



Padilla Bay

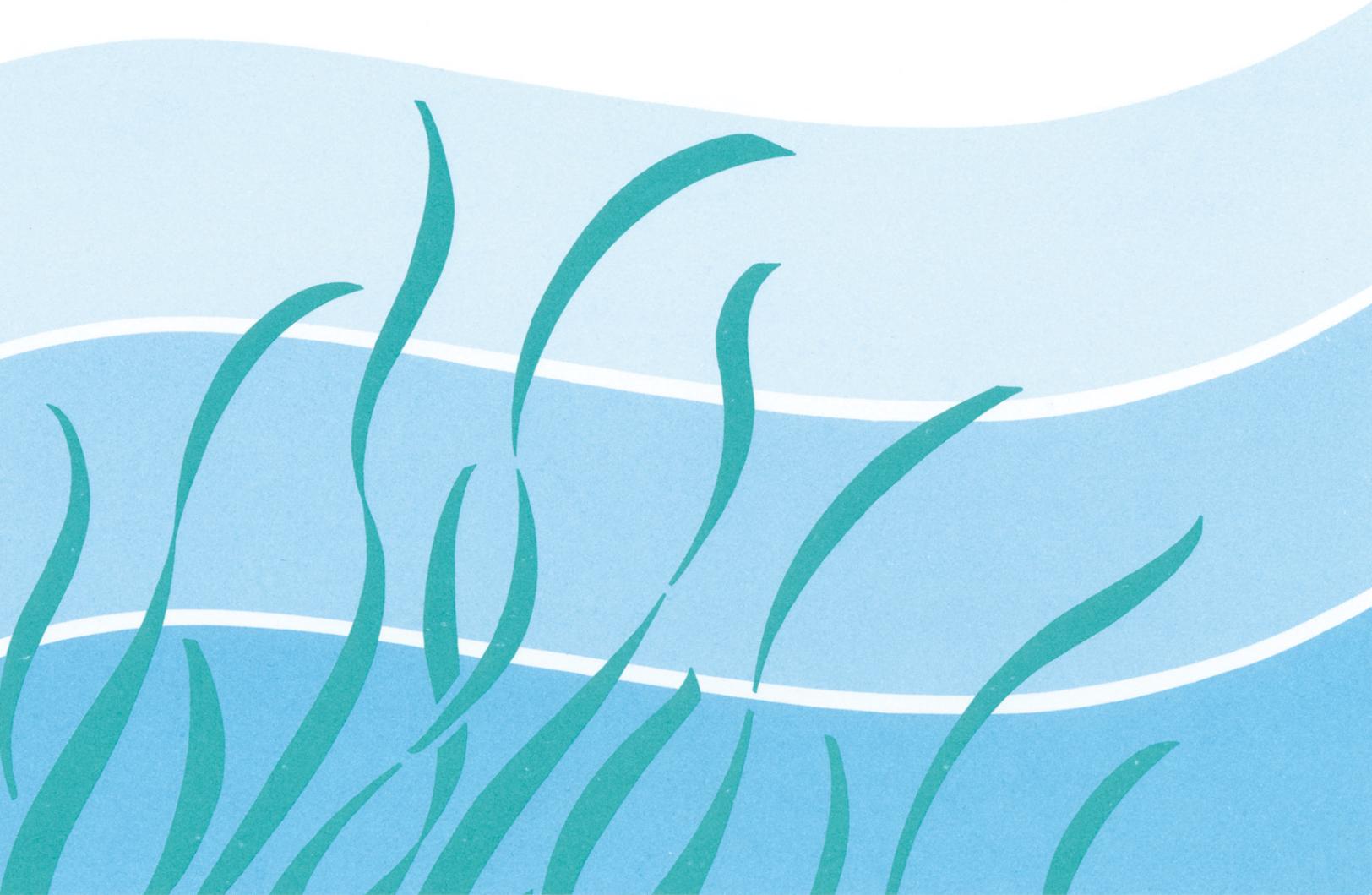
National Estuarine Research Reserve

Reprint Series No. 17
Reprinted November 1992

**THE EFFECT OF LANDFILL LEACHATE FROM PADILLA BAY
ON THE ABUNDANCE OF EPIBENTHIC HARPACTICOID
COPEPODS AND SEDIMENT TOXICITY MEASURED WITH THE
AMPHIPOD BIOASSAY (*Rhepoxinius abronius*)**

James R. Wiggins

1992



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Padilla Bay National Estuarine Research Reserve is managed by the Shorelands and Environmental Assistance Program, Washington State Department of Ecology, in cooperation with the Estuarine Reserves Division, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce. The preparation of this document was financially aided through a grant to the Washington State Department of Ecology with funds obtained from NOAA/Office of Ocean and Coastal Resource Management, and appropriated for Section 306 or 315 of the Coastal Zone Management Act of 1972, as amended.



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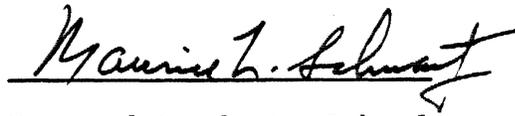
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By

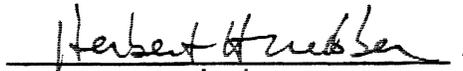
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Accepted in Partial Completion
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Master of Science

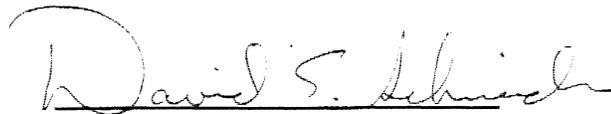


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Bibliographic citation: Wiggins, James R. 1992. The effect of landfill leachate from Padilla Bay on the abundance of epibenthic harpacticoid copepods and sediment toxicity measured with the amphipod bioassay (*Rhepoxinius abronius*). M.S. Thesis. Western Washington University. 58 pp. Bellingham, Washington. Padilla Bay National Estuarine Research Reserve Reprint Series No. 17 (Reprinted 1992).

MASTER'S THESIS

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(Rhepoxinius abronius)

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
James R. Wiggins

April 1992

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ABSTRACT

Landfills are disposal sites for municipal and industrial waste. Landfill leachate, whether from seepage through the landfill as a result of rainfall or groundwater or as direct runoff from discarded liquid wastes, affects various marine communities in coastal waters. Combining chemical analyses, sediment bioassays for toxicity analyses, and *in-situ* community sampling allows for a thorough determination of the degree of impact leachate has on a nearshore marine system.

Landfill leachate enters a small embayment in the southwest corner of Padilla Bay. Acting for the EPA, the Washington Department of Ecology (WDOE) found no significant contamination of priority pollutants in this area in 1986.

After visiting this site in 1989, I became concerned about the WDOE results and decided to incorporate a sediment quality triad approach to complete a site assessment. Using the WDOE chemistry results for the first portion of the triad, I performed a sediment bioassay using the amphipod (Rhepoxinius abronius) and analyzed for the abundance of

epibenthic harpacticoid copepods. The area exposed to the landfill leachate runoff showed no significant decline in harpacticoid copepod abundance compared to the control. The amphipod sediment bioassay for this location showed a significantly higher mortality than the control. The analysis of another area where leachate showed visible signs of oil contamination at depth had a significantly higher abundance of harpacticoids on the surface than the control and landfill leachate affected area and had the highest mortality in the amphipod bioassay (100% mortality using the top 2 cm of sediment). The top 2 mm of the sediment column for the oil contaminated site showed similar bioassay results as the control, indicating a natural sediment "cap" over toxic sediments.

Local harpacticoid copepod species, predominantly Harpacticus sp. and Tisbe sp. (a portion of the epibenthos) reside in the sediment surface layer. The sediment is oxidized, high in humic content, and well mixed. This study indicates that the sediment surface layer provides adequate conditions to support an epibenthic community by separating it from toxic benthic sediments.

ACKNOWLEDGEMENTS

I wish to thank the following persons for their assistance to complete this project:

Dr. H.H. Webber, editing, advisement, and support;
Elizabeth Binney, editing, advisement, patience and support;
Jeff Cordell, identification of harpacticoids;
Gene Mckeen, skipper of the ANOVA;
Caryl Dunavan, Huxley lab technician;
Dr. David Schneider, committee advisor;
Dr. Stephen Sulkin, committee advisor;
Dr. Robin Matthews, advisement;
Dr. J. Richard Mayer, advisement;
Dr. William Summers, advisement, financial assistance;
Doug Doolittle, Biology technician, microscope and boat use;
Dennis Boherer, Biology technician, microscope and boat use;
Tim Hall, NACSI, use of dredge net and advisement;
Doug Bulthuis, PBNERR, financial support;
Terry Stevens, PBNERR, financial support;
Don Norman, site advisement;
Art Johnson, W.D.O.E., literature;
Dr. Patsy McCloughlin, staining procedures;
Audubon Society, financial support;
Adrian Klaver, technical support;
Staff of Huxley College, Geology Department, and W.W.U.;
T. DeWitt, support and assistance with amphipod bioassay;
R. Swartz, support and assistance with amphipod bioassay.

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INTRODUCTION

POLLUTION ASSESSMENT:

My academic and professional interests are how anthropogenic pollutants affect biological systems, specifically wetlands. Wetlands, the transition zone where aquatic and terrestrial systems meet, have the capacity to decontaminate and filter pollutant discharges from a variety of sources (EPA 1988a, Mitsch & Jorgensen 1989). Various combinations of sediments, plants, and bacteria within a wetland reduce pollutant toxicity (Wiedner et al. 1988, Wiedner et al. 1989, Henrot et al. 1989) but the wetland pollutant carrying capacity is unknown. Wetlands also function as important habitat providing essential breeding, spawning, rearing, feeding, nesting, and wintering areas for fish and wildlife. (PSWQA 1986, Gardner 1990). However, fifty percent of the fresh water wetlands and ninety percent of the saltwater wetlands of the state of Washington have been eliminated (Boule et al. 1983, PSWQA 1986, Gardner 1990). It is therefore important to understand the risk to ecological structure and function of remaining wetlands from the contaminants entering them and the adverse impacts contaminants may have on biota. By determining the impact pollutants have on wetlands, scientists and regulators can prioritize the problem areas.

Aquatic habitats, including the estuaries, wetlands, and coastal waters of Washington State, receive many pollutants, such as synthetic chemicals, metals, and

nutrients, that adversely effect freshwater and marine biota (Malins et al. 1982, Horner 1988). These pollutants enter marine waters from a variety of sources including spills, sediment dredging and dumping operations, urban runoff from point and nonpoint sources, landfills, and municipal and industrial discharges (Dexter et al. 1981). Some common contaminants that enter aquatic systems are petroleum hydrocarbons, chlorinated compounds, pesticides, metals, and treated and untreated sewage. (Malins et al. 1984).

