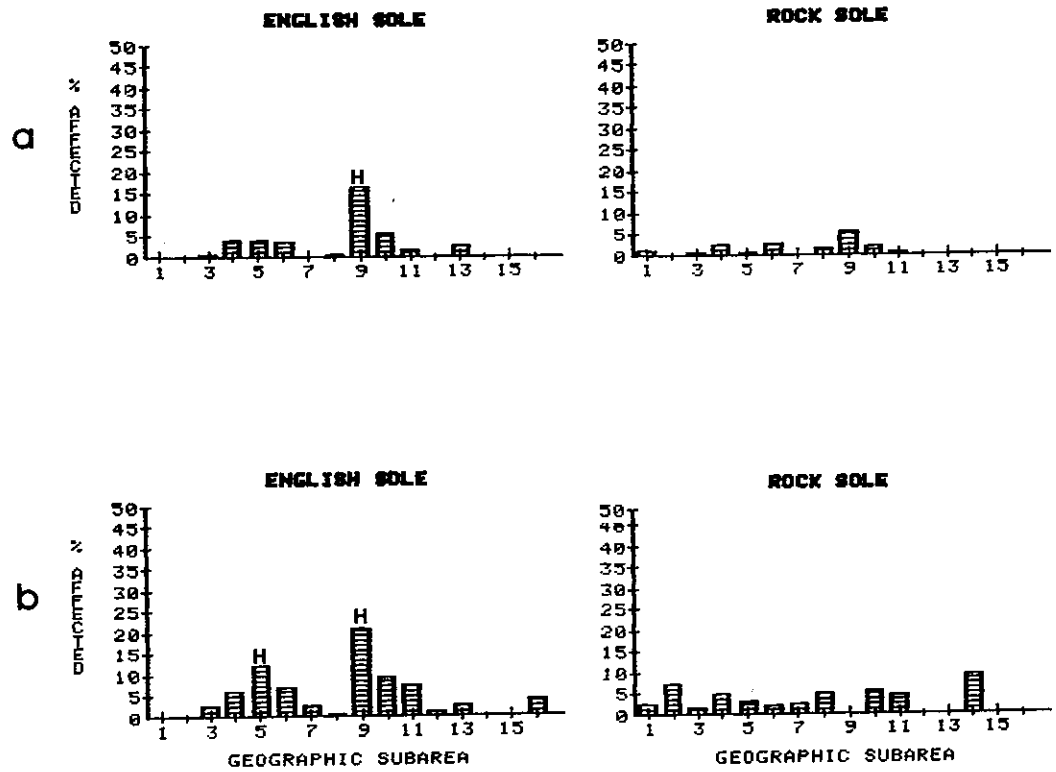
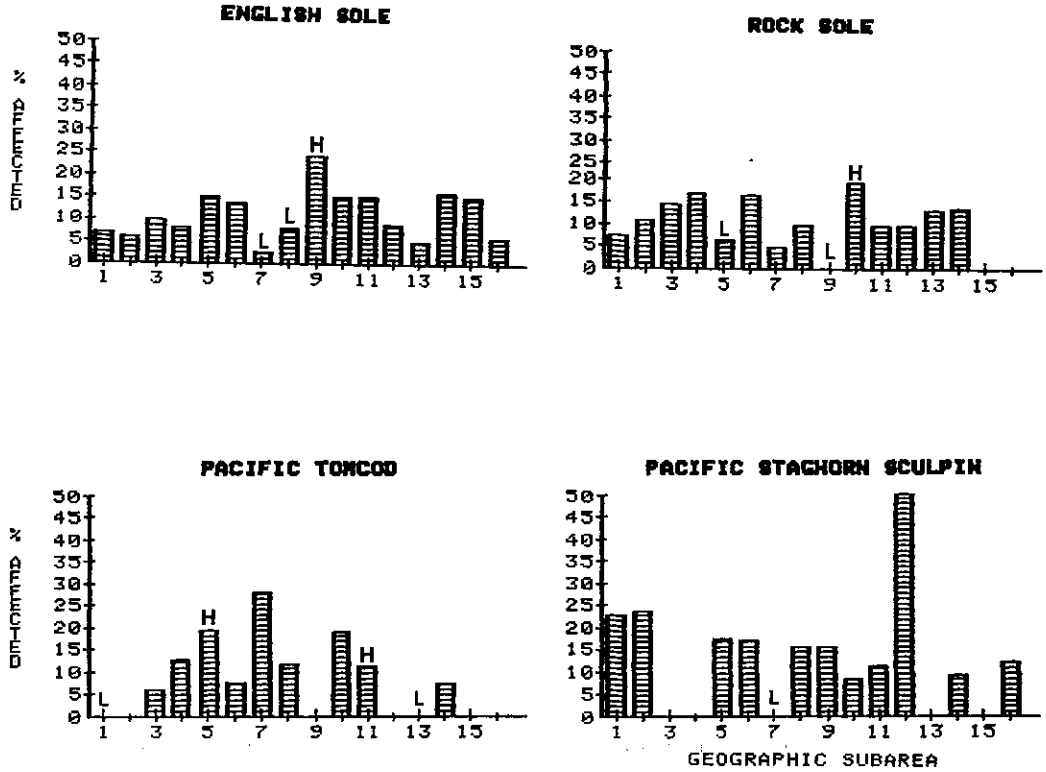


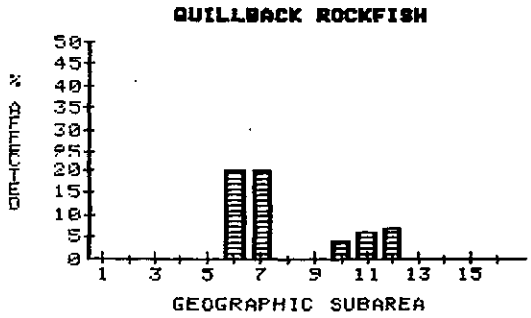
Figure 19. Gas chromatograms of chlorinated compounds in extracts of clams (Macoma nasuta) from caged study experiments. (A) Clams from Quartermaster Harbor (10g, wet weight), and (B) clams after being caged in the Hylebos Waterway for 71 days (10g, wet weight).



NUMBER	GEOGRAPHIC SUBAREA	NUMBER EXAMINED				
		English sole	Rock sole	Pacific tomcod	Pacific staghorn sculpin	Quillback rockfish
1	Budd Inlet	103	88	18	40	0
2	Case Inlet	34	28	7	17	0
3	SW Commencement Bay	152	153	35	6	9
4	Old Tacoma	50	42	8	0	0
5	Commencement Bay Waterways	276	125	95	98	2
6	Hylebos Waterway	297	159	120	116	10
7	Brown's Point	37	43	18	16	5
8	Sinclair Inlet	201	82	52	71	3
9	Upper Duwamish River	136	18	0	60	0
10	Lower Duwamish River	399	300	65	37	25
11	Seattle Waterfront	161	138	63	18	53
12	Outer Elliott Bay	116	119	2	2	31
13	West Point	40	38	18	1	21
14	Port Madison	38	22	29	11	14
15	Port Susan	33	1	0	1	0
16	Discovery Bay	51	10	0	17	0

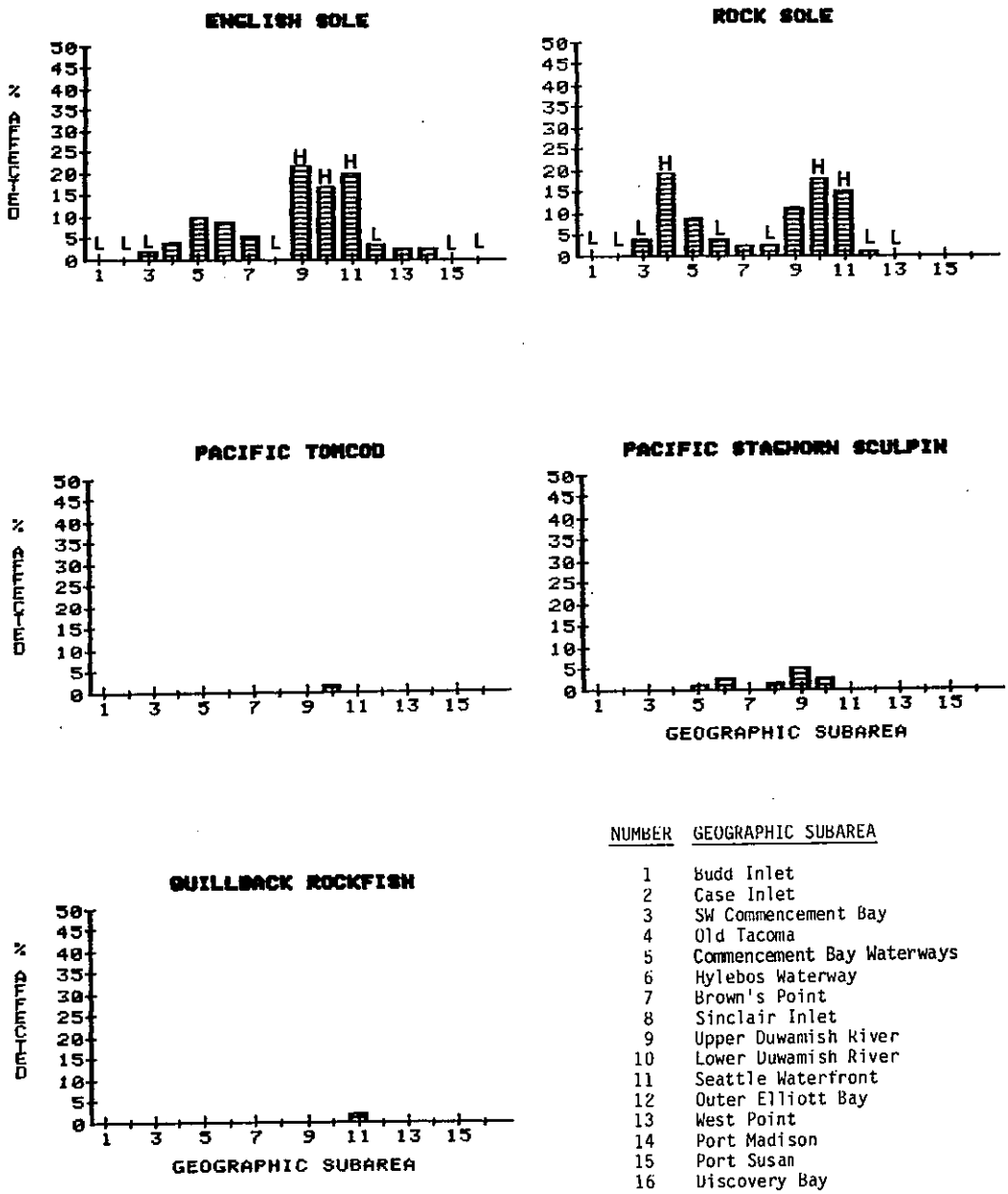
Figure 20. The geographic distribution by subarea (See Table 28) of fish species with (a) neoplasms and (b) "preneoplasms" of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated.





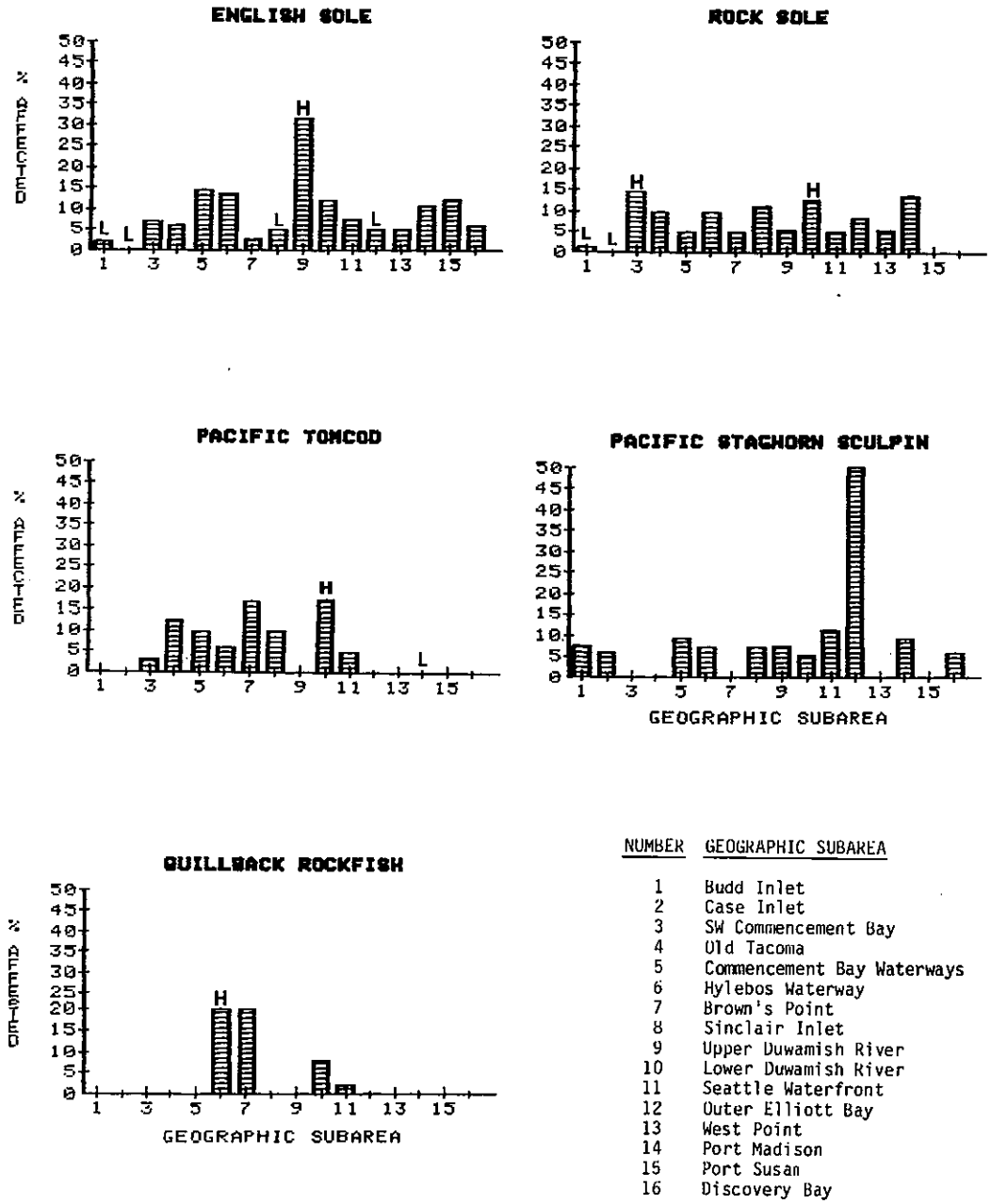
NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

Figure 21. The geographic distribution of fish species with nonspecific degenerative/necrotic lesions of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.



