



*Padilla Bay*

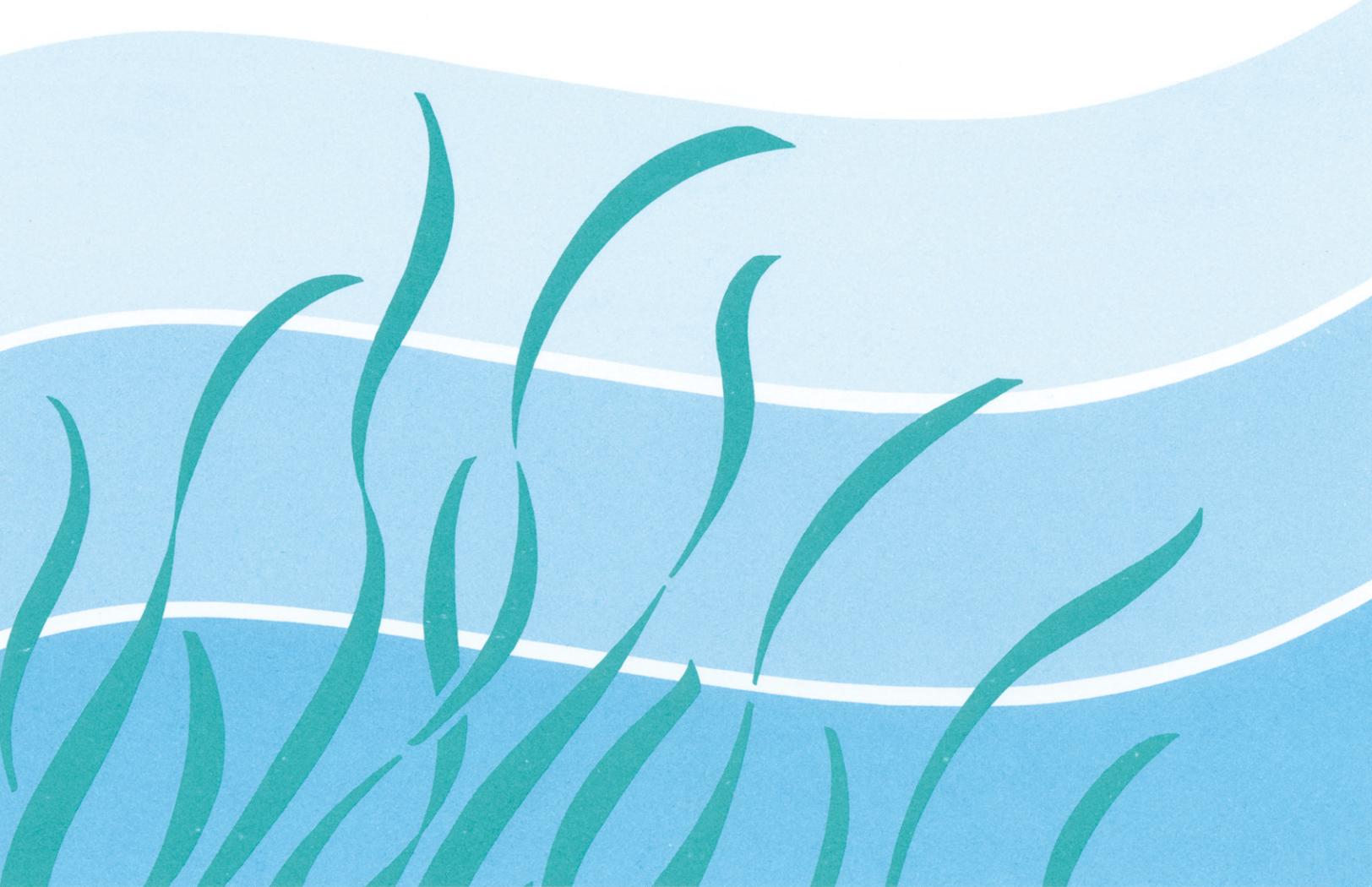
National Estuarine Research Reserve

Reprint Series No. 16  
Reprinted November 1992

**SEA SURFACE FILMS: DEPOSITION AND TOXICITY IN  
INTERTIDAL HABITATS**

**William Wood Gardiner**

**1992**



The Padilla Bay National Estuarine Research Reserve is one of the reserves in the National Estuarine Research Reserve System. One of the purposes of the Reserve is to facilitate research and monitoring at Padilla Bay to provide information for the conservation and management of the nation's estuaries, in particular greater Puget Sound and other estuaries in the Pacific Northwest. The Padilla Bay National Estuarine Research Reserve assists the dissemination of this information from research and monitoring by publishing a Reprint Series and a Technical Report Series.

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**SEA SURFACE FILMS:  
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**A Thesis  
Presented to the Faculty of  
Western Washington University**

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

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**William Wood Gardiner**

**Spring, 1992**

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## ABSTRACT

Sea surface films are a concentration point for organic and inorganic compounds, bacteria, phytoneuston, zooneuston, and detritus. Atmospheric, terrigenous, and marine anthropogenic contaminants are also enriched at the surface, causing lethal and sub-lethal toxic effects in marine vertebrates and invertebrates. In coastal regions, wind, waves, and surface currents drive surface films shoreward, making them available for littoral interaction. Beaching of drift cards and sea foam indicates the potential for surface film deposition, or stranding, as the tide recedes. The objectives of this study were to demonstrate sea surface film deposition during tidal ebb and to determine the relative toxicities of these deposits in rural, semi-rural, and urban embayments.

A modified Garrett screen sampler was designed to collect surface film deposits. Stranding was demonstrated in a series of five *in situ* labeling experiments using surface active *Lycopodium* spores. Mean spore recoveries in surface film (SL) samples (6,896 spores/ml) and surface deposit (SD) samples (12,711 spores/ml) were significantly higher than those of bulkwater samples (0 spores/ml). A mean SD:SL spore density ratio of 3.7 suggested a magnification effect during deposition. This effect may have been a natural concentration process as surface film materials strand during the tidal ebb.

Surface deposit, surface film, and bulkwater toxicity was evaluated for Discovery, Padilla, and Commencement Bays using three echinoderm (*Dendraster excentricus*) bioassays. The 48-h development test measured percent mortality (M) and percent abnormality (A). The sperm cell test (SCT) assayed sperm viability (S) and a cytogenetic bioassay measured alterations in embryonic mitosis. Commencement Bay surface deposits produced significantly higher rates of mortality, larval deformity, and SCT response (M-37.0%; A-30.6; S-39.9%) than deposit samples from either Padilla

(M-12.8%; A-10.0%; S-5.4%) or Discovery Bay (M-2.2%; A-3.1%; S-8.7%). Larvae incubated in Padilla Bay surface deposits had significantly higher mortalities than those of Discovery Bay. Significant increases in percent anaphase aberrations were detected in larvae incubated in both Commencement (36.2% abnormal anaphase) and Padilla Bay (8.0% abnormal anaphase) surface deposits, while Commencement Bay surface deposit samples had a significantly reduced mitotic index (8.0 mitoses/embryo).

Larvae incubated in Commencement Bay surface films had significantly higher rates of mortality and abnormality than larvae in either Padilla or Discovery Bay surface film samples. Bulkwater responses in all tests were considerably lower than both surface film and surface deposit responses for all three embayments.

The spore labeling experiments demonstrated that free floating surface films are deposited onto intertidal substrates during tidal ebb. In coastal regions, such as Puget Sound, this stranding of organically enriched surface films represents a potential transfer of marine surface materials into littoral habitats. *Dendraster excentricus* bioassays indicated that contaminated surface deposits can produce developmental, reproductive, and mutagenic effects. A conceptual model based on surface current, wind, demographic, and drift card data predicted that Puget Sound shorelines which are downwind or downcurrent from contaminant sources are especially at risk from these effects.

Parkwell, Heather Mayhew, Terry Siebens, Julie Schindler, Ann Drum, Pat Fallon, Dave Erikson, and Gary Thomas. Without these people, I'd be just another guy standing in the mud.

I will be forever grateful to Dennis Dacey and Helen Sherk, who work in the fascinating realm of the vertebrate retina and visual cortex, and who attack everything with an unbridled enthusiasm and a caring finesse. Thanks for sharing your passion for science. It can only be contagious.

I am thrilled to have such a medium to thank my parents and my sister, Lee, for their undying support. With roots like that, one can only grow upwards.

---

This work is dedicated to the memory of Thomas Kulak, who will never know the profound influence he has had on so many lives.

## ACKNOWLEDGEMENTS

I stand knee deep in Padilla Bay mud watching the midnight tide slowly ebb. The crisp white polyethylene fibers of my screen sampler pulse yellow and orange in the dark night air. What should be a quiet seagrass bed of *Zostera marina* and Great Blue Herons is interrupted by violent and somewhat surreal bursts of flame from the March Point refineries. A solitary human in this bizarre scene, I am alone in this act of science. Upon further rumination (waiting for Padilla Bay to drain at 1 a.m. can be very thought provoking), I realize that the origins of my actions are not singular, but a product of many wonderful people, to whom I am indebted.

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## INTRODUCTION

Encompassing the upper one thousand microns of the water column, sea surface films are a concentration point for natural organic and inorganic compounds, neuston, detritus, and anthropogenic contaminants. The combination of high chemical and biological activity and a distinctive position at the air-sea interface creates a physically and biologically unique environment. In coastal regions, surface films can move shoreward in response to winds, waves, and currents. Shoreward transport of surface active materials makes them available for deposition during tidal ebb and represents a conveyance of living and detrital material from a marine ecosystem to a littoral system. In urban areas, this depositional process may also facilitate the shoreward transport and integration of a complex mixture of anthropogenic contaminants into intertidal habitats. The objectives of this study were to demonstrate intertidal surface film deposition and to determine the toxicity associated with these deposits in urban, semi-rural, and rural embayments.

### *The Surface Film Environment*

Although two dimensional in appearance, the sea surface is composed of several layers (Figure 1), defined by both their physical location and their chemical and biological constituents (Hardy and Word, 1986). The surface nanolayer, from the surface to a depth of several hundred nanometers, typically contains hydroxylated and carboxylated carbohydrates and proteins, waxes, esters, bacteria, and viruses (Baier, 1972; Guscinski, 1986; Hardy, 1982). The surface microlayer, to a depth of several hundred microns, is a gooey matrix of lipids, particulate and dissolved nutrients, carbohydrates, bacteria, protozoans, phytoneuston, small neustonic metazoans ( $<500 \mu\text{m}$ ), and detritus (Word et al., 1986). The surface millilayer extends to a depth of about one millimeter. Larger particulates, zooneuston ( $>500 \mu\text{m}$ ), and ichthyoneuston are found in the millilayer. A

more general term for these layers is sea surface film, an organically enriched layer several angstroms to one thousand microns thick. When films become heavily enriched, they may damp capillary waves and are classified as surface slicks (Dietz and LaFond, 1950). The transition from non-slick to visible slick is a continuum of surface film thickening (Smith, 1991).

Relative to bulkwater, surface films are often enriched in dissolved and particulate organic carbon, nitrogen, phosphorus, ATP, phenolics, and detritus. High levels of detritus and dissolved and particulate organics increase sea surface film viscosity. This viscous matrix creates a stable, carbon rich environment, inhabited by dense populations of phytoneuston and zooneuston (Word et al., 1986).

Phytoneustonic populations may be 1,000 times more abundant per unit volume than underlying bulkwater phytoplankton populations; however, large temperature fluctuations and extreme ultraviolet exposure limits species diversities (Hardy, 1973; Word et al., 1986; Zaitsev, 1971). Marine phytoneustonic communities are dominated by small pennate diatoms, microflagellates, and blue-green algae (Hardy and Valett, 1981). Cellular phytoneustonic carbon fixation rates may be photoinhibited by the high light intensities of the surface, but their photosynthetic productivity per unit volume can be enhanced up to 52 times that of phytoplanktonic productivity in the waters below (Hardy and Apts, 1989). Chlorophyll standing crop and its breakdown products are also enhanced at the surface (Cullen et al., 1989; De La Giraudiere et al., 1989; Hardy and Apts, 1989).

The zooneustonic community has both permanent (euneuston) and facultative (meroneuston) members, representing nearly every phyla, including rotifers, pontellids, cladocerans, polychaetes, decapods, and fish larvae and eggs (Grant, 1985; Zaitsev, 1971). Microbial populations, including bacterioneuston, yeasts, and molds, are highly active in

the metabolism and turnover of amino acids (Carlucci et al., 1991; Kjelleberg et al., 1979). Bacterioneuston populations alone may be 10,000 times more dense than bacterioplankton (Hardy, 1982; Sieburth, 1971). It is clear that surface films represent a complete and unique ecosystem, with each link of the food web represented in a distinct physical environment.

Sea surface films receive material input from atmospheric, terrigenous and marine sources. Atmospheric sources include wet and dry deposition and gaseous diffusion. Terrigenous inputs are facilitated by river run-off and eolian dusts (Liss, 1975; Zaitsev, 1971). Allochthonous and autochthonous processes inject surface active marine materials. Marine sources include upwelling, resuspension, buoyancy, and allochthonous and autochthonous biotransformation (Word et al., 1986). Perhaps the most important marine source is bubble transport from underlying waters. Bubbles collect compounds from the water column by material adsorption to the rising bubble's air water interface (Blanchard, 1986; Liss, 1975). Materials are removed from the surface by evaporation, aerosol ejection by bursting bubbles, dissolution, sinking, capture by organisms, degradation, and downwelling (Liss, 1975; Word et al., 1986).

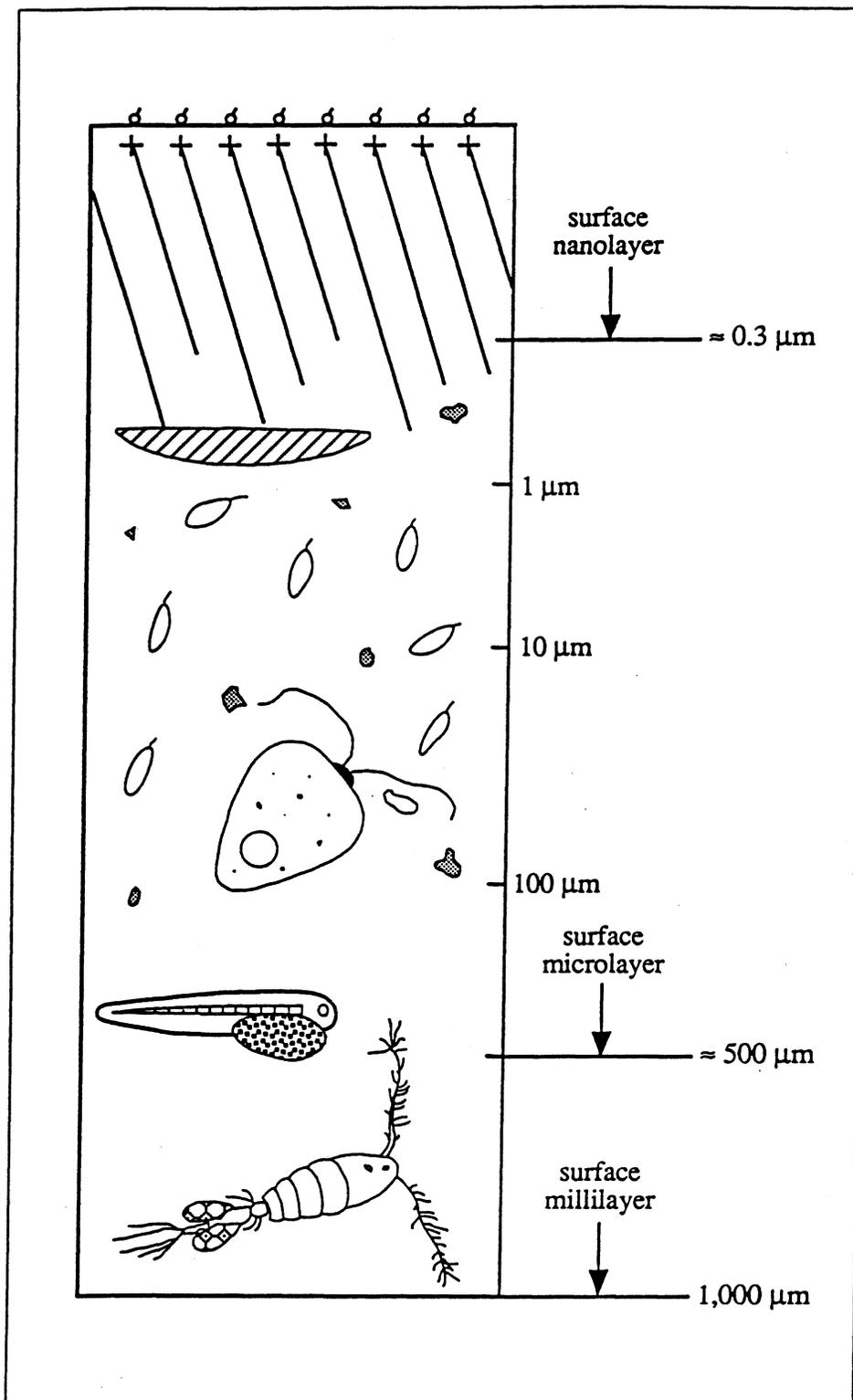


Figure 1. Schematic of surface film structure (adapted from Hardy, 1982).