Non-point source pollutants are difficult if not impossible to regulate and quantify. Non-point sources have vague areas of origin such as landfill leachate, stormwater runoff, domestic septic systems, and cattle and dairy farms. These contaminants may originally enter upland lakes, streams, and waterways, but locally are finally deposited in the inland marine waters.

Landfills, whether urban or industrial, are a non-point source of contamination because of the production of leachate. As precipitation percolates through the fill, the incoming water picks up organic and inorganic pollutants from physical, hydrolytic and fermentative processes (Lema et al. 1988) producing leachate. The leachate then transports the dissolved contaminants into the surrounding environment e.g. surface waters or ground water.

When contaminants such as leachate enter a wetland or an open water system, various physical, chemical, and biological processes occur. Contaminants may form particulates by adsorbing onto sediments (Helz et al. 1974, Guy et al. 1978). Contaminants, such as metals and organic compounds may dissolve in open water and interstitial water (Evans 1989), and bioconcentrate and bioaccumulate into resident organisms (Crececius et al. 1980, Negilski et al. 1981, Sullivan et al. 1983, Ahsanullah & Florence 1984, Ahsanullah et al. 1984, Davies-Colley et al. 1984). This may chronically and/or acutely affect the resident organisms.

The toxicity and bioavailability of contaminants in sediments is dependent on many variables, including speciation of metals that changes with pH, the presence or absence of oxygen (redox layer), organic content, and the presence or absence of humic acids (Karickhoff et al. 1979, EPA 1984, Evans 1989). Contaminants may be bound in the sediments and not be available to the resident fauna. If toxic sediments are disturbed through processes such as dredging, the contaminants may become biologically available.

Most pollutants upon entering marine waters, adsorb and absorb onto minerals and humic substances to form particulates that settle to the bottom (Johnson 1974, Karickhoff et al. 1979, Malins et al. 1984). Dexter et al. (1981) states there is a net sedimentation rate of 6.1 mm/yr

to the main basin of the Puget Sound from sediments delivered by fresh water input of adjacent rivers. This indicates most dissolved and particulate pollutants remain in the sediments of the inland marine waters of Washington State. Therefore most contaminants from leachate and the other various forms of point and non-point source pollution attach to suspended particulates and settle to the bottom sediment.

EVALUATION OF IMPACTS ON BIOTA:

Biotic community systems and individual organisms have different tolerances to various pollutants. Whether the sources of contamination are from organic enrichment, lack of oxygen, hydrocarbons, or high levels of a metal like cadmium, biotic response characteristics vary in regards to various levels of toxicant concentration. Some organisms have a high tolerance to specific contaminants allowing them to dominate a community. As an example, the polychaete Capitella capitata is tolerant of high levels of organic enrichment and may dominate an area where wood waste has settled into the marine sediment (EPA 1986). Various micro- and meiofauna such as Protozoa, Turbellaria, Nematoda and Gastrotricha can tolerate long periods of anoxia (Pomeroy et al. 1977, Wiebe et al. 1981), within areas of high organic enrichment.

When a system is analyzed for an adverse response to a contaminant, compensation for the potential of individual

and community variation in tolerance to particular contaminants must be accounted for. Various chemical analyses, bioassays, and inspection of fauna are therefore used to complete an impact assessment. Long and Chapman (1985) suggest using each of these three factors (chemical, bioassay and fauna), the "Sediment Quality Triad" to give a more thorough analysis of a potentially impacted system. This triad method includes: 1. a numerical assessment of specific chemicals (e.g. EPA priority pollutants), 2. whole effluent or sediment bioassay, and 3. a biosurvey or bioassessment of a resident benthic or epibenthic community. Using the triad system to evaluate effects from contamination does not require assumptions about specific mechanisms of chemical interaction between an organism and a contaminant. Presence and response are the only two qualities observed (chemical presence, biota presence, and biotic response). Each aspect, (chemical, biological, and faunal) is evaluated separately (Long and Chapman 1985, Chapman 1989).

Methods used to determine the presence of a particular contaminant, chemical concentrations, and biological effects are discussed in various publications and manuals [the Puget Sound Estuary Program (PSEP) 1986a protocols, Standard Methods (APHA 1989), and Environmental Protection Agency (EPA 1983) protocols]. Using common and accepted procedures

in a study allows for reproducibility, credibility and cohesion between studies.

Chemical analysis looks for specific toxicants and their concentrations allowing the researcher to ascertain if predetermined acute or chronic quantities are present. However, if samples are analyzed just for specific metals and chemical pollutants that are suspected to be present, the contaminants not analyzed for will obviously be missed. Detection limits will also miss low concentrations of various contaminants that have potential cumulative and synergistic effects on biota. The bioassay and faunal analyses are intended to compensate for this.

Bioassays analyze cumulative and synergistic effects for low pollutant concentrations, and acute effects for high pollutant concentrations, to determine sediment and water toxicity on an organism. Different organisms are used depending on the medium analyzed (water or sediment) and the tolerance of the organism to variations within the medium (fresh, marine, or estuarine water; and sand, mud, or cobble sediments). Availability of the bioassay organism and the facilities to do the testing are also a consideration when choosing which bioassay organism to use.

There are several bioassay methods for sediment toxicity depending upon (as discussed above) organism tolerance, medium used, and availability of the organism and facilities. Typical and accepted bioassays for estuarine

sediments are: sediment amphipod (Swartz et al. 1979, Swartz et al. 1984a, Swartz et al. 1988, Swartz et al. 1989), juvenile Neanthes (Pesch 1979, Johns et al. 1989), bacterial luminescence and oyster embryo (Williams et al. 1986), bivalve larvae, microtox and anaphase aberrations (PSEP 1986b).

This study utilizes the sediment bioassay using the phoxocephalid amphipod, (Rhepoxinius abronius, Figure 1). These organisms are readily available, I had familiarity with the bioassay, and the facilities, although limited, were available. Included in my reasoning to use this bioassay were the types of sediments found at my study site. Some of these sediments are high in silt/clay content and some have a high organic content. Swartz et al. (1984b), and Ott (1986) have shown that R. abronius is tolerant to a broad range of sediment grain sizes and levels of organic enrichment. Dewitt et al. (1988) have further determined R. abronius sensitivity to natural sediment size and established prediction limits for survival as a function of percent fines (silt/clay).

This sediment/amphipod method is an acute, static, bioassay. The bioassay may be used alone as a screening tool in broad-scale sediment quality surveys, in combination with sediment chemistry and in-situ biological indices (the triad approach), and in laboratory experiments addressing a variety of sediment and water quality manipulations

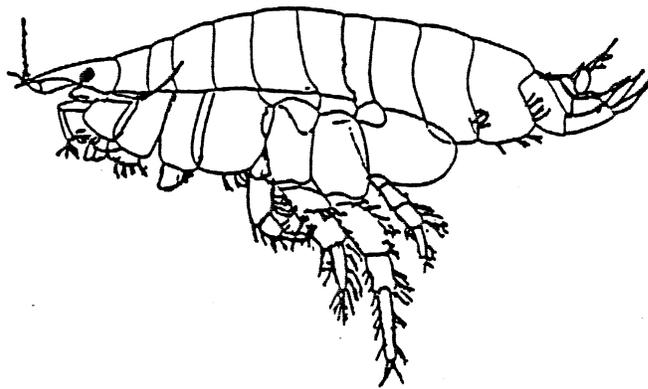


Figure 1. The Phoxocephalid amphipod,
Rhepoxinius abronius.

(Swartz et al. 1974, Swartz et al. 1979, Swartz et al. 1984a, Oakden et al. 1984a, Oakden et al. 1984b, Kemp et al. 1985, Ott 1986, Swartz et al. 1988). The amphipod sediment bioassay has been used to compare toxicity in contaminated sediments with uncontaminated sediments (Swartz et al. 1976, Swartz et al. 1989), and laboratory experiments using known concentrations of various contaminants within sediments (Oakden et al. 1984a, Oakden et al. 1984b, Swartz et al. 1988).

Chemical data were available for a portion of Padilla Bay (the site chosen for this study) (Milham 1986) and to complete a triad approach to assess for sediment contamination, I quantified epibenthic harpacticoid copepods (Figure 2) for the biological component. Quantification of a faunal component evaluates for acute effects from lethal concentrations of a contaminant and/or chronic and synergistic effects of sublethal concentrations from bioaccumulation.

Harpacticoid copepods are the most abundant epifauna of intertidal mud and sand beaches in the inland marine waters of Washington State. (Simenstad et al. 1980a, Sibert 1981, Cordell 1986, Cordell 1987, Simenstad 1987, WA. State Dept. of Fish. 1988). They occur from high inter-tidal to subtidal elevations and are an important prey species to out-migrating juvenile salmonids (Mason 1974, Feller and Kaczynski 1975, Feller 1977, Simenstad and Kinney 1978).

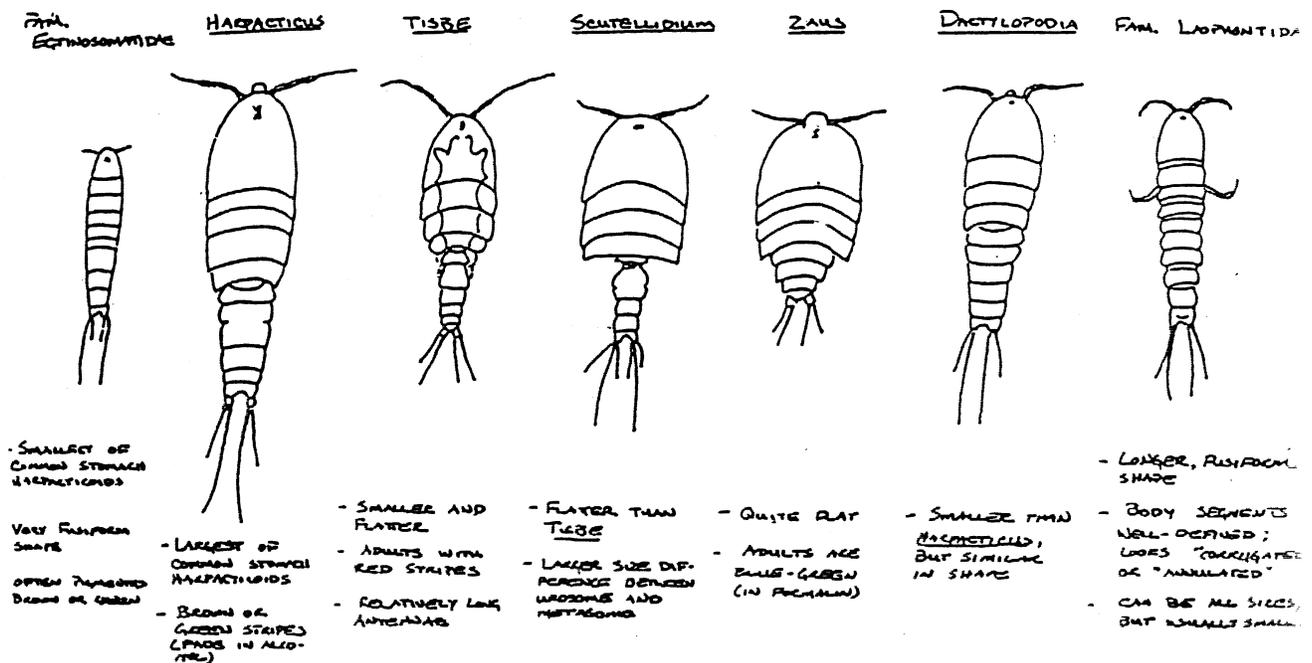


Figure 2. Predominant epibenthic harpacticoid copepods within Padilla Bay, Washington. (From J. Cordell 1990).