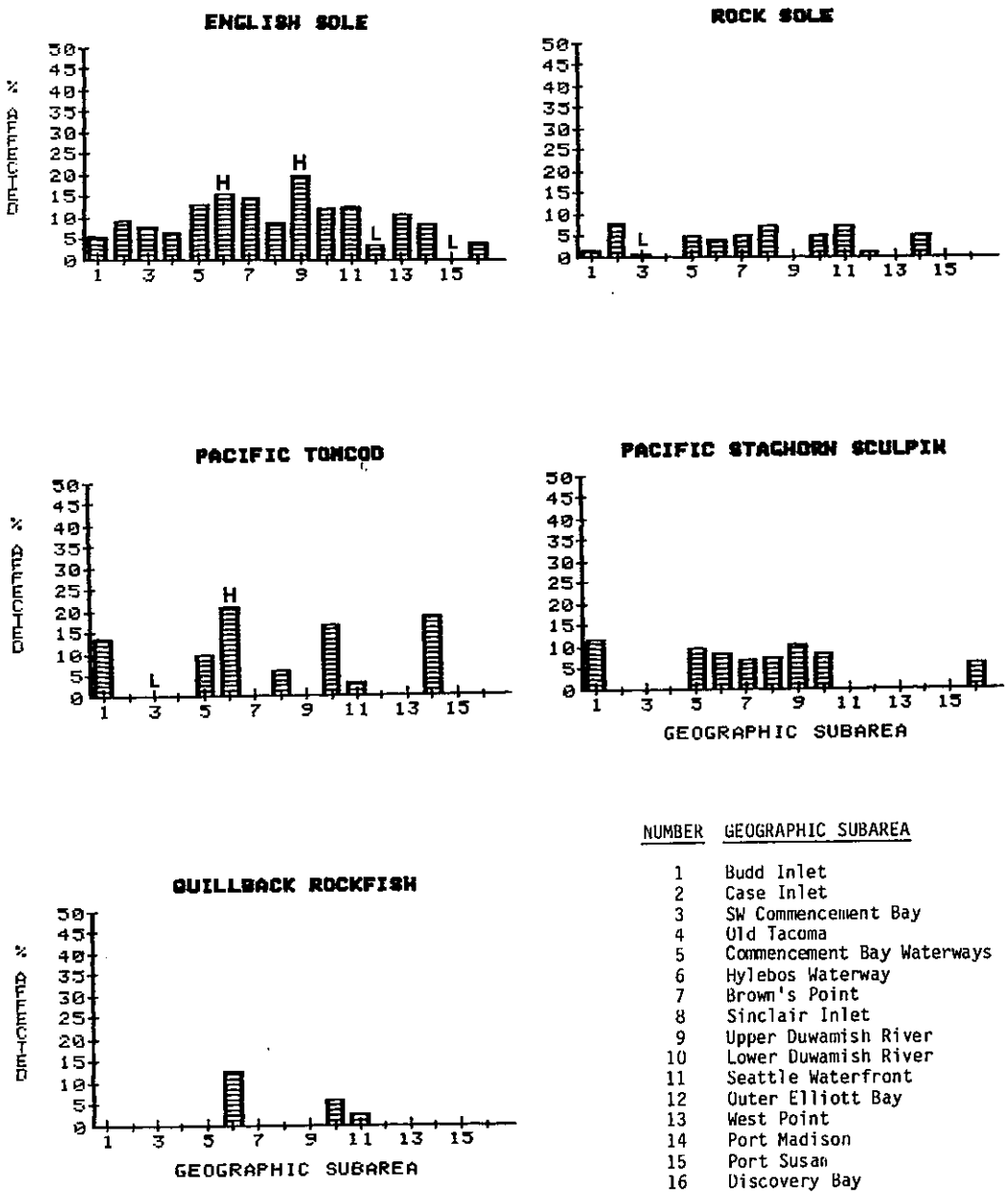
NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

Figure 22. The geographic distribution of fish species with specific degenerative/necrotic lesions of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.



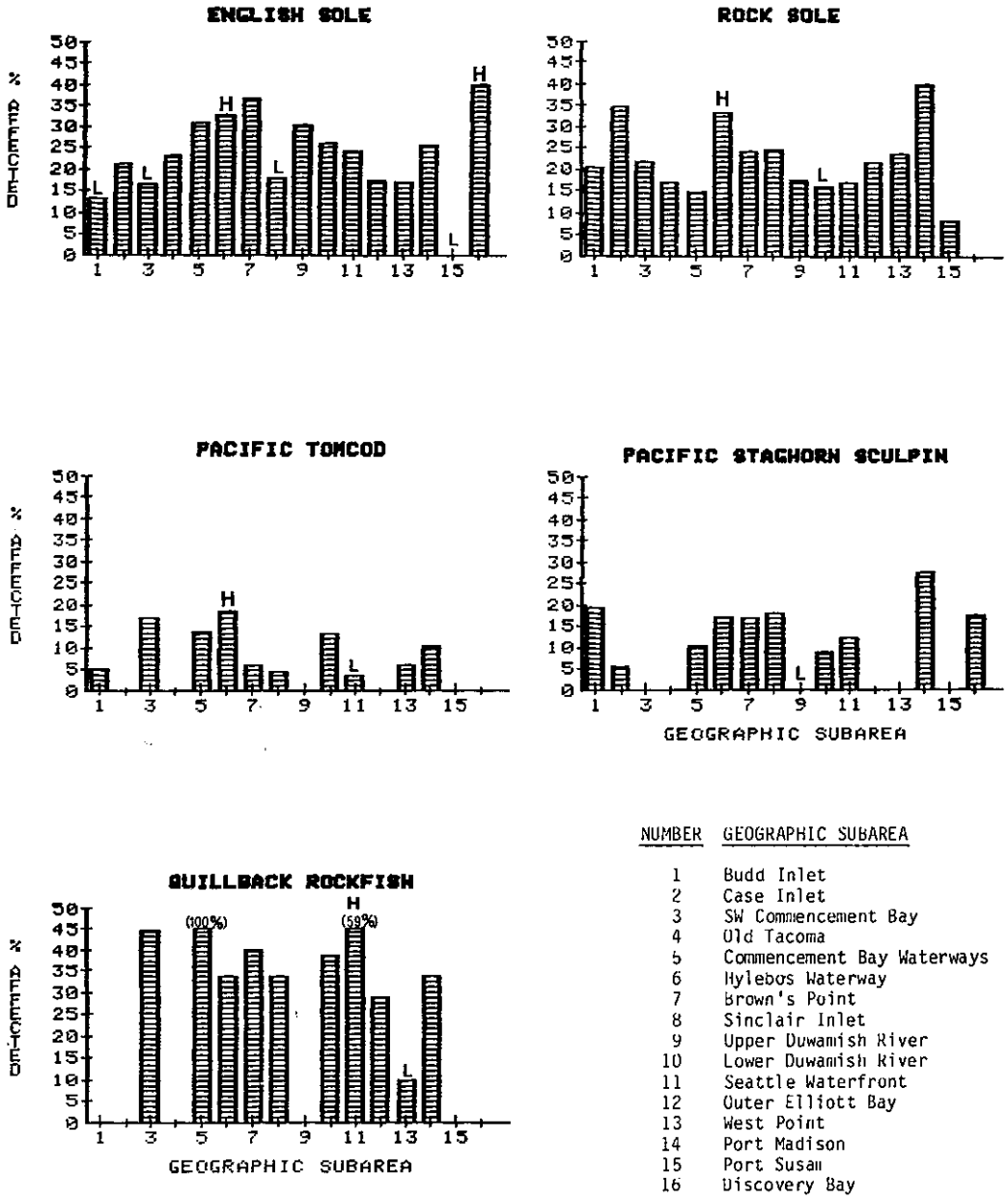
NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

Figure 23. The geographic distribution of fish species with intracellular storage disorders of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.



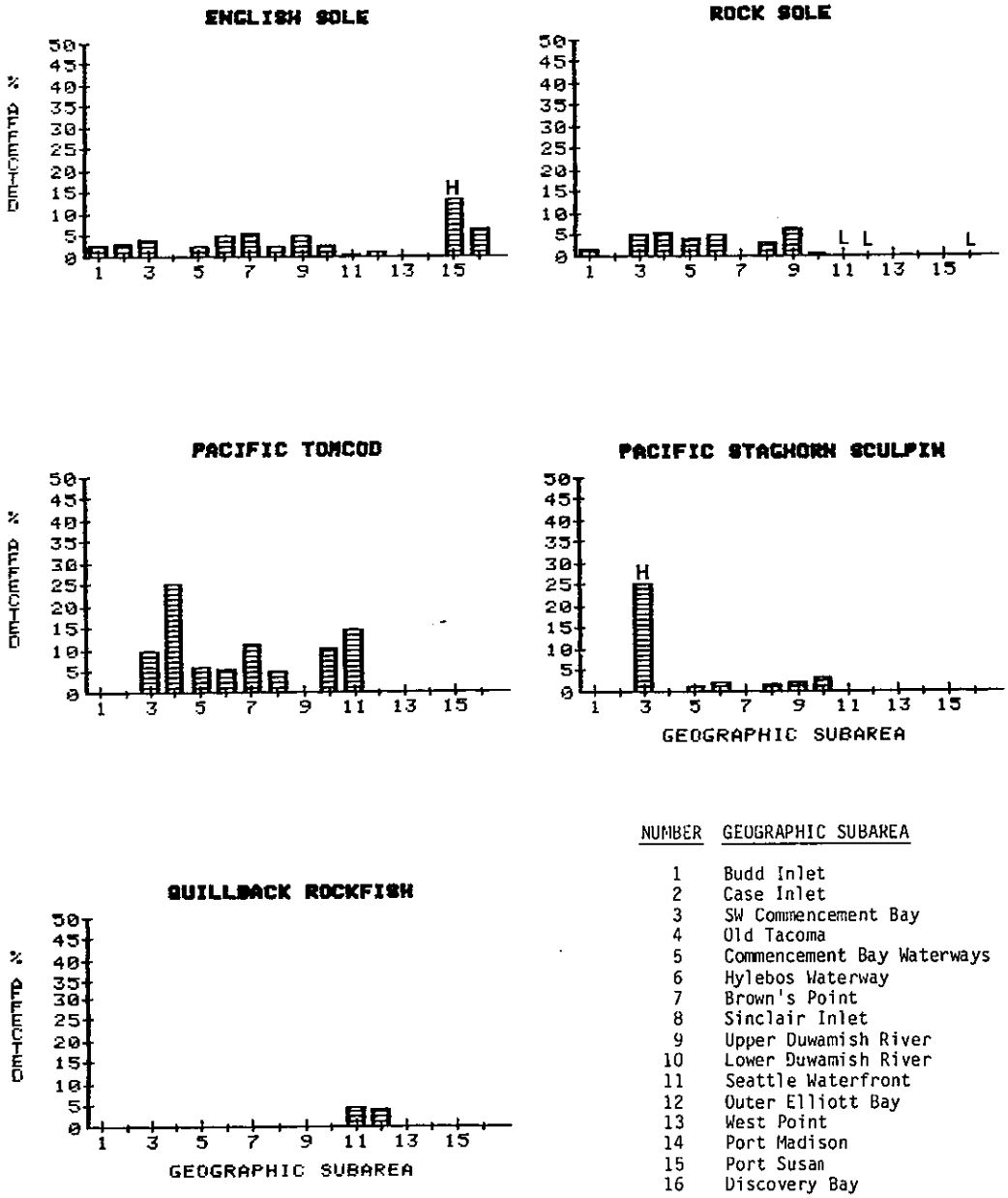
NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

Figure 24. The geographic distribution of fish species with kidney necrosis. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.



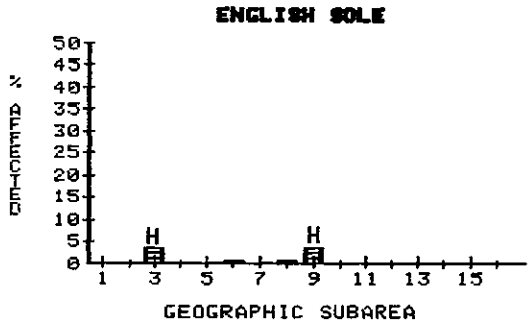
NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

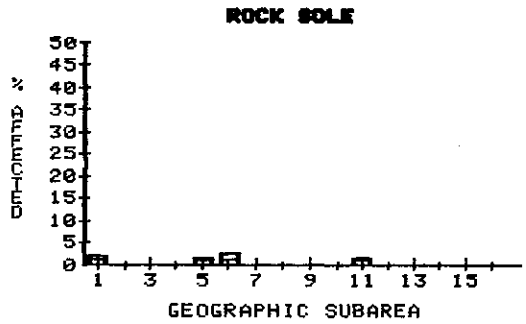
Figure 25. The geographic distribution of fish species with proliferative conditions of the gill. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.

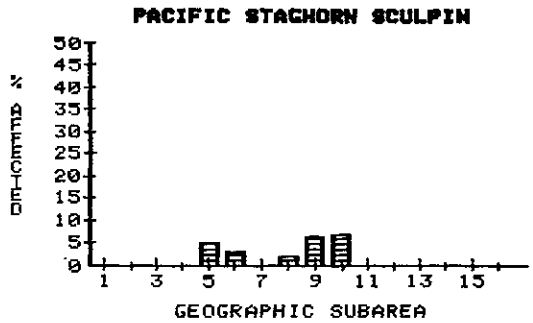


NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

Figure 26. The geographic distribution of fish species with splenic hypoplasia. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.

