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Concentration and Toxicity of Sea-Surface Contaminants in Puget Sound

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April 1986

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CONCENTRATION AND TOXICITY OF SEA-SURFACE
CONTAMINANTS IN PUGET SOUND

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SUMMARY

The water-sediment interface is a well-known area for the concentration of contaminants in aquatic environments. The air-water interface is a less well-known, but highly effective, collection point for a variety of organic and metal contaminants. Sources include wastewater and industrial discharges as well as atmospheric deposition. The sea-surface microlayer (SSM) (upper 50 μm of the sea surface) is an important biological habitat, especially for the reproductive stages of many fish and shellfish. Neustonic (surface living) eggs and larvae may be subjected to stress from exposure to sea-surface contaminants.

The Marine Research Laboratory conducted studies during CY 1985 to evaluate the effects of sea-surface contamination on the reproductive success of a valued marine species. Microlayer and bulk water samples were collected from a rural bay, central Puget Sound, and three urban bays and analyzed for a number of metal and organic contaminants as well as for densities of neuston and plankton organisms. Fertilized neustonic eggs of sand sole (Psettichthys melanostictus) were exposed to the same microlayer samples during their first week of embryonic and larval development. Also, we evaluated the effects of microlayer extracts on the growth of trout cell cultures.

Compared to rural sites, urban bays generally contained lower densities of neustonic flatfish eggs during the spawning season. Also, in contrast to the rural sites or the one central Puget Sound site, approximately half of the urban bay microlayer samples resulted in significant increases in embryo mortality (up to 100%), kyphosis (bent spine abnormalities) in hatched larvae, increased anaphase aberrations in developing embryos, and decreased trout cell growth. The toxic samples generally contained high concentrations of polycyclic aromatic and/or chlorinated hydrocarbons and/or potentially toxic metals. In some cases, concentrations of contaminants on the sea surface exceeded water-quality criteria by several orders of magnitude. Several samples of subsurface bulk water collected below highly contaminated surfaces showed no detectable contamination or toxicity.

The percent of embryos that developed and hatched into live larvae decreased with increasing microlayer contamination according to

$$\ln \% \text{ live larvae} = 4.43 - 0.000007 \text{ PAH} - 0.006 \text{ metal}$$
$$R^2 = 0.89, P = 0.0004$$

where PAH is the total concentration of polycyclic aromatic hydrocarbons (ng/L), and metal is the total concentration of Cu+Pb+Zn+Ag+Hg ($\mu\text{g/L}$).

Monitoring the SSM provides a sensitive indicator of relatively recent environmental contamination. The spatial and temporal extent of sea-surface pollution and its effects on overall fishery resources remain to be determined.

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This work was supported by the National Oceanic and Atmospheric Administration (NOAA), Ocean Assessments Division. We greatly appreciate the helpful suggestions and direction of Edward Long (NOAA) at the inception of and throughout this study. Invaluable technical assistance in the field sampling and bioassays was provided by Alan Stubin and Steven Kiesser. Kathleen Sabo conducted much of the cell growth and anaphase aberration effects studies. Charles Apts and James Gurtisen performed the metal and organic chemical analyses. David Carlile's statistical treatment of the data greatly increased the utility of the results. We thank Walter Pearson for his perceptive review of the original draft report.

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INTRODUCTION

The sea-surface microlayer (SSM) (upper 50 μm or less of the sea surface) is a vital biological habitat. Many fish and shellfish, including cod, sole, flounder, hake, anchovy, crab, and lobster, have egg or larval stages that develop in this upper layer. Contaminants from atmospheric deposition, urban runoff, wastewater outfalls, industrial point sources, and ocean dumping partition after entering coastal waters. A large portion of these contaminants associate with suspended particles and deposit in the bottom sediments. However, contaminants that have low water solubility or that associate with floatable particles concentrate at the air-water interface. Consequently, several studies have documented high concentrations of potentially toxic polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals in the SSM (Hardy 1982). Currently, the spatial distribution of this SSM contamination or its effects on the reproduction of valuable marine species remains unknown.

The Marine Research Laboratory operated by Pacific Northwest Laboratory (PNL), in response to a request from the National Oceanic and Atmospheric Administration (NOAA), conducted studies to characterize the types and concentrations of sea-surface contaminants at several sites in Puget Sound and to evaluate the toxicity of surface microlayer samples to fish eggs that float on the surface during embryonic and larval development.

This report provides a review of past studies dealing with the SSM as a habitat for fish and shellfish reproduction and as a concentration point for contaminants. Results are given for the first year of research on the concentrations and toxicity of contaminants in the sea surface at several sites in Puget Sound. Conclusions and recommendations are also given.

BIOLOGY AND CHEMISTRY OF THE SEA SURFACE

THE SEA-SURFACE MICROLAYER HABITAT

The eggs of certain marine species occur predominantly or exclusively in the upper millimeter of the water surface where they may be exposed to sea-surface contaminants. The SSM in most areas is a highly productive interface. Natural hydrophobic biogenic organic compounds, produced by plankton in the water column, collect at the surface and provide a rich substrate for dense metabolically active bacterial populations (Carlucci et al. 1985). Tintinnids, protozoa, and small crustaceans feed on these bacteria. Primary productivity of phytonuston (microalgae) is frequently 10 to 100 times greater per unit volume than that of phytoplankton (Hardy and Apts 1984; Hattori et al. 1983; Souza-Lima 1982).