Merrell and Koski (1978), Simenstad et al. (1980b), Sibert (1981), and Cordell (1986) have stated that the most abundant prey species to pink and chum salmonids occurring in the waters from Washington to Alaska are harpacticoid copepods, particularly Harpacticus sp., Tisbe sp., and Zaus sp.. Harpacticoid copepods are abundant (approximately 6,000 to 14,000/m²), but are spatially patchy in distribution (Simenstad 1987), and locally temporally restricted to a spring bloom (Jewett & Feder 1977, Cordell 1990). Their position in the trophic level as detrital feeders (Jewett & Feder 1977), and epibenthic nature make them a good potential source as an indicator of surface sediment contamination and as an indicator of wetland fitness (Simenstad 1987, Cordell & Simenstad 1988). Although harpacticoid copepods do not bury in the sediments (Cordell 1990), they are epibenthic and come in contact with surficial sediments. This exposes them to water borne contaminants and contaminated particulates that have settled out of the water column onto the sediment surface.

STUDY SITE:

Padilla Bay (Figure 3) is an estuary located within the inland waters of Washington State. It has the status of being a National Estuarine Research Reserve. It is comprised of about 4,000 hectares of intertidal and subtidal sand and mud flats, and seagrass meadows (Webber 1986). On the western shore lies March Point, site of two petroleum

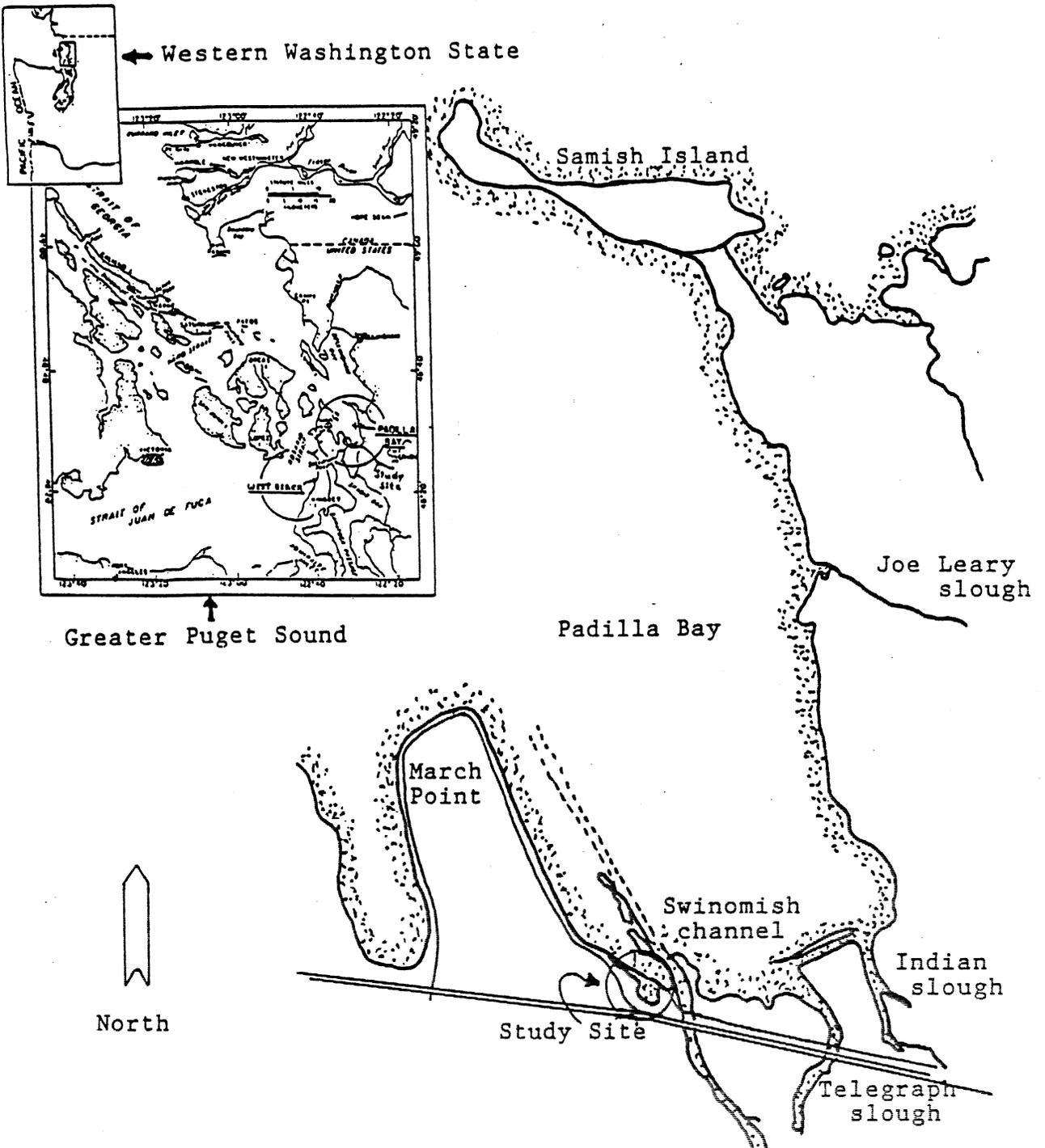


Figure 3. Study site location in the southwest corner of Padilla Bay.

refineries (Texaco and Shell Oil), the Northwest Petrochemical Corporation, and the Allied Chemical Company (EPA 1988b). To the east and south are extensive agricultural lands in the Skagit Valley. Fresh water influences come out of the north from the Fraser River and out of the south from the Skagit river (Webber 1986). There are also three local minor sources of fresh water; Joe Leary, Indian, and Telegraph sloughs.

This estuary supports migrant populations of waterfowl including 26 species of ducks such as Barrows golden eye (Bucephala islandica), bufflehead (Bucephala albeola) and old squaw (Clangula hymelis). There is also a large abundance of black brant (Branta bernicla) and dunlin (Calidris alpina) waterfowl. Mammals such as harbor seals (Phoca vitulina), and fish species such as herring (Clupea harengus), smelt (Hypomesus pretiosus), Chinook salmon (Oncorhynchus tshawytscha), Coho (O. kisutch), Pink (O. gorbuscha) and Chum (O. keta) also migrate through Padilla Bay (PBNERR 1984).

The bay supports resident populations of Great Blue herons (Ardea herodias), shiner perch (Cymatogaster sp.), Dungeness crab (Cancer magister) and many sand and mud marine organisms including harpacticoid copepods.

The salinity is around 27 ‰, although it varies to lower levels around freshwater seeps and higher levels during hot weather in shallow pools (Cassidy and Mckeen

1986). Cassidy and Mckeen (1986) also indicate Padilla Bay has a water temperature that ranges from 4.5 to 18.5° C and has an average depth of 2 meters. Sixty five percent of the bay is exposed at low tide.

This shallow habitat acts as a refugia from predation for salmonids and crabs (Simenstad 1987). Padilla Bay is also supplied with high quantities of detrital material from its extensive sea grass beds (Zostera marina, Z. japonica). This serves as a food source for mieofauna, such as harpacticoid copepods that are prey species for higher trophic organisms (Simenstad 1987).

The sampling area for this study was established adjacent to the now abandoned Skagit County March Point landfill (Figure 4). This landfill was opened in the mid 1950's, operating as a municipal and mixed industrial dump and closed and capped in 1974 (EPA 1988b). There were a variety of metallic wastes, inorganic and organic compounds, and household and construction wastes disposed at the site (EPA 1988b):

The area examined in this study (Figure 4) is located in the southwest corner of Padilla Bay. Landfill leachate is present at two sites, sites A (Figure 5) & B (Figure 6). The third site C (Figure 7), is the control and assumed "clean" site.

Site A has a mud substrate with a 0.5 meter deep channel running parallel to the beach with salt marsh