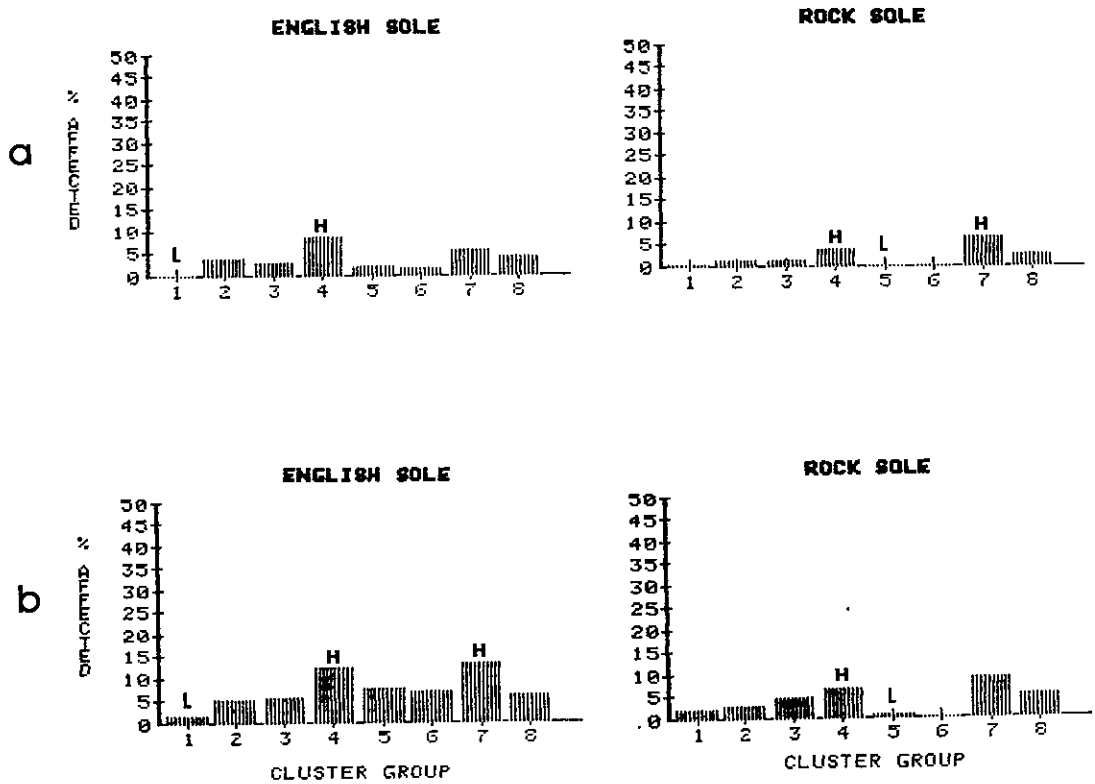






NUMBER	GEOGRAPHIC SUBAREA
1	Budd Inlet
2	Case Inlet
3	SW Commencement Bay
4	Old Tacoma
5	Commencement Bay Waterways
6	Hylebos Waterway
7	Brown's Point
8	Sinclair Inlet
9	Upper Duwamish River
10	Lower Duwamish River
11	Seattle Waterfront
12	Outer Elliott Bay
13	West Point
14	Port Madison
15	Port Susan
16	Discovery Bay

Figure 27. The geographic distribution of fish species with inclusion cysts of the gall bladder. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 20.



NUMBER EXAMINED

CLUSTER GROUPS NUMBER	English sole	Rock sole	Pacific tomcod	Pacific staghorn sculpin	Quillback
1.	261	267	64	36	50
2.	508	372	119	89	33
3.	434	215	107	125	52
4.	339	178	52	72	2
5.	212	162	90	91	29
6.	75	37	37	8	7
7.	163	47	53	68	0
8.	50	42	8	22	0

Figure 28. The distribution in cluster groups (See Table 25) of fish species with (a) neoplasms and (b) "preneoplasms" of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated.

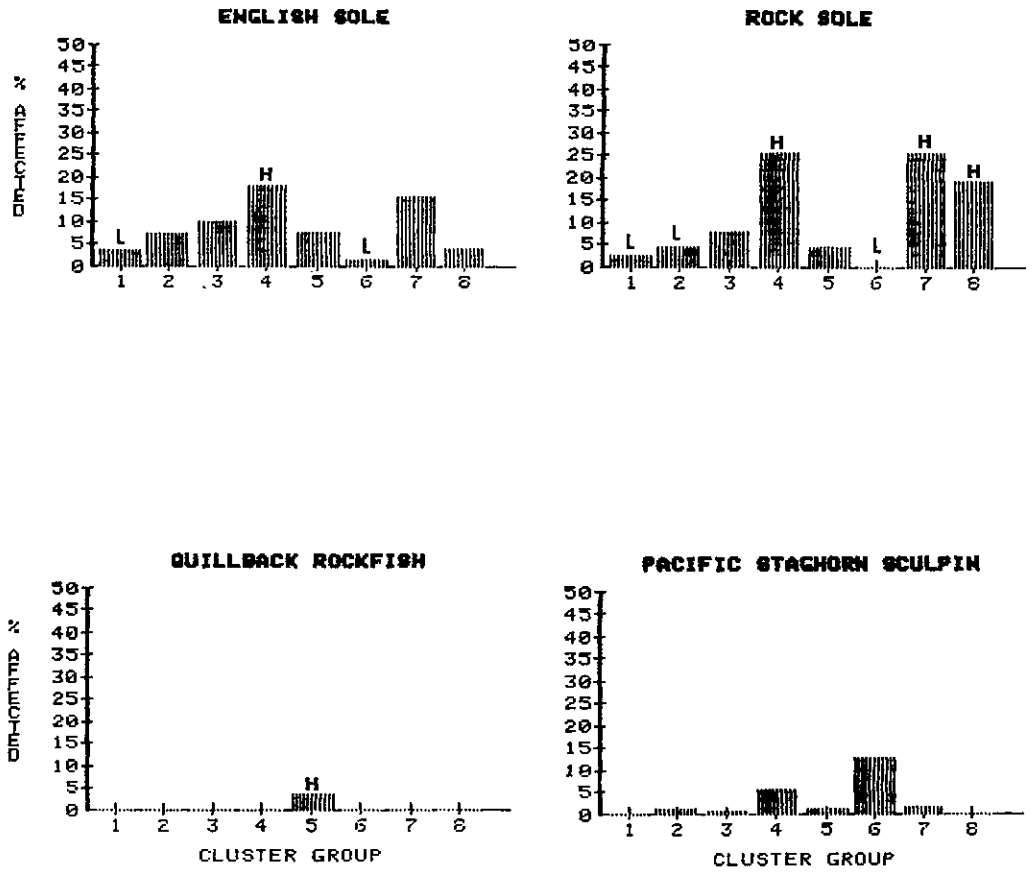


Figure 29. The distribution in cluster groups of fish species with specific degenerative/necrotic lesions of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 28.

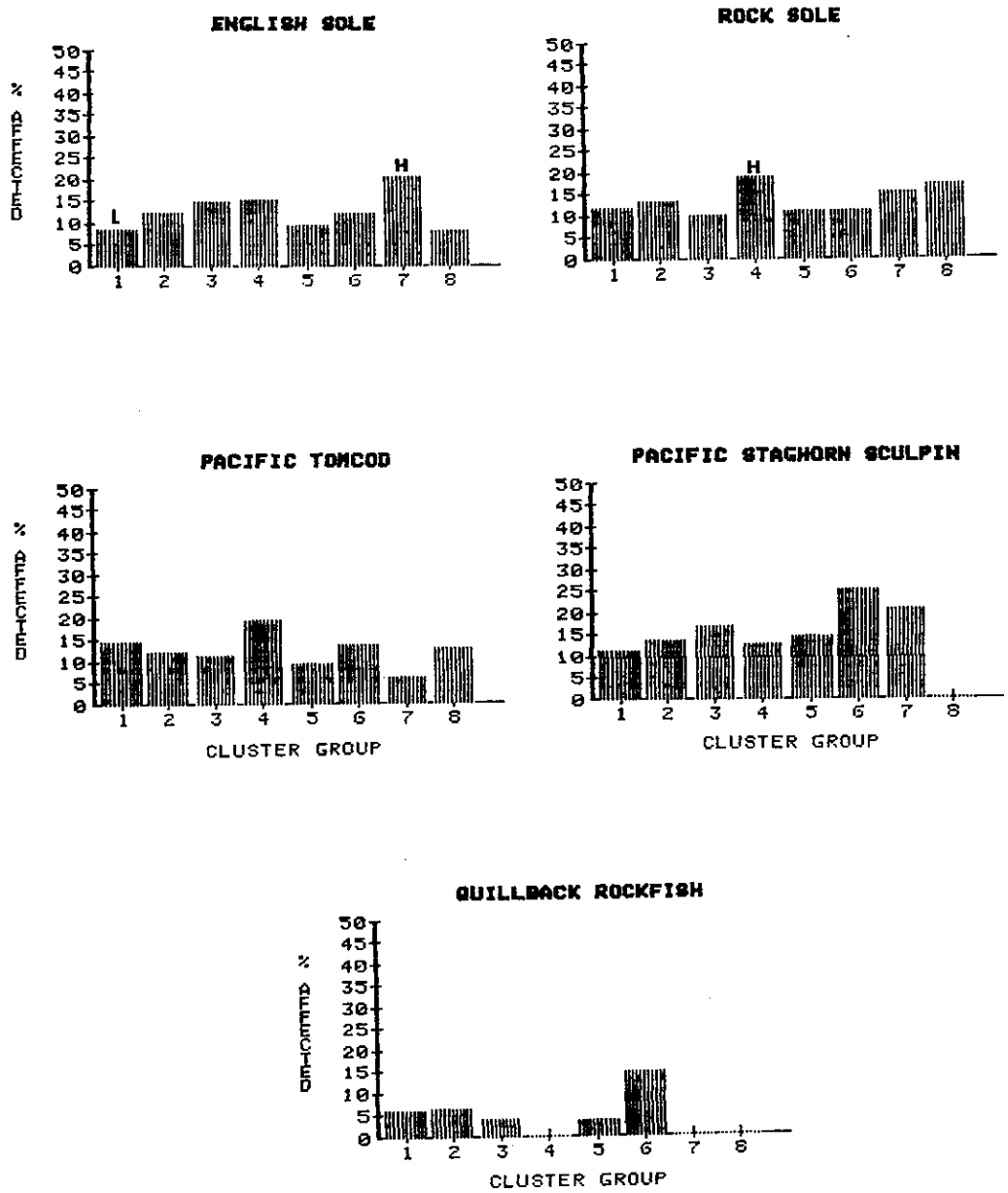


Figure 30. The distribution in cluster groups of fish species with nonspecific degenerative/necrotic lesions of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 28.

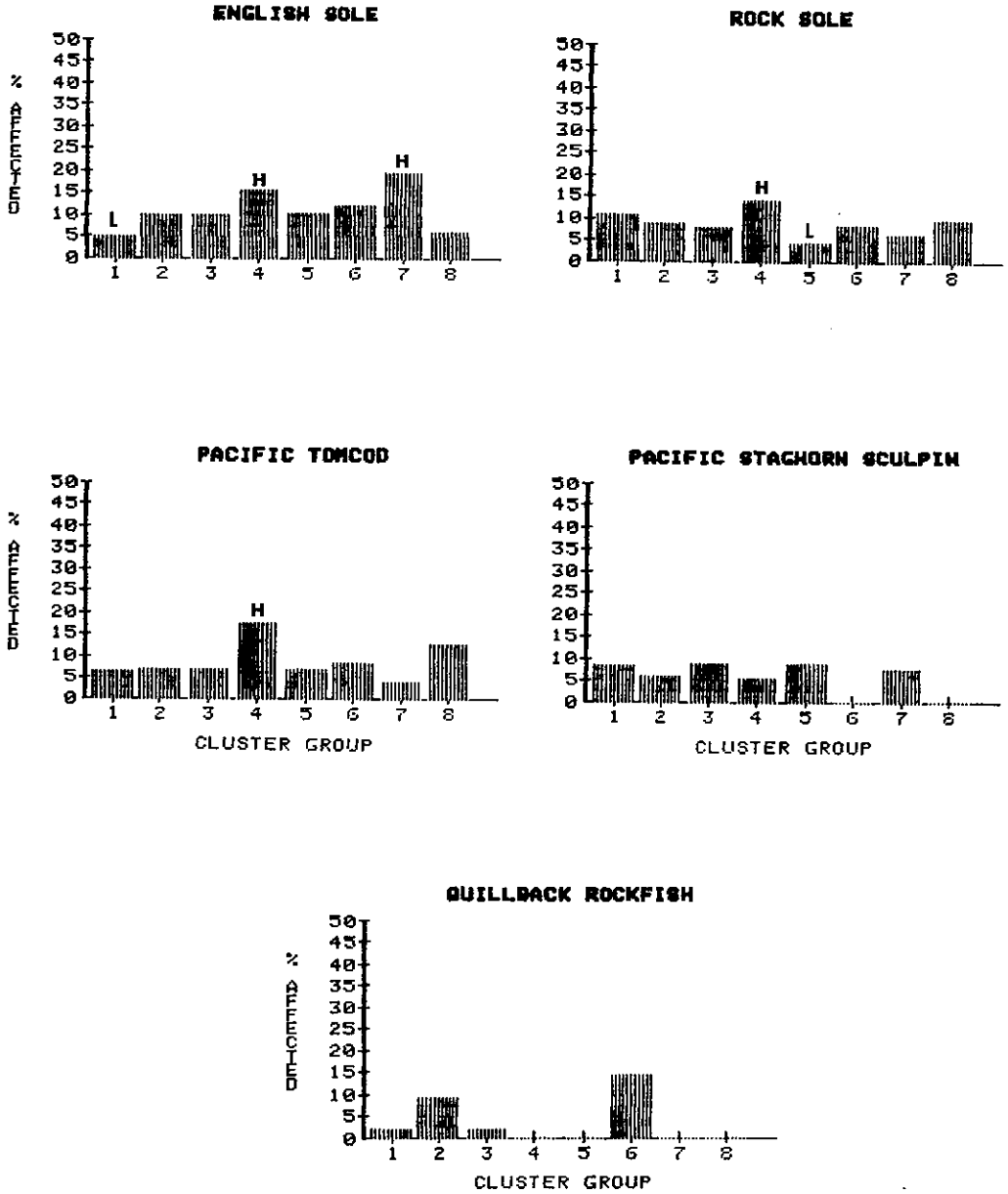


Figure 31. The distribution in cluster groups of fish species with intracellular storage disorders of the liver. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 28.