Marine zooneuston communities are often dominated by copepods of the genera Anomalocera, Pontella, and Labidocera. They presumably feed on the high densities of microneuston (Zaitsev 1971). Many species of epineuston, attached to buoyant particles, are widespread throughout the world's oceans. Over 50 species were found in the Indian Ocean, Red Sea, Mediterranean Sea, and Biscay Bay. The euneuston were most numerous in the open tropical waters of the central Indian Ocean. Sea-surface contamination with floating tar and plastic particles results in a decrease in taxonomic diversity but an increase in abundance of epifauna attached to floating particles over widely dispersed geographic areas (Holdway and Maddock 1983a,b).

Diurnal vertical migration often provides an important link between the production of the microlayer and planktonic organisms that move from the water column into the surface layer to feed (Gladyshev and Malyshevskii 1983). For example, the early larval stages of the Pacific octopus exhibit a distinctly neustonic behavior pattern characterized by an association with and apparent feeding on the surface film (Marliave 1981).

Data on the vertical distribution of atherinids in waters off the southern California coast show that, during the day, larvae were collected only at the surface, with a shift to lower depths occurring at night (Kauffman et al. 1981). Many other studies have documented the vertical migration of planktonic

organisms that feed directly on the SSM. Thus, the time of day that neuston sampling occurs can be an important determinant of organism density.

Vertical migrations of dinoflagellates result in high densities of some species in the surface microlayer and reveal the continuity between surface film and the underlying water column (Wandschneider 1979). Thus, if surface contaminants reduce the productivity of the microlayer, a secondary effect could occur on planktonic organisms that feed on the water surface.

However, the microlayer's most significant role may be its importance as a habitat for pelagic eggs and larvae. Many species of fish have eggs and larvae that are exclusively neustonic. Neuston nets generally collect the upper 4 to 15 cm of water surface and, thus, the number of eggs or larvae contained in only the upper millimeter of the water cannot be differentiated. However, our experience (described in results below) indicates that, when collected eggs are placed directly into beakers or pans of seawater, they rapidly float to the water surface where they concentrate in the upper millimeter (Figure 1). Therefore, in the natural environment, they are in contact with SSM contaminants.

Crab larvae are an important constituent of the zooneuston community found in our late spring samples (see the Results Section). Megalopae of the Dungeness crab (Cancer magister) display positive phototaxis and tend to concentrate directly in the surface film during midday (Jacoby 1982).

In the Strait of Juan de Fuca, we have observed Dungeness crab larvae concentrating in the surface microlayer in such extreme densities that they partly protrude through the surface film giving the water surface a bristling brush-like appearance. This occurrence has also been observed off the Oregon/Washington shelf area^(a). Time of day was an important factor in determining the vertical distribution of the blue crab's first-stage zoeae at the mouth of Chesapeake Bay, where 90 to 99% of all larvae collected were taken in the neuston (upper 0 to 15 cm) layer. Neuston concentrations were particularly great

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(a) S. Berry, personal communication, Washington State Department of Fisheries, Olympia, Washington, to J. T. Hardy, Marine Research Laboratory, Sequim, Washington.

FIGURE 1. Fertilized Eggs of Sand Sole Floating in Contact with the Microlayer. (Eggs are about 0.9 mm dia)

in late-night or morning samples. In midday and afternoon samples, only 5 to 20% of the total larvae were collected by the neuston net. Peak abundance at the surface occurred between 0100 and 0800 hours. If only daytime samples were collected, which do not include the neuston layer, the total abundance of crab larvae would be underestimated by up to 3 orders of magnitude when compared to nighttime neuston samples. The surface concentration did not appear to result from vertical migration but from a synchronized hatch corresponding to the time of high slack tide (Provenzano et al. 1983). Even 65 km offshore in the mid-Atlantic Bight, relatively high densities of crab zoeae (13 larvae/m^3) have been found (Smyth 1980).

Many fish have pelagic eggs that float on the sea surface during embryogenesis. In most cases, neustonic fish eggs float on the surface because of their high lipid content and positive buoyancy. As Zaitsev (1971) noted, "...most of the eggs must rise to the surface film and remain suspended there. Although the specific weight of the egg increases during development, it remains low enough to hold the egg close to the surface film." Surface enrichments of organisms were found to be stable even at sea states of about 20 knots.

In San Diego Bay and Los Angeles Harbor, 68% of the fish eggs collected in June and October were found in the neuston samples. Oblique tows yielded lower estimates of egg densities and neustonic larvae relative to the combined discrete depth samples (Brewer 1981).

In the Northwest Pacific Ocean, neuston collections from the upper 0- to 10-cm surface layer contained more than 45 invertebrate species and their eggs and larvae, as well as fish larvae and fry. The biomass was often greater than 4 g/m³ in the coastal zone and decreased seaward. Samples were dominated by four species of copepods and fish larvae (Safronov 1981).

Early studies of the California Cooperative Fisheries Investigation (CalCOFI) in the Southern California Bight did not quantitate the neuston tows; however, fish larvae and/or juveniles of several fish species were collected exclusively in the neuston net and were completely absent from the oblique-hauled plankton samples. Fish having exclusively neustonic larvae were herring (Opisthonema sp.), flying fish (Exocoetidae several sp.), slender snipefish (Macrorhamphosus gracilis), mullet (Mugil sp.), sablefish (Anoplopoma fimbria), greenling (Hexagrammos sp.), painted greenling (Oxylebius pictus), and saury (Cololabis spp.) (Ahlstrom and Stevens 1975). Quantitative analysis of the neuston samples collected from 1978 to 1984 is under way and should be completed in 1986.^(a)

(a) G. Moser, personal communication, National Marine Fisheries Services, LaJolla, California, to J. T. Hardy, Marine Research Laboratory, Sequim, Washington.