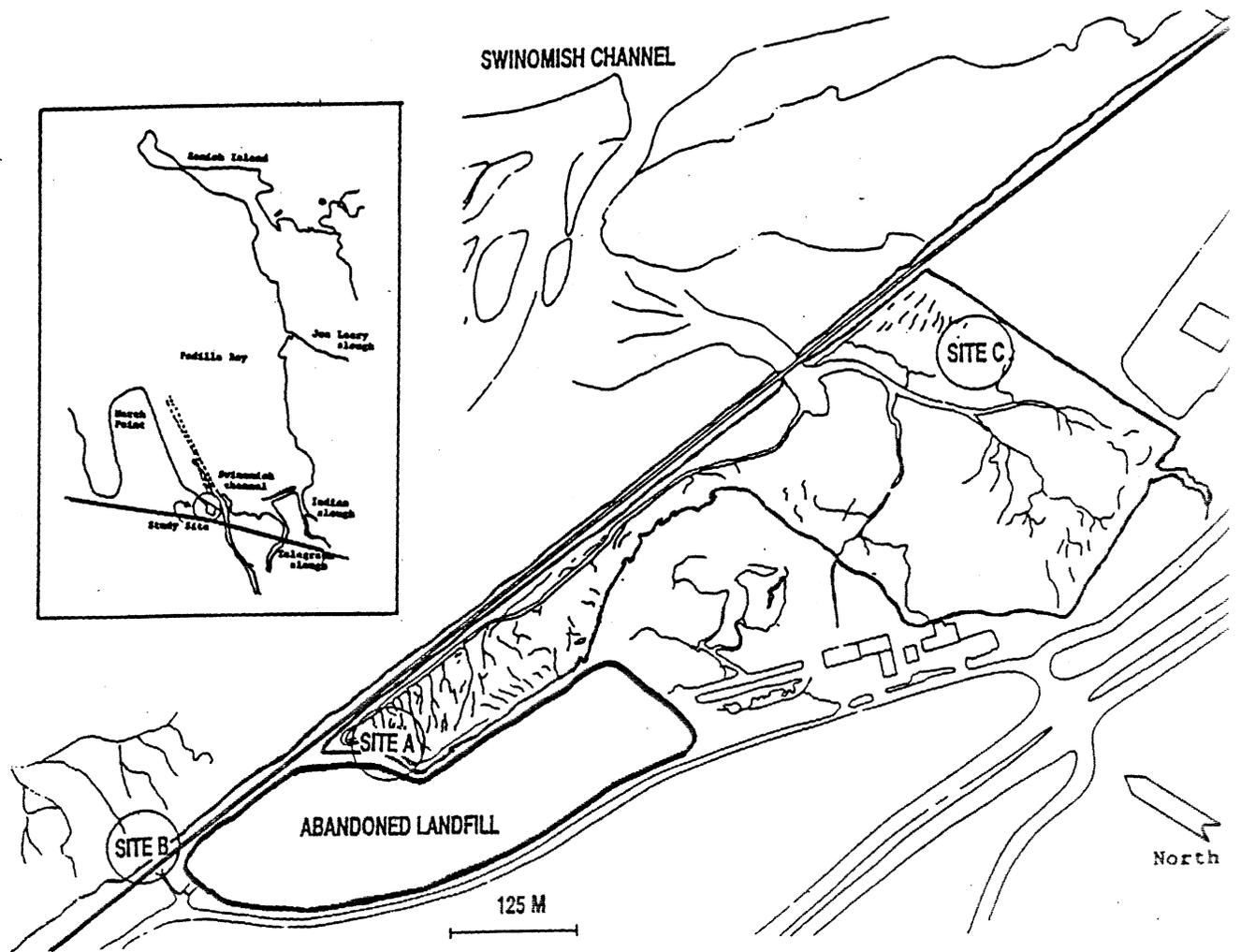


Figure 4. Study sites A, B, C, in the southwest corner of Padilla Bay.

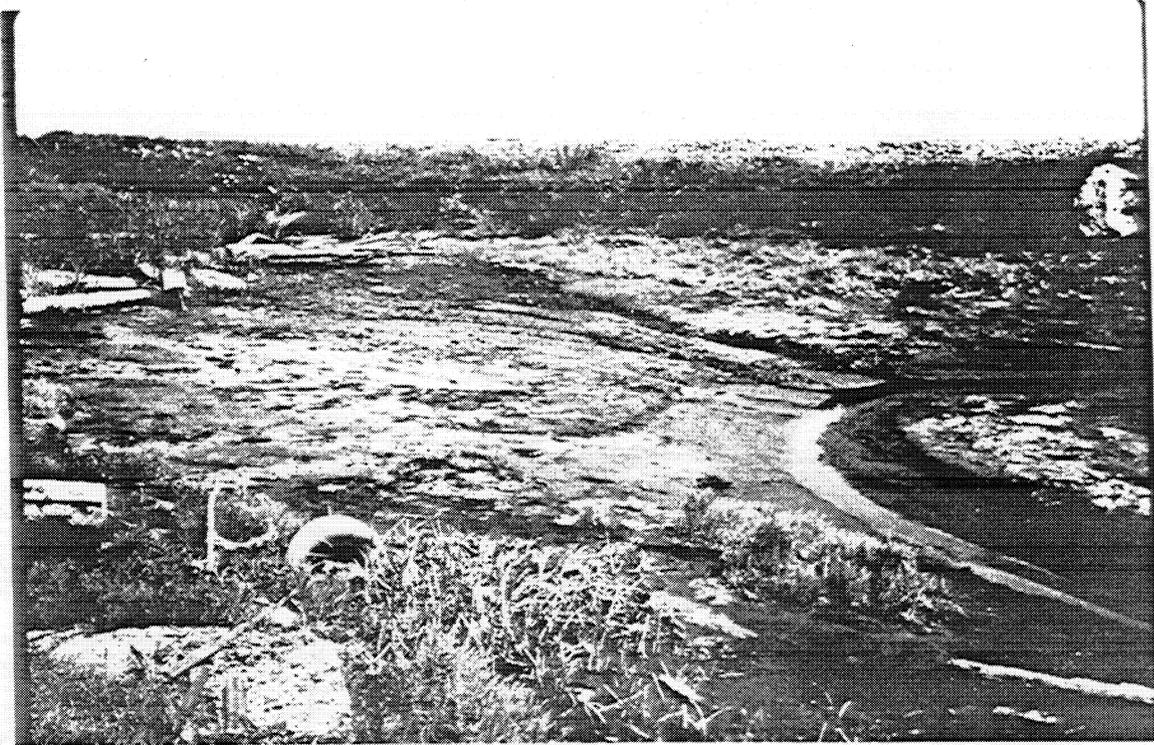


Figure 5. Study site A, southwest corner of Padilla Bay.



Figure 6. Study site B, southwest corner of Padilla Bay.

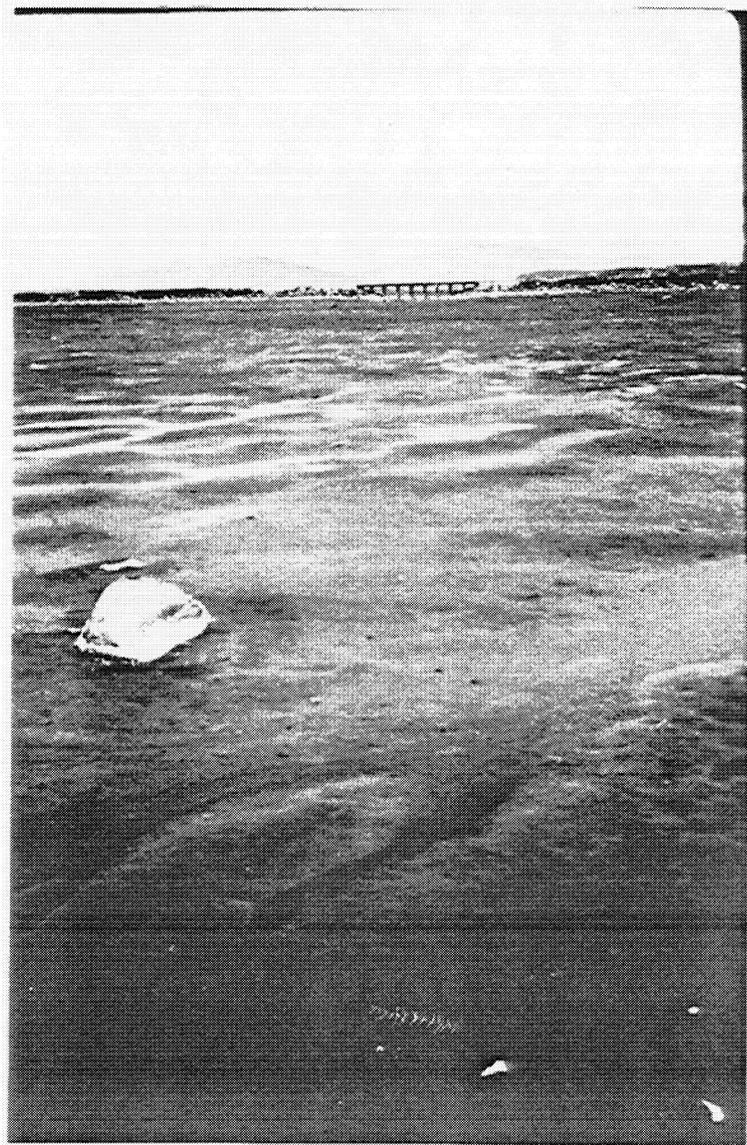


Figure 7. Study site C, southwest corner of Padilla Bay.

vegetation (Salicornia sp.) on the edges. An orange precipitate laden leachate is seeping at various locations from the landward side into the bay coating most vegetation and debris. A slight odor of petroleum also pervades. There is a 2 meter tall pile of rip/rap stacked at the upper tide line with logs and litter scattered about. The landward side is where the old landfill site is. The landfill has been covered with dirt and is now the site of an operating log mill.

Site B has a sand/gravel upper tidal substrate with mud at lower elevations. Some debris (old tire, car door, etc.) is scattered around on the beach and a very strong petroleum odor exudes from the upper portion of the beach. Visual signs of oil contamination are also present at this site. There is no observable flow of leachate here but an oily product emanates from within the rocks at the upper tide line. Site B is also lined with rip/rap at the upper tide line and has a railroad track running adjacent to it. The abandoned landfill is inland from the railroad tracks.

Sample site C is characterized as a clean site due to its distance from sites A & B and its use in previous epibenthic harpacticoid copepod abundance studies (Cordell 1986). This site has a mud substrate with grasses and forbes above the high tide line. There is a 2 meter embankment of fill material up gradient and a few meters

beyond the waters edge with a paved parking area farther inland.

OBJECTIVE:

In 1986 the Washington State Department of Ecology (DOE) under the Superfund Multi-site Cooperative Agreement Preliminary Assessment/Site Inspection (PA/SI) Program analyzed four water samples and two sediment samples in and around sites A and C (Milham 1986), but did not include site B. Their conclusion was, based on chemical analysis, that no significant concentrations of EPA priority pollutants occurred at the site (Milham 1986).

My objective was to determine if the sediments associated with the landfill leachate had toxic effects on biota, i.e. was the abundance of epibenthic harpacticoids significantly different between experimental sites and did sediments from these sites increase mortality on amphipods during a bioassay.

I did this by applying a sediment quality triad. My procedure was to sample for abundance of epibenthic harpacticoid copepods in the areas where landfill leachate is present (sites A & B, Figure 4) and compare the abundance results with an area where leachate is assumed to not be present, (site C). I also performed a sediment bioassay for the three sites. Samples from the top 2 cm of the sediment column of all three sites plus the top 2 mm surface layer of

site B were used. Incorporating the existing chemical data I then completed the triad.

MATERIALS AND METHODS

AMPHIPOD BIOASSAY:

Procedures for the amphipod bioassay followed Swartz et al. (1984b), with a few variations as follows. The amphipods (Rhepoxinius abronius) were obtained off Whidbey Island at West Beach (Figure 3), on 10 May 1990 using the Shannon Point Marine Lab vessel, RV ANOVA. Samples were taken with a modified 0.25 m² dredge taking tows in 5 to 10 meters of water with a three minute haul duration. Some sediments were examined in the field using a 2 mm sieve to aid in determination of the total count of amphipods. Most large organisms and macrophytes were removed from the sediments in the field. The amphipods were kept at 15°C, in well oxygenated seawater within an environmental chamber for 36 hours. After 36 hours the bioassay was started.