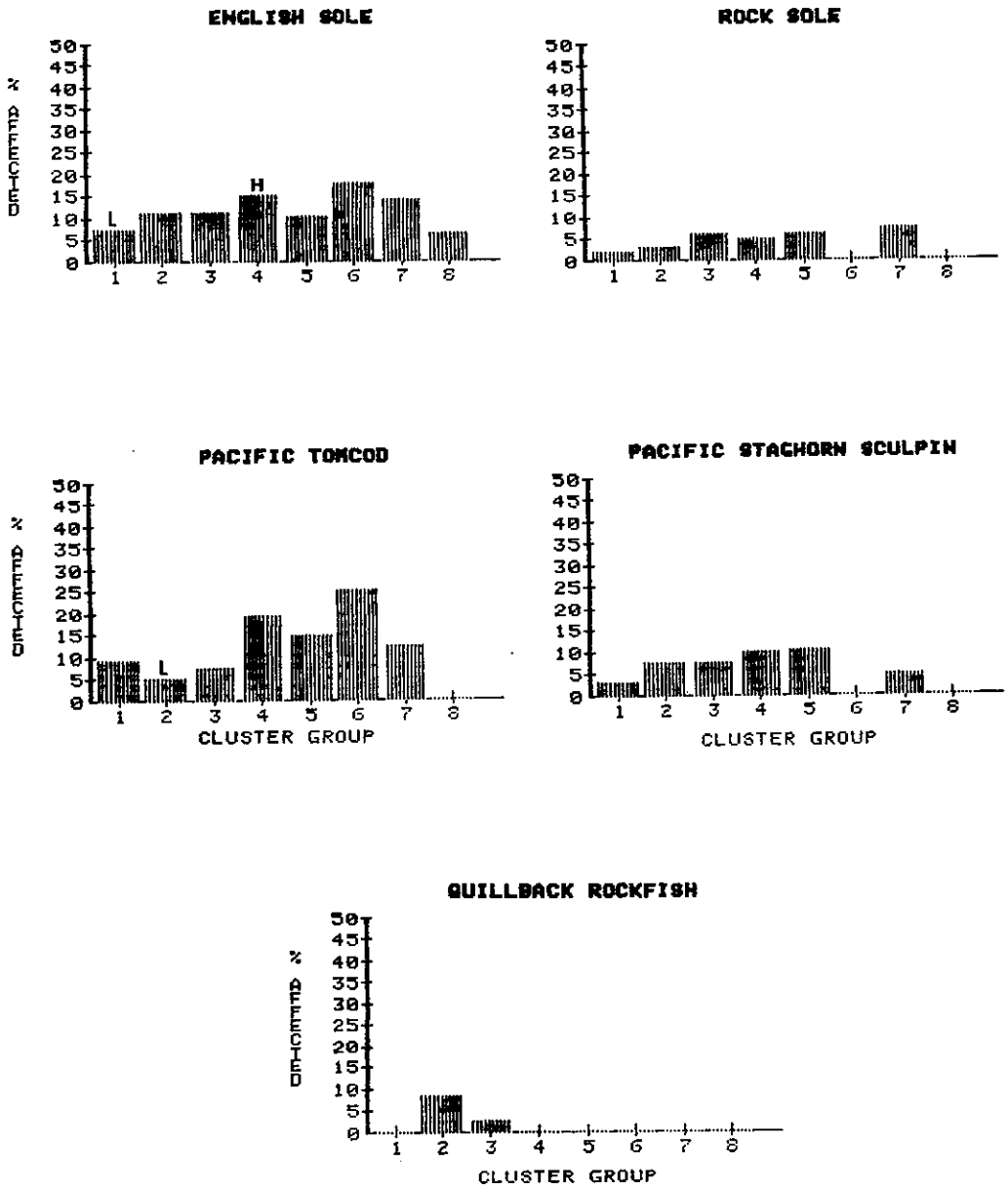


Figure 32. The distribution in cluster groups of fish species with kidney necrosis. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. A number of fish examined is given in Figure 28.

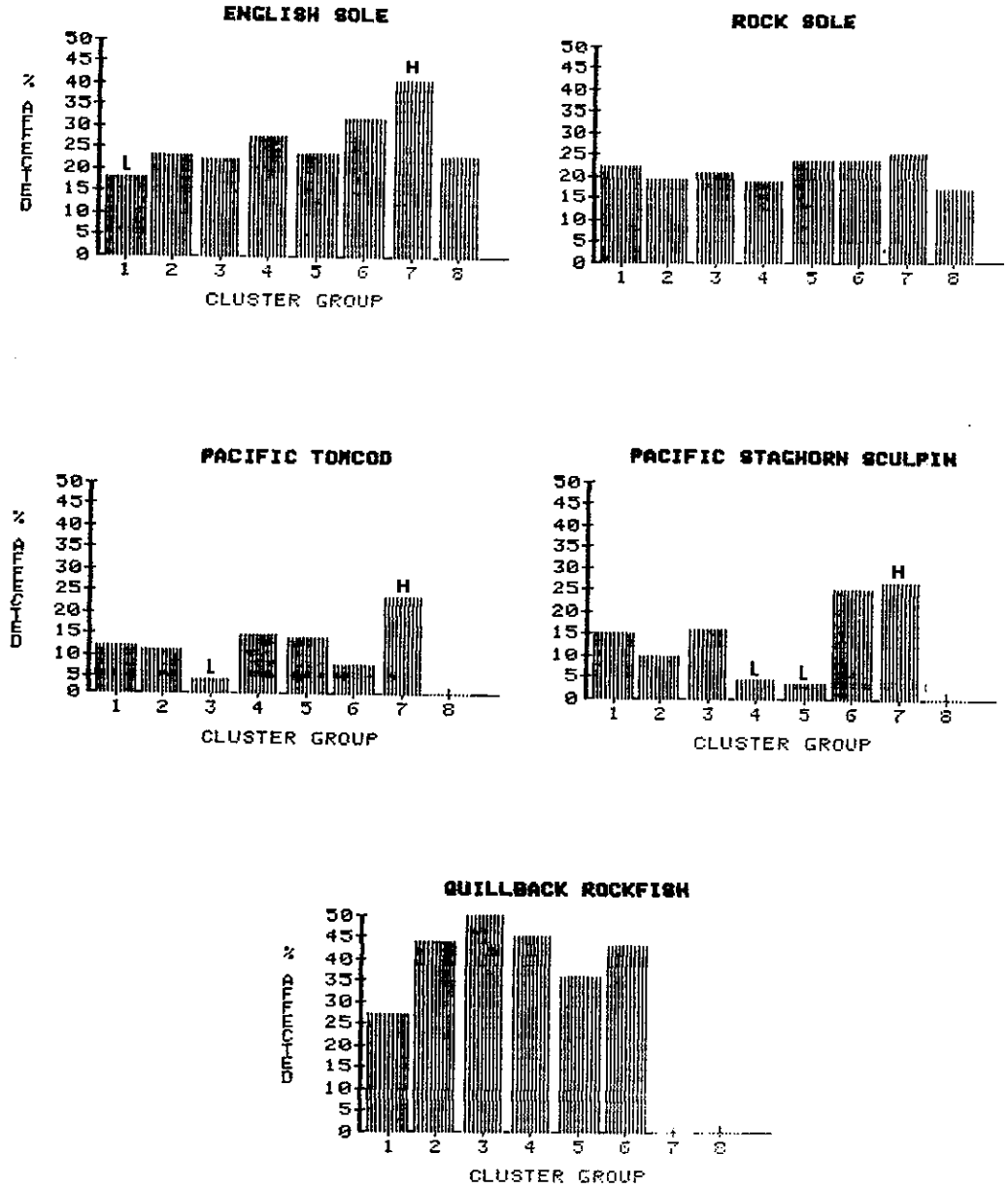


Figure 33. The distribution in cluster groups of fish species with proliferative conditions of the gill. A significantly ($p \leq 0.05$) higher (H) or lower (L) prevalence is indicated. The number of fish examined is given in Figure 28.

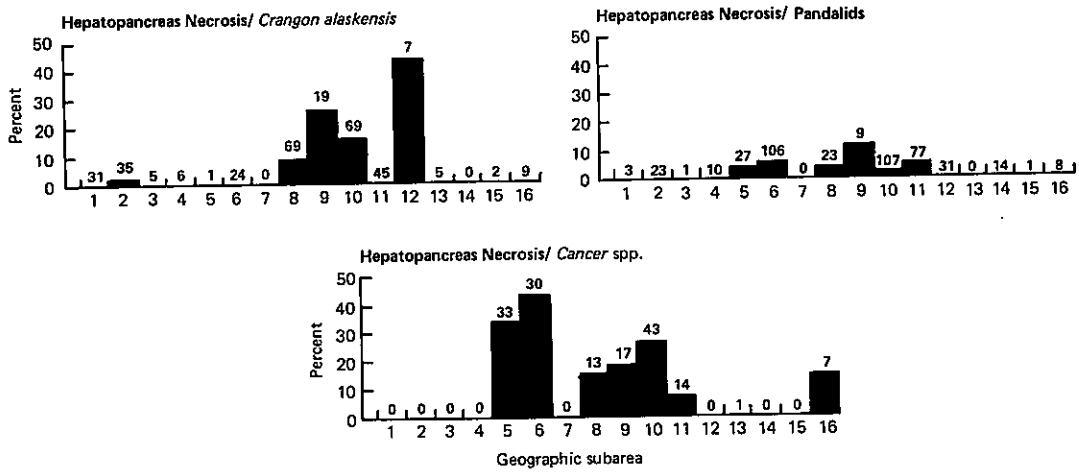


Figure 34. The geographic distribution of hepatopancreas necrosis observed in Crangon alaskensis, pandalid shrimp, and Cancer spp. The number of animals examined for each subarea is given. The subareas are described in Table 28.

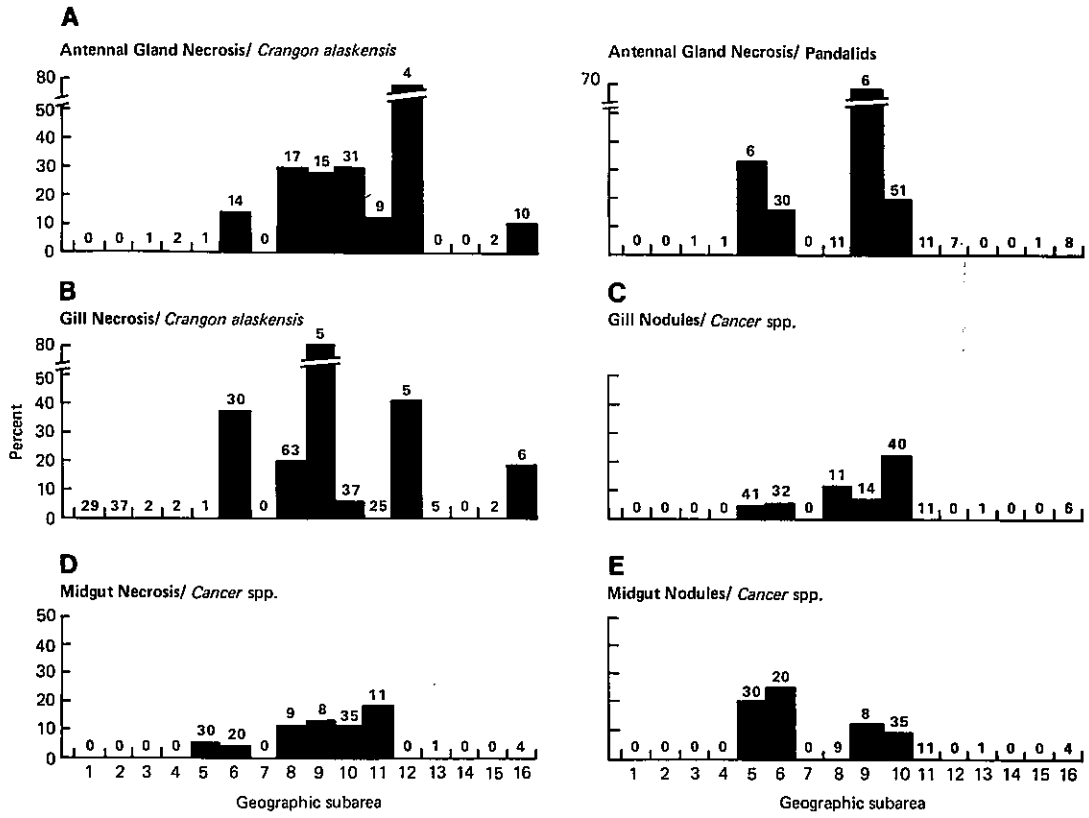
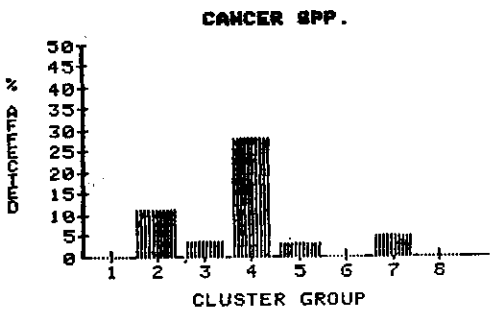
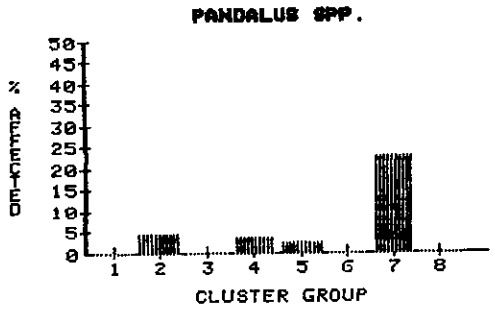
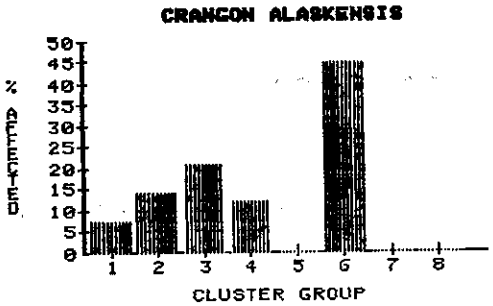


Figure 35. The geographic distribution of necrotic and nodular lesions observed in shrimp and crab. (A) Antennal gland necrosis in C. alaskensis and pandalid shrimp, (B) gill necrosis in C. alaskensis, (C) gill nodules in Cancer spp., and (D) midgut necrosis and (E) midgut nodules in Cancer spp. The number of animals examined for each subarea is given. The subareas are described in Table 28.







NUMBER EXAMINED

CLUSTER GROUP NUMBER	CANCER Spp.	PANDALUS Spp.	CRANGON ALASKENSIS	CRANGON FRANSCISCORUM
1	9	15	28	0
2	37	78	58	0
3	30	90	82	0
4	43	57	17	8
5	32	69	14	0
6	0	19	9	0
7	21	49	13	0
8	0	3	4	0

Figure 36. The distribution in cluster groups of crustacea with necrotic lesions of the gills.