### ***Anthropogenic Contamination***

The same processes that enrich natural compounds at the surface also concentrate anthropogenic contaminants. Surface films have been found with high levels of metals, hydrocarbons, pesticides, and PCBs. Surface film enrichments of organic and inorganic metals have been found in U.S. harbors and waterways, coastal Monaco and Florida, and in the North Sea (Cross et al., 1987; Duce et al., 1972; Hardy et al. 1985, 1987a, and 1990; Hardy and Cleary, 1992; Harvey et al., 1982; Lion et al., 1979). Often these levels exceed established water quality criteria (EPA, 1986). Petrogenic hydrocarbon concentrations are elevated throughout the world's coastal and offshore waters and have been found in measurable levels in tissues of neustonic organisms (Butler and Sibbald, 1987; Cross et al., 1987; Hardy et al., 1987a and 1990; Marty et al., 1979; Morris, 1974). Pesticides and PCBs have not only been found in nearshore urban waters, such as Los Angeles Harbor and Commencement Bay, but also offshore, in the Sargasso Sea and the Gulf of Mexico (Bidleman and Olney, 1974; Burns et al., 1985; Duce et al., 1972; Eganhouse et al., 1990; Ofstad and Lunde, 1978; Seba and Corcoran, 1969). More recent measurements in the Gulf of Mexico indicate that PCB and DDT levels may be decreasing due to their reduced usage (Sauer et al., 1989). High surface film concentrations of toxic organotin compounds, used in anti-fouling boat paints, have not only been measured in English and North American marinas, but also in offshore sites in the North Sea (Cleary and Stebbing, 1987; Hall et al., 1987; Hardy and Cleary, 1992; Templeton and Gardiner, 1991).

Nearly 25% of Freon-extractable and 10% of Freon-nonextractable materials and bacteria from secondary sewage effluents are floatable fractions (Word et al., 1990). Some of these surface sewage effluent fractions have been detected in U.S. and Australian surface waters (Nichols and Espey, 1991; Selleck, 1975; Word and Ebbesmeyer, 1984).

From the above studies, it is clear that surface film contamination is widespread, encompassing a wide variety of xenobiotics in both coastal and offshore waters.

Biological effects have been directly linked to surface film contamination. When sole eggs were incubated in urban Puget Sound microlayer samples, hatching success and larval development were significantly reduced (Hardy et al., 1987b). Kelp bass embryos exposed to Los Angeles Harbor microlayer samples had greatly increased mortality and chromosomal aberrations (Cross et al., 1987). Herring and turbot embryonic mortalities and abnormalities increased when placed in North Sea surface film samples (von Westernhagen et al., 1987). Echinoderm larvae, exposed to samples from Puget Sound, the Persian Gulf following the 1991 oil spill, and offshore waters in the North Sea, showed increased mortality and abnormality (Hardy and Cleary, 1992; Hardy and Gardiner, 1991; Hardy et al., 1987b). Decreased zooneuston and sole egg densities have been found in urban, compared to rural, Puget Sound surface films (Hardy et al., 1987b, Hardy and Antrim, 1988). It is difficult to link specific contaminants to these effects due to the complex mixture of xenobiotics in many areas. Furthermore, contaminant concentrations below chronic or acute levels, may exhibit synergistic effects to create toxic mixtures (Rand and Petrocelli, 1985).

#### *Surface Film Transport*

Surface film movement is governed by both marine and atmospheric processes. Internal waves, encountering shallow shorelines or rigid thermoclines, rise to the sea surface, transporting materials vertically (Ewing, 1950). Shore-parallel slick and non-slick bands are then formed and transported horizontally, above the wave trough, at the internal wave velocity (Shanks and Wright, 1987). Wind, gravity waves, and surface currents can then modify internal wave-driven film movements (Ewing, 1950; Lange and

Huhnerfuss, 1978). In laboratory and field trials, slick speeds were measured at 2.6 to 5.5% of the prevailing wind speed, while direction was controlled by both wind and gravity waves (Lange and Huhnerfuss, 1978). Surface current eddies have been correlated with the stranding locations of drift cards and shipwreck-generated floatable materials, indicating their role in transporting surface materials (Ebbesmeyer et al., 1991). Internal waves and wind, modified by gravity waves and surface currents, appear to be the predominant processes transporting surface films (Dietz and LaFond, 1950; Lange and Huhnerfuss, 1978). Net surface film movement is, thus, a complex sum of these component forces.

In coastal areas, films often move shoreward in distinct bands, a result of internal wave periodicity. Internal wave-mediated shoreward transport of sea surface films has been demonstrated with styrofoam surface drogues and has been linked with the shoreward migration of intertidal gammarid amphipods and cyprid, crab, and reef fish larvae (Kingsford and Choat, 1986; Shanks, 1983; Shanks and Wright, 1987). Furthermore, longshore differences in intertidal barnacle settlement rates were correlated with onshore movements of surface drogues in a rocky intertidal embayment (Shanks and Wright, 1987). Numerous drift card studies demonstrate both shoreward movement of surface materials and stranding of these materials onto intertidal substrates during tidal recession (Cox et al., 1980; Ebbesmeyer et al., 1984). Deposition of offshore surface films onto beaches is also suggested by similarities in pentane-extractable material concentrations in offshore slicks and beach interstitial waters (Word and Ebbesmeyer, 1984). The above studies indicate the potential for surface films to deposit during tidal recession, but they have either depended upon drogues, which behave differently than surface films, or have been based on indirect measurements. To our knowledge no one

has directly labeled surface films and measured their deposition in intertidal habitats.

### **Objectives**

The objectives of this study were to demonstrate surface film deposition and to compare the toxicity of surface deposits in an urban, semi-rural, and rural embayment. A surface deposit screen sampler was developed to effectively collect stranding surface films. In a series of *in situ* manipulative experiments, surface films labeled with surface-active *Lycopodium* spores were shown to deposit onto intertidal substrates during tidal recession. In echinoderm (*Dendraster excentricus*) 48-hour, sperm cell, and cytogenecity bioassays, the toxicities of surface deposits, free floating surface films, and bulkwaters were determined for Discovery Bay (rural), Padilla Bay (semi-rural), and Commencement Bay (urban). In light of these new findings, and current oceanographic, topographic, and demographic information within Puget Sound, potential shorelines at risk from contaminated surface deposits were predicted in a conceptual model. Both urban and rural shorelines may receive contaminated surface film deposits.

## METHODS

Surface film deposits (SD) were collected with a modified Garrett screen sampler (Garrett, 1965). Monodour polyethylene mesh (1,000  $\mu\text{m}$  mesh size, 500  $\mu\text{m}$  thread diameter, 1  $\text{m}^2$  area) was stretched across a polycarbonate plexiglas frame and attached to plexiglas frame legs by nylon screws. The screen was displaced from the frame by 15 cm to prevent sample contamination by bulkwater associated with the frame. Thirty centimeter legs anchored the sampler above the intertidal substrate. Further strength was added to the frame by diagonal threads of Steelon, nylon coated stainless-steel wire (Figure 2). The entire sampler was aged for four days in flowing seawater, and all materials conformed to ASTM (1990) specifications for sampling water. Each 1- $\text{m}^2$  screen collected 200-350 ml samples. The depth sampled, calculated from the volume collected and sampler area, was 200-350  $\mu\text{m}$ .

Surface deposit samples (SD) were collected by submerging the sampler at a 45° angle, then reorienting horizontally, and anchoring the legs to the intertidal substrate. The deposit sampler was positioned parallel to the shoreline. After the tide had receded across the sampler, the screen was removed from the substrate, held vertically, and the water drained into an acid-cleaned, solvent rinsed 500-ml glass jar.

Free floating surface films (SL) were collected with the same type of sampler. The screen was submerged at a 45° angle, reoriented horizontally, brought slowly to and then lifted from the surface, and drained as above. Subsurface bulkwater (BW) was collected by plunging an acid-washed, solvent-rinsed 500-ml glass jar approximately 50 cm below the surface, holding for 60 seconds, unsealing, then resealing at depth.

Sampler efficiency was determined in laboratory simulated tidal recessions. The

sampler was placed in a fiberglass 100-gallon tank filled with sand-filtered seawater. Four replicate experiments were conducted with spores of the *Lycopodium* plant, representing particulates, and freon-extractable plankton oil dissolved in sunflower oil, representing surface-active lipids. After the spores or oil had spread evenly (10-15 min), the water was drained from below, depositing surface materials onto the screen sampler. The deposit sample was drained into an acid and solvent-rinsed jar, then the screen was rinsed with deionized water into a second rinse jar. A sample of the drained bulkwater was also collected. *Lycopodium* spores were enumerated by transferring 1-ml subsamples with a volumetric autopipette into a 1-ml counting chamber, then visually enumerating them using a light microscope (400X). Spore density (spores/ml) was then multiplied by the sample volume to yield total spores collected.

Oils from the tank samples were extracted in Freon, with the extracts being analyzed by infra-red spectrophotometry (Standard Methods, 1989). A concentration series of plankton/sunflower oil was also analyzed to create a standardization curve. Total spores and oil collected were calculated for deposit, rinse, and bulkwater samples. The deposit and rinse values were then combined to calculate collection efficiency. Spore and oil collection efficiencies were expressed as percent recovery of material available to the screen for collection. This was estimated by the formula below:

$$\text{material available to sampler} = \text{material released} - \text{loss to walls} - \text{non-screen water surface}$$

Material lost to the walls and occupying "non-screen water surfaces" was estimated by multiplying the wall area contacted by the surface film and the unsampled surface area by the spores or oil per unit area. This assumed even spreading of surface materials.

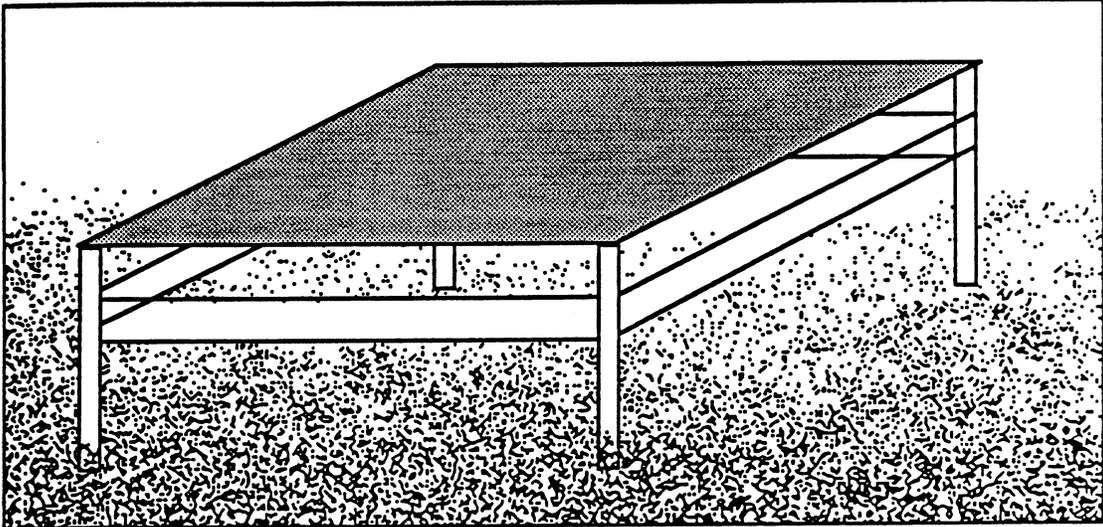


Figure 2. Sea surface film deposit sampler.

### *Deposition Experiments*

Five replicate deposition experiments were conducted sequentially during the same tidal cycle at five different sites on the northeast shore of Sequim Bay, Washington. The SD samplers were positioned during a high tide. A light wind of 0-1  $\text{ms}^{-1}$  was present during the experiments. Ten to twenty meters upwind of the samplers, approximately 2 g of surface-active *Lycopodium* spores were released over the surface. The spores were allowed to spread and drift. As the tide receded across the SD samplers, surface film and bulkwater samples were collected adjacent to, but not interfering with, the surface deposit samplers. After exposure, the SD samples were collected in a clean glass jar. Both the SL and SD samplers were rinsed with 300 ml of deionized water into a second sample jar. Spores were enumerated as in the screen efficiency experiments and expressed as spores/ml. Wind velocity, water temperature, and salinity, as well as qualitative observations, were recorded during the sampling period.

### *Toxicity Tests*

*Study sites.* Based on previous demographic and toxicity data (EPA, 1987) and shoreline accessibility, Discovery, Padilla, and Commencement Bays were selected for toxicity testing (Figure 3). These embayments represented Puget Sound rural, semi-rural, and urban environments, respectively.

Discovery Bay (Figure 4), is a rural embayment on the Olympic Peninsula, near Gardiner, Washington. Shorelines are predominantly cobble and coarse sand, although the southern bay substrates are sand and mud. The majority of the bay shorelines are conservancy designated, with rural communities at Diamond Point, Beckett Point, Cape George, and Discovery. Previous toxicity studies showed very low levels of contaminants.

There are no point-source discharges in the bay, and sediment chemistry levels are consistently low (EPA, 1987).

Padilla Bay (Figure 5), is a semi-rural estuarine bay, near the town of Anacortes. Padilla Bay is an important fish and shellfish resource area, with extensive seagrass beds and mud flats exposed during low tides. Much of the shoreline is rural, dominated by farmland. Samish Island, in the north bay, is designated as residential rural and March Point, to the southeast, is an urban region. Two oil refineries are located on March Point, which also has a dredge disposal site and a point source refinery effluent discharge of over 10,000 million gallons per year. Sediment levels of high molecular weight hydrocarbons have been measured at 730 ppb at March Point (EPA, 1987).

Commencement Bay (Figure 6), near Tacoma, is an urban embayment with cobble and sandy shorelines. Commencement Bay is considered a Superfund site, with point source and non-point source discharges along all of its shorelines. High sediment contaminant burdens and intertidal toxicities occur throughout the bay (EPA, 1987). Commencement Bay also has an accessible urban shoreline.