Continuing studies are being conducted by the National Marine Fisheries Services (NMFS) on the ichthyoplankton and ichthyoneuston offshore from Washington, Oregon, and northern California. Preliminary results indicate that neuston net collections (upper 15 cm) contain abundant eggs of northern anchovy (Engraulis mordax), Dover sole (Microstomus pacificus), and king-of-the-salmon (Trachipterus altivelis) and larvae of sablefish (Anoplopoma fimbria), yellow Irish lord (Hemilepidotus spinosus), northern anchovy (E. mordax), cabezon (Scorpaenichthys marmoratus), rockfish (Sebastes sp.), and Pacific saury (Cololabis saira). During April-May 1980, 1.38 million curlfin sole (Pleuronichthys decurrens) eggs were in the surface layer of the survey area; none were found in the subsurface water (Kendall and Clark 1982).

During May-June 1981, the total number of eggs in the survey area's surface layer was 20.6 million, 30.8 million, and 16.4 billion for northern anchovy, Pacific saury, and Sebastes sp., respectively. No eggs were found in the subsurface layers for the three species (Clark 1984). Several species of fish larvae occurred in the millions in neuston tows but were completely absent from subsurface tows. These included saury (C. saira), sabelfish (A. fimbria), Opiodon elongatus, cabezon (Scorpaenichthys marmoratus), ronquil (Bathymaster sp.), speckled sanddab (Citharichthys stigmaeus), starry flounder (Platichthys stellatus), and curlfin sole (Pleuronichthys decurrens) (Clark 1984).

Budd (1940) describes the development of the eggs and early larvae of English sole and sand sole. Pelagic eggs that require buoyancy, such as sole, have comparatively thin and light shells or zona radiata. They generally have a rapid development time of 1 to 6 days compared to much longer time periods for demersal eggs. The eggs of sand sole are very similar to those of English sole (Budd 1940). The very buoyant eggs are pelagic, their specific gravity being considerably less than that of seawater; they are spherical and transparent with a diameter of 0.9 mm (see Figure 1). The egg membrane is thin, slightly less than 0.014 mm in thickness. Several hours before hatching, the embryo begins to sink slowly and its movements become increasingly violent. Hatching time depends on temperature; in English sole, hatching time is approximately 90 h at 13°C. For sand sole, hatching time is approximately 120 h at

10°C. Previous studies have noted, and we confirm, that newly hatched larvae often float helplessly upside down at the surface of the water because of the buoyancy of their large yolk sacks (Budd 1940).

For English sole, 90% of viable hatch normally occurs within a range of 20 to 32 parts per thousand (ppt) salinity and 6.5° to 10°C; 50% viable hatch occurs within the range 12 to 39 ppt and 4.5° to 12.5°C. The salinity of neutral buoyancy rises from 27.8 ppt at fertilization to 30.8 ppt at hatching. Temperature is the more significant factor in its effect on larval production (Alderdice and Forrester 1968).

THE SEA SURFACE AS A COLLECTION POINT FOR POLLUTANTS

Mixtures of contaminants entering coastal areas generally concentrate at two interfaces--the water-sediment interface and the air-sea interface. The sea surface is an important concentration point for both natural biogenic organic films (Baier et al. 1974), and organic and metal contaminants (Hardy 1982). Compared to subsurface bulk water only a few centimeters deep, the SSM (the upper 50 μm) often contains high enrichments (microlayer concentration/bulk water concentration) of both natural and anthropogenic organics and metals. In contrast to benthic sediments, which serve as accumulators and integrators of contamination over a relatively long time period, contamination of the microlayer results mainly from recent influxes and, therefore, serves as an indicator of contamination over short time spans.

Sources and pathways leading to the observed microlayer enrichments include industrial and power plant effluents, riverine and wastewater discharges, tanker discharges or spills and direct and indirect atmospheric deposition. Organic and metal pollutants are often associated with hydrophobic particles or air bubbles (Wallace and Duce 1978) in the water column and are transported to the surface. An indirect source originates from the recycling of microlayer organics: bubbles form in the bulk water (e.g., during whitecap conditions); these bubbles break at the sea surface and inject film droplets into the atmosphere which again deposit on the sea surface (Blanchard 1964; Garrett 1967, 1968; Baier 1972).

Atmospheric deposition may contribute up to half of the total input of PAHs and particulate metals entering coastal areas (Hardy 1982). Polycyclic aromatic hydrocarbons are widely distributed common products of fossil fuel combustion and are present in emissions from gasoline- and diesel-powered vehicles, refuse incineration, coal-fired power plants, and many other anthropogenic sources. Studies have shown that aerosol particles are generally coated with organic films that often contain several different metals and surface-active organic compounds (Ketseridis et al. 1976; Kesteridis and Eichman 1978; Graedel 1978; Graedel and Weschler 1981; Gill et al. 1983). The more volatile hydrocarbons, up to the tricyclic stage, may occur largely in the gaseous state in the atmosphere, but the higher molecular weight members are associated with airborne particulate matter and are transported long distances (Atlas and Giam 1980). Results suggest that "aerosols are a source of these compounds (e.g., benz[a]anthracene and benz[a]pyrene) and that the microlayer is a repository until the PAHs are removed...The PAH in aerosols originate from manmade combustion processes...Their buildup in the microlayer seems evident, until absorption and sedimentation...remove them" (Strand and Andren 1980).