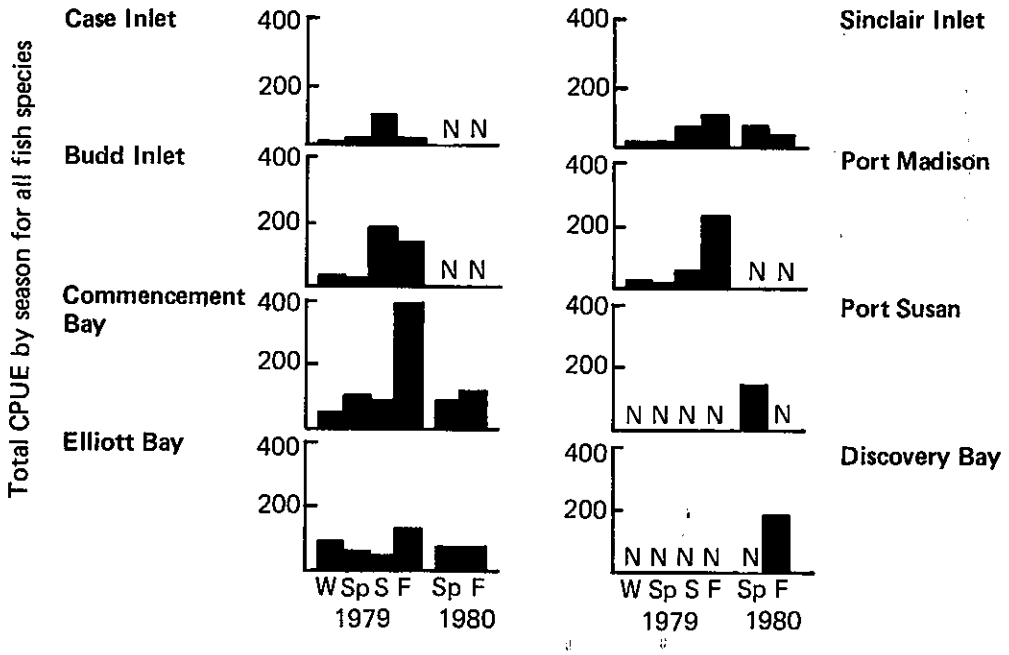


Figure 37. Total Catch Per Unit Effort values for all fish species for geographic areas (N=not samples).

W - winter
Sp - spring
S - summer
F - fall

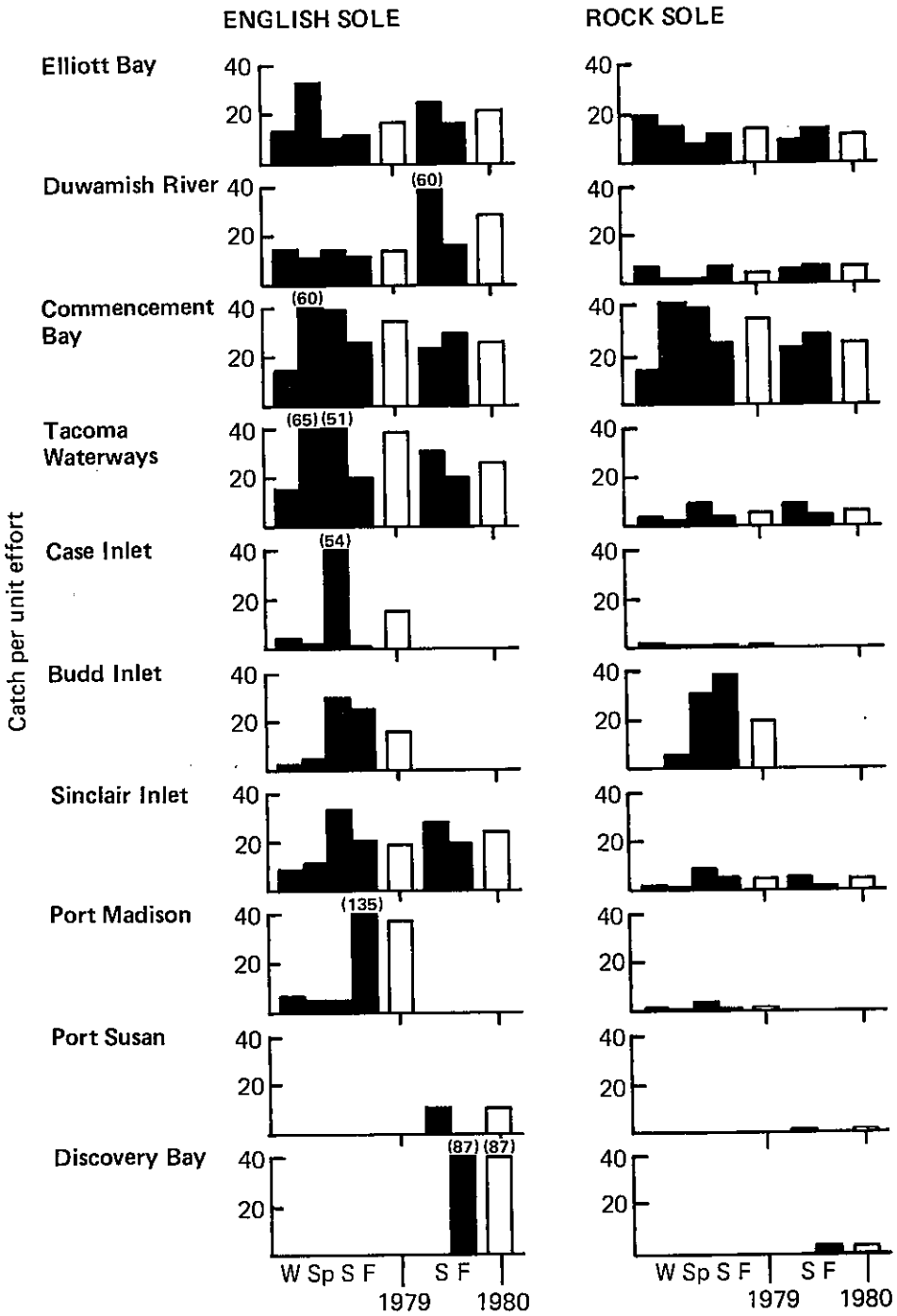


Figure 38. Catch Per Unit Effort values of English sole and rock sole for geographic areas. Closed columns signify seasonal averages and open columns signify annual averages. If values were higher than 40, the actual value is presented on top of the column in ( ).

W - winter
Sp - spring
S - summer
F - fall

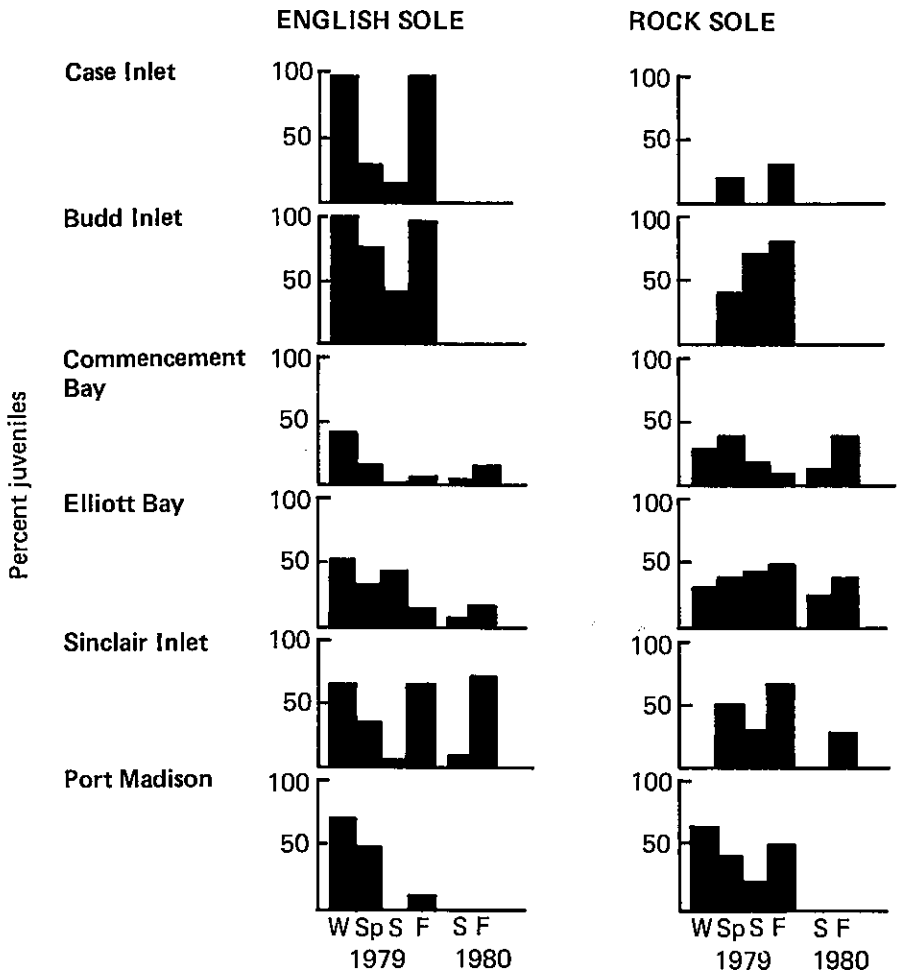


Figure 39. Catch Per Unit Effort values for young-of-the-year (less than 150 mm) English sole and rock sole for geographic areas.

W - winter
Sp - spring
S - summer
F - fall

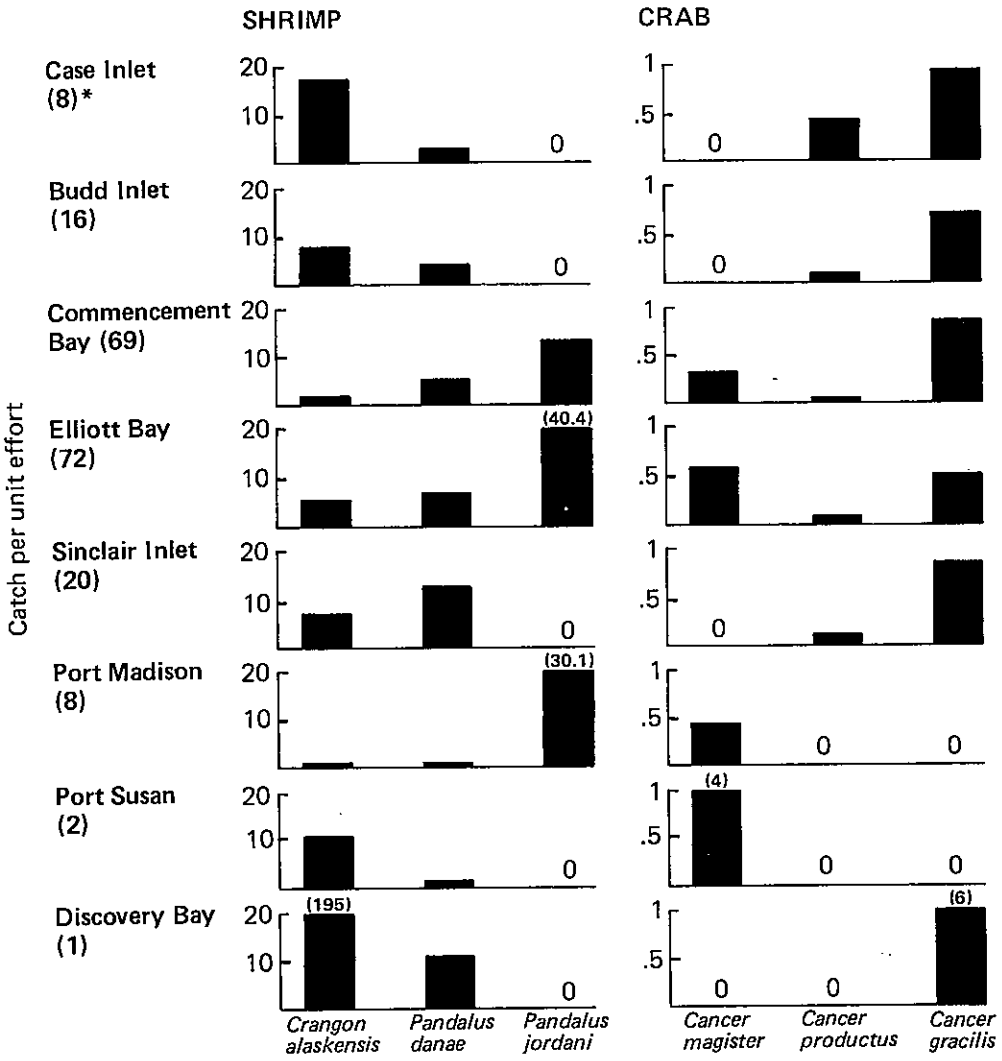


Figure 40. Catch Per Unit Effort values of six species of crustacea for geographic areas. *The number of stations sampled in 1979 and 1980.

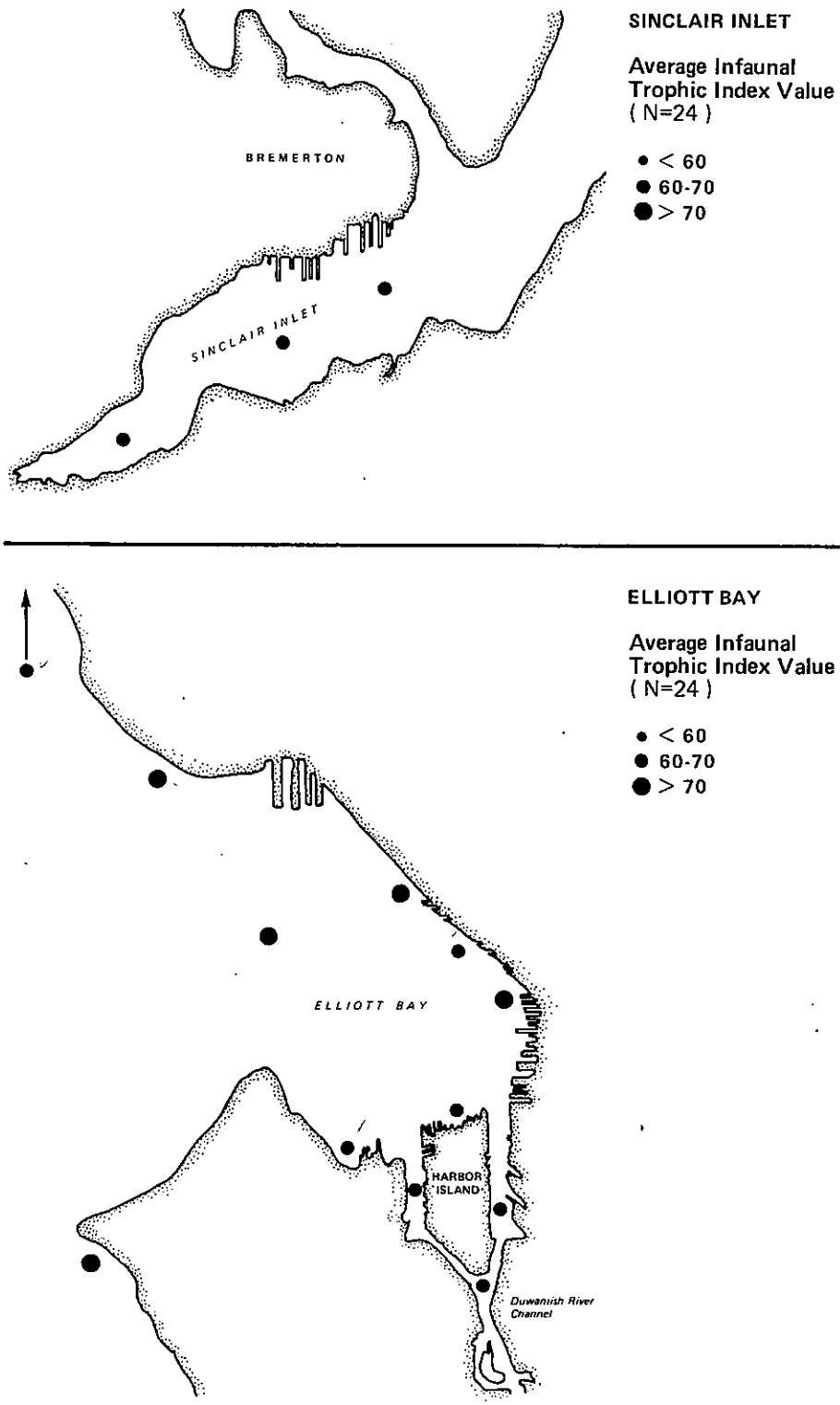


Figure 41. Average Infaunal Trophic Index values (average of 24 sediment samples collected in the winter, spring, summer, and fall of 1979) for each sampling station in the sampling areas. Sinclair Inlet and Elliott Bay.