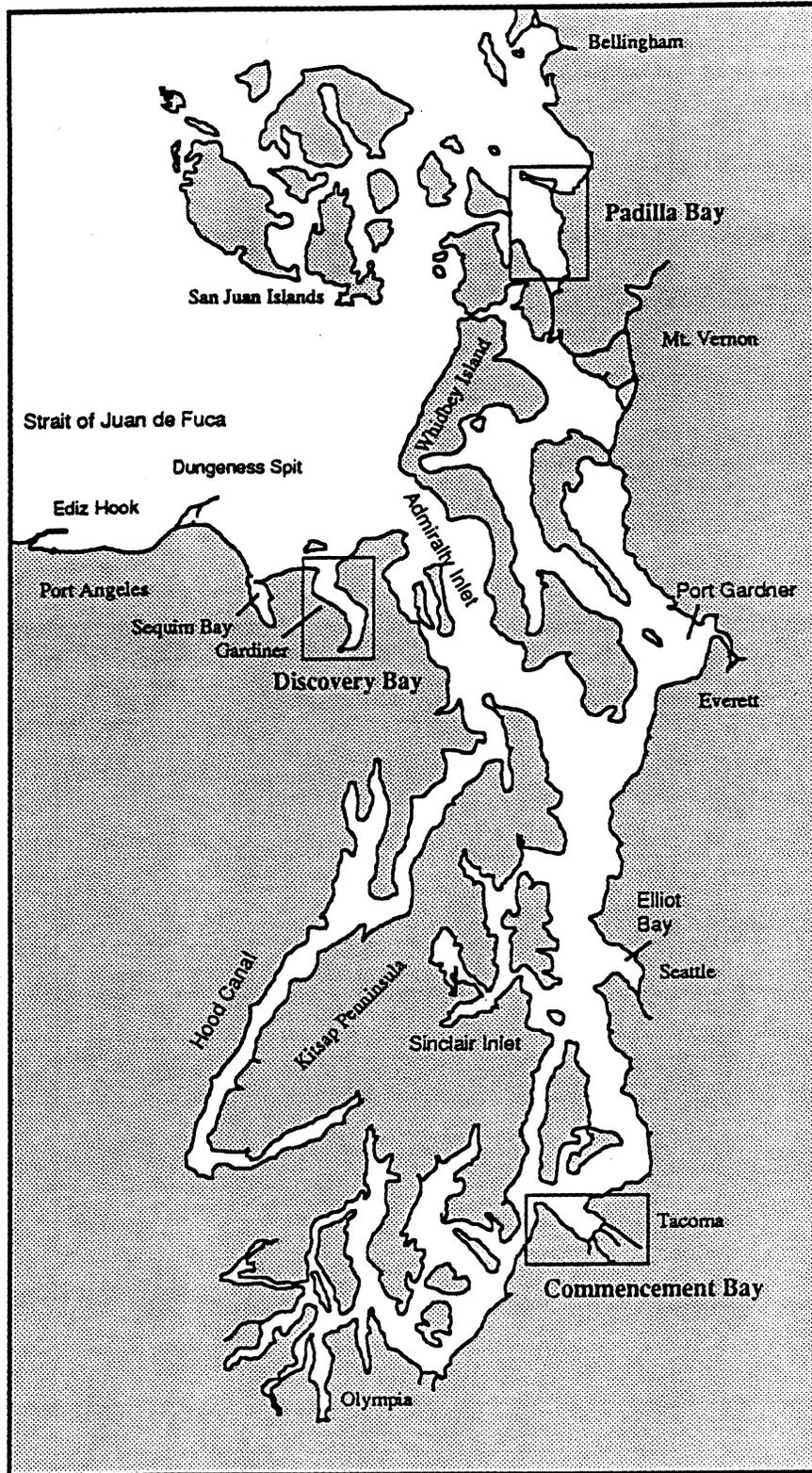


Figure 3. Puget Sound and sampled embayments.

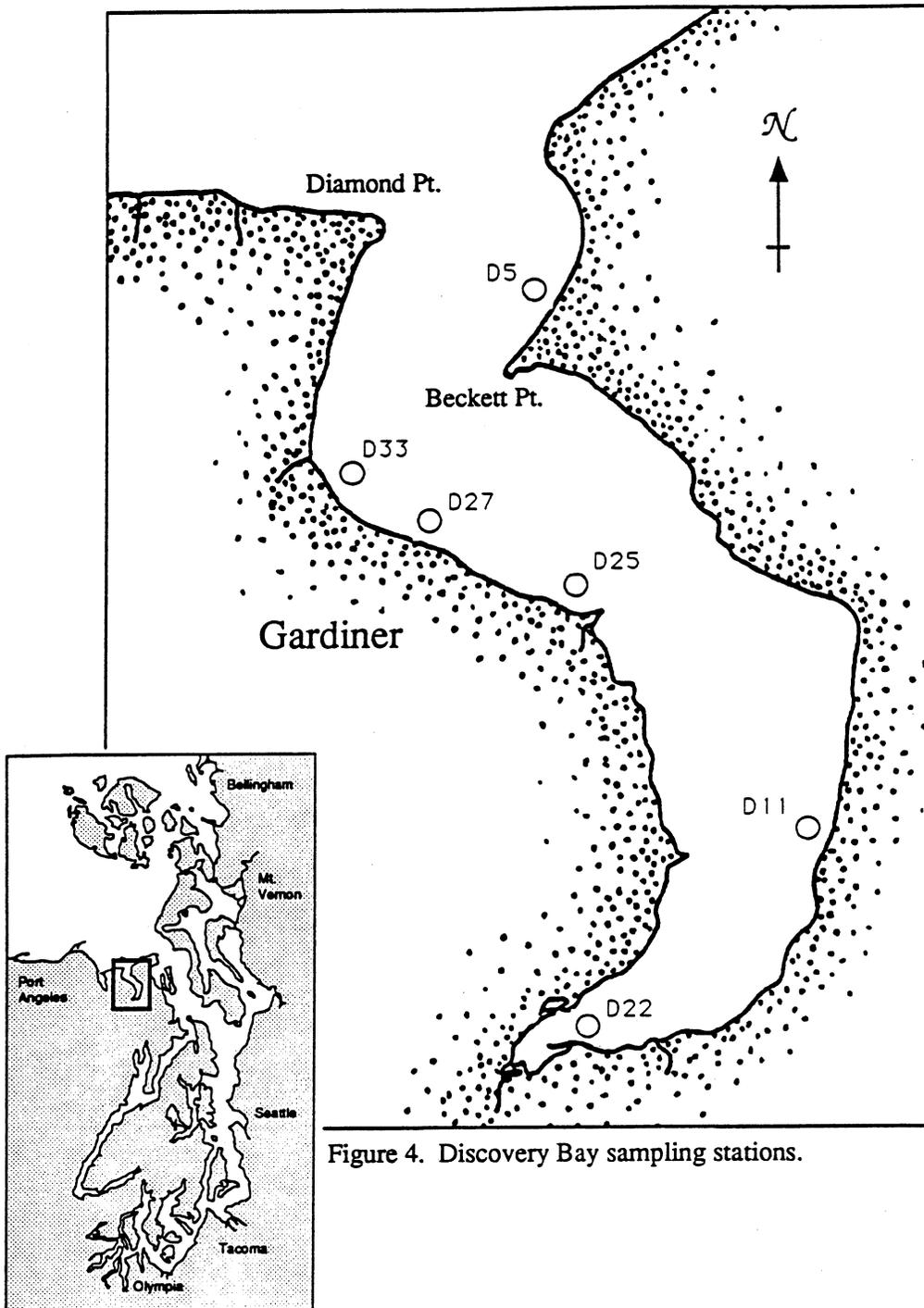


Figure 4. Discovery Bay sampling stations.

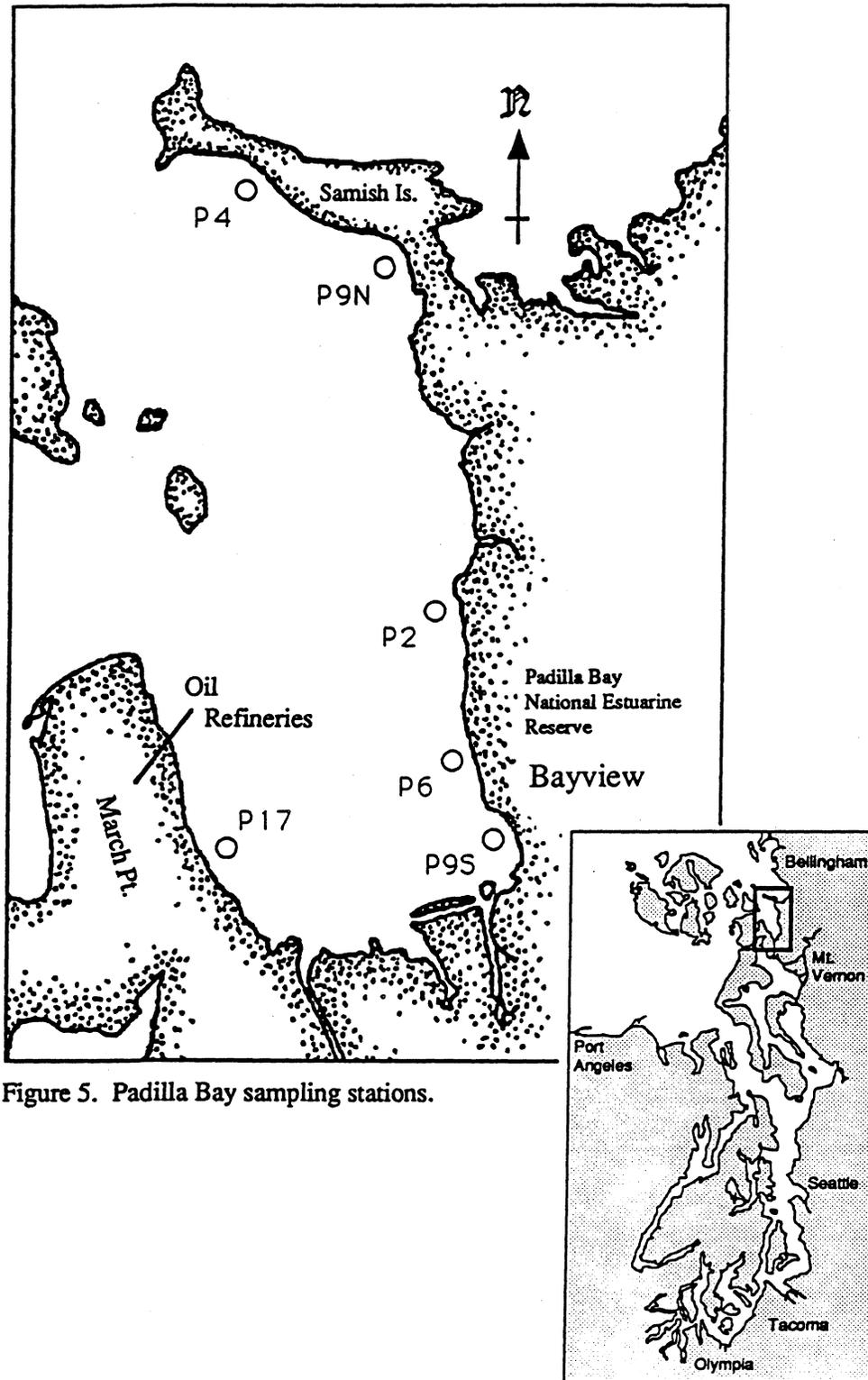


Figure 5. Padilla Bay sampling stations.

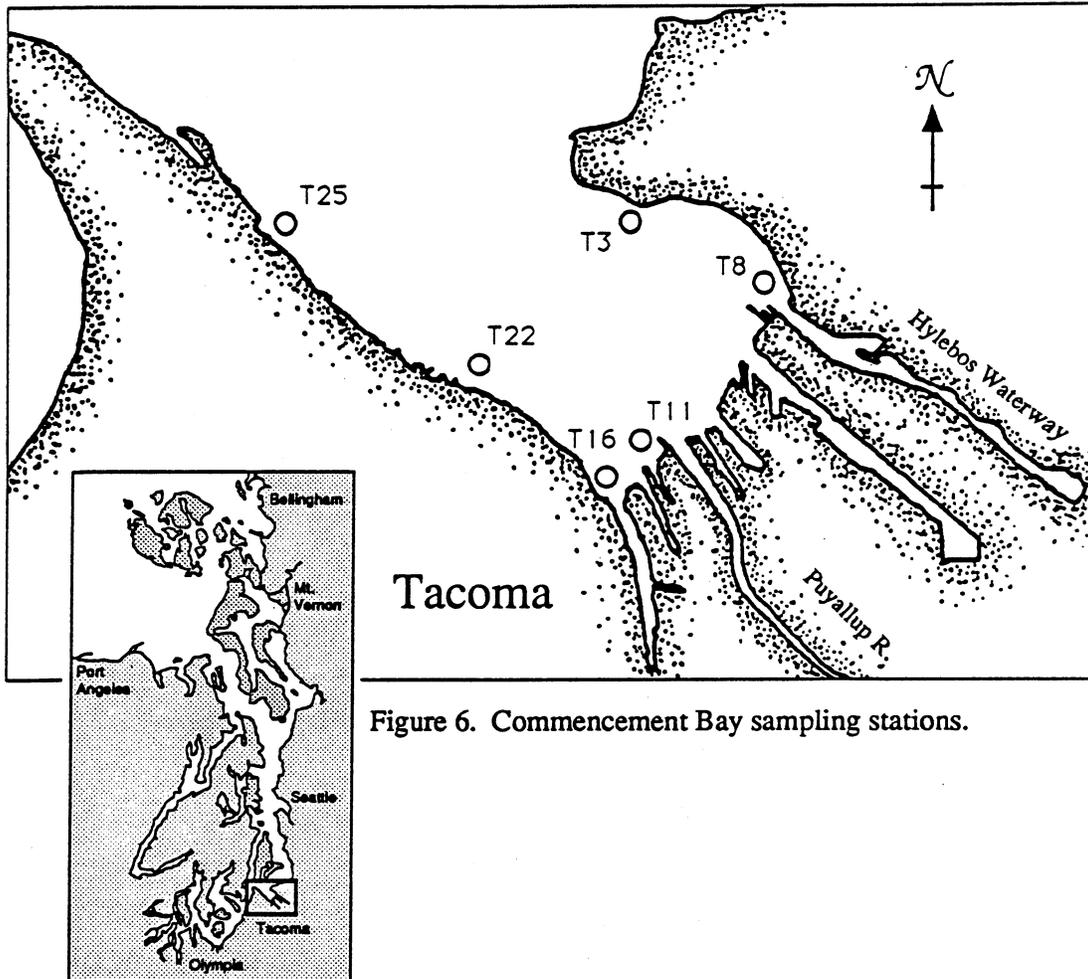


Figure 6. Commencement Bay sampling stations.

*Sample Collection.* Based on previous Commencement Bay microlayer toxicity data with *Strongylocentrotus purpuratus* (Hardy and Antrim, 1988), it was determined that six stations within each bay were necessary to determine a statistical difference of 10% toxicity response (Peterman, 1990). Shorelines for each embayment were divided into 500-m linear sections and were assigned sequential numbers. Six shoreline segments were then selected using a random number generator.