METHODS

COLLECTION OF MICROLAYER SAMPLES

Microlayer samples were collected from 13 stations in Puget Sound (points 1 through 13 in Figure 2). Stations included relatively clean, uncontaminated rural reference areas more remote from documented areas of contamination (i.e., Sequim Bay and Central Sound) (Long 1982); three urban bays (Elliott Bay near Seattle, Commencement Bay near Tacoma, and Port Angeles Harbor); and near the West Point sewage treatment discharge diffuser (Station 12). Subsurface bulk water samples were collected only at Stations 8, 12, and 13. Samples were collected four times during 1985 with the following designations: A (February 12), B (March 3 to 6), C (March 10 to 12), and D (May 9). At each station, an integrated sample of surface film material was collected using the glass-plate microlayer sampler developed by the Marine Research Laboratory and reported in Hardy et al. 1985b. A glass plate was dipped vertically through the water surface and withdrawn. Water was allowed to drip from the plate, and the plate was then pushed through a silicon squeegee; the removed microlayer drained into a collecting tube. To reduce any contamination from the sampling procedure, the microlayer sampler and all sample containers were acid cleaned in 10% Ultrex^(a) and the glass plate in 50% Ultrex nitric acid. All the apparatus was then rinsed with deionized water and dried in a laminar-flow filtered air hood. A sample blank from the surface microlayer of distilled water was sampled in the same way as the field samples and, after analysis, showed no significant contamination from the sampling apparatus. The surface pressure (an indicator of the presence of surface-active organic films) was measured at each station using the spreading oil method (Adam 1937). Samples of the bulk water were collected from a depth of 30 cm for metals analysis and bioassays by subsurface opening of 2-L widemouth, acid-cleaned, polyethylene bottles, and for organic analysis by pumping to the surface through a Teflon-coated pump into solvent-cleaned 4-L glass bottles.

(a) Ultrex is a tradename of the J. T. Baker Chemical Company, Phillipsburg, New Jersey 08865.

FIGURE 2. Location of Sampling Stations 1 to 13

CHEMICAL ANALYSIS

Subsamples of each sample were placed on ice and returned to the laboratory within 24 h to conduct the biotoxicity tests. Subsamples for metals analysis were treated and analyzed (Hardy et al. 1985a). Samples for organic analysis were treated as follows: at each station, about 300 mL of microlayer were placed in Teflon bottles and approximately 4 L of bulk water were pumped

into glass jugs; bacterial degradation was halted in samples by adding HgCl_2 . In the laboratory, each sample was filtered through 0.5- μm ashed glass fiber filters. The filtrate was adsorbed on an Amberlite^(a) (purified XAD-2) resin column and then eluted with methyl t-butyl ether. Organics from the particulate fraction were soxhlet extracted. A separate experiment was conducted to determine extraction efficiencies.

Samples showing statistically significant or, in some cases, even marginal toxicity as well as reference stations from Central Sound and Sequim Bay were selected for chemical analysis. In addition, microlayer and bulk water samples collected above the sewage treatment discharge diffuser near West Point, Puget Sound were also analyzed.

Sample extracts were analyzed by capillary gas chromatographic techniques described previously (Riley et al. 1981) for aromatic and PAHs, pesticides, and PCBs (Arochlor 1254). Metals were analyzed by atomic absorption spectrophotometry (Hardy et al. 1985a,b) for Pb, Zn, Cu, Ag, Cd, and Hg.

LABORATORY BIOASSAYS

Toxicity tests were conducted using sand sole eggs (Psettichthys melanostictus), which occur abundantly in the microlayer of Puget Sound during the late winter and spring. Tests from sampling B (March 3 to 6) were conducted using eggs collected by neuston net from Sequim Bay. All other tests used laboratory-fertilized eggs produced from gametes of one male and one female sand sole. Between 4 and 10 replicate 100-mL Pyrex beakers containing about 15 eggs each, and 30 mL of microlayer or bulk water sample were incubated at $9^\circ\text{C} \pm 1^\circ\text{C}$ in the laboratory. Treatments were arranged in a random investigator-blind manner.

To avoid the effects of salinity differences, the salinity of each sample was adjusted to 30 ppt using American Society of Testing and Materials (ASTM) sea salts. A parallel test was done in which the reference Sequim Bay seawater was diluted to 20 ppt with deionized water and then reconstituted to 30 ppt

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(a) Amberlite is a tradename of Supelco, Inc., Bellefonte, Pennsylvania 16823.

with sea salts. The results showed that no significant acute toxicity resulted from the addition of the sea salts. The addition of 10 ppt sea salts to Sequim Bay bulk water caused an increase in the concentration of Cu to 14.4 $\mu\text{g/L}$ (compared to a mean of 3.8 $\mu\text{g/L}$ Cu in the Sequim Bay microlayer). However, the total concentration of analyzed metals (Cu+Pb+Zn+Cd+Ag+Hg) in the salt-adjusted sample was still less than the total metals in the Sequim Bay microlayer. Most samples received much smaller salt additions, and no toxicity would be expected from the small quantities of metals added with the salt.

At the end of the bioassay incubation period, dishes were examined (blindly) and the number of live larvae recorded. Live larvae were completely free from the egg shell, showed active motility, and appeared to have normal morphology. Final data was expressed as the percentage of initial eggs in each treatment that hatched into live larvae.