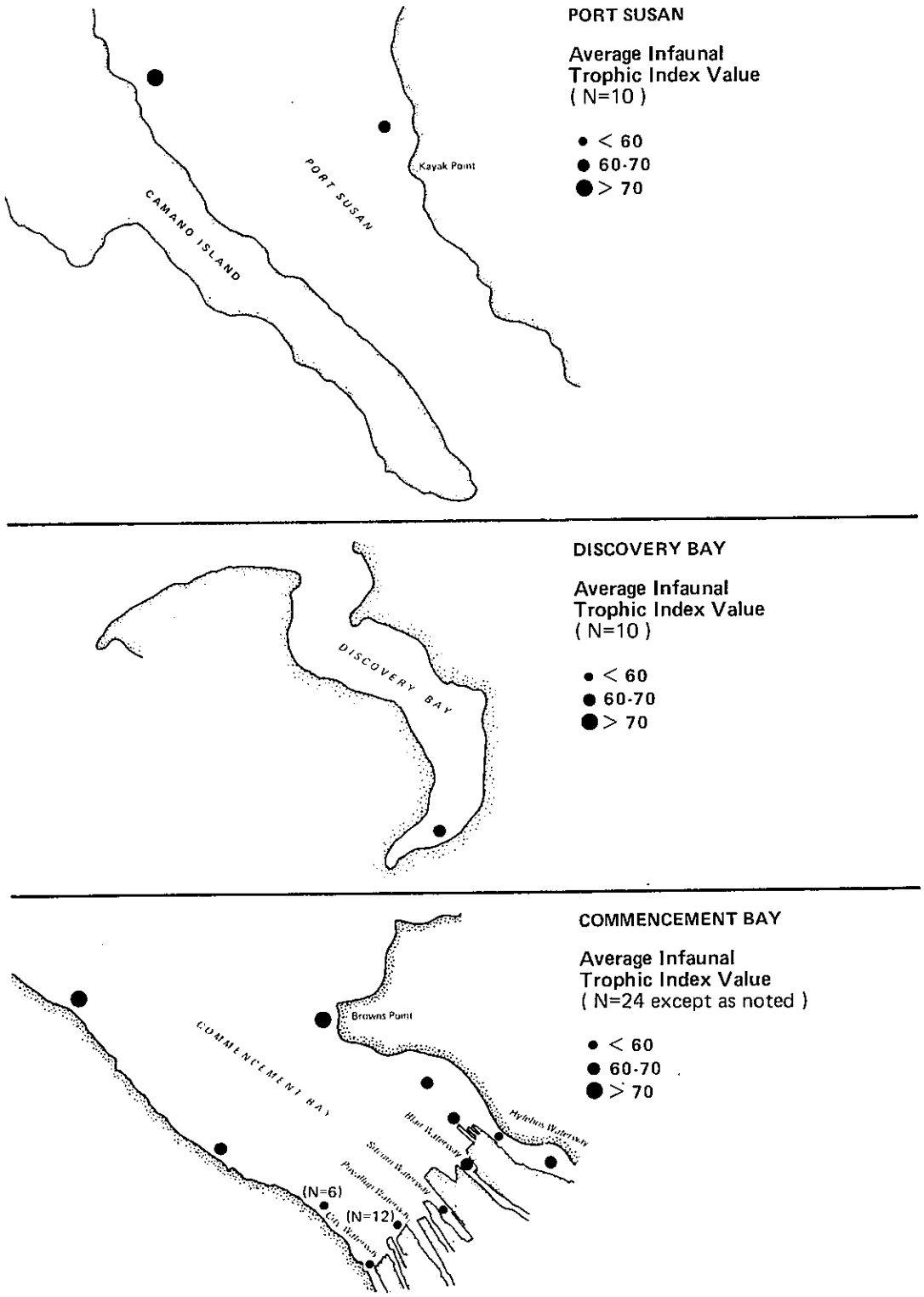
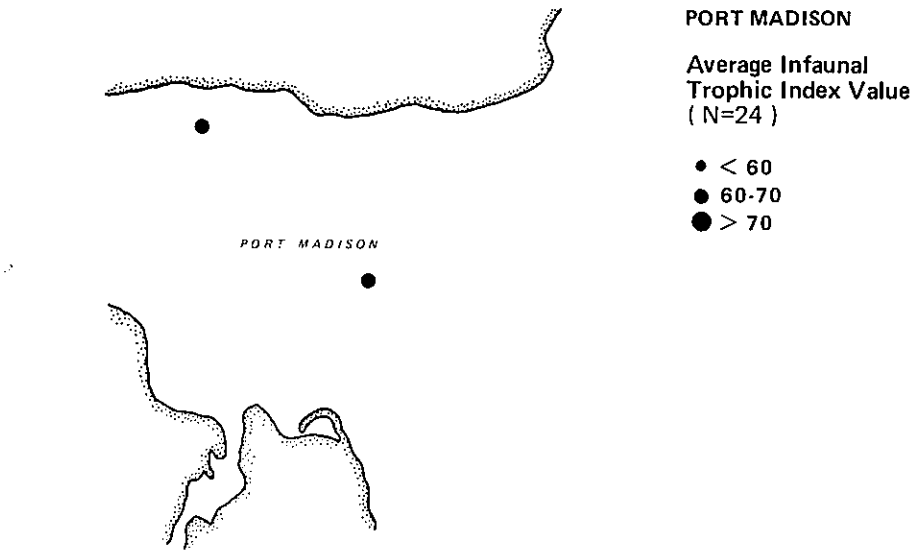


Figure 41. (continued) Port Susan, Discovery Bay, and Commencement Bay.



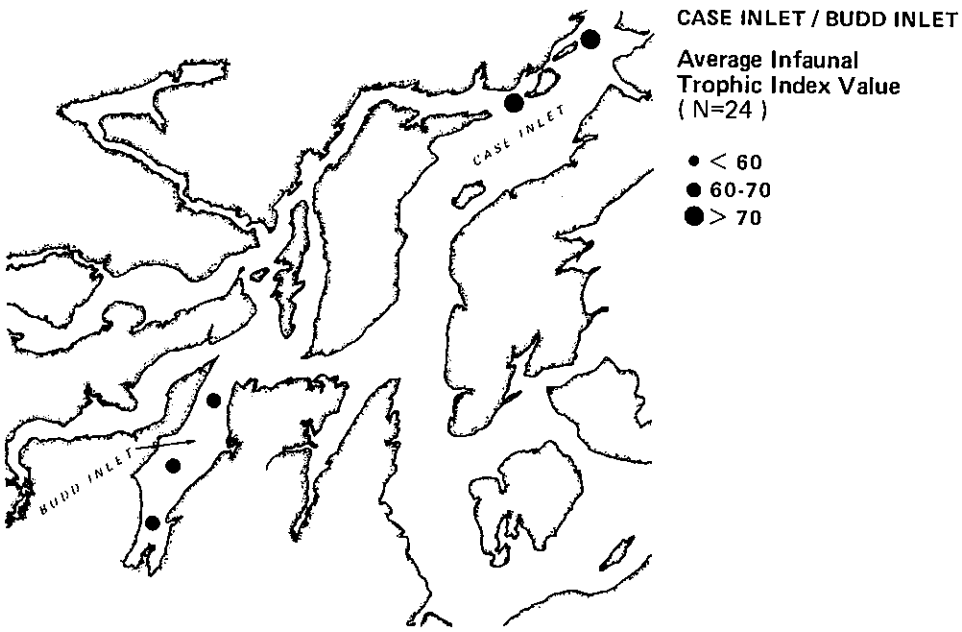


Figure 41. (continued) Port Madison, Case Inlet, and Budd Inlet.

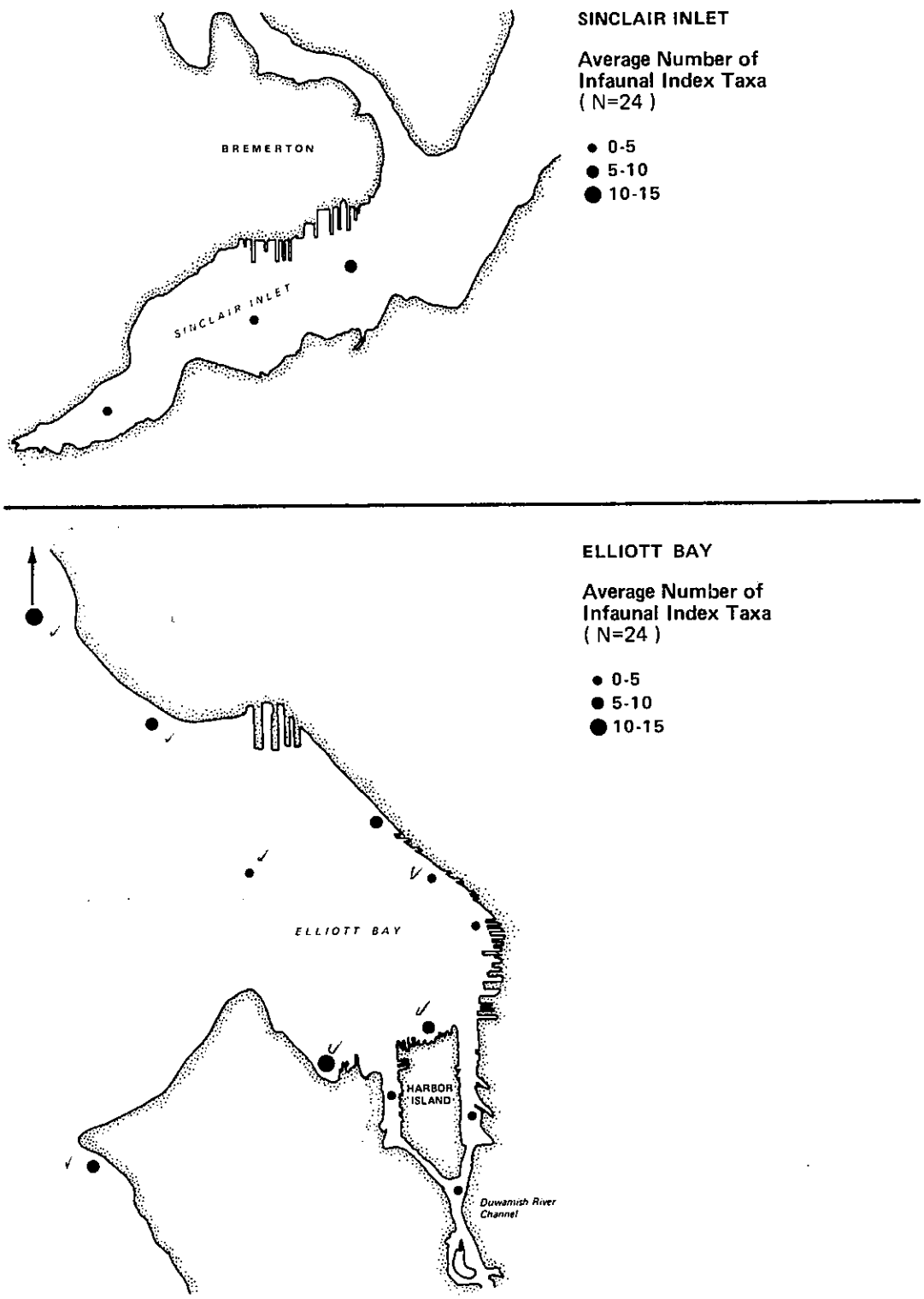


Figure 42. Average number of Infaunal Trophic Index Taxa (taxon richness) values (average of 24 sediment samples collected in the winter, spring, summer, and fall of 1979) for each sampling station in the sampling areas. Sinclair Inlet and Elliott Bay.