Because of logistical constraints, testing all stations within one bay or one station in each of the three bays at one time was not feasible. Therefore, a partially-balanced incomplete block sampling design (Cochran and Cox, 1957) was chosen for sampling and testing (Table 1). Six rounds of sampling and testing were performed, with each round, or block, including two stations from one bay and one station from another. Each bay was paired with the other two bays twice and each bay and station assignment was completely random. This design was selected to account for the variance associated with block-specific weather or test conditions.

Table 1. Partially balanced incomplete block design used for sampling and testing. Each round represents one bioassay, which includes three stations. A=Discovery Bay, B=Padilla Bay, C=Commencement Bay.

Round 1	Round 2	Round 3	Round 4	Round 5	Round 6
A1	A3	B2	A5	A6	B6
A2	A4	B3	B4	C3	C5
B1	C1	C2	B5	C4	C6

Pooling five or more screens at each station was necessary to collect the 1.5 l of water needed for testing and chemistry. Screens were placed at 10-m intervals along the shore at three to five tidal heights. Thus, pooling of screens within a station served, not

only to collect the larger volume of water necessary, but also to reduce spatial variability among stations. Each surface deposit screen location had a corresponding surface film and bulkwater sample. Therefore, surface deposit, surface film, and bulkwater samples from each station were composed of pooled samples from five or more screen locations.

Samples were placed in a cooler for transport and kept dark at 4°C until testing. Samples were used within 36 hours of collection (ASTM, 1990).

Environmental conditions were recorded for each station. Wind speed ( $\pm 0.25 \text{ ms}^{-1}$ ) and direction were measured with a hand-held anemometer and compass. Water temperature ( $\pm 0.05^\circ\text{C}$ ), dissolved oxygen (DO) ( $\pm 0.05 \text{ mg/l}$ ), and pH ( $\pm 0.005$  units) were measured with a Cole-Parmer model 5566 probe. A refractometer was used to measure salinity ( $\pm 0.025$  ppt), and beach characteristics and weather conditions were recorded at each site. These data are presented in Appendix 13.

*Test Organism Collection.* All tests were conducted with the echinoderm, *Dendraster excentricus*, collected from Sequim Bay and Semiahmoo Spit, Washington. Organisms were collected between June and September during -1 ft. or lower tides and were held in flow-through tanks supplied with Sequim Bay seawater at ambient temperatures. Sand dollars were allowed to feed in Sequim Bay sediments during the holding period.

*Toxicity Test Preparation.* Dilutions of 6.25%, 12.5%, 25.0%, 50.0%, and 100.0% of each sample were mixed with sand-filtered Sequim Bay seawater. Sand-filtered seawater was used because earlier studies have shown high echinoderm mortalities associated with membrane-filtered (0.45 $\mu\text{m}$ ) seawater (Antrim, 1991; McLean-Pinza, 1990). Toxicity tests were performed between 28 and 32 ppt salinities. Salinities below 28 ppt were adjusted with saline brine. Three 150-ml dilution replicates were placed in 1-l wide mouth jars for the 48-h egg and post-fertilization embryo exposure. Three 50-ml dilution replicates were

placed in beakers for the 48-h test sperm exposure. For the sperm cell test, three 10-ml replicates of each dilution were placed in 6 X 100 mm borosilicate test tubes with a Gibson volumetric micropipette. All exposure chambers were then placed in random positions in a 15°C water bath.

*48-hour Bioassay.* The methods for the 48-h bioassay closely followed those of Oshida and Goochey (1981). Gametes were collected from *D. excentricus* by injecting 0.5 ml of 0.5 M KCl into the coelomic cavity through the peristomal membrane. Females, releasing reddish-pink eggs, were inverted over beakers filled with unfiltered seawater. Males, releasing yellowish sperm, were inverted over dry petri dishes and kept "dry" until dilution. Eggs from no fewer than two females were rinsed and combined, and the egg concentration determined by counting the eggs in 0.5 ml of the concentrated solution. The concentrated egg solution was then diluted to 10,000 eggs/ml, and 1 ml of the dilution was added to 150 ml of each treatment with an autopipette and maintained for 30 min prior to the fertilization. Concentrated sperm from at least two males was adjusted to 1 drop in 5 ml unfiltered seawater. Fifteen minutes after egg exposure initiation, 1.2 ml of the sperm dilution was added to the sperm exposure beakers with 50 ml of treatment. At this point both eggs and sperm were simultaneously exposed to the treatments. Fifteen minutes after sperm exposure initiation the sperm solution was added to the egg solution, resulting in 200 ml of test solution in each mason jar. Five minutes after initiation a subsample was taken from a control jar to check percent fertilization. Fifteen minutes after initiation, a 4-ml subsample was taken from all jars and fixed in Lugol's solution. These samples were used to determine stocking densities. Forty-eight hours after initiation, a 4-ml subsample from each treatment was removed with an autopipette and fixed in 10% formalin in scintillation vials.

The 48-h subsamples were analyzed for pluteus development by observing the entire subsample in the scintillation vial on an inverted compound light microscope. This non-intrusive technique allowed the sample to be used later for cytogenicity/cytotoxicity tests. Samples were scored for blastula, gastrula, pluteus, abnormal, and unfertilized embryos. Normal pluteus had well formed spicules, arms, and guts. Pluteus abnormalities included exogastrulation, incomplete or irregular gut formation, and incomplete or irregular spicule development (Figure 7). To determine percent mortality, stocking densities were determined by counting and averaging a ten percent random sample of the 15-min subsamples for each test.

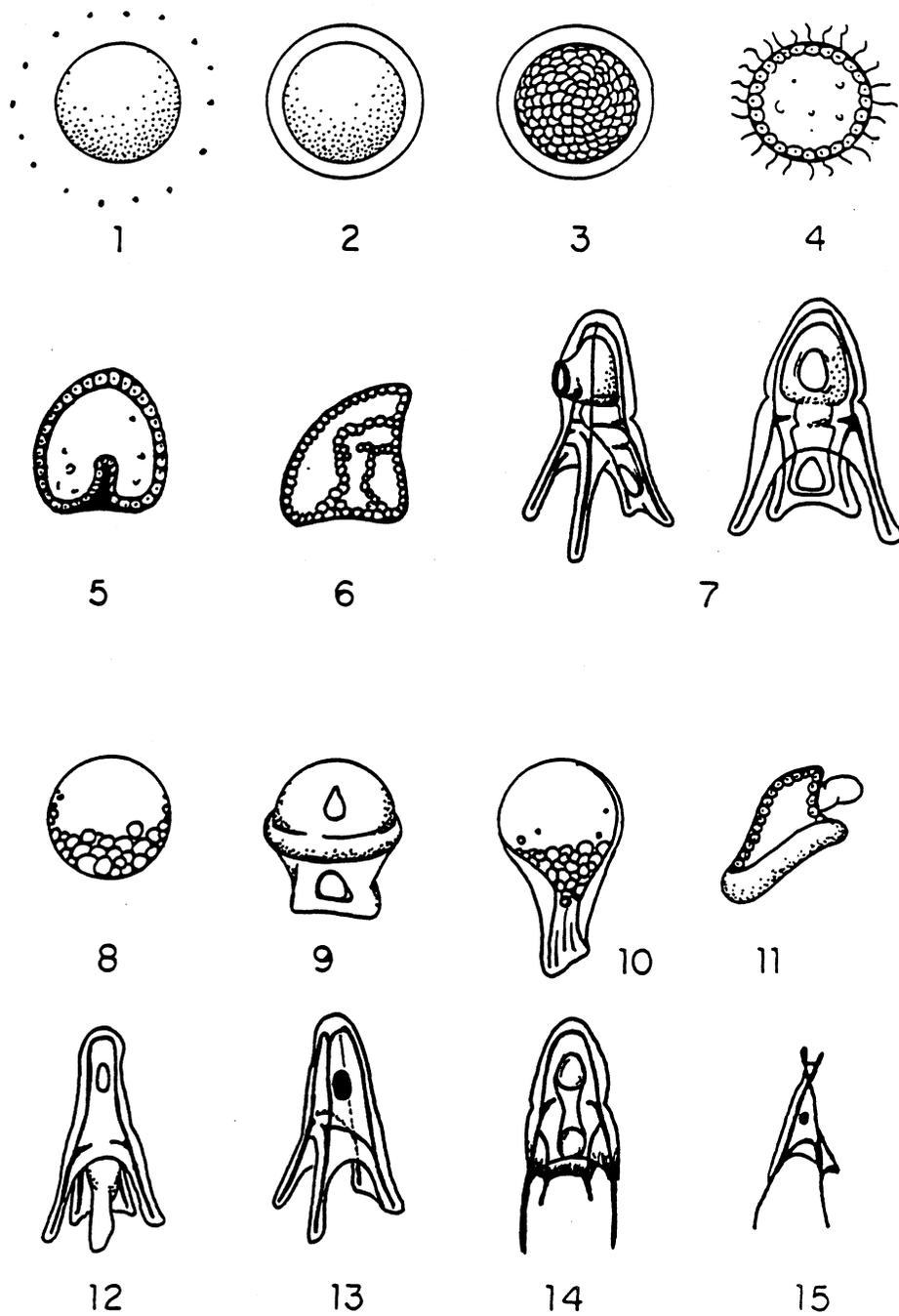


Figure 7. Normal and abnormal echinoderm development. 1: unfertilized egg; 2: fertilized embryo; 3: morula; 4: blastula; 5: early gastrula; 6: late gastrula; 7: pluteus; 8-10: early embryo abnormalities; 11-12: exogastrulation; 13: detached gut; 14-15: spicule abnormalities.

*Sperm Cell Test (SCT).* The echinoderm sperm cell test followed the protocol developed by Dinnel et al. (1987). Dry sperm, collected as above, were diluted with 45 ml of sand-filtered Sequim Bay seawater. The sperm density was then determined by adding a 1-ml subsample of the mixture to 10 ml glacial acetic acid in a 100-ml stoppered graduated cylinder, and bringing to volume with seawater. The contents were mixed and a subsample removed with a pasteur pipette, placing a drop in a Neubauer hemocytometer counting chamber. Sperm in the middle 400 small squares were counted at 400X. Sperm density (sperm/ml) was equal to this count  $\times 10^6$ . The sperm solution was then diluted to the concentration needed to yield a 1,200:1 sperm to egg ratio. Following sperm dilution, 0.1 ml of sperm was added to each treatment with an autopipette and the sperm exposed for one hour. Egg density was adjusted to 2,000 eggs/ml, and one hour after sperm exposure initiation, 1.0 ml of egg suspension was added to each treatment, resulting in an egg concentration of 200 eggs/ml in each test tube. Twenty minutes after the egg addition, the test was terminated and the embryos fixed with five drops of Lugol's solution.

For each treatment, 100 embryos were checked for fertilization in each treatment test tube. Percent fertilization was determined by observing the presence or absence of a fertilization membrane.

*Cytogenicity/Cytotoxicity Test.* The procedure for staining and analyzing mitotic figures closely followed that of Hose (1985). *D. excentricus* pluteus larvae from the 48-h test, fixed in 10% formalin, were placed on a microscope slide with the formalin removed. Larvae were then post-fixed in 45% glacial acetic acid for 5 min. The acetic acid was removed, and the pluteus larvae were immersed in aceto-orcein stain (0.10 g orcein, 5 ml 45% acetic acid, 0.25 ml propionic acid; filtered in #4, then #2 Whatman filter paper) for 30 min. Following staining, larvae were squashed and smeared to a monolayer with a

coverslip. Analyses were performed on a light microscope with a 100X objective.

Visualization of mitotic figures was enhanced by either a green filter or Nemarsky optics.

Embryo preparations were surveyed for mitotic figures and cytologic and cytogenetic aberrations (Figure 8). Prophase was recognized by a 1-2  $\mu\text{m}$  round nucleus with a stippled appearance. Cells in metaphase were identified by an elongated and heavily stained opaque nucleus. In anaphase, the condensed chromatin was separated into two parallel sister chromatids, which were elongated and heavily stained. Both metaphase (pro-metaphase and metaphase) and anaphase (anaphase and telophase) cells were enumerated.

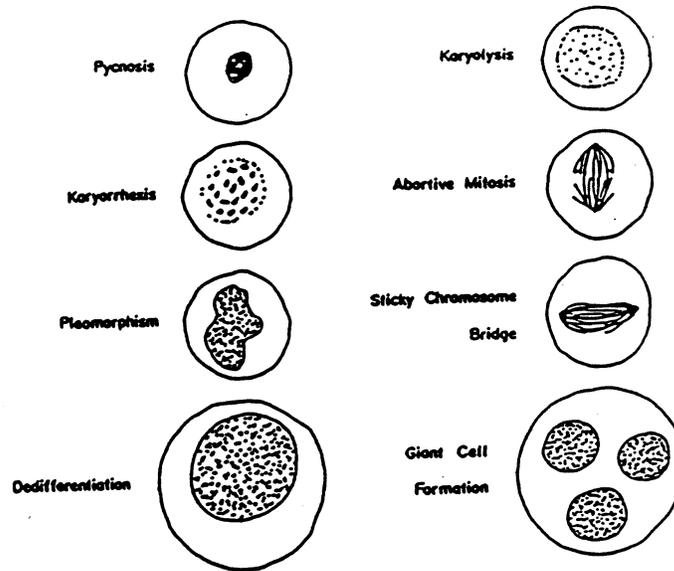
Cytologic abnormalities included:

- Pycnosis- nucleus shrunken to a dense mass of chromatin.
- Karyolysis- dissolution of cell nucleus.
- Karyorrhexis- fragmentation of nucleus.
- Abortive mitosis- abnormal chromosome configurations unable to complete their mitosis.
- Pleomorphism- occurrence of different cellular forms.
- Sticky chromosome bridge- telophase bridge consisting of more than just chromosome.
- Dedifferentiation- appearance of large, undifferentiated cells in an advanced embryo.
- Premature differentiation- appearance of advanced cell differentiation in an early embryo.
- Giant cell formation- large cell containing multiple normal-sized nuclei.

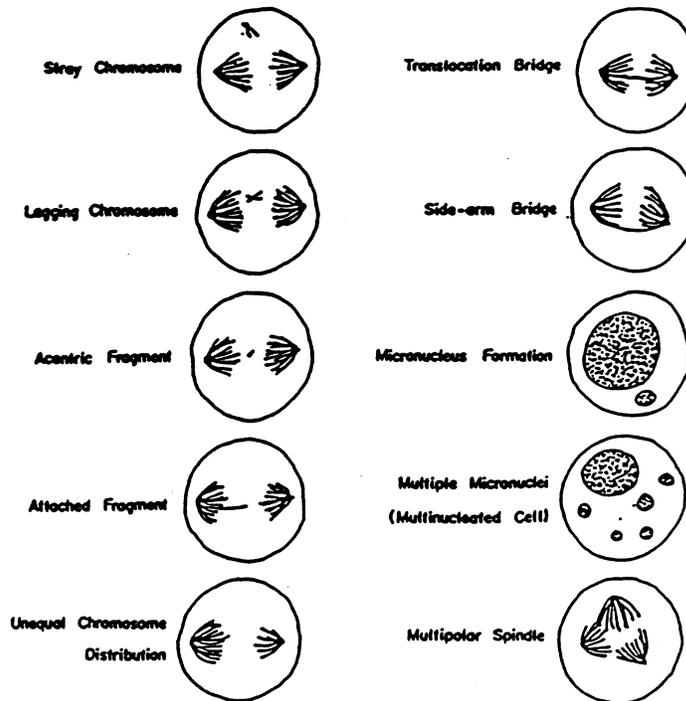
Cytogenetic abnormalities included:

- Stray chromosome- chromosome left at the equator or outside the spindle during mitosis.
- Lagging chromosome- chromosome which lags behind the main body of chromosomes.
- Acentric fragment- chromosomal fragment left at equator during division.
- Attached fragment- chromosomal fragment that lags behind the main body of chromosomes and appears to be attached by a thin strand of chromatin.
- Translocation bridge- chromatin bridge stretched between the two groups of anaphase chromosomes.
- Side-arm bridge- pseudochiasma.
- Unequal chromosome distribution- chromosome non-disjunction.
- Multipolar spindle- tri- or multipolar anaphase spindle.