IN SITU BIOASSAYS

Similar in situ toxicity tests were conducted. The field bioassay system consisted of duplicate rectangular polyethylene containers held in a 0.09- m^2 styrofoam floatation board. The bottoms of the dishes were open for water exchange through a 0.5-mm mesh nylon screen. The apparatus was submerged below the water surface and slowly brought to the surface to trap the surface film within each dish. One exposure system was placed in a laboratory table containing flowing Sequim Bay seawater, and the other was located within Port Angeles Harbor moored in an area of visible surface films. Eggs from one female sand sole were fertilized with sperm from one male. After a 2-hours incubation period, approximately 50 embryos were added to each dish and allowed to incubate for 6 days. In situ exposures were also started on March 28 near Station 2 in Elliott Bay (near the Seattle Aquarium) and at Station 4 in Commencement Bay (Hylebos Waterway).

ORGANISM DENSITIES

Samples were collected by neuston net tows and vertical plankton net tows at two reference sites (Sequim Bay and Central Sound) and at two urban bay sites (Elliott Bay and Commencement Bay) (see Figure 2), and were preserved in

formalin. Ninety-seven field net samples of neuston and plankton collected between February and June were analyzed. In each sample, the number of individuals of 12 major taxonomic divisions were enumerated using an inverted compound plankton microscope.

DILUTION EXPOSURES AND CYTOTOXICITY

Five samples that showed significant toxicity from previous tests were used for dilution series bioassays. In addition, a microlayer sample from Sequim Bay was also tested. The previously frozen samples were thawed and diluted with fresh filtered Sequim Bay seawater to produce concentrations of 100%, 10%, 1%, and 0.1% microlayer. During the dilution series assays, one of the replicate beakers from each treatment was preserved in 4% unbuffered formalin after only 35 h of incubation. A total of 25 samples were examined for chromosomal aberrations in the blastodisc stage embryos. The ether extracts of the microlayer samples were evaporated to dryness and resuspended in a dimethyl sulfoxide (DMSO) carrier. Extracts of ten selected microlayer samples were tested for their effects on growth and their ability to induce chromosomal aberrations in in vitro rainbow trout cell cultures.

EMBRYO ANAPHASE ABERRATIONS

Both treated and control embryos of sand sole in the blastodisc stage of development (35 h post fertilization) were removed from the exposure beakers and fixed in 4% neutral-buffered formalin. The embryos were then dissected to remove the chorion and yolk, transferred to 60% acetic acid for 24 h, then placed into aceto-orcein stain for an additional 24 to 48 h. Once staining was complete, the embryos (blastodisc) were destained in 60% acetic acid until the condensed chromosomes of the mitotic figures were easily distinguished from the surrounding cellular material. Each embryo was then removed from the destain solution with fine forceps, placed onto a cleaned microscope slide in a drop of acetic acid, and covered with a glass coverslip. Gentle pressure was then applied to the coverslip to cause the cells to spread into a monolayer for microscopic examination. Each coverslip was then sealed with clear nail polish to prevent evaporation of the acetic acid. The cells were then examined at

400X to 1000X magnification on a compound microscope, and the percentage of abnormal anaphase figures was determined. The types of abnormalities commonly encountered are chromosome bridges, lagging fragments, attached fragments, and multipolar cells. The mean number of abnormal cells in the treated groups was compared to that seen in the control groups (baseline) by means of a two-sample Student's t-test. Thirteen control embryos were examined, and at least three to five treated embryos were examined when possible. Because of embryo death and occasional inhibition of mitotic activity by the treatments, some of the treated groups had less than the optimum number of embryos that could be used for anaphase evaluation. The techniques used in this portion of the study were from Longwell and Hughes (1980) as modified by Liguori and Landolt (1985).

CYTOTOXICITY

Rainbow trout gonad cells (RTG-2) were grown at 18°C in Leibovitz L-15 medium supplemented with 10% deactivated fetal bovine serum and antibiotics. pH was maintained at 7.1 to 7.3 by means of an internal phosphate buffer (1.3 mM disodium phosphate), which equilibrates with the atmosphere, thus eliminating the need for additional carbon dioxide. The cell line is routinely checked and is free of contaminating mycoplasma. Before their use in these tests, the cells were grown to near confluence, removed from the culture flasks with versene/trypsin, and transferred to the appropriate culture vessel for exposure to the microlayer sample.

Cells were placed into multiwell culture dishes (24 wells per dish and 2 cm² surface area per well) at a density of 20,000 viable cells per well, and allowed to settle and attach for 18 h. Cells from five wells were then removed and counted on an electronic particle counter. These counts were used to establish the number of cells per culture at the beginning of the exposure period (time 0). Simultaneously, cells were exposed to three dilutions of each microlayer extract dissolved in 0.5% DMSO as a carrier. Following incubation in the presence of the microlayer extract for 96 h, cultures were washed with buffered saline to remove dead cells and harvested for counting. Control cultures consisted of cells exposed only to 0.5% DMSO (4 replicates), whereas exposed cultures consisted of 3 replicates of each dilution of each microlayer

extract. The exposed cultures were compared to the controls by means of a two-sample Student's t-test to determine if a significant effect on growth/survival existed as a result of exposure to the microlayer extract.