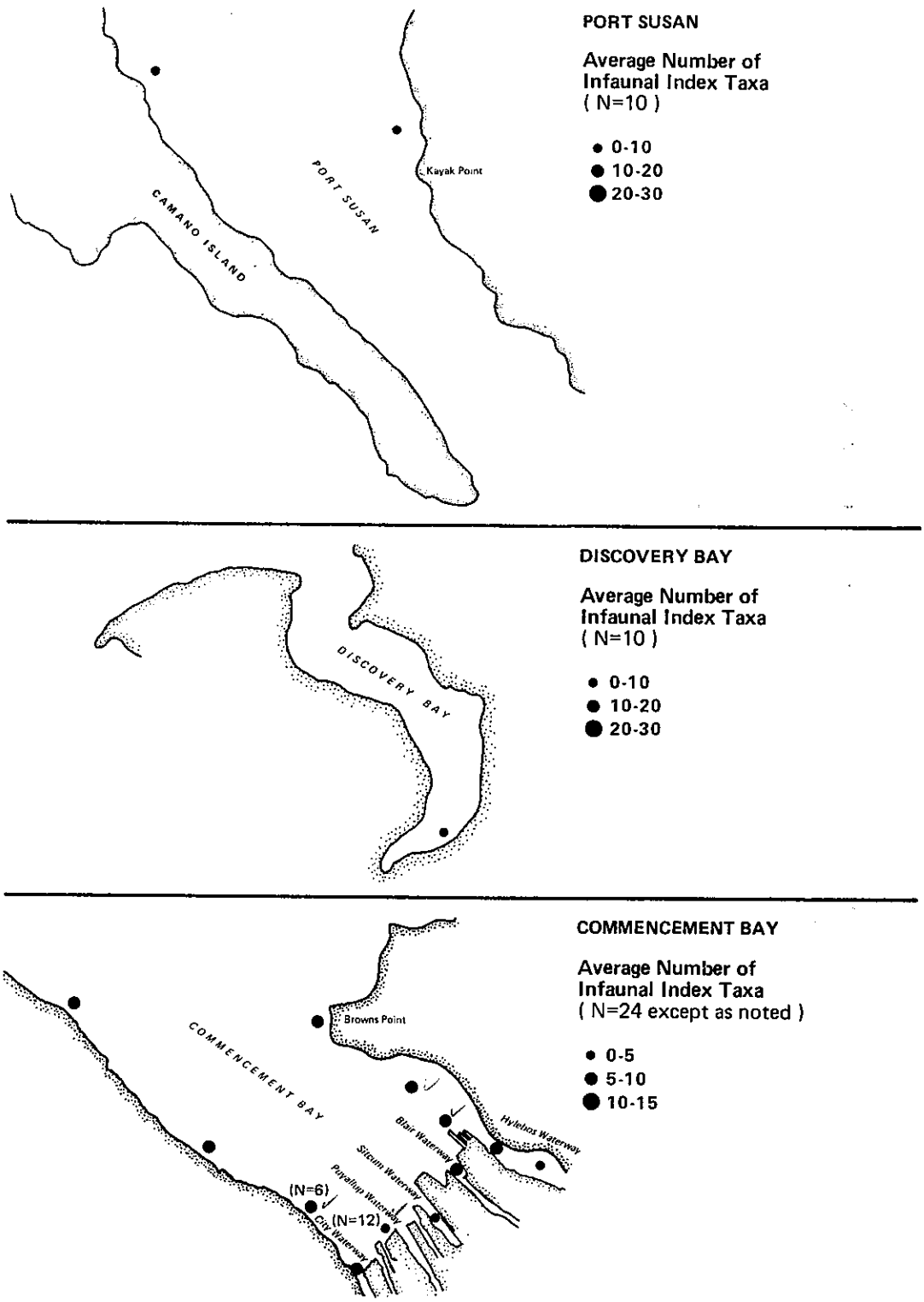
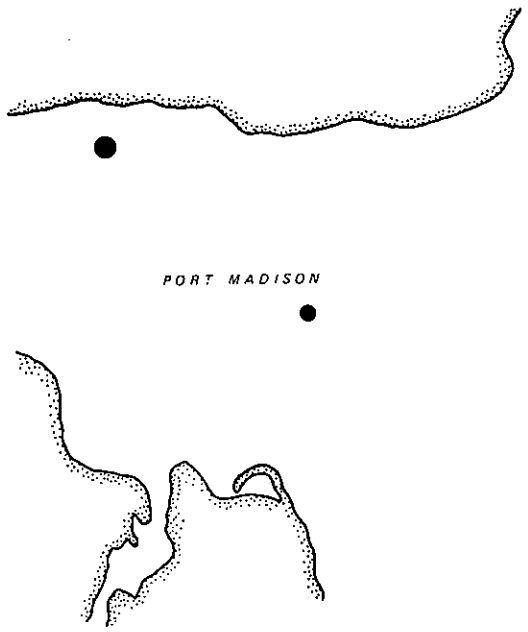


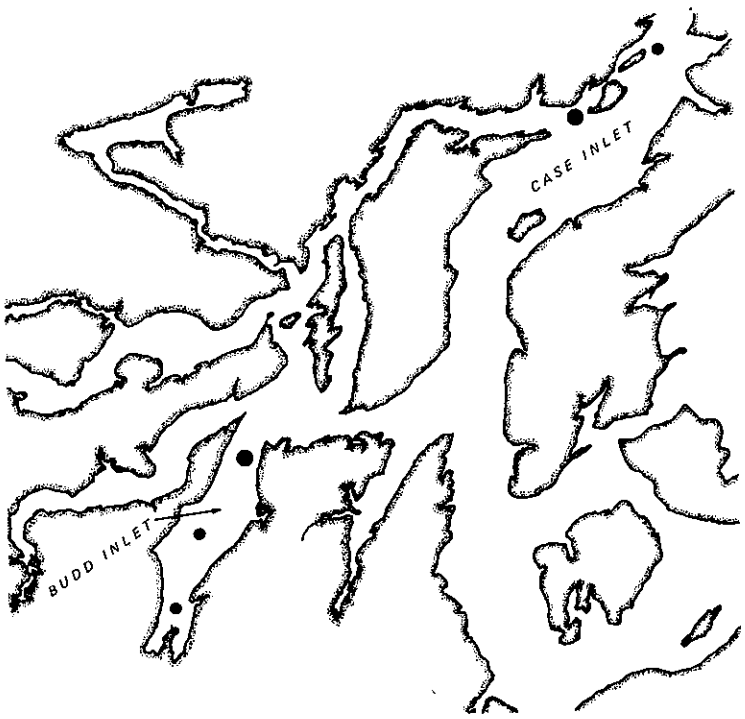
Figure 42. (continued) Port Susan, Discovery Bay, and Commencement Bay.



PORT MADISON

Average Number of
Infaunal Index Taxa
(N=24)

- 0-5
- 5-10
- 10-15



CASE INLET / BUDD INLET

Average Number of
Infaunal Index Taxa
(N=24)

- 0-5
- 5-10
- 10-15

Figure 42. (continued) Port Madison, Case Inlet, and Budd Inlet.

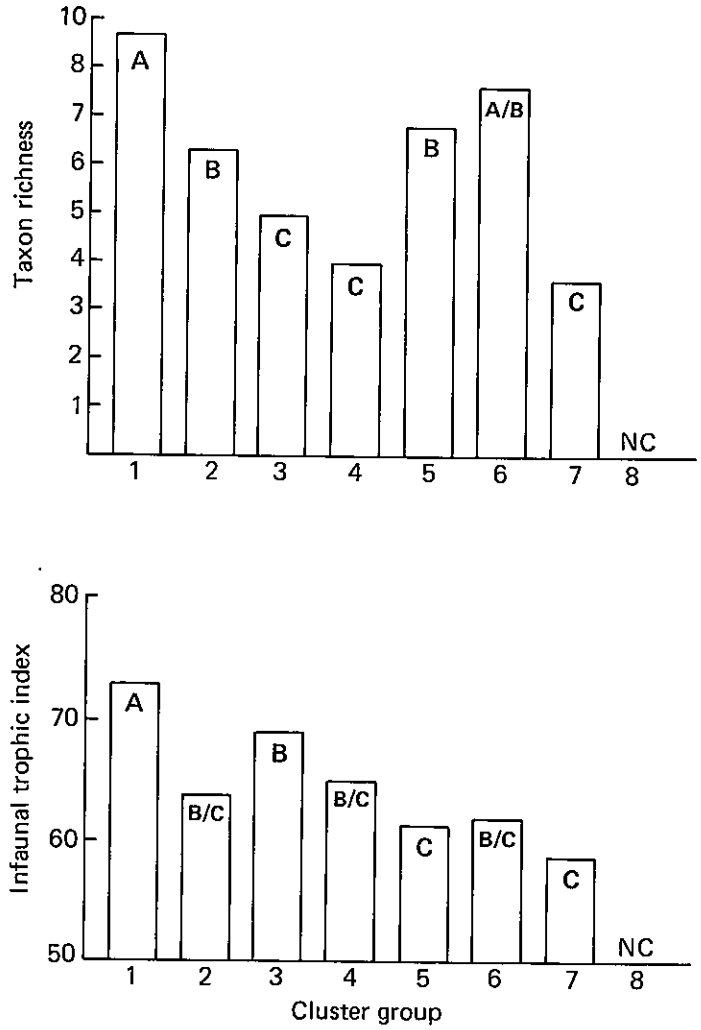
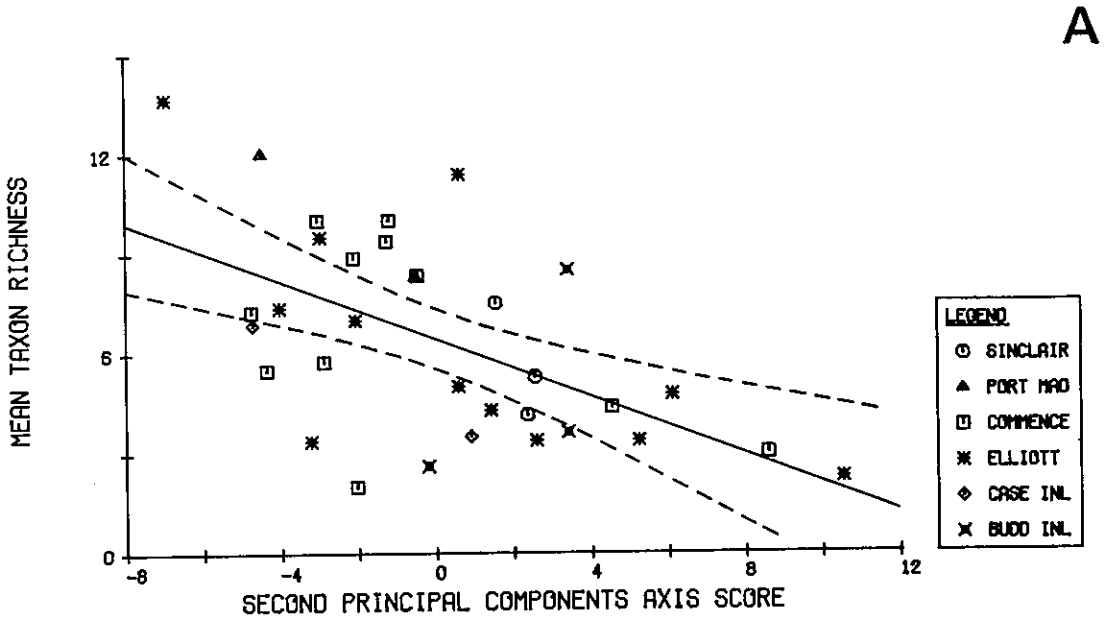


Figure 43. Average taxon richness values (upper histogram) and average Infaunal Trophic Index values (lower histogram) for the sampling stations within each cluster group. Cluster Group Stations which have values that are significantly different ($p \leq 0.05$) are identified by different letters (A,B, or C). Cluster Group stations with the same letter or two letters (A/B, B/C) have values which are statistically ($p \leq 0.05$) indistinguishable.



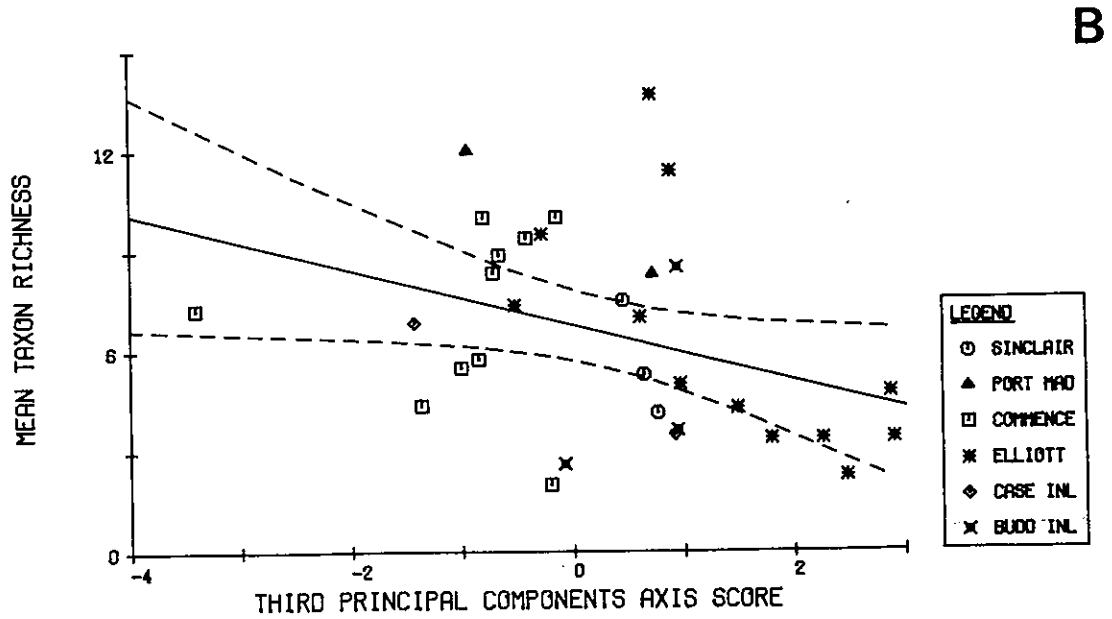


Figure 44. Regression lines and 95% confidence limits for mean taxon richness values for 1979 benthic invertebrate samples as a function of (A) relative concentration of metals (Spearman rank correlation coefficient -0.52; $p<0.001$), and (B) relative concentration of PCBs (Spearman rank correlation coefficient -0.45; $p<0.001$).

APPENDIX A

Compounds identified in sediment from two stations in Commencement Bay and one in Elliott Bay.

Organic compounds determined in three Puget Sound Sediments, in ng/g (dry weight basis)

Compound Name	Tacoma Commencement Bay		Seattle Elliott Bay
	Old Tacoma	Hylebos Waterway	Pier 54
Dichlorobutadienes	10	6	trace[1]
Trichlorobutadienes	4000	90	8
Tetrachlorobutadienes	3000	300	40
Pentachlorobutadienes	1000	90	4
Hexachlorobutadiene[4]	1000	90	1
Tetrachloroethanes	0.2	--	--
Hexachloroethane	3	40	--
Chlorobenzenes	--	--	trace
Dichlorobenzenes[4]	0.6	20	1
Trichlorobenzenes	70	50	0.3
Tetrachlorobenzenes	50	20	0.09
Pentachlorobenzenes	20	10	0.02
Hexachlorobenzene[4]	300	20	0.03
Dichlorocyclohexadienes	0.03	--	--
Trichlorocyclohexadienes	10	--	--
Tetrachlorocyclohexadienes	20	--	--
Pentachlorocyclohexadienes	40	--	--
Hexachlorocyclohexadienes	50	3	--
Heptachlorocyclohexadienes	6	--	--
Octachlorocyclohexadiene	30	--	--
Trichlorostyrenes	1	--	--
Tetrachlorostyrenes	10	--	--
Pentachlorostyrenes	50	--	--
Hexachlorostyrenes	40	--	--
Heptachlorostyrenes	10	--	--
Octachlorostyrene	20	--	--
Hexachlorocyclopentadiene	6	--	--
Trichlorocyclopentenes	0.04	--	--
Tetrachlorocyclopentenes	100	60	--
Pentachlorocyclopentenes	20	--	--
Hexachlorocyclopentenes	60	20	--
Heptachlorocyclopentenes	10	--	--
Pentachloromethylpropanes	3	--	--