Aberrations were scored as cytologic (CL) or cytogenetic (CG). Specific CL and CG categories were noted, but not tallied individually. Mitotic index (# mitoses/embryo), % mitoses with cytologic aberrations, % anaphase with cytogenetic aberrations, and % mitoses with cytogenetic aberrations were calculated. Twenty whole embryos were analyzed for each replicate. Only the 100% surface deposit samples and controls were analyzed in this test.



Types of cytotologic aberrations.



Types of cytogenetic aberrations.

Figure 8. Cytologic and cytogenetic aberrations (from Hose, 1985).

### *Metals Analysis*

To assess the contaminant levels in the toxicity test samples, 100-ml subsamples were removed for metals analysis. Analyses for other contaminants could not be conducted due to the small quantity of sample available.

Unfiltered, acidified surface deposit, surface film, and bulkwater samples were analyzed for silver (Ag), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), and zinc (Zn) at the Skidaway Institute of Oceanography, Georgia. Induction coupled plasma-mass spectrometry (ICP/MS) analysis was performed on 1/10 dilutions of each sample. Two control spikes and four standards (1/10 dilutions of National Institute of Standards and Technology standard (NIST) 1643C) were included in the analysis to assess method accuracy and precision.

### *Data Reduction and Analysis*

All raw data were entered into a computer spreadsheet program. Calculations were made to determine percent survival, percent mortality, and percent abnormality for each replicate. Percent survival was calculated as the number of normal pluteus divided by the stocking density (an estimate of embryos/ml at 15 min) times 100. Percent mortality was (100 - % survival). Percent abnormality was calculated for the surviving larvae and was the sum of blastulae, gastrulae, and abnormal larvae divided by the total larvae surviving at 48 h, multiplied by 100. Percent response for the sperm cell test was expressed as % unfertilized, and was calculated as (100 - % fertilized). Sample replicates were averaged and the mean converted to adjusted response relative to the controls with Abbott's formula (Dinnel et al., 1987):

$$\text{Adjusted test response} = \frac{\% \text{ Test response} - \% \text{ Control Response}}{100 - \% \text{ Control response}} (100)$$

For statistical comparisons, the mean adjusted response of all six stations within each bay were used (n=6).

*Statistical Comparisons- Mortality, Abnormality, and SCT.* Mortality, abnormality, and sperm cell test results were compared by analysis of variance (ANOVA,  $\alpha=0.05$ ) F-tests on the arcsine-square root of the mean bay or mean layer (n=6) adjusted responses. F-test comparisons of responses among bays/within layers were made using the partially-balanced incomplete block design. For comparisons among layers/within bays, a randomized complete block design was used. If the F statistic was in the critical region, a one-tailed t-test ( $\alpha=0.05$ ) comparison was performed to determine significance between individual means.

*Statistical Comparisons-Cytogenicity Test.* Mitotic index, cytologic abnormalities per embryo, and % anaphase aberrations comparisons were made using an ANOVA F-test ( $\alpha=0.05$ ), completely randomized design. If the F statistic was in the critical region, a one-tailed t-test ( $\alpha=0.05$ ) comparison was performed to determine significance between individual means.

#### ***Quality Assurance/Quality Control Procedures/Waste Minimization***

Both positive and negative controls were included in each round of testing. The negative controls were sand-filtered Sequim Bay seawater. For 48-h endpoints, negative control mortalities and abnormalities should not have been above 10% (EPA, 1992). For the sperm cell test the target fertilization rate was 60-90% (Dinnell et al., 1987). Control fertilization should have been within this range. Positive controls, indicating organism sensitivity, were copper ( $\text{CuSO}_4$ ) dilutions of 2.7, 9.0, 30.0, and 100.0  $\mu\text{g/l}$ . EC50 determinations for copper, using the trimmed Spearman-Kärber method (Hamilton et al., 1977), were included in each bioassay and the results compared to previous *D. excentricus*

EC50s in the literature to determine test sensitivity. Acceptable copper control LC50 ranges were 0.1 - 9226.3  $\mu\text{g Cu/l}$  for mortality and abnormality and 18.7 - 37.3  $\mu\text{g Cu/l}$  for the sperm cell test (Dinnel et al., 1989; EPA, 1986). Brine controls were included for assays including brine corrected samples. Immediately following initiation of the 48-h test, control fertilization was checked. If fertilization was less than 90%, the test was rejected.

Water quality parameters were measured for 100% dilutions in each test prior to initiation and daily throughout the test to maintain proper test conditions. Water quality instruments were calibrated daily (DO, pH) or monthly (T, S). Acceptable ranges for water quality parameters were:

temperature (T)	15°C±1.0°C
dissolved oxygen (DO)	≥4.0 mg/l
pH	ambient ±0.5 units
salinity (S)	26.0-32.0 ppt

Data were entered onto a computer spreadsheet, and all entries were independently reviewed. Means with standard deviations higher than 10% had sample counts reviewed. Any station value which visually appeared to be an outlier and had cause for rejection was substituted with the bay mean to preserve the balanced statistical design. Sites D-33 and P-2 both had samples which may have been contaminated and were substituted with the bay mean.

Wastes were disposed of in accordance with Battelle Northwest Hazardous Waste Disposal protocols (Battelle Northwest Laboratories, 1990). Wastes were held to a minimum, with fixatives and control toxicants being chosen both for their effectiveness and relative safety. Copper was selected as the reference toxicant because it is effective at concentrations below water quality criteria (EPA, 1986). Wherever possible, Lugol's solution was substituted for formaldehyde, and the concentration of Lugol's solution used

was reduced to 3 drops/10 ml with echinoderm embryos. Using scintillation vials and the inverted microscope for analyzing 48-h embryos was a non-intrusive technique, allowing the same larvae to be used for later cytogenicity analysis, thus reducing the amount of formaldehyde necessary.

## RESULTS

In laboratory trials, the screen sampler efficiency was 64% for the plankton extract in sunflower oil and 55% for *Lycopodium* spores (Appendix 1 and 2). These results are consistent with previous studies using screen samplers (van Vleet and Williams, 1980).

### *Deposition Experiments*

Five spore dispersal experiments were performed (Table 2). Prior to dispersal, no spores were found in surface films (SL-before dispersal) or in the bulkwater (BW-before dispersal). Following dispersal, no spores were found in bulkwater samples (BW-after dispersal); however, in surface film samples (SL-after dispersal) the mean spore count was 6,896 spores/ml. This indicates successful labeling of the surface film. The mean spore density for surface deposit samples was 12,711 spores/ml and the mean SD:SL ratio was 3.7. Both the surface deposit and surface film spore densities were significantly higher than in the bulkwater ( $t_{SL}=5.609$ ,  $t_{SD}=6.323$ ,  $\alpha < 0.05$ ), and the surface deposit spore densities were significantly greater than the surface film spore densities ( $t=2.468$ ,  $\alpha < 0.05$ ). During the deposition experiments, spores were observed moving shoreward and stranding on the beach, adjacent to the sampler.

Table 2. Spores recovered by site (spores/ml) and surface deposit:surface film ratio.  
 S.E.: standard error; (before): before dispersal; (after): after dispersal.

SITE	BULK WATER (before)	BULK WATER (after)	SURFACE FILM (before)	SURFACE FILM (after)	SURFACE DEPOSIT	SD/SL
1	0	0	0	8,103	27,355	3.4
2	0	0	0	896	9,353	10.4
3	0	0	0	8,869	15,006	1.7
4	0	0	0	14,454	5,734	0.4
5	0	0	0	2,157	6,109	2.8
<b>MEAN (S.E.)</b>	<b>0 (0)</b>	<b>0 (0)</b>	<b>0 (0)</b>	<b>6,896 (1,200)</b>	<b>12,711 (1,798)</b>	<b>3.7 (0.8)</b>

### ***Toxicity Tests***

Percent response for 48-h mortality, abnormality, and sperm cell viability were determined for all dilutions (Appendices 3-8). Because responses were seldom above 50%, statistical analyses were performed on data from the undiluted samples (100% dilutions) rather than calculated EC50s. F statistics and t statistics for all comparisons are presented in Table 3.

***Comparisons Among Bays-Mortality.*** Significant differences in percent mortality were found among bays in both surface deposit and surface film samples, while no significant differences were found in bulkwater samples (Table 4, Figure 9). Commencement Bay surface deposit and surface film samples had significantly higher *D. excentricus* larval mortality than either Padilla Bay or Discovery Bay samples. Padilla Bay surface deposit mortalities were significantly higher than in Discovery Bay deposits. Surface films in Discovery Bay caused a higher response than Padilla Bay surface films. Overall, the highest larval mortalities were found in samples from Commencement Bay's inner harbor (T-16, T-11, T-8) and north shore (T-3), while the lowest mortalities were in Discovery Bay samples (Table 5).

Table 3. Table of F and t statistics for toxicity tests.

Comparison	Statistic	CR	Comparison	Statistic	CR
<b>3a. Among Bay Comparisons</b>					
48-M-SD	F= 8.96	4.10	CB-PB	t= 3.950	1.812
			PB-DB	t= 3.613	1.812
48-M-SL	F= 6.38	4.10	CB-PB	t= 5.930	1.812
			PB-DB	t= 2.227	1.812
48-M-BW	F= 1.52	4.10			
48-A-SD	F= 4.45	4.10	CB-PB	t= 3.045	1.812
			PB-DB	t= 1.768	1.812
48-A-SL	F= 7.50	4.10	CB-PB	t= 3.774	1.812
			PB-DB	t= 1.732	1.812
48-A-BW	F= 0.24	4.10			
SCT-SD	F=14.65	4.10	CB-DB	t= 8.213	1.812
			DB-PB	t= 0.318	1.812
SCT-SL	F= 3.14	4.10			
SCT-BW	F= 9.25	4.10	CB-PB	t= 3.227	1.812
			PB-DB	t= 3.794	1.812
GEN-MI	F=14.32	3.41	CSW-PB	t= 1.161	2.447
			PB-DB	t= 0.243	2.447
			DB-CB	t= 5.268	2.447
GEN-CL	F= 3.26	3.34			
GEN-AA	F=15.35	3.34	CSW-DB	t= 1.414	2.447
			DB-PB	t= 2.754	2.447
			CB-PB	t= 5.767	2.447

48-M: 48-h test (mortality); 48-A: 48-h test (abnormality); SCT: sperm cell test; GEN: cytotoxicity bioassay; SD: surface deposit; SL:surface film; BW: bulkwater; MI: mitotic index; CL: % cytologic abnormalities; AA: % anaphase aberrations; DB: Discovery Bay; PB: Padilla Bay; CB: Commencement Bay; CSW: control seawater.

Comparison	Statistic	CR	Comparison	Statistic	CR
<b>3b. Among Layer Comparisons</b>					
48-M-CB	F= 5.03	4.10	SD-SL	t= 1.100	1.812
			SL-BW	t= 13.932	1.812
48-M-PB	F= 0.53	4.10			
48-M-DB	F= 5.59	4.10	SD-SL	t= 1.065	1.812
			SL-BW	t= 2.136	1.812
48-A-CB	F= 9.23	4.10	SD-SL	t= 0.771	1.812
			SL-BW	t= 3.342	1.812
48-A-PB	F= 1.15	4.10			
48-A-DB	F=10.02	4.10	SD-SL	t= 0.134	1.812
			SL-BW	t= 1.315	1.812
SCT-CB	F= 8.94	4.10	SD-SL	t= 0.423	1.812
			SL-BW	t= 1.351	1.812
SCT-PB	F= 5.33	4.10	SD-SL	t= 1.125	1.812
			SL-BW	t= 4.401	1.812
SCT-DB	F= 3.33	4.10			

48-M: 48-h test (mortality); 48-A: 48-h test (abnormality); SCT: sperm cell test; SD: surface deposit; SL: surface film; BW: bulkwater; DB: Discovery Bay; PB: Padilla Bay; CB: Commencement Bay.

Table 4. Statistical results for station mean % response comparisons for three bioassays among bays, within layers. DB: Discovery Bay; PB: Padilla Bay; CB: Commencement Bay.

LAYER	MORTALITY			ABNORMALITY			SPERM CELL TEST		
	DB	PB	CB	DB	PB	CB	DB	PB	CB
BULKWATER	<u>0.0</u>	<u>4.9</u>	<u>9.9</u>	<u>0.6</u>	<u>7.0</u>	<u>4.8</u>	0.7	2.1	16.4
SURFACE FILM	5.1	0.3	24.2	<u>3.9</u>	<u>10.1</u>	21.8	<u>9.3</u>	<u>7.9</u>	<u>32.2</u>
SURFACE DEPOSIT	2.2	12.8	37.0	<u>3.1</u>	<u>10.0</u>	30.6	<u>8.7</u>	<u>5.4</u>	39.9

values joined by underlines: no significant difference among bays (within layers)

## ECHINODERM MORTALITY INTER-BAY COMPARISON

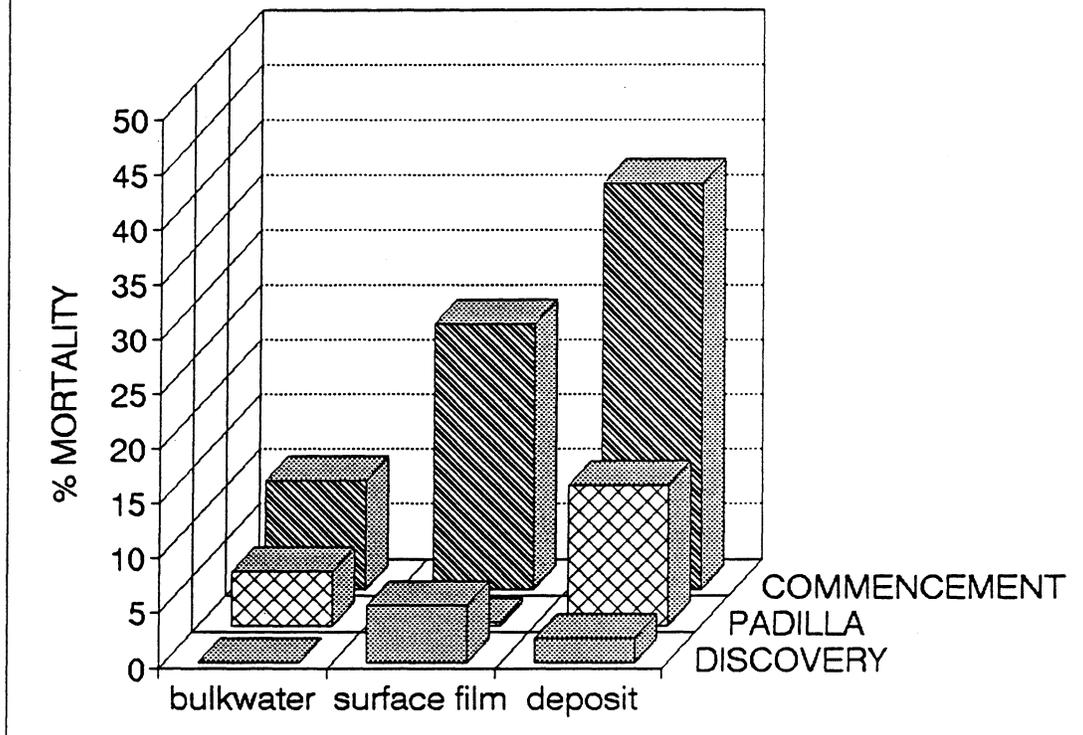


Figure 9. Inter-bay comparison of percent mortality.