Four experimental sites and one control for the sediments were used. Sediments suspected of being contaminated were taken from areas A and B (Figure 4). Sample A "leachate" came from site A within the leachate affected area in the upper 2 cm of the sediment column. Samples B1 "oily" and B2 "oily surface" came from site B. Sample B1 from the upper 2 cm and sample B2 from the upper 2 mm of the sediment column were used. The fourth sample was taken from an environmentally similar area located across the embayment with no visual leachate effect, site C. Sediments from site C were taken from the upper 2 cm of the

sediment column. The fifth sample "Whidbey control" was from the area where the amphipods reside to act as a method control. The study site sediment samples were taken at the 1.0 to 1.5 m tidal height from 5 random locations at each site within the top 2 cm of the sediment column. Approximately 2 liters of sediment were collected at each site, pooled and then subsampled into 10 containers for each bioassay. Sediments were collected two days before the start of the bioassay when the beach was exposed (Figure 8). The sediment samples were collected using a stainless steel spoon, transported in polyethylene bags and kept at 4°C.

Twenty four hours before the experiment began, fifty, 750 ml jars were loaded with 100 ml of the respective sediments, approximately 3 cm deep, and then filled with filtered seawater. The salinity of the interstitial water from each pooled sample varied with some falling below 15‰. Sediments in all jars were therefore mixed with 30‰ seawater to ensure consistent salinity. Each jar had an air source attached to it to ensure continual oxygen saturation (Figure 9). The air supply did not disturb the sediments. The air supply first went through a glass fiber packed, side arm flask to filter moisture and particulates. The air supply then went into a 5.1 cm header pipe that has 60, 0.32 cm rubber tubing lines attached, leading to each jar. Glass pipets were then attached to the ends of the tubing and inserted into each jar. All glassware and

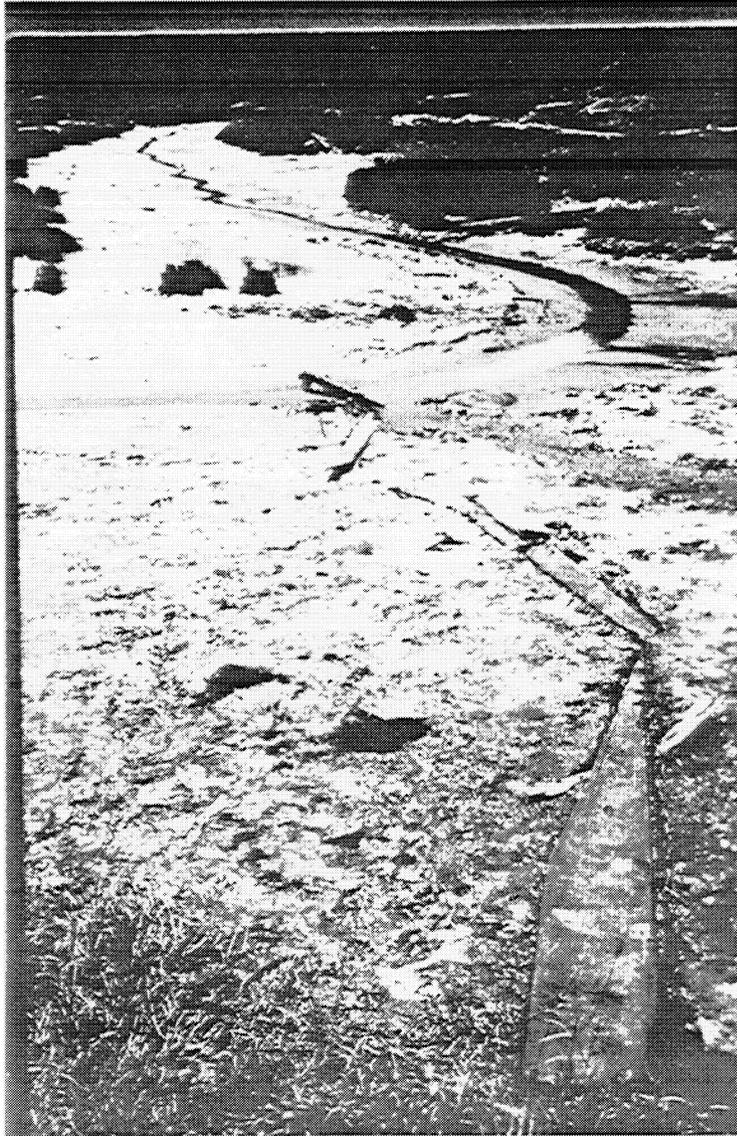


Figure 8. Sediment sampling technique to reach site A.

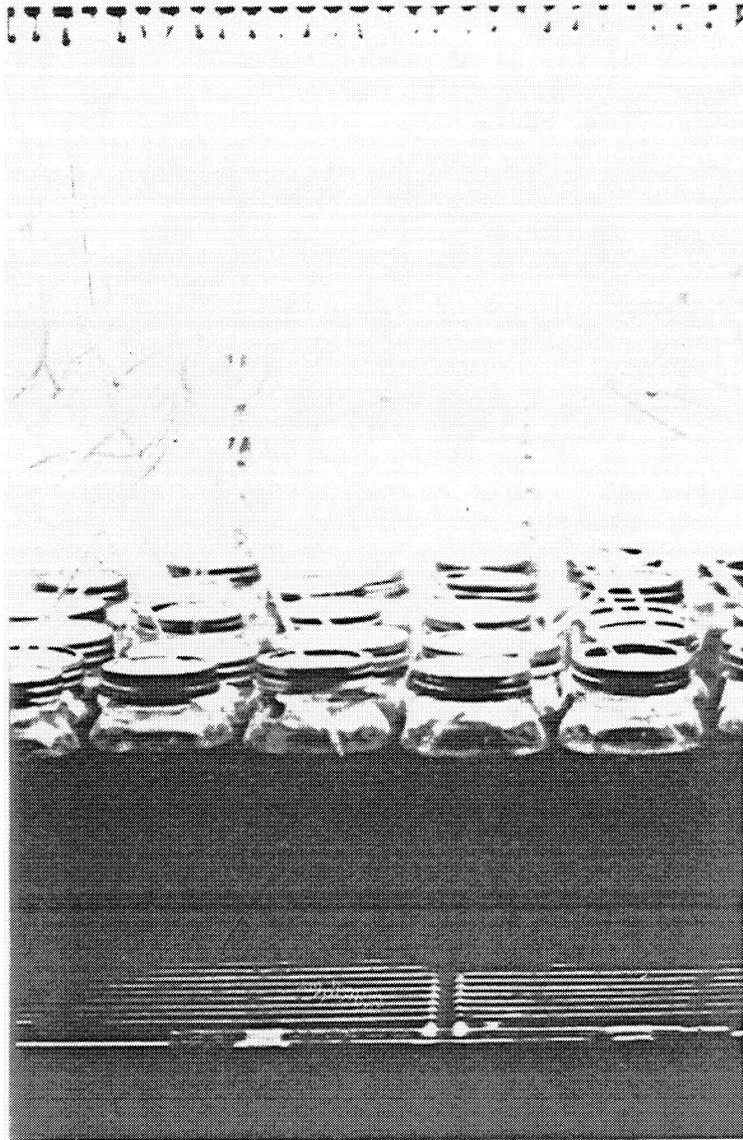


Figure 9. Sediment toxicity amphipod bioassay showing jars, air attachments, and header, all within an environmental chamber.

associated equipment were prewashed with soap and hot water, rinsed with 2 N HCl and then rinsed three times with distilled water.

On day zero of the bioassay, 13 May 1990, the amphipods were resieved using a 1 mm sieve, with 20 amphipods placed into each jar. Amphipods that were 1 mm to 3 mm in length were used. Random numbers were generated for each jar for position in the environmental chamber. The jars were covered with Parafilm to reduce evaporation and an aerator reattached. Since the amphipods are nocturnal (Swartz et al. 1984a), the lights were kept on constantly to ensure they remain buried. This allowed for full sediment exposure.

Bioassay observations were made twice a day to ensure the equipment was functioning properly. After 10 days of exposure the amphipods were resieved and counted. Only the live amphipods were tabulated. Life was determined by any small movement resulting from gently probing. Salinity was also determined for each jar using an optical refractometer.

HARPACTICOID COPEPODS:

Thirty one replicates were taken from each sample site, sites (A, B, and C) using a 182 cm² epibenthic pump (Figure 10, Cordell & Simenstad 1988). The total area sampled for each site was 0.5642 m². Samples were collected during the flood tide at site A on 31 March 1990, site B on 28 & 29 March 1990, and site C on 30 March 1990. Sites were

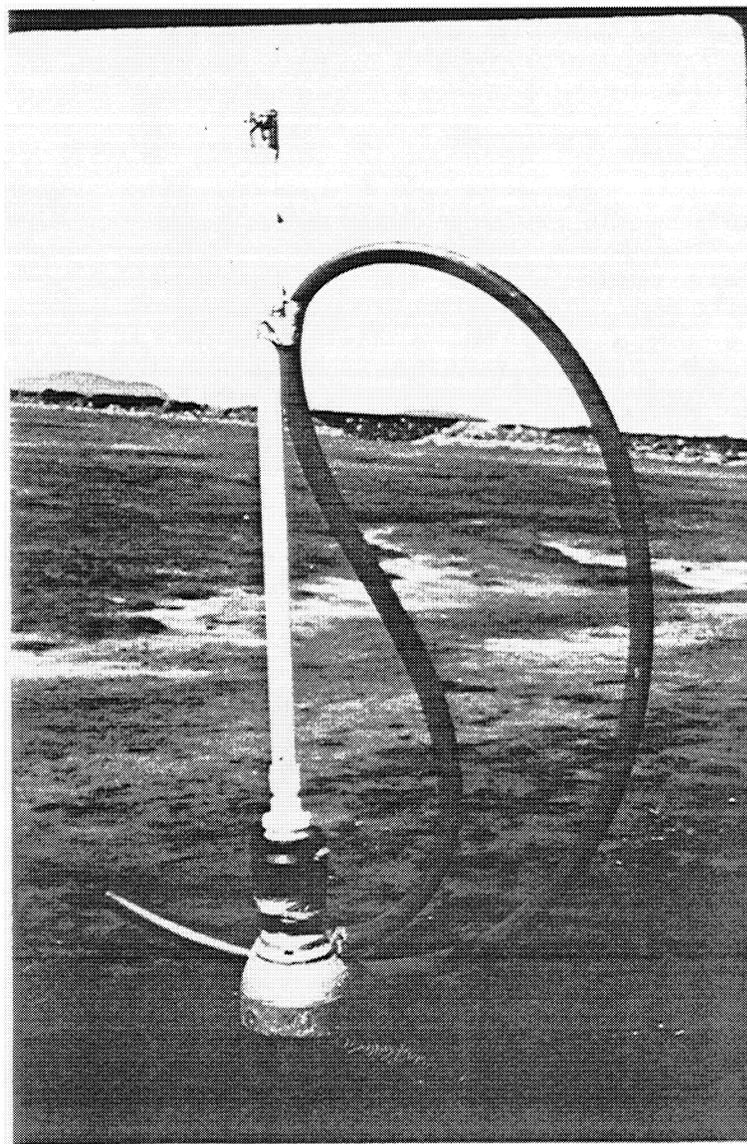


Figure 10. Epibenthic pump (182 cm²), for sampling epibenthic harpacticoid copepods.

sectioned into 100, 4 m² grids, numbered, then randomly selected for in-field sampling. Actual field sampling was haphazard due to wind and difficulty locating predetermined locations. Samples were sieved in the field through a 0.125 mm screen and stored in a 5% buffered formalin solution. Within 48 hours samples were resieved and placed into methanol stained with 1.0 g/L Rose Bengal. All samples were then rinsed, species identified when possible and enumerated using a Wild dissecting scope.