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Dichloropentenes/Dichlorocyclopentanes	30	--	--
Trichloropentenes/Trichlorocyclopentanes	60	--	--
Tetrachloropentenes/Tetrachlorocyclopentanes	200	20	0.03
Pentachloropentenes/Pentachlorocyclopentanes	200	20	0.3
Trichloroheptatrienes/Trichlorocycloheptadienes	3	--	--
Tetrachloroheptatrienes/Tetrachlorocycloheptadienes	6	--	--
Pentachloroheptatrienes/Pentachlorocycloheptadienes	30	--	--
Hexachloroheptatrienes/Hexachlorocycloheptadienes	10	--	--
Dichlorobiphenyls[4]	20	1	2
Trichlorobiphenyls[4]	100	60	10
Tetrachlorobiphenyls[4]	300	200	50
Pentachlorobiphenyls[4]	200	300	100
Hexachlorobiphenyls[4]	200	400	100
Heptachlorobiphenyls[4]	100	100	100
Octachlorobiphenyls[4]	70	100	30
Nonachlorobiphenyls	2	--	20
Decachlorobiphenyl	8	--	--
Chloronaphthalenes	--	--	0.03
Dichloronaphthalenes	2	--	0.04
Trichloronaphthalenes	10	1	0.2
Tetrachloronaphthalenes	10	10	0.3
Pentachloronaphthalenes	3	10	0.6
Hexachloronaphthalenes	0.7	9	0.08
Heptachloronaphthalenes	2	--	--
Octachloronaphthalene	0.6	--	--
Chlorothiophenes	0.6	--	0.02
Dichlorothiophenes	2	--	--
Trichlorothiophenes	0.06	--	--
Chlorobenzothiophenes	0.5	--	--
Dichlorobenzothiophenes	2	--	--
Trichlorobenzothiophenes	0.6	--	--
Chlorohexanes	0.2	--	--
Chlorocyclohexanes	0.02	--	--
Dichlorocyclohexanes	0.4	--	trace
Dichlorobenzofurans	0.04	--	--
Chlorotoluenes	--	--	0.03
Tetrachlorotoluenes	0.5	--	--
Chloroxylenes	0.03	--	--
Dichloroxylenes	0.3	--	--

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Bromo(C_3H_7) benzenes[2]	0.7	--	--
Tetrachlorocyclohexenes	0.1	--	--
Pentachloropropanes	--	4	--
Heptachloropropanes	--	2	--
Octachloropropane	3	--	--
Hexachloropropene	--	40	--
Dichlorobutenals	1	--	--
Chlorofluoranthenes/ Chloropyrenes	0.8	80	0.07
Dichlorofluoranthenes/ Dichloropyrenes	0.7	50	--
Trichlorofluoranthenes/ Trichloropyrenes	--	6	--
Tetrachlorofluoranthenes/ Tetrachloropyrenes	0.2	30	--
Pentachlorofluoranthenes/ Pentachloropyrenes	--	30	--
Bromochlorofluoranthenes/ Bromochloropyrenes	1	20	--
Bromofluoranthenes/ Bromopyrenes	60	50	--
Dibromofluoranthenes/ Dibromopyrene	30	200	--
Dibromomethylfluoranthenes/ Dibromomethylpyrenes	0.1	--	--
Bromobiphenyls	1	--	--
Dibromobiphenyls	1	--	--
Chlorophenanthrenes/ Chloroanthracenes	0.4	30	--
Dichlorophenanthrenes/ Dichloroanthracenes	0.3	40	--
Trichlorophenanthrenes/ Trichloroanthracenes	--	10	--
Tetrachlorophenanthrenes/ Tetrachloroanthracenes	--	2	--
Bromophenanthrenes/ Bromoanthracenes	--	0.6	--
Dichloro-bis(chlorophenyl ethylene(DDE)[4]	10	30	2
Bis(chlorophenyl)dichloroethane (DDD)/ Trichloro-bis (chlorophenyl)20ethane(DDT)[4]	20	100	20
Chloro-bis(chlorophenyl)ethylene (DDMU)	--	1	--

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Bromofluorenes	0.2	--	--
Dibromofluorenes	4	--	--
Trichloroterphenyls	2	60	--
Tetrachloroterphenyls	2	3	--
Pentachloropyridine	--	0.3	--
Dibromodihydropyrenes	--	2	--
Octachlorodibenzodioxin	0.1	--	--
Iodohexanes	--	--	2
(C_2H_5) Benzenes[4]	10	600	7
(C_3H_7) Benzenes[4]	20	40	20
(C_4H_9) Benzenes[4]	100	10	20
(C_5H_{11}) Benzenes	7	--	8
(C_6H_{13}) Benzenes	1	--	2
(C_7H_{15}) Benzenes	0.3	--	--
(C_8H_{17}) Benzenes	0.3	--	--
Styrene	0.1	>50	0.6
Methyl Styrenes	--	--	0.3
(C_2H_5) Styrenes	--	--	1
(C_3H_7) Styrenes	--	--	0.6
(C_4H_9) Styrenes	--	--	5
(C_5H_{11}) Styrenes	--	--	1
Indan[4]	3	0.4	30
Methyl Indans	3	0.9	5
(C_2H_5) Indans	0.4	--	5
(C_3H_7) Indans	0.7	--	2
(C_4H_9) Indans	0.5	--	3
(C_5H_{11}) Indans	--	--	0.3
Indene	0.2	--	1
Naphthalene[4]	400	100	8
Methyl Naphthalenes[4]	400	80	2000
(C_2H_5) Naphthalenes[4]	300	100	>600
(C_3H_7) Naphthalenes[4]	500	200	>1000
(C_4H_9) Naphthalenes	200	60	50
(C_5H_{11}) Naphthalenes	10	--	50
(C_6H_{13}) Naphthalenes	--	--	5
(C_7H_{15}) Naphthalenes	--	--	2
Phenyl Naphthalenes	100	200	30
Methyl Phenyl Naphthalenes	40	400	4
(C_2H_5) Phenyl Naphthalenes	--	100	--
(C_3H_7) Dihydronaphthalenes	1	--	0.6
(C_4H_9) Dihydronaphthalenes	0.6	--	1
Tetralin	0.3	--	0.4

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Methyl Tetralins	0.3	--	4
(C_2H_5) Tetralins	2	--	5
(C_3H_7) Tetralins	0.5	--	4
(C_4H_9) Tetralins	--	--	4
(C_5H_{11}) Tetralins	--	--	0.3
Phenyl Tetralins	--	30	--
Cyclohexyl Benzene	2	--	0.7
Methyl Cyclohexyl Benzenes	1	--	2
(C_4H_7) Alkenyl Benzenes	2	--	--
(C_5H_9) Alkenyl Benzenes	3	--	0.7
(C_6H_{11}) Alkenyl Benzenes	0.4	--	1
(C_7H_{13}) Alkenyl Benzenes	0.3	--	1
(C_8H_{15}) Alkenyl Benzenes	--	--	0.5
(C_9H_{17}) Alkenyl Benzenes	--	--	2
(C_4H_5) Alkadienyl Benzenes	10	--	--
(C_7H_{11}) Alkadienyl Benzenes	--	--	0.4
(C_8H_{13}) Alkadienyl Benzenes	--	--	0.2
Biphenyl[4]	40	20	10
Acenaphthene[4]	300	40	1000
Methyl Biphenyls/ Methyl Acenaphthenes	20	30	30
(C_2H_5) Biphenyls/ (C_2H_5) Acenaphthenes	3	6	10
(C_3H_7) Biphenyls/ C_3H_7) Acenaphthenes	5	--	4
(C_4H_9) Biphenyls/ (C_4H_9) Acenaphthenes	60	40	30
(C_5H_{11}) Biphenyls/ (C_5H_{11}) Acenaphthenes	3	--	10
(C_6H_{13}) Biphenyls/ (C_6H_{13}) Acenaphthenes	--	--	5
Acenaphthalene	20	3	200
Fluorene[4]	300	40	1000
Methyl Fluorenes	100	20	20
(C_2H_5) Fluorenes	50	80	--
(C_3H_7) Fluorenes	30	--	4
(C_4H_9) Fluorenes	--	--	0.4
(C_5H_{11}) Fluorenes	9	--	3
Phenyl Fluorenes	--	20	--
Phenylmethylene Fluorenes	--	100	--

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Phenanthrene[4]	2000	400	7000
Anthracene[4]	600	200	2000
Methyl Phenanthrenes/ Methyl Anthracenes[4]	500	400	100
(C_2H_5) Phenanthrenes/ (C_2H_5) Anthracenes	300	300	30
(C_3H_7) Phenanthrenes/ (C_3H_7) Anthracenes	300	300	10
(C_4H_9) Phenanthrenes/ (C_4H_9) Anthracenes	400	--	70
(C5H11) Phenanthrenes/ (C_5H_{11}) Athracene	20	--	--
Cyclopenta (def) phenanthrene	200	200	30
Phenyl Phenanthrenes/ Phenyl Anthracenes	--	40	--
Fluoranthene[4]	3000	2000	8000
Pyrene[4]	2000	2000	10000
Methyl Fluoranthenes/ Methyl Pyrenes	700	3000	100
(C_2H_5) Fluoranthenes/ (C_2H_5) Pyrenes	90	200	20
(C_3H_7)Fluoranthenes/ (C_3H_7) Pyrenes	20	200	--
(C_4H_9)Fluoranthenes/ (C_4H_9) Pyrenes	--	40	--
Dihydropyrenes	0.09	--	20
Benz(a)anthracene(BAA)$_4$	2000	2000	7000
Chrysene(CHR)$_4$	2000	2000	6000
Other MW228 Aromatics	400	70	20
Methyl BAA/CHR/etc.	100	1000	40
(C_2H_5) BAA/CHR/etc.	60	200	--
Methylene BAA/CHR/etc.	--	80	--
Benzo(ghi)fluoranthene$_4$	--	600	30
Binaphthyls	60	300	--
Methyl Binaphthyl	--	70	--
Tetrahydrobinaphthyls	10	--	0.5
(C_2H_5) Dihydro Phenanthrenes/ (C_2H_5) Anthracenes	--	20	--
Phenyl Dihydromethanonaphthalene/ Phenyl Acenaphthene	--	3	--
Dihydrobbenzenoanthracene	--	400	--
Terphenyl	70	--	--

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Benzofluoranthenes(BFLA)[4]	1000	3000	5000
Benzo(e)pyrene(BP)[4]	800	1000	4000
Benzo(a)pyrene(BP)[4]	300	500	2000
Perylene(PER)[4]	100	200	2000
Methyl BFLA/BP/PER	60	200	8
(C_2H_5) BFLA/BP/PER	10	30	--
Indeno (1,2,3-cd)pyrene(IDP)	300	400	3000
Benzo(ghi)perylene(BPER)	60	200	10
Methyl IDP/BPER	--	10	--
(C_2H_5) IDP/BPER	--	20	--
Benzo(b)chrysene	--	100	--
Dibenz(a,h)anthracene	--	200	--
Other MW278 aromatics	20	200	2
Methyl Dibenzanthracenes	6	20	--
Quaterphenyl	--	6	--
Coronene	--	3	--
Dibenzopyrenes (MW304)	--	20	--
Diphenylacenaphthalene	1	--	--
Benzofluorene	--	--	30
Phenalene	--	--	0.3
Other MW226 Aromatics	--	--	40
(C_3H_7) Tetrahydropicene	--	--	7
Dihydroethanoanthracene	--	--	0.2
1,4-Ethanotetralin	--	--	0.9
Benzothiophene	50	6	80
Methyl Benzothiophenes	0.9	0.6	0.5
(C_2H_5) Benzothiophenes	0.7	3	1
(C_4H_9) Benzothiophenes	--	--	0.2
(C_5H_{11}) Benzothiophenes	1	--	--
Dibenzothiophene[4]	300	90	500
Methyl Dibenzothiophenes/ Methyl Naphthothiophenes	100	50	--
(C_2H_5) Dibenzothiophenes/ (C_2H_5) Naphthothiophenes	60	90	--
Benzo(def)dibenzothiophene	--	--	2
Benzonaphthothiophene	80	200	1
Methyl Benzonaphthothiophenes	--	100	--
Bis(dimethylpropyl)disulfide	--	4	--
Carbazole	9	2	8
Methyl Carbazoles	5	--	10
(C_2H_5) Carbazoles	1	--	6
(C_3H_7) Carbazoles	3	--	5
(C_4H_9) Carbazoles	1	--	1
Benzocarbazole	0.6	--	--
Dibenzocarbazole	0.3	--	--

Compound Name	Old Tacoma	Hylebos Waterway	Pier 54
Phenylethenyl aniline	0.8	--	5
Azapicene/Azadibenzanthracene	0.8	--	1
Methyl Benzacridines	0.5	--	0.3
Phenyl Indoles	0.04	--	--
Diphenyl pyridines	0.5	--	0.9
Methyl Diphenyl pyridines	--	--	0.5
Phenyl Cinnoline	0.1	--	--
Nitrobenzene	--	--	trace
Phenylaniline	--	--	0.3
Phenyl Isoquinolines/ Phenyl Quinolines	--	--	0.2
Styrl Quinolines/ Styrl Isoquinolines	--	--	1
Benzofuran	--	--	0.6
Dibenzofuran	100	60	40
Methyl Dibenzofurans	50	60	10
(C_2H_5) Dibenzofurans	90	70	10
Xanthene	--	--	10
Dibenzofuran amine	--	--	0.05
Benzonaphthofuran	100	--	40
Dinaphthofuran/Dibenzo-naphthofuran	30	--	--
Methyl Naphthaldehydes	3	--	--
(C_3H_7) Phenols	10	--	--
Fluorenone	0.2	--	0.07
(C_2H_5) Fluorenones	0.5	--	0.3
Anthracenone	5	--	--
Diphenyl cyclopropenones	7	--	50
Benzanthracenone	0.5	--	0.2
Indenoanthracenone	0.6	--	--
Methoxymethylstyrenes	3	--	0.4
Acetophenone	--	0.6	--
(C_2H_5) Acetophenone	--	--	0.02
Terphenyl-ols	--	100	--
Dibutyl Phthalate	--	7	0.5
Butyl Benzyl Phthalate	--	1	--
Dioctyl Phthalate	--	9	3
Methoxyphenanthrenes	--	--	5
Benzodioxole	--	--	trace
Nonanal	--	--	0.06
Decanal	--	--	0.1
Dodecanal	--	--	trace
Dibenzoxepin	--	--	2
(C_5H_{11}) Decalins	0.3	--	--
(C_2H_5) Cyclohexanes	trace	--	--
(C_4H_9) Alkenyl Cyclohexenes	--	--	0.9
(C_4H_{11}) Cyclohexadiene	--	--	0.2

FOOTNOTES

1. Trace indicates that the compound was detected but present at <=0.01 ng/gm dry weight.

2. (C_xH_y) indicates compounds which have a total of x alkyl carbons added to the parent compound in one or more side chains; i.e. (C_3H_7) could be one of trimethyl, methyl ethyl, isopropyl or n-propyl side chains.

3. -- indicates the compound was not detected.

4. Compounds were quantitated based on the ratio of the response for standard compounds compared to internal standard. All other quantities were determined using the mass spectrometer and assumed response ratio of 1 because standard compounds were not available. These quantities are only relative abundances between samples; however, the absolute values may be off by as much as a factor of ten.

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