Table 5. Dendraster percent mortality by station and means. S.E.: Standard error.

STATION	BULKWATER	SURFACE FILM	SURFACE DEPOSIT
<b>DISCOVERY BAY</b>			
D-27	0.0	7.0	6.6
D-33	*0.0	*5.1	*2.2
D-11	0.0	0.0	0.0
D-22	0.0	6.5	4.4
D-5	0.0	0.0	0.0
D-25	0.0	12.0	0.0
MEAN (S.E.)	0 (0.0)	5.1 (0.8)	2.2 (0.5)
<b>PADILLA BAY</b>			
P-2	0.0	0.0	41.7
P-6	0.0	0.0	0.9
P-17	19.9	0.0	4.5
P-9N	0.0	0.0	29.5
P-4	0.0	0.0	0.0
P-9S	9.4	1.9	0.0
MEAN (S.E.)	4.9 (1.4)	0.3 (0.8)	12.8 (3.0)
<b>COMMENCEMENT BAY</b>			
T-8	0.0	3.9	31.7
T-11	0.0	21.0	29.3
T-22	21.3	41.8	15.3
T-16	0.0	44.4	85.0
T-25	21.2	3.1	23.3
T-3	1.3	30.8	37.1
MEAN (S.E.)	9.9 (2.6)	24.2 (3.0)	37.0 (4.1)
* mean substituted for value			

*Abnormality.* Significant differences in percent abnormality were found among bays in both surface deposit and surface film samples, while no significant differences were found in bulkwater samples (Table 4, Figure 10). Commencement Bay surface deposit and surface film samples had significantly higher rates of larval abnormality than in either Padilla or Discovery Bay samples. Padilla Bay had intermediate rates of abnormality in both surface deposits and surface film samples; however, no significant differences were found when compared to Discovery Bay samples. Overall, the highest rates of larval abnormalities were found in samples from Commencement Bay's inner harbor (T-16, T-11, T-8) and north shore (T-3) and the Bayview sample in Padilla Bay (P-9S), while the lowest rates of abnormality were in Discovery Bay (Table 6). The most common abnormalities were skeletal; however exogastrulae, and underdeveloped larvae were also seen.

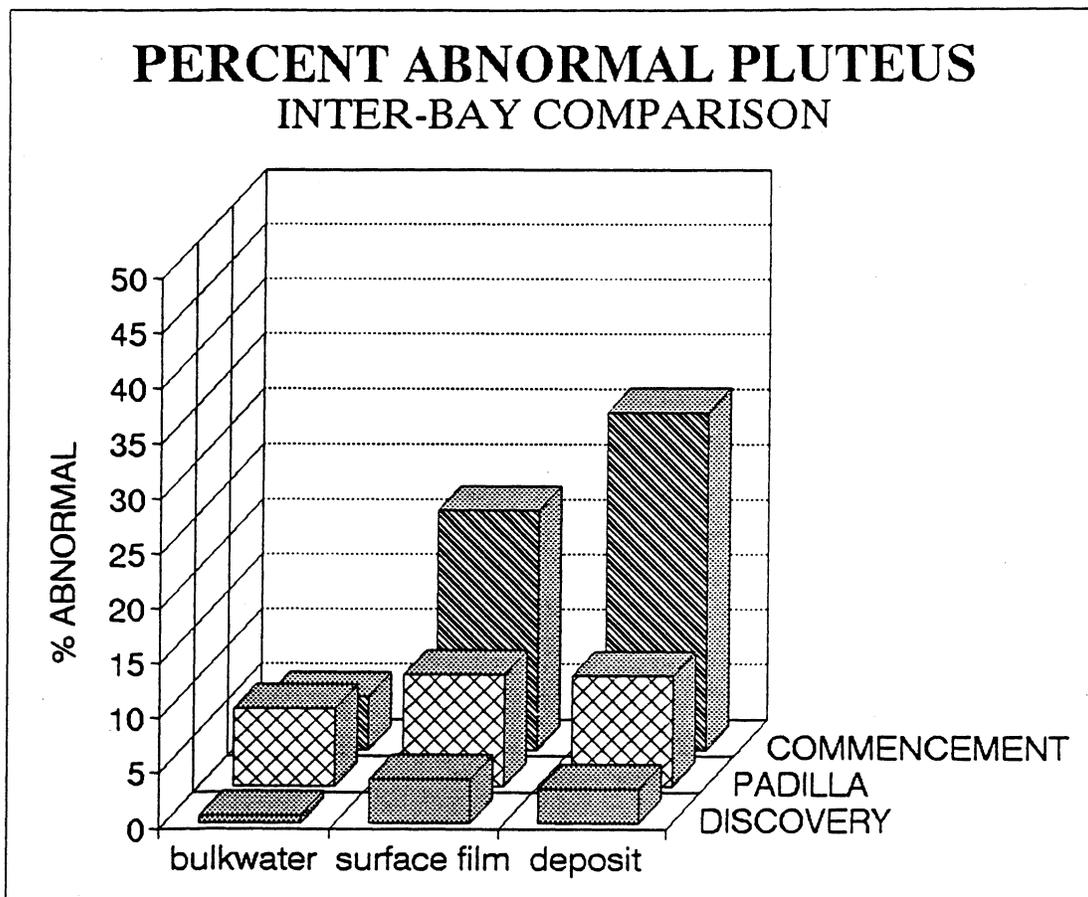


Figure 10: Inter-bay comparison of percent abnormal pluteus.

Table 6. Dendraster percent abnormality by station and means. S.E.: Standard error.

STATION	BULKWATER	SURFACE FILM	SURFACE DEPOSIT
<b>DISCOVERY BAY</b>			
D-27	2.0	11.4	6.4
D-33	*0.6	*3.9	*3.1
D-11	0.0	0.0	0.0
D-22	0.0	0.9	2.2
D-5	0.7	7.2	6.9
D-25	0.0	0.0	0.0
MEAN (S.E.)	0.6 (0.2)	3.9 (0.8)	3.1 (0.5)
<b>PADILLA BAY</b>			
P-2	5.2	8.7	*10.0
P-6	2.8	5.1	4.8
P-17	9.2	5.9	9.4
P-9N	0.0	14.2	5.5
P-4	10.4	6.7	4.9
P-9S	14.1	20.3	25.5
MEAN (S.E.)	7.0 (0.9)	10.1 (1.0)	10.0 (1.3)
<b>COMMENCEMENT BAY</b>			
T-8	0.0	26.4	32.0
T-11	13.8	29.2	37.0
T-22	0.0	6.1	6.1
T-16	0.0	28.3	69.9
T-25	6.9	9.2	12.6
T-3	8.1	31.7	25.8
MEAN (S.E.)	4.8 (1.0)	21.8 (1.9)	30.6 (3.8)
* mean substituted for value			

*Sperm Cell Test.* Significant differences in percent response (% unfertilized) were found among bays in surface deposit, surface film, and bulkwater samples. Commencement Bay consistently had the highest response (Table 4, Figure 11) and was significantly different in surface deposit and bulkwater samples. Both Padilla Bay and Discovery Bay surface deposit and surface film samples had similar responses. Bulkwater samples had significant responses in both Commencement and Padilla Bays. Overall, the highest responses were seen in the samples from the Puyallup River (T-16) and on the south shore (T-22) in Commencement Bay, near March Point in Padilla Bay (P-17), and in the southern tip (D-22) of Discovery Bay (Table 7).

# ECHINODERM SPERM CELL TEST INTER-BAY COMPARISON

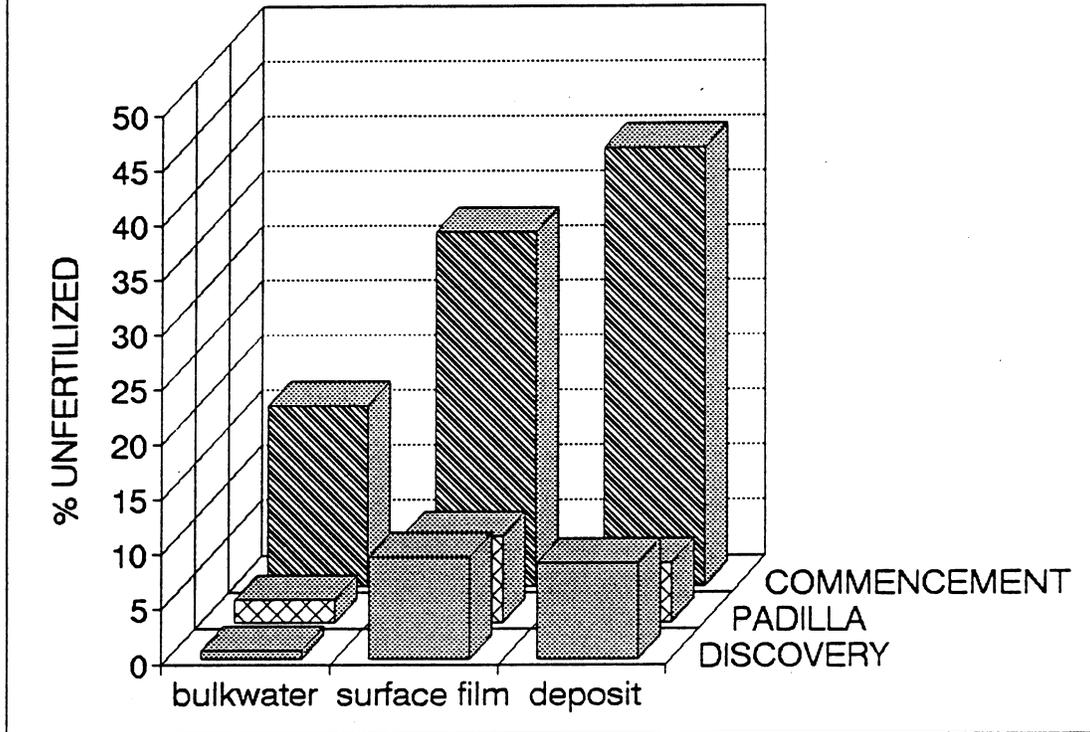


Figure 11. Inter-bay comparison of sperm cell test response.

Table 7. Dendraster sperm cell test response (% unfertilized) by station and means S.E.: Standard error.

STATION	BULKWATER	SURFACE FILM	SURFACE DEPOSIT
<b>DISCOVERY BAY</b>			
D-27	1.0	1.0	12.9
D-33	*0.7	*9.3	*8.7
D-11	1.4	4.1	6.3
D-22	1.1	21.0	12.3
D-5	0.0	1.5	0.0
D-25	0.0	18.8	11.9
MEAN (S.E.)	0.7 (0.1)	9.3 (1.6)	8.7 (0.9)
<b>PADILLA BAY</b>			
P-2	*2.1	*7.9	*5.4
P-6	0.0	9.9	9.2
P-17	2.0	14.5	10.2
P-9N	0.0	1.5	0.0
P-4	6.5	10.5	7.5
P-9S	2.2	3.0	0.0
MEAN (S.E.)	2.1 (0.4)	7.9 (0.9)	5.4 (0.8)
<b>COMMENCEMENT BAY</b>			
T-8	16.1	17.2	24.0
T-11	1.3	11.5	17.4
T-22	35.2	49.9	79.3
T-16	37.8	81.9	94.8
T-25	4.4	18.5	16.7
T-3	3.7	14.1	7.0
MEAN (S.E.)	16.4 (2.7)	32.2 (4.7)	39.9 (6.2)
* mean substituted for value			

*Cytogenicity/Cytotoxicity.* Commencement Bay surface deposit samples had significantly higher rates of mitotic and anaphase abnormalities than Padilla and Discovery Bay surface deposits and Sequim Bay controls (Table 8). The mitotic index (mitoses/embryo) was reduced from 17.9 in the controls to 8.0 in Commencement Bay samples (Figure 12a), while percent cytogenetic aberrations per anaphase increased from 2.6% in the controls to 36.2% (Figure 12c). Although the number of cytologic abnormalities per embryo increased from 2.7 in the controls to 5.2 in Commencement Bay samples, the difference was not significant (Figure 12b). Padilla Bay had significantly higher rates of anaphase aberrations (8.0%) than the controls. Little or no cytogenetic effects were seen in Discovery Bay. The most common cytogenetic aberrations seen were translocation bridges and side arm bridges. The most common cytologic abnormalities were pycnotic and dedifferentiated cells.

Table 8. Cytotoxicity test results for surface deposit samples and controls; mean (S.E.), n=5.

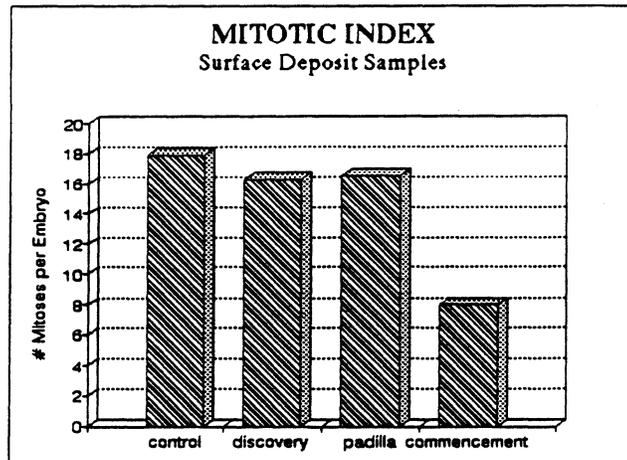
EXPOSURE	% MORT	% ABN	$\bar{x}$ M/emb	$\bar{x}$ CL/emb	% CG/ana	% M w/CG
CONTROL SW	NA	NA	17.9 (0.8)	2.7 (0.2)	2.6 (0.1)	0.6 (0.1)
DISCOVERY	2.2 (0.5)	3.1 (0.5)	16.3 (0.3)	2.8 (0.3)	2.3 (0.3)	0.6 (0.1)
PADILLA †	12.8 (3.0)	10.0 (1.3)	16.4 (0.6)	3.0 (0.5)	8.0 (3.1)	1.9 (0.7)
COMMENCEMENT	37.0 (4.1)	30.6 (3.8)	8.0 (0.1)	5.2 (0.4)	36.2 (3.1)	11.3 (1.2)

†: n=3; NA: not applicable, calculated from Abbot's formula;

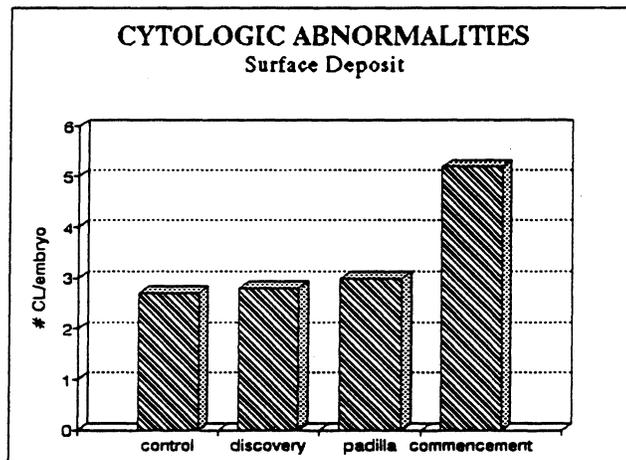
% MORT: % mortality; % ABN: % abnormality;  $\bar{x}$  M/emb: mean mitoses per embryo;

$\bar{x}$  CL/emb: mean cytologic abnormalities per embryo; % CG/ana: percent anaphase aberrations;

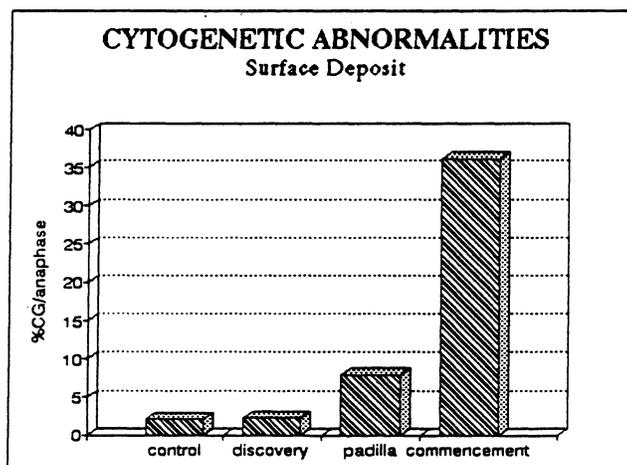
% M w/CG: % mitoses with cytogenetic aberrations.



a.



b.



c.