IN VITRO ANAPHASE ABERRATIONS

Genotoxic damage was evaluated with the anaphase aberration (aa) test (Nichols et al. 1977; Kocan et al. 1982). Rainbow trout gonad cells were grown on 1 x 5 cm cover slips in Leighton tubes at a density of 40,000 cells per tube. Once the cells settled and began to grow, the culture medium was replaced with medium containing one of three different concentrations of microlayer extract. The cells were allowed to grow for 48 h. After this period, the cells were fixed with methanol:acetic acid (3:1) for 1 h, then allowed to air dry before staining. Once dry, the cover slips were placed into 3% Gurr's Giemsa stain in Sorenson's buffer (pH 6.8) for 10-15 min, then washed in tap water, air dried, and mounted cell-side-down on a clean glass microscope slide. Slides were coded to prevent operator bias and each set of slides evaluated at 470X magnification by two different observers. Scoring of the anaphase cells was by the method of Nichols et al. (1977). Each of the slides was examined until 100 anaphase cells were observed and the percent with abnormalities determined. Therefore, each exposure consisted of two slides X 100 cells per slide (= 200 cells per exposure). Controls consisted of four cultures exposed to DMSO only (0.5%) and five untreated cultures. Because the dry weight of the microlayer extracts was not known at the time they were dissolved into DMSO, some sample-to-sample variation occurred in the amount of extract present during exposure.

MULTIVARIATE ANALYSIS

We initially conducted stepwise regressions using the data on biotic responses (percent live larvae in bioassays, flatfish egg density at field stations, trout cell growth assay) to a variety of potential toxicants (total metals, total PAHs, pesticides, and PCBs) found in the SSM samples. The best set of independent variables (including analysis for two-way interaction) was determined based on the criterion (Neter and Wasserman 1974) of a significant

($\alpha \leq 0.05$) relationship between the dependent and one or more independent variables. We then conducted multicollinearity diagnostics (Belsley et al. 1980) on the most appropriate model. A plot of the residuals was examined to determine if an unexplained source of variance was present. When a significant relationship was found between a dependent and one or more independent variables, the observed significance level (P), the model (including regression coefficients), and the R^2 value were reported; otherwise, the range of observed significance levels for all models tested were shown along with a "nonsignificant" designation.

RESULTS

ORGANISM DENSITIES

Major differences in species composition and abundance occurred between the neuston top (nt) (0- to 4-cm layer), neuston bottom (nb) (23- to 43-cm layer), and vertical (v) (150-m water column) collections (Table 1). Only a few taxa were exclusively or mostly neustonic. These included an unidentified abundant amphipod, crab larvae in the Central Sound in June (e.g., sample number 850613-7), and flatfish eggs in February and early March. Flatfish eggs were found almost exclusively in the top neuston net at three sites (Sequim Bay, Point Pully, and Elliott Bay) during the peak of the spawning season. In mid-March to April, some eggs were found in the subsurface water, and by mid-May the spawning season was over and eggs were absent (Table 2). In Commencement Bay, egg numbers were often lower in the microlayer than in the water column. In this bay, the low salinities in some of the samples (resulting from the Puyallup River inflow) would not provide floatation for the eggs.

Flatfish egg densities tended to be much greater in the reference areas of Sequim Bay and Central Sound than in the urban bays. In fact, the number of eggs in the surface layer of the urban bays during the spawning period of February and March averaged only 1% of the number at the reference sites (Table 2). However, from these small number of samples, we cannot determine the percentage of the Puget Sound area at which such low egg densities may occur. Does the low egg density area cover the entire urban bay area? Does it radiate out from the urban bay area? What percentage of the total Puget Sound sea surface does it represent? To find the answers, a more detailed sampling grid approach will be conducted during the height of the spawning season in late February or early March.

During the mid-April sampling, strong winds of about 20 knots were encountered, and in the Central Sound eggs were not restricted to the surface layer (Table 2). Crab larvae were often abundant in May and June samples, sometimes at or below the surface. These results indicate that 1) in the absence of