STATISTICAL ANALYSES:

All data were tabulated and statistics were performed using SPSSX to review possible differences between the samples for toxicity in the bioassay and abundance for the epibenthic harpacticoid copepod. Analysis of Variance (ANOVA) and Student-Newman-Keuls (SNK) (multiple comparison test to examine between pairs of means) procedures with a 5% significance level of confidence was used. Homogeneity of variance was checked using Bartlett's procedure. If sample data were not homogeneous the data were log transformed and the Bartlett's procedure run again. NOTE: [The Bartlett's test is affected by non-normality, "...the analysis of variance is robust, operating well even with considerable heterogeneity of variances, as long as all N_i are equal..." (Zar, 1984)]. ANOVA was performed on both the epibenthic harpacticoid copepod abundance and the amphipod bioassay mortality results.

PHYSICAL CHARACTERISTICS:

Aliquots of the pooled sediment from each site were analyzed for total solids (TS), total volatile solids (TVS) and particle size (sand/silt/clay, S/S/C) using Puget Sound Estuary Protocols (1986 a,b).

Total Solids is the dry weight of the sediments and includes both inorganic matter (sand, silt, and clays) and organic matter (e.g. detritus, humic substances).

Total Volatile Solids estimates the organic fraction lost during ignition. TVS gives you a crude estimate of both the organic and inorganic fractions.

Sediment size was determined for the sample sites. Particle size gives the sand, silt and clay fraction which is important in determining the viability of R. abronius during the bioassay. Various percentages of organic matter and silt/clay affect the survival rates of R. abronius in the bioassay (Dewitt et al. 1988, Swartz et al. 1984a). The various physical characteristics tested for, such as organic matter (humic substances) and clay content also affect the adsorption and absorption capabilities of particles for a variety of pollutants.

The following is a description of lab procedures for
TS, TVS, and particle size pipet analysis (PSEP 1986a):

TOTAL SOLIDS (TS) = $(A-B)(100)/C-B$

A = wt of dish + dry (95 C) sample residue.
B = wt of dish.
C = wt of dish and wet sample.

TOTAL VOLATILE SOLIDS = $(A-C)(100)/A-B$

A = wt of dish and dry (95 C) sample residue.
B = wt of evaporation dish.
C = wt of dish and ignition (550 ° C) residue.

PARTICLE SIZE, PIPET ANALYSES = $50[(A-C)-(B-C)]$
(aliquot and dish dried at 95 ° C)
(phi size used, <4.0 sand, 4.0 silt, 8.0 clay)

A = wt of residue in a 20 ml aliquot for given phi
boundary.
B = wt residue in a 20 ml aliquot for next larger
phi size.
C = mean wt of dispersant (if used).

size fractions, sand = > 63 um
silt = 63 um to .039 um
clay = < 3.9 um

(all sand fractions were dry sieved and were less than 1 mm)

RESULTS

SEDIMENT BIOASSAY:

The bioassay results showed all experimental sites were significantly different from the Whidbey control site (Table 1, Figure 11), i.e. the experimental sites had an elevated mortality over the control. Site B1 was also significantly different from all other sites.

The top 2 cm of surficial sediment from site B, sample (B1) caused the highest mortality of all sites. These sediments killed all the amphipods in all jars, i.e. 100% mortality (Figure 11). Site A, the area with numerous surficial flowing streams of leachate, also had a significantly higher amphipod mortality than all other sites. Site A had a 63% mean mortality (mean mortality per 10 jars), a minimum mortality (least dead per jar) of 20%, a maximum mortality (most dead per jar) of 90%, and a standard deviation (SD) of 19.5. Tables 1 and 2 describe the results of the bioassay.

The ANOVA results of the bioassay showed the other two sites, site C the control, and site B(2), the surficial 2 mm of area B, had the lowest mortality of the experimental sites (Figure 11, Table 2). Sediment sample B2, indicated the top 2 mm of the sediment column of the oily site B, had a 31% mean mortality per 10 jars, a 10% minimum mortality per jar, a 75% maximum mortality jar, and a SD of 22.6. The clean site, C, had a 43% mean mortality per 20 jars, a

Table 1. SNK multiple comparison test for the amphipod sediment bioassay, pooled sediment samples were used for each site. Ten jars with 20 amphipods per jar were used. Listed below are: (1) site, A, B1, B2, C, & Whidbey Control W.C., (2) percent mean mortality of the 20 amphipods per jar, (3) standard deviation (SD), (4) percent minimum mortality of the 20 amphipods per jar, (5) percent maximum mortality of the 20 amphipods per jar, and (6) (*) denotes pairs of groups significantly different at the 0.05 level.

(1) Site	(2) Mean Mort.	(3) S D	(4) Min. Mort.	(5) Max. Mort.
A	63.0	19.5	20	90
B1	100.0	0.0	100	100
B2	31.5	22.6	10	75
C	43.0	11.8	25	65
W.C.	5.5	6.0	0	15

(6)	grp WC	grp C	grp B2	grp A	grp B1
grp WC					
grp C	*				
grp B2	*				
grp A	*	*	*		
grp B1	*	*	*	*	

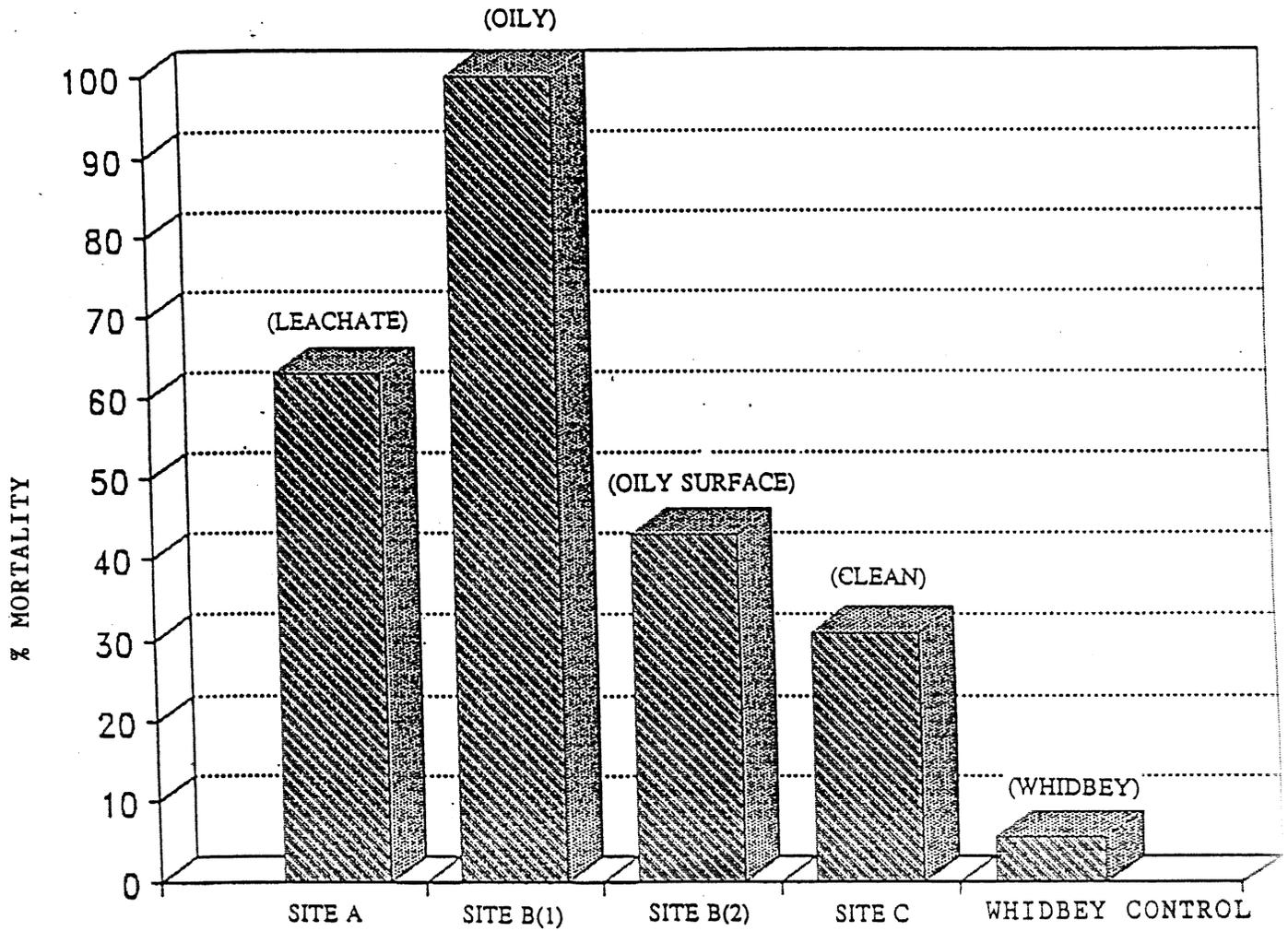


Figure 11. Sediment toxicity bioassay results, percent mean mortality by sediment site using pooled sediment samples from each site.

Table 2. Amphipod sediment bioassay, percent mortality per site (A, B1, B2, C, & Whidbey Control W.C.), per jar (1...10), using 20 amphipods per jar. Mean (X) and standard deviation (SD) summarize data.