Figure 12. Cytotoxicity/cytogenicity test results.

*Comparisons Among Layers.* There were no significant differences between surface deposit and surface film samples within bays for all three bioassays (Table 9). Surface deposit and surface film samples had significantly higher mortalities than bulkwater samples in both Commencement and Discovery Bays, while Padilla Bay had no significant differences in either percent mortality or percent abnormality among the layers. Commencement Bay surface deposits and films had significantly higher rates of larval abnormality than the bulkwater samples. Surface deposit sperm cell test responses were significantly higher than bulkwater samples in all three bays.

Table 9. Statistical results for station mean comparisons for three bioassays among layers, within bays. BW: bulkwater; SL: surface film; SD: surface deposit.

EMBAYMENT	<u>MORTALITY</u>			<u>ABNORMALITY</u>			<u>SPERM CELL TEST</u>		
	BW	SL	SD	BW	SL	SD	BW	SL	SD
DISCOVERY	0.0	<u>5.1</u>	<u>2.2</u>	<u>0.6</u>	<u>3.9</u>	<u>3.1</u>	0.7	<u>9.3</u>	<u>8.7</u>
PADILLA	<u>4.9</u>	<u>0.3</u>	<u>12.8</u>	<u>7.0</u>	<u>10.1</u>	<u>10.0</u>	2.1	<u>7.9</u>	<u>5.4</u>
COMMENCEMENT	9.9	<u>24.2</u>	<u>37.0</u>	4.8	<u>21.8</u>	<u>30.6</u>	<u>16.4</u>	<u>32.2</u>	<u>39.9</u>

values joined by underlines: no significant difference among layers (within bays)

*Positive and Negative Controls.* Positive and negative control data are presented in Appendixes 10 and 11, respectively. Copper control mortality and abnormality LC50s ranged from 13.3 to 38.5  $\mu\text{g Cu/l}$  and were within control limits of 0.1 - 9,226.3  $\mu\text{g Cu/l}$ . Sperm cell test copper EC50s ranged from 9.5 to 52.6  $\mu\text{g Cu/l}$  and were higher than the control limits of 18.7 - 37.3  $\mu\text{g Cu/l}$  in rounds 2, 3, and 4. Negative seawater control responses were above 10% in some of the 48-h tests; however, in most cases it was less than 15% and did not appear to compromise results. Control response in the SCT was acceptable in all rounds except round 1, which had 89.7% response. Test SCT-1 did appear to be more sensitive.

#### **Metals Analysis**

Results from the metals analysis are presented in Appendix 12. Although some individual stations had higher concentrations of some metals, there were no overall trends.

## DISCUSSION

The modified screen sampler proved to be an effective collector of surface films as they deposited onto intertidal substrates. In laboratory tank trials, screen efficiency was 64% for oils and 55% for *Lycopodium* spores. This is consistent with previous efficiencies observed for screen samplers (van Vleet and Williams, 1980). The deposit screen sampler offers a relatively easy method for collecting materials as they deposit onto intertidal habitats and promises to be a useful tool in assessing natural depositional processes, such as carbon and material transport from enriched surface films to biologically active intertidal sediments. The surface deposit sampler could also be used effectively in tracking xenobiotic movement from aquatic to intertidal ecosystems. Transport and fate of floatable contaminants following spills or accidental releases, such as oil spills, may be studied with the screen sampler, as well.

### *Surface Film Deposition*

The surface film labeling experiments clearly showed that surface films, driven by onshore winds, deposit onto intertidal substrates during tidal ebb. The *Lycopodium* spores proved to be a surface specific material, with no spores being found in bulkwater samples. Significantly higher spore densities in both surface film and surface deposit samples suggested that spores stranding on the deposit samplers and intertidal substrates originated from the surface film. This finding is consistent with earlier studies in which Word and Ebbesmeyer (1984) found similar levels of pentane extractable materials (PEM) in both sewage-influenced shoreline slicks and neighboring beach sediment interstitial waters. Coupling the PEM enhancements with drift card strandings, Word and Ebbesmeyer concluded that floatable fractions of sewage effluent move shoreward to deposit onto and

percolate through beach sediments. Shanks (1983) found that surface slicks transported styrofoam drogues shoreward 1 to 2 km in 2 to 3 hours. Associated with these slicks were large populations of gammarid amphipods, and cyprid, crab, and reef fish larvae (Shanks and Wright, 1987; Kingsford and Choat, 1986), which ride internal waves to the surface and then migrate shoreward within the surface slicks. Shanks (1987) also found that tar balls, associated with internal wave-driven slicks, move shoreward, eventually becoming stranded.

Surface deposits had higher spore densities than did the labeled film samples. Patchy distributions during spore dispersal may account for this disparity. Although adjacent surface areas were sampled to reduce variability, some differences in sampled films may exist. Experiment 4 did have higher surface film spore densities than the corresponding surface deposit sampler. A more probable explanation is that the deposit sampler was collecting a larger surface area than the surface film sampler. The surface film sampler was lifted from the water, capturing a discrete area, whereas the deposit sampler collected films as the tide receded, integrating surface films over a longer time period. During this period, films were continually being driven shoreward and stranding on the sampler. The mean SD:SL ratio of 3.7 (Table 2) may have approximated a depositional magnification factor. This magnification was not necessarily an artifact of sampler design, but a simulation of natural depositional processes onto intertidal substrates.

#### *Echinoderm Toxicity*

Depositing and free floating surface films in Commencement Bay were significantly more toxic than those of either Padilla or Discovery Bays. This trend was consistent for *Dendraster excentricus* mortality, abnormality, sperm cell fertilization, and

cytogenetic/cytologic abnormalities.

Commencement Bay surface deposit responses ranged from 31-40% for mortality, abnormality, and the SCT. Embryos developing in Commencement Bay surface deposits had significantly reduced mitotic indices and significant increases in anaphase aberrations. Commencement Bay surface film responses ranged from 22-32% for mortality, abnormality, and the sperm cell test. Bulkwater samples produced significantly lower responses, ranging from 5-16% for mortality, abnormality, and the sperm cell test. Overall, the inner harbor of Commencement Bay had the highest levels of response; however, sites throughout the bay exhibited some toxicity.

Padilla Bay samples showed some surface deposit and surface film response, and were intermediate in their overall toxicity. Padilla Bay surface deposit responses ranged from 5-13% for mortality, abnormality, and the SCT. Embryos developing in Padilla Bay surface deposit samples displayed a significant increase in embryos with anaphase aberrations. Padilla Bay surface film responses ranged from 1-10% for mortality, abnormality, and the SCT. Bulkwater samples exhibited little toxicity, with responses ranging from 2-5% for the 48-h and SCT bioassays.

Discovery Bay, while having low toxicities, did show some response in surface film and deposit samples. Discovery Bay surface deposit responses ranged from 2-8% for mortality, abnormality, and the SCT. No significant cellular abnormalities were found in embryos incubated in Discovery Bay deposits. Discovery Bay surface film responses ranged from 4-9% for mortality, abnormality, and the SCT. Bulkwater responses were 0-1% for the 48-h and SCT bioassays.

The results of this study are consistent with earlier microlayer studies in Puget Sound. Hardy et al. (1987b) found zero percent normal urchin larvae in tests with glass

plate collected microlayer samples from Commencement Bay. In Sequim Bay controls, normal development was eighty percent. Higher mortalities than this study may be due to the thinner surface film (35-50  $\mu\text{m}$ ) collected by the glass plate sampler. Our screen sampler collects depths of 200-350  $\mu\text{m}$ . Decreased sampling depth is generally associated with higher contaminant enrichments. Alternatively, the lowered response in our surface film bioassays may be due to an actual reduction in surface film toxicity since 1987.

This study found mean percent *D. excentricus* anaphase aberrations of 36.2% in Commencement Bay and 2.0% in Sequim Bay controls. Padilla Bay had a mean percent anaphase aberration of 8.0%; however, this may be due primarily to one high value at site P-6. Hardy et al. (1987b) found 24.4% sole embryo abnormal anaphase in bioassays with Commencement Bay microlayer samples and 8.0% in Sequim Bay samples. Cytogenetic abnormalities may lead to cellular or larval death. While cellular mutations are not necessarily lethal when seen in the post-gastrulae stage, they do indicate the presence of mutagens in the microlayer samples (Hose, 1985). Cytologic abnormalities are a result of toxic insult (Hose, 1985) and were observed in tests with Commencement Bay surface deposits. The most common abnormalities were pycnotic and dedifferentiated cells. Pycnosis indicates irreversible cellular injury. Dedifferentiated cells in late stage embryos indicate cellular tissue disorganization or disintegration (Hose, 1985; Rosenthal and Alderdice, 1976). A wide variety of contaminants have been linked to echinoderm and fish anaphase aberrations, including cadmium, arsenic, styrene, benzo(a)pyrene, and sewage effluent constituents (Cross et al., 1987; Hose, 1985; Hose et al., 1983; Long et al., 1990; Pagano et al., 1982 a and b). Our results indicate the presence of both mutagenic and cytologic agents in Commencement Bay surface deposits, and the possibility of mutagenic agents in Padilla Bay.

Although *D. excentricus* may be exposed to surface deposits by extreme low tides, they are not the primary species at risk from surface film deposits. Sand dollar sensitivity to toxicants is, however, comparable to other intertidal species which may routinely be exposed to surface deposits. Several studies have directly compared the echinoderm larval, sperm cell, and genotoxicity bioassays with other intertidal invertebrate bioassays. Long et al. (1990) evaluated five toxicity tests with California sediments, and found the echinoderm cytogenicity test to produce an intermediate evaluation of toxicity. Among the most sensitive tests were *Mytilus edulis* abnormality and survival, and *Rhepoxynius abronius* survival. Echinoderm abnormality was considered less sensitive; however, it was similar in response to *Ampelisca abdita* survival and the *Dinophilus gyrociliatus* egg laying test. A comparison of sediment bioassays in Puget Sound (Pastorok and Becker, 1989) used the relative rankings of low, medium, and high dose response and found *D. excentricus* developmental and chromosomal abnormality endpoints to have a medium response. *R. abronius* mortality, *Eohaustorius estuarius* mortality and reburial, *Neanthes arenaceodentata* biomass, *Crassostrea gigas*/*M. edulis* abnormality, and Microtox had high dose responses, while *N. arenaceodentata* mortality had medium dose response. The majority of contaminants tested were low and high molecular weight PAHs. Hose (1985) notes that the sensitivity of the sea urchin anaphase aberration test is comparable to the more commonly used aquatic genotoxicity tests, the sister chromatid exchange (SCE) and fish chromosomal aberrations. A comparison of LC50 and EC50 data provides a further evaluation of the *D. excentricus* bioassays relative to other intertidal invertebrates. Acute and chronic copper and acute DDT LC50s are reported in Table 10.

From the above studies, it is apparent that *Dendraster excentricus* is a sensitive species to a variety of toxicants, and that the sand dollar responses are comparable to

other intertidal species. Results from this study, therefore, do indicate that toxicity is introduced by surface film deposits into intertidal habitats. The three echinoderm bioassays utilized in this study may not only demonstrate specific developmental, reproductive, and cellular effects, but also more general deleterious effects experienced in the intertidal region.

Table 10: Acute and chronic copper (EPA, 1985) and acute DDT LC50 data (EPA, 1980).

Species	LC50 ( $\mu\text{g Cu/l}$ )		DDT ( $\mu\text{g/l}$ )
	ACUTE	CHRONIC	
<i>Dendraster excentricus</i> (SCT)	26.4	-	-
<i>Dendraster excentricus</i> (embryo)	33.3	24.5(48h)	>17.4
<i>Crassostreas gigas</i> (SCT)	6.3	-	0.4
<i>Crassostreas spp.</i> (embryo)	26.5	46.0(12d)	>4.6
<i>Mytilus edulis</i> (embryo)	5.8	-	>17.2
<i>Neanthes arenoceodentata</i>	150.6	56.0(28d)	-
<i>Phyllodoce maculata</i>	120.0	40.0(26d)	-
<i>Mya arenaria</i>	39.0	35.0(7d)	-
<i>Arcatia spp.</i>	41.5	102.0(48h)	-
<i>Cancer magister</i> (larvae)	49.0	-	1.1
<i>Mercenaria mercenaria</i>	-	30.0(8d)	-
<i>Macoma inquinata</i>	-	17.5(30d)	-
<i>Ampelisca abdita</i>	-	90.0(7d)	-
<i>Euphasia pacifica</i>	-	22.0(24h)	-
<i>Crangon septumspinosa</i>	898.3	-	0.6

### Implications

Considering the transport and fate of surface film materials in coastal regions, the deposition of film materials during tidal recession plays an important role. As discussed in the introduction, surface slicks concentrate marine, atmospheric, and terrigenous materials. Internal waves, gravity waves, wind, and surface currents drive these highly concentrated surface materials toward coastlines. Internal waves and wind appear to be the primary

forces, modified by gravity waves and currents (Lange and Huhnerfuss, 1978). This study shows that a coating of surface film materials is then deposited onto the substrate. Potentially high amounts of marine carbon, nutrients, lipids, proteins, detritus, zooneuston, phytoneuston, bacteria, larvae, and anthropogenic contaminants could then become incorporated in the littoral ecosystem.

Chemical behavior in sediments is extremely complex and is dependent upon a variety of factors, such as sediment grain size and carbon content, chemical partitioning, bioturbation, biotransformation, temperature, and synergistic interactions with other chemicals. Over time, an equilibrium between sediment-bound and free interstitial pore-water concentrations is established (van der Kooij et al., 1991). Equilibrium concentrations will vary depending upon sediment grain size and carbon content, and the octanol-water partitioning coefficient of the chemical (DiToro et al., 1991). In intertidal regions, chemical behavior is made more complex by tides and wave action. However, if we assume partitioning between pore water and sediment adsorption in intertidal sediments, we can discuss deposit behavior and bioavailability in a variety of substrate types.

In soft substrates, deposits may percolate through sediments and adhere to the granules, with both the sediments and the interstitial water becoming enriched with organic, inorganic, and anthropogenic materials (Word et al., 1988). Muddy substrates do not readily drain and may trap those deposits which are able to penetrate (Gray, 1981). Clay may act much as a hard substrate, repelling liquid penetration. Rocky intertidal substrates may become coated with deposits, remaining until subsequent tidal cycles. During periods of diminishing tidal heights, deposit-enhanced interstitial waters and coated substrates could remain for 24 or more hours.