TABLE 1. Densities of Organisms in Field Samples

DATE - STATION NUMBER	WEATHER	WIND	TEMP.	SAL.	SP. TYPE	SAMPLE TYPE	TAXON																TOTAL # INDIV. PER M ²																																																																																																																																																																																																																														
							A	B	C	D	E	F	G	H	I	J	K	L	M	N	O																																																																																																																																																																																																																																
850221-3C	RAIN	4	8	28	12	V	394	14	2	0	0	5	0	13	0	0	0	0	0	0	0	428																																																																																																																																																																																																																															
850221-3D						NT	50	7	0	0	0	0	0	2	0	0	1	1	7	0	0	47																																																																																																																																																																																																																															
850221-3E						NB	39	1	0	0	0	0	0	8	0	0	0	0	10	0	0	57																																																																																																																																																																																																																															
850222-6C	HEAVY OVERCAST	0	8	10	19	V	299	2	17	0	0	3	0	20	0	0	2	3	0	0	0	346																																																																																																																																																																																																																															
850222-6D						NT	39	0	0	0	0	0	0	0	0	1	2	0	0	0	0	44																																																																																																																																																																																																																															
850222-6E						NB	18	0	0	0	0	0	7	0	0	0	0	2	0	0	0	28																																																																																																																																																																																																																															
850222-7C	HEAVY OVERCAST	0	9	28	>22	V	1957	5	0	0	16	0	27	0	0	0	11	0	0	0	0	1637																																																																																																																																																																																																																															
850222-7D						NT	149	0	0	0	0	1	0	0	22	8	0	0	0	0	0	178																																																																																																																																																																																																																															
850222-7E						NB	190	0	0	0	1	0	0	0	4	3	2	0	0	0	0	156																																																																																																																																																																																																																															
850222-8C	OVERCAST	2	8	30	-	V	730	411	160	42	28	0	0	56	0	0	0	0	0	0	0	1447																																																																																																																																																																																																																															
850222-8D						NT	142	26	18	109	0	0	0	0	8	1	3	0	0	0	0	307																																																																																																																																																																																																																															
850222-8E						NB	194	84	6	99	4	2	8	2	0	0	9	0	0	0	0	407																																																																																																																																																																																																																															
850305-1C	OVERCAST CLEARING	1	8.0	27	12	V	711	915	33	0	0	5	11	0	0	0	5	0	0	0	0	1280																																																																																																																																																																																																																															
850305-1D						NT	31	0	1	0	0	0	0	0	1	2	1	0	0	0	0	39																																																																																																																																																																																																																															
850305-1E						NB	40	1	0	0	0	1	4	0	2	0	3	0	0	0	0	56																																																																																																																																																																																																																															
850305-2C	OVERCAST CLEARING	1	?	24	18	V	705	76	36	0	0	14	14	0	0	4	14	0	0	0	0	864																																																																																																																																																																																																																															
850305-2D						NT	3	0	0	0	0	0	0	0	0	0	1	1	0	0	0	4																																																																																																																																																																																																																															
850305-2E						NB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3																																																																																																																																																																																																																															
850305-3C	OVERCAST DRIZZLE	3	7	25	10	V	608	450	16	0	0	0	53	0	0	9	0	0	0	0	0	1112																																																																																																																																																																																																																															
850305-3D						NT	40	7	0	0	0	1	0	1	2	1	0	0	0	0	0	52																																																																																																																																																																																																																															
850305-3E						NB	28	2	0	0	0	0	0	3	0	0	0	0	0	0	0	34																																																																																																																																																																																																																															
850306-4D	SUNNY	0	8	17	19	NT	27	0	0	0	0	0	0	0	1	0	1	3	0	0	0	32																																																																																																																																																																																																																															
850306-4E						NB	23	7	0	0	0	0	0	2	2	1	0	1	0	0	0	36																																																																																																																																																																																																																															
850306-5C	SUNNY	0	8	16	8	V	560	30	15	0	0	2	7	0	0	4	0	4	0	0	0	623																																																																																																																																																																																																																															
850306-5D						NT	7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	8																																																																																																																																																																																																																															
850306-5E						NB	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3																																																																																																																																																																																																																															
850306-6C	FOG BUT CLEARING	0	?	19	8	V	523	29	22	0	?	?	3	10	2	0	17	0	0	0	0	622																																																																																																																																																																																																																															
850306-6D						NT	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6																																																																																																																																																																																																																															
850306-6E						NB	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13																																																																																																																																																																																																																															
850309-7C	OVERCAST	3	8	27	6	V	501	20	13	0	17	0	46	0	0	0	0	0	0	0	0	599																																																																																																																																																																																																																															
850309-7D						NT	82	0	0	0	0	0	0	1	0	4	0	2	0	0	0	90																																																																																																																																																																																																																															
850309-7E						NB	145	1	1	0	0	0	2	0	10	1	0	0	0	0	0	199																																																																																																																																																																																																																															
850303-8C	OVERCAST	2	4	30	14	V	319	76	42	63	14	17	31	14	0	0	11	0	0	0	0	610																																																																																																																																																																																																																															
850303-8D						NT	182	0	0	0	0	0	0	0	4	0	0	0	0	0	0	1018																																																																																																																																																																																																																															
850303-8E						NB	208	0	3	24	0	1	0	0	100	0	0	0	0	0	0	338																																																																																																																																																																																																																															
850303-9C	OVERCAST	2	4	30	>22	V	936	239	143	112	94	24	6	18	12	0	47	0	0	0	0	1649																																																																																																																																																																																																																															
850303-9D						NT	308	7	9	63	2	0	0	0	99	0	13	0	0	0	0	502																																																																																																																																																																																																																															
850303-9E						NB	300	29	21	133	38	18	3	0	79	0	6	0	0	0	0	647																																																																																																																																																																																																																															
850311-1C	SUNNY	1	6	27	18	V	535	35	28	0	2	2	9	2	2	2	2	0	0	0	0	416																																																																																																																																																																																																																															
850311-1D						NT	12	0	0	0	0	0	0	0	0	0	0	1	4	0	0	18																																																																																																																																																																																																																															
850311-1E						NB	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	9																																																																																																																																																																																																																															
850311-2C	FOG BUT CLEARING	1	8.0	26	14	V	302	52	29	0	0	9	2	0	2	0	2	0	0	0	0	398																																																																																																																																																																																																																															
850311-2D						NT	25	1	3	0	0	0	0	0	0	1	1	4	0	0	0	34																																																																																																																																																																																																																															
850311-2E						NB	6	0	2	0	0	0	0	0	0	1	0	0	0	0	0	9																																																																																																																																																																																																																															
850311-3C	FOG	2	7	25	12	V	403	52	34	0	3	3	20	2	3	0	3	0	0	0	0	525																																																																																																																																																																																																																															
850311-3D						NT	13	0	0	0	0	0	2	0	0	1	9	1	0	0	0	56																																																																																																																																																																																																																															
850311-3E						NB	12	2	1	0	0	0	1	1	0	1	0	0	0	0	0	19																																																																																																																																																																																																																															
850312-4D	SUNNY	1	9	26	16	NT	810	0	0	0	0	0	0	0	0	0	8	4	0	0	0	821																																																																																																																																																																																																																															
850312-4E						NB	30	0	3	0	0	1	0	0	3	0	0	0	0	0	0	37																																																																																																																																																																																																																															
850312-10C	SUNNY	0	7	28	20	V	293	59	27	1	0	0	22	4	10	0	10	0	0	0	0	421																																																																																																																																																																																																																															
850312-10D						NT	64	0	1	0	0	0	0	0	2	0	1	0	0	0	0	42																																																																																																																																																																																																																															
850312-10E						NB	4	0	0	0	0	0	1	0	7	0	1	0	0	0	0	34																																																																																																																																																																																																																															
850312-11C	SUNNY	1	8.0	8	20	V	523	13	65	0	2	2	9	2	2	0	4	0	0	0	0	623																																																																																																																																																																																																																															
850312-11D						NT	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11																																																																																																																																																																																																																															
850312-11E						NB	10	0	0	0	0	0	0	0	6	0	0	0	0	0	0	19																																																																																																																																																																																																																															
850311-7C	OVERCAST	2	8	27	18	V	336	95	26	2	2	2	26	0	4	9	13	0	0	0	0	516																																																																																																																																																																																																																															
850311-7D						NT	129	10	0	0	0	5	0	0	21	898	10	0	0	0	0	1073																																																																																																																																																																																																																															
850311-7E						NB	102	8	11	1	0	4	11	0	6	1	4	0	0	0	0	148																																																																																																																																																																																																																															
850310-8C	OVERCAST CLEARING	3	8	29	6	V	1031	43	60	54	3	0	22	38	5	0	11	0	0	0	0	1270																																																																																																																																																																																																																															
850310-8D						NT	176	0	13	13	0	0	0	0	21	0	35	0	0	0	0	296																																																																																																																																																																																																																															
850310-8E						NB	37	1	6	7	0	0	0	0	17	0	1	0	0	0	0	69																																																																																																																																																																																																																															
850410-2C	OVERCAST	3	9.0	30	6	V	741	18	54	7	1	54	7	4	0	4	7	0	0	0	0	892																																																																																																																																																																																																																															
850410-2D						NT	8	0	0	0	1	0	0	0	0	0	0	0	0	0	0	14																																																																																																																																																																																																																															
850410-2E						NB	12	0	0	0	1	0	0	0	0	0	0	0	0	0	0	15																																																																																																																																																																																																																															
850411-10C	OVERCAST	7	9.0	18	12	V	528	53	25	7	0	43	24	0	0	11	11	199	0	0	0	68																																																																																																																																																																																																																															
850411-10D						NT	124	13	0	3	0	2	0	0	0	0	0	0	0	0	0	25																																																																																																																																																																																																																															
850411-10E						NB	33	18	1	3	1	4	7	0	5	0	0	0	0	0	0	12																																																																																																																																																																																																																															
850410-7C	OVERCAST CLEARING	4	9.0	29	6	V	559	60	35	0	3	49	16	0	4	11	11	0	0	0	0	735																																																																																																																																																																																																																															
850410-7D						NT	99	2	6	3	0	1	0	0	4	1	31	0	0	0	0	15																																																																																																																																																																																																																															
850410-7E						NB	290	9	12	3	0	7	0	0	9	0	9	0	0	0	0	41																																																																																																																																																																																																																															
850409-8C	SUNNY	1	11	31	10	V	2821	633	121	136	60	90	18	211	0	0	467	0	0	0	0	3755																																																																																																																																																																																																																															
850409-8D						NT	1392	4	125	54	0	2	0	0	0	0	0	0	0	0	0	168																																																																																																																																																																																																																															
850409-8E						NB	104	0	27	7	0	0	0	0	1	19	15	0	0	0	0	12																																																																																																																																																																																																																															
850516-3C	SUNNY	5	12	27	12	V	1386	578	275	14	0	130	0	24	0	0	116	886	542	0	0	0	2266																																																																																																																																																																																																																														
850516-3D						NT	93	4	119	2	2	0	2	0	0	0	100	123	0	0	0	56																																																																																																																																																																																																																															
850516-3E						NB	85	0	142	0	0	2	2	0	0	0	4	168	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2. Densities of Flatfish Eggs (number/m³)