Site/jar	% mort.	Site/jar	% mort.
A 10	70	B1 20	100
A 11	75	B1 21	100
A 12	65	B1 22	100
A 13	60	B1 23	100
A 14	45	B1 24	100
A 15	20	B1 25	100
A 16	90	B1 26	100
A 17	65	B1 27	100
A 18	80	B1 28	100
A 19	60	B1 29	100
X=	63	X=	100
SD=	19.5	SD=	0.0

Site/jar	% mort.	Site/jar	% mort.
B2 30	20	C 40	40
B2 31	35	C 41	25
B2 32	20	C 42	40
B2 33	25	C 43	65
B2 34	10	C 44	50
B2 35	20	C 45	55
B2 36	75	C 46	45
B2 37	15	C 47	35
B2 38	70	C 48	30
B2 39	25	C 49	45
X=	31.5	X=	43
SD=	22.6	SD=	11.8

Site/jar	% mort.
W.C. 50	15
W.C. 51	0
W.C. 52	0
W.C. 53	15
W.C. 54	5
W.C. 55	0
W.C. 56	0
W.C. 57	10
W.C. 58	5
W.C. 59	5
X=	5.5
SD=	6.0

minimum mortality of 25% per jar, a maximum mortality of 65% per jar, and a SD of 11.8. These two sites, (B2 and C) are the only two sites not significantly different from each other (Table 1).

The Whidbey site had the lowest mortality. This site had a mean mortality of 5.5% per 20 jars, a minimum mortality of 0.0% per jar, a maximum mortality of 15% per jar, and a SD of 6.0.

The salinity for each jar at the completion of the bioassay went from a low of 30_{0/00} to a high of 35_{0/00} with a mean for each set of jars at approximately 33_{0/00}.

(Table 3). Salinity variation due to evaporation during the bioassay is assumed not to be a factor affecting mortality (Swartz et al. 1984a).

HARPACTICOID COPEPODS:

The epibenthic harpacticoid copepods that were identified at all 3 sites within the study area are listed and illustrated in Figure 2. These are the predominant epibenthic harpacticoid copepods in Padilla Bay (Simenstad et al. 1988, Cordell personal communication (1990)) and the only ones identified at the study site. The number for areas A (leachate affected area) and C (clean site) were not significantly different. Of the 3 sites, area B (the oily area) had a significantly greater number than areas A and C (Tables 4 and 5, Figure 12).

Table 3. Bioassay salinity (‰) per treatment (pre & post)

Leachate (A)	Oily (B1)	Oily surface (B2)	Clean (C)	Whidbey Control
jar-pre-post	jar-pre-post	jar-pre-post	jar-pre-post	jar-pre-post
10..30..32	20..30..33	30..30..34	40..30..34	50..30..35
11..30..32	21..30..33	31..30..33	41..30..33	51..30..32
12..30..35	22..30..33	32..30..34	42..30..33	52..30..32
13..30..33	23..30..33	33..30..32	43..30..33	53..30..32
14..30..32	24..30..33	34..30..33	44..30..33	54..30..32
15..30..36	25..30..33	35..30..33	45..30..35	55..30..32
16..30..32	26..30..33	36..30..33	46..30..35	56..30..35
17..30..35	27..30..33	37..30..33	47..30..34	57..30..34
18..30..35	28..30..32	38..30..35	48..30..32	58..30..30
19..30..33	29..30..35	39..30..35	49..30..32	59..30..32
X 33.5	X 33.1	X 33.5	X 33.4	X 32.6
SD 1.6	SD 0.7	SD 1.0	SD 1.1	SD 1.6

Table 4. Total abundance (31 samples/site) sampled for epibenthic harpacticoid copepod density (0.5642 m²) as taxa (genus and family) abundance and percentage.

taxa	SITE					
	(A)		(B)		(C)	
	abundance	%	abundance	%	abundance	%
<u>genus</u>						
<u>Harpacticus</u>	2438	34.1	5043	36.0	725	9.5
<u>Tisbe</u>	4382	61.3	8331	59.5	6770	88.9
<u>Dactylopodia</u>	205	2.8	406	2.9	29	0.04
<u>Zaus</u>	35	0.5	104	0.7	95	1.2
<u>Scutellidium</u>	13	0.2	14	0.1	2	0.02
<u>family</u>						
Ectinosomatidae	80	1.1	55	0.4	0	0.0
Laophontidae	1	0.01	46	0.3	4	0.05
Total	7154		13,999		7625	

Table 5. Abundance of epibenthic harpacticoid copepods per sample for each site. Taxa are enumerated in Table 4. Each sample size is 182 cm², total area 0.5642 m².

SAMPLE	SITE A	SITE B	SITE C
1	201	314	184
2	113	228	175
3	74	159	452
4	187	185	245
5	66	336	463
6	36	455	124
7	20	122	26
8	45	252	116
9	145	93	227
10	65	232	183
11	314	178	91
12	90	863	273
13	117	2031	123
14	116	1752	108
15	318	387	143
16	236	1652	167
17	130	917	124
18	226	420	203
19	666	286	300
20	52	177	163
21	258	472	260
22	35	464	435
23	398	152	123
24	354	91	258
25	93	65	240
26	47	130	187
27	572	263	933
28	68	435	531
29	200	222	531
30	1669	505	327
31	102	287	236

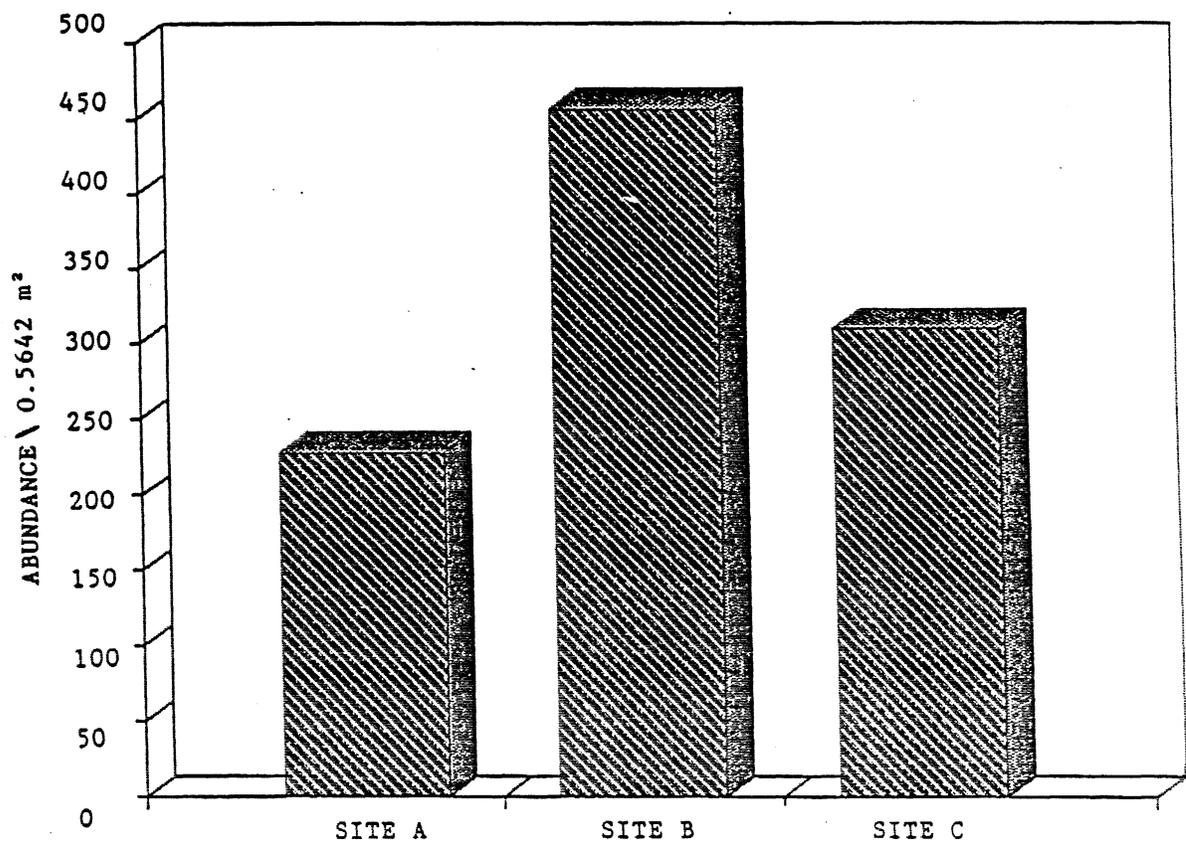


Figure 12. Mean abundance of epibenthic harpacticoid copepods per site. Total area = 0.5642 m²/site. Density of site B is significantly different from sites A and C. Sites A and C are not significantly different.

The total abundance and percentage for each taxa, sample, and location is listed in Tables 4 and 5. Table 6 lists the results of the ANOVA for the total number of epibenthic harpacticoid copepods per epibenthic sample for each site.

Sites A and C are nearly equal in epibenthic harpacticoid copepod abundance. Site A had a total abundance of 7154 epibenthic harpacticoid copepods per 0.5642 m², a mean sample abundance of 228 per 182 cm², a minimum sample abundance of 20 per 182 cm², a maximum sample abundance of 1669 per 182 cm², and a SD of 310. Site C had a total abundance of 7625 epibenthic harpacticoid copepods per 0.5642m², a mean sample abundance of 243 per 182 cm², a minimum sample abundance of 26 per 182 cm², a maximum sample abundance of 933 per 182 cm², and a SD of 175.

Site B had the greatest abundance of epibenthic harpacticoid copepods nearly doubling sites A and C. Total abundance was 13,999 epibenthic harpacticoid copepods per 0.5642 m². Site B had a mean sample abundance of 456 per 182 cm², a minimum sample abundance of 65 per 182 cm², a maximum sample abundance of 2031 per 182 cm², and a SD of 495.

SEDIMENTS:

One composite sediment sample from each sampling site (samples A, B1, B2, C, and Whidbey) was used for the physical characterization of sediments [total solids (TS),

Table 6. SNK multiple comparison test for the number of epibenthic harpacticoid copepods, each site (A, B & C) = 0.5642 m².

Listed below are:

- (1) site,
- (2) total abundance of 31 samples,
- (3) mean abundance per sample,
- (4) standard deviation, SD,
- (5) minimum count per 182 cm² sample,
- (6) maximum count per 182 cm² sample, and
- (7) (*) denotes pairs of groups significantly different at the 0.050 level.

(1) site	(2) total	(3) mean	(4) SD	(5) min.	(6) max.
A	7,154	228	310	20	1669
B	13,999	456	495	65	2031
C	7,625	243	175	26	933

(7)	grp A	grp B	grp C
grp A			
grp B	*		
grp C		*	

total volatile solids (TVS) and particle size as sand/silt/clay (S/S/C), Table 7]. Statistical analyses were not applied to the sediment characteristics because all subsamples were pooled leaving one sample per site.