Both intertidal epifauna and infauna are potentially exposed to surface deposits. Direct exposure is possible in hard substrates and fast draining sediments, such as cobble. In soft sand and mud sediments, routes of exposure to deposit materials may include contact, respiration of deposit-enhanced interstitial waters, and feeding on deposits adsorbed onto interstitial particulates or sediment granules (Levinton, 1982). Intertidal marine fauna, living and feeding between sedimentary granules, are most likely to contact deposits. These species include ciliates, hydrozoans, turbellarians, nematodes, polychaetes, harpacticoid copepods, and ostracods (Kozloff, 1983). Intertidal hard and soft substrate grazers move and feed on the substrate and may feed directly on deposit-coated surfaces (Levinton, 1982). In coastal wetlands, a variety of algal species could become coated with deposits when exposed at low tides. Seagrass species and their associated fauna have been found to be sensitive to various anthropogenic materials (Reish et al., 1988). Coral reefs, exposed during low tides, may also interact with surface deposits. Flora and fauna which do contact organically enriched surface deposits may exploit a rich food source. In urban-influenced intertidal habitats they are also exposed to a source of toxicity, through direct contact, ingestion, or respiration.

### ***Shorelines at Risk***

Wind, surface current, and drift card data were combined with demographic information to predict shorelines at risk from contaminated surface slick deposition (Figures 13 and 14). A westerly wind flow dominates the Puget Sound region throughout most of the year, creating westerlies along the Strait of Juan de Fuca, northerlies in central and southern Puget Sound and southerlies in northern Puget Sound (Downing, 1983; Kopenski and Long, 1981). Low pressure cells off the Washington coast may result in reversals of this pattern to a more southerly flow (Ebbesmeyer et al., 1991). Winter

marine air enters the sound from the south, creating southerly winds in the north and south Sound and easterly air flow through the Strait of Juan de Fuca (Downing, 1983; Kopenski and Long, 1981). These dominant wind patterns may be modified by local topography. Both winter and summer patterns are represented by large arrows. Net surface patterns (0-10 m) are shown with small arrows (EPA, 1987).

Drift card data have been collected for several Puget Sound regions. Ebbesmeyer et al. (1979) released drift cards and drift sheets in Port Angeles harbor and from the tip of Ediz Hook during April, 1978, and found cards stranded predominantly on Dungeness Spit, its western shores, and on southern Vancouver Island, Canada. Cards were also found in Sequim and Discovery Bays, the northwestern shores of Whidbey Island, and the southwest shores of the San Juan Islands. In addition, Ebbesmeyer et al., (1979) found pulp mill effluent from Port Angeles in Dungeness Spit waters. Cox et al. (1980) released cards at points along two proposed oil pipeline routes in July, 1980. Release points included Port Angeles harbor to Dungeness Spit and east near Protection Island to the western shore of Whidbey Island, near Marrowstone Island and northern Camano Island. Cards released from Port Angeles to Whidbey Island and Marrowstone Island were recovered from Ediz Hook to Dungeness Spit, Sequim and Discovery Bays, western Whidbey Island, Fidalgo Head, throughout the San Juan Islands, and southern Vancouver Island. Camano Island releases were recovered on eastern Whidbey Island shores, Camano Island, and shorelines near Mt. Vernon and Everett. Ebbesmeyer et al. (1984) released cards at the site of the proposed Seahurst outfall, south of Elliott Bay. Recoveries were along the eastern shores of the East Passage, Vashon and Maury Island, Bainbridge Island, Whidbey Island, Elliott Bay and shorelines near Edmonds. These drift card observations were factored into the predicted surface contaminant movements in

Figures 13 and 14.

This conceptual model estimates shorelines at risk from "chronic" sources of pollutants. These sources include both urban areas and EPA Regions of Concern (EPA, 1987). Event-related contaminants, such as oil spills or non-point source pollution, are not included in these predictions. The model also assumes that the residence times of surface contaminants are equivalent to those of drift cards. Risks in remote areas requiring long transport times, such as the San Juan Islands, may be overestimated.

The shorelines-at-risk conceptual model represents the broader significance of contaminated surface film deposition. Based upon the deposition and toxicity test results from this study and historic Puget Sound drift card and wind data, both urban and rural shorelines may receive contaminated surface deposits. Those Puget Sound embayments which have previously been shown to have contaminated surface films, such as Elliott Bay, Commencement Bay, Port Gardner, Port Angeles (Hardy et al., 1987a and b), and those which could be predicted to have contaminated surface films, such as Budd Inlet, Sinclair Inlet, Eagle Harbor, March Point, and Bellingham Bay, would be most likely to experience contaminated surface film deposition on their shorelines. Beaches adjacent to or downwind of these regions are also predicted to receive contaminated surface film deposition, due to the responsiveness of surface films to wind and surface influences. It is interesting to note that regions which are predicted to receive contaminated surface film deposition overlap those shorelines which have conditionally certified, decertified, or uncertified shellfisheries, and bivalve advisories (EPA, 1987).

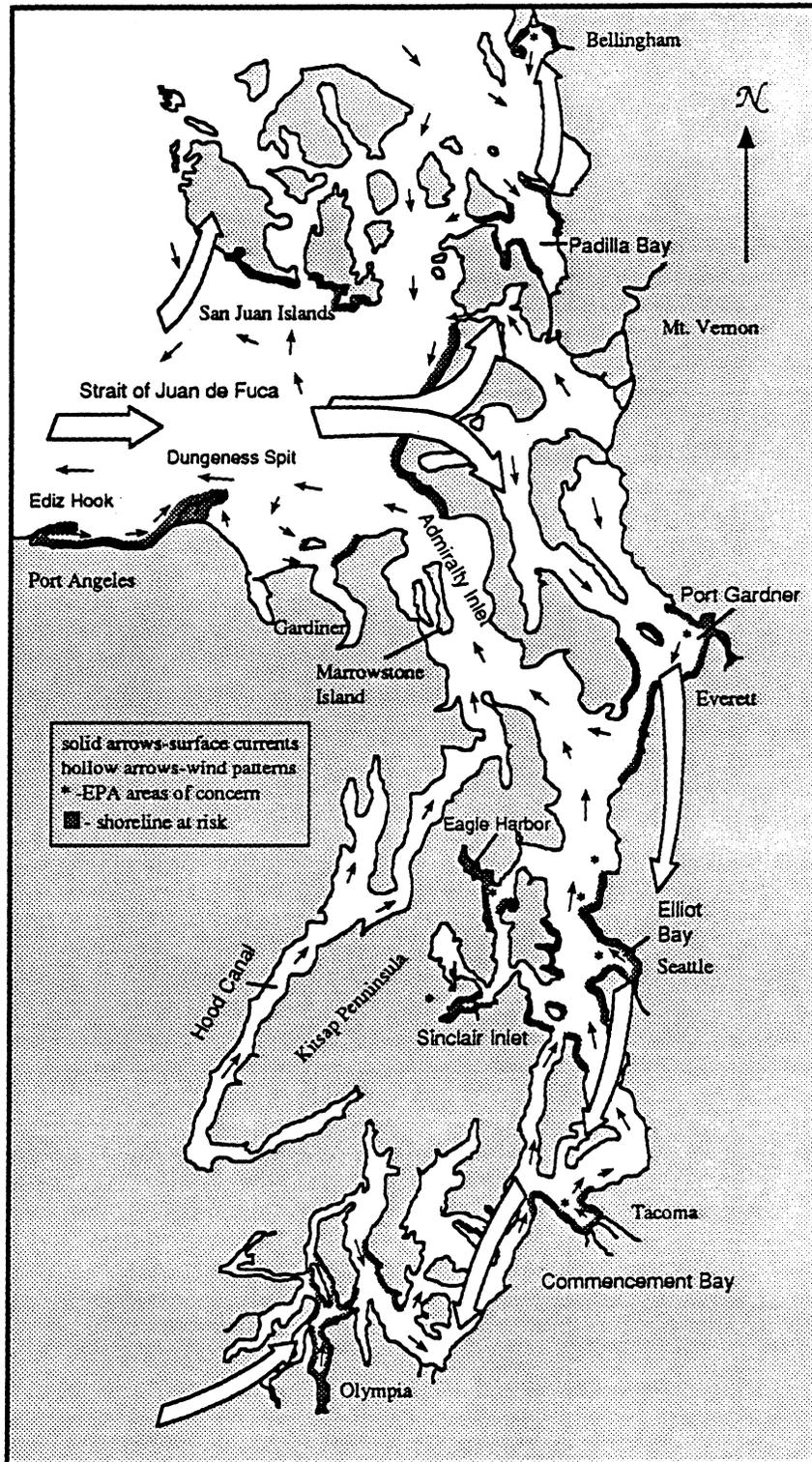


Figure 13. Puget Sound shorelines at risk- summer wind patterns.

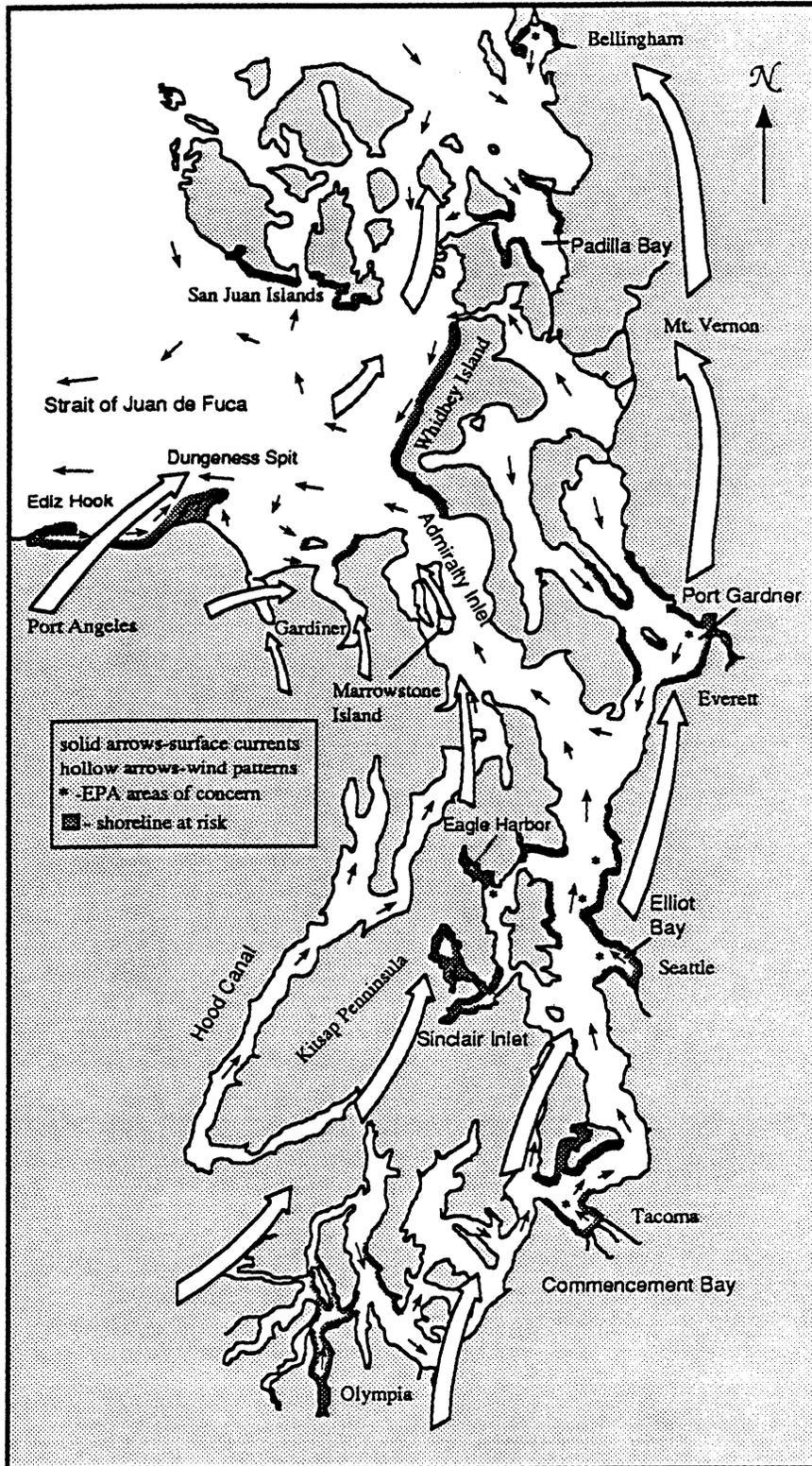


Figure 14. Puget Sound shorelines at risk- winter wind patterns.

## CONCLUSIONS

This study begins to fill an important gap in surface film research and in determining the fate and transport of surface materials. Previous studies have demonstrated that atmospheric, terrigenous, and marine materials concentrate in surface films. In some nearshore and offshore regions, surface materials may include anthropogenic contaminants, which have been shown to exhibit toxic effects to marine organisms. Winds, waves, and currents transport these films laterally across the sea surface. In coastal areas, this often results in shoreward transport. This study demonstrated that surface films, loaded with natural and anthropogenic materials, then deposit onto intertidal substrates. These surface deposits may then become integrated into the intertidal habitat. In regions with sources of surface contamination, these deposits also represent a source of toxicity to intertidal organisms.

Although this scenario may seem complete, there are many areas which require further research. The processes of shoreward transport and deposition need to be further investigated. Rates of deposition and the processes which govern film stranding would aid in understanding the degree of material transport from the marine system to the littoral zone. Chemical fate and partitioning behavior are both critical to our understanding of contaminant bioavailability in sediments, and are areas of current research (DiToro et al., 1991; van der Kooij et al., 1991). Finally, further toxicological studies with a variety of test organisms are needed to better establish the extent and nature of surface deposit contamination throughout the year.

The major findings of this study were:

1. The deposition screen sampler is effective at collecting surface films during tidal ebb. The monodour screen collected 64% of available plankton/sunflower oil and 53% of available *Lycopodium* spores in simulated tidal recessions.
2. Sea surface films, successfully labeled by surface-active *Lycopodium* spores, deposit onto the deposit screen sampler and adjacent intertidal substrates. The mean surface deposit:surface film spore density ratio was 3.7, indicating a magnification of surface materials during deposition. This most likely represents a natural concentration process during stranding.
3. Significant increases in mortality, abnormality, anaphase aberrations and decreases in sperm cell viability and mitoses per embryo were seen in Commencement Bay surface deposit samples. Commencement Bay surface films had significantly higher rates of mortality and abnormality than either Padilla or Discovery Bay surface films. Commencement Bay bulkwater samples only showed significant toxicity in the sperm cell test. Commencement Bay surface deposits clearly exhibit significant toxicity, indicating that this bay would be an appropriate site to further study depositional toxicity.

4. Padilla Bay surface deposit samples had significant increases in *D. excentricus* mortality and significant reductions in mitoses. Padilla Bay bulkwater samples only showed significant toxicity in the sperm cell test. Overall, Padilla Bay samples were intermediate in their toxicity. Further study is necessary to evaluate the extent of surface deposit contamination within Padilla Bay.

5. No significant response was seen in any of the Discovery Bay samples, except surface film mortality. It appears as if Discovery Bay is currently not at risk from contaminated surface film deposition.

6. Within embayments, there were no significant differences among surface deposit and surface film samples. Surface deposit responses were significantly greater than bulkwater responses in all Commencement Bay bioassays. Commencement Bay surface film had higher responses than bulkwater in percent mortality and percent abnormality.

7. Based on wind, drift card, and demographic data, both urban and rural beaches in Puget Sound may receive contaminated surface deposit input.

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