Site A is on the prevailing windward end of a long fetch on the small embayment illustrated in Figure 4. Site A had a TVS of 12.1 and was composed primarily of silt, 92% (S/S/C = 3/92/5).

Sediment sample B2, taken from the upper 2 mm of the sediment column of site B was composed primarily of silt S/S/C = 13/78/9. It had a TVS of 5.8%. Sediment sample B1, the upper 2 cm of the sediment column of site B was composed primarily of sand S/S/C = 70/26/4 and had a lower TVS of 3.2%. This indicates the surficial 2 mm of the sediment column are siltier than the underlying sediments.

The sediment from area C, 92% silt/clay (S/C), S/S/C = 8/86/6, with the highest TVS of 18.9%, is on the leeward side of the embayment and had no visible collection of detritus.

The Whidbey Control sediment is from West Beach on Whidbey Island. The Whidbey control sediment was sieved through a 2 mm screen to separate large macrophytes and fauna. This sample had a high sand content at S/S/C = 99.8/0.2/0, with a TVS of 0.025 (an unknown amount of silt was lost during the collection process using the dredge).

Table 7. Physical characteristics of composite sediment sample for each site: Total solids (TS), total volatile solids (TVS) and sand/silt/clay (SSC) as a percentage.

	Leachate (A)	Oily (B1)	Oily surface (B2)	Clean (C)	Whidbey control
TS	27.9	67.8	40.2	40.3	73.0
TVS	12.1	3.2	5.8	18.9	0.03
SSC	2.5/92/4.5	70/26/4	13.3/78/8.4	8/86/6	99.9/.1/0

DISCUSSION

BIOASSAY:

Rhepoxinius abronius resides primarily in sediments of clean sandy beaches in the shallow subtidal zone, at an abundance of 2,000 to 4,000 individuals per m² (Swartz et al. 1984a, Oakden 1984). Swartz et al. (1984a) has collected specimens from well sorted, fine sand in Yaquina Bay, Oregon (sand/silt/clay, S/S/C = 97/1/2), to more silty sediments as in Commencement Bay, Washington, with a S/S/C of 23/68/9 (Swartz et al. 1982). These data indicate R. abronius is naturally tolerant to a broad range of sediment sizes. However, as indicated below in bioassay tests, R. abronius mortality increases with decreasing particle size that shows a predictable relationship.

In laboratory experiments, R. abronius had a survival of >90% within sediment having a silt/clay content at >90% (Swartz et al. 1984b). Swartz et al. (1984b) showed a survival rate of 90% in a sediment with a (S/S/C) content of 9.7/36.8/53.5 from Poverty Bay, Washington. Dewitt et al. (1988) found that "...using a static laboratory microcosm, the mean amphipod survival in fine uncontaminated field sediments (>80% S/C) can be 15% lower than survival in native sediment". The above data led DeWitt et al. (1988) to calculate a Lower 95% Prediction Limit (LPL) for R. abronius within sediments of increasingly greater percent fines (silt and clay). Figure 13 indicates predicted

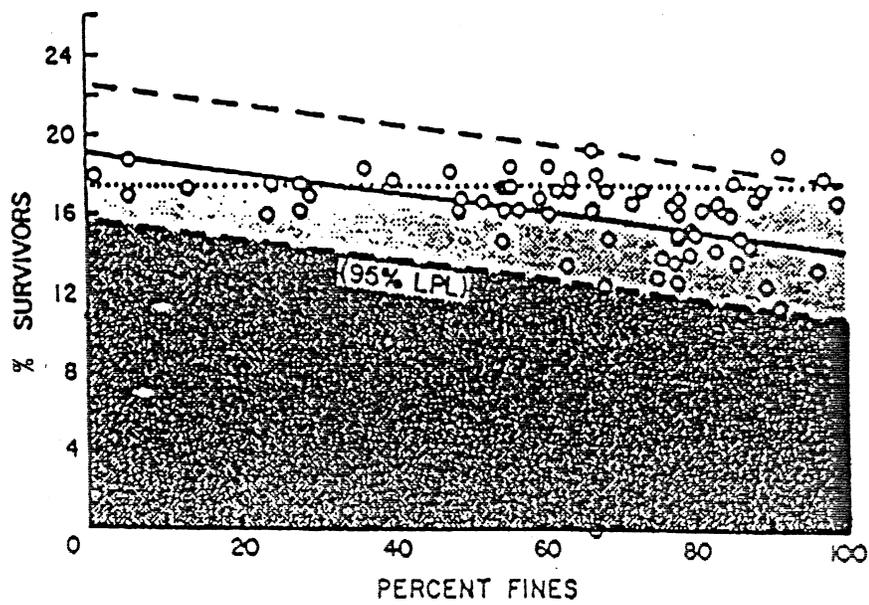


Figure 13. Amphipod sensitivity to natural sediments.
 (Taken from DeWitt et al. 1988, page 119).

survival of R. abronius in sediments with percent fines content ranging from 0 to 100%.

Swartz et al. (1984a) states R. abronius is also tolerant to high percentages of organic enrichment as measured by total volatile solids (TVS). Swartz (1984a) found R. abronius survival to stay within his calculated control range (<10% mean mortality) from sediments with a TVS up to 18.2%. Swartz et al. (1984a) also found R. abronius had a 30% mortality in a sediment sample with a TVS of 39.8%. The total volatile solids content for this study site should therefore not increase mortality (highest TVS was 18.9 at site C and had less than predicted mortality).

Swartz et al. (1985) and Ott (1986) obtained a 45% mortality when subjecting R. abronius to Kaolin clay (<5 micrometers) in a 10 day bioassay.

Mortality from particle size is predictable using Dewitt et al. (1988) (Figure 13). The expected mortality using Dewitt et al. (1988) and my results for each area are as follows:

Site	% silt/clay	actual % mort.	expect % mort.
A	96.5	63.0	45
B1	30.0	100.0	25
B2	86.0	31.5	40
C	92.0	43.0	45
W.C.	<1.0	5.5	20

This chart shows sediment samples A and B1 had higher than expected mortalities and are concluded to be contaminated.

Milham (1986) however did not find significant amounts of EPA priority pollutants at site A, therefore, the high mortality bioassay results were not anticipated. A probable answer for the high mortality bioassay results at site A may come from Swartz et al. (1988). He found typical mortalities in an amphipod bioassay when zinc, mercury, polychlorinated biphenyls and fluoranthene were present in very low concentrations. When combined with varying combinations, additive toxicological effects occurred. Swartz et al.'s (1988) conclusion of additive effects may be one reason for the increased mortality in the amphipod bioassay using sediment from site A (leachate). For site B, sediment sample B1 (oily 2 cm), I will assume the high concentration of hydrocarbons, (visual inspection) is the cause of the 100% mortality.

High numbers of epibenthic harpacticoid copepods were found at each site, but the top 2 cm of the sediment column at sites A and B had a higher than expected amphipod bioassay mortality. The oxidized sediment surface layer of my study site is less biologically toxic than the underlying benthic sediments at sites A and B. This is illustrated by the bioassay results and the abundance of epibenthic harpacticoid copepods.

HARPACTICOID COPEPODS:

All three sites in this study showed typical or greater abundance for epibenthic harpacticoid copepods when compared

to similar areas within Padilla Bay (Cordell, personal communication 1990, Cordell, 1986). The total abundance for sites A, and C are consistent with those found by Cordell (1986) for this area of Padilla Bay. Cordell found 7,000 to 13,600 epibenthic harpacticoid copepods per m^2 or 3949 to 7673 per $0.5642 m^2$ at locations near site C. Site B nearly doubles the abundance Cordell typically found at sites in Padilla Bay.

The greatest abundance of epibenthic harpacticoid copepods occurred at site B. However, site B also had the highest mortality, 100%, in the bioassay using the top 2 cm of the sediment column but had expected and typical mortalities using the top 2 mm of the sediment column.

Even though the abundance results were typical or higher than expected for Padilla Bay, variation between the sites and within each site occurred. Tables 4 and 5 list the variations between and within each site.

One reason for the variation in site abundance of epibenthic harpacticoid copepods is a natural heterogenous spatial distribution (Simenstad 1987), i.e. patchiness. A more site specific possibility is a source of food. Since detrital-feeders obtain some of their energy from associated microbiota (Levinton 1982), it may be possible that the leachate at site A directly and indirectly adds to the microflora and fauna as a food source of the epibenthos by supplying nutrients and bacteria. It therefore may add to

the food/energy content available to the epibenthic harpacticoid copepods, increasing their abundance.

Another reason for spatial variability between sites may be my sampling procedure. On the day site B was sampled, there was a strong wind out of the north which may have washed "some" epibenthic harpacticoid copepods closer to shore where sampling took place. Simenstad et al. (1988) found a higher abundance of epibenthic harpacticoid copepods on the leading edge of an inundating tide. This area, site B, also has the longest fetch of the three sites, potentially adding to the abundance of epibenthic harpacticoid copepods with the leading edge of the inundating tide. Sites A and C were sampled in calm weather while the water was turbid when sampling site B.

Hicks and Coull (1983) suggest that some harpacticoid copepod species may be more pollution tolerant than others. Marcotte (1974) showed Tisbe sp. to be the dominant species in an area effected by raw municipal sewage. Hoppenheit (1977) has also shown that Tisbe holothuriae may be tolerant to high concentrations of NO₂ and can respond positively in population density when exposed to high concentrations of cadmium. This indicates Tisbe spp. may be tolerant to pollutants present at site A. Tisbe sp. is the dominant harpacticoid copepod at all three sites, 61%, 59.5%, and 88.8% at sites A, B, and C respectively (Table 4).

The reason for the high abundance of epibenthic harpacticoid copepods in an area where benthic sediments are toxic as indicated by the bioassay results for sites A and B is uncertain. This study shows epibenthic harpacticoid copepods may occur in the thin surface/water interface that overlies a toxic deeper sediment. This study indicates there is a sediment cap in area B that acts as a barrier from the underlying contaminated sediments. Also site A has typical epibenthic harpacticoid numbers in an area that displays toxic effects as indicated the sediment bioassay.

The results of this study show that the deposition of uncontaminated sediments and/or natural processes of detoxification of pollutants by aerobic sediments, humic substances, and biodegradation (albeit assumed) forms a barrier of protection from potential toxic sediments lower in the sediment column at site B. I will assume, although no data were collected for the upper 2 mm of the sediment column at site A, that conditions similar to area B exist at site A.

To perform only a chemical analysis of an area (such as done by Milham 1986) may not give a true picture of pollutant effects and omits additive and/or synergistic effects. Therefore when examining a system for toxicity, enumeration of a portion of the biota and a bioassay are necessary to assess additive effects and the possible separation of toxic sediments within the sediment column.

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