DATE	SEQUIM BAY		CENTRAL SOUND		ELLIOTT BAY		COMMENCEMENT BAY	
	NEUSTON	BULK	NEUSTON	BULK	NEUSTON	BULK	NEUSTON	BULK
FEBRUARY 21	8.18	0	21.84	0	.65	0	1.20	0
MARCH 5-6	405.45	0	3.80	0	.99	0	.35	2.41
MARCH 10-12	20.89	5.41	20.63	4.34	.5	1.05	2.06	10.13
APRIL 11-12 (WINDY)	0	0	8.32	8.13	0	0	0	0
MAY 16-17	0	0	0	0	0	0	0	0

strong winds flatfish eggs and, to a large extent, crab larvae are restricted to the surface microlayer and 2) egg densities appear much reduced in urbanized bay areas.

LABORATORY BIOASSAYS

The percent of larvae hatching at the end of a 6-day exposure period ranged from 0 to 96% (Table 3). When samples were analyzed by analysis of variance (ANOVA) and ranked by a Newman-Keul's multiple range test, six samples were found to be significantly toxic to flatfish eggs. All toxic samples were from urban bay sites. These included Stations 1 and 2 (Elliott Bay), 4 and 10 (Commencement Bay), and 13 (Port Angeles Harbor). Only microlayer samples from the urban bays showed significant toxicity. No toxicity was found in bulk water samples from either urban or rural bay stations. The greatest survival of larvae, 86 to 96%, occurred in samples from Central Sound and Sequim Bay. Of the 13 microlayer samples tested from nonreference (i.e., neither Sequim Bay nor Central Sound) areas, 6 showed significant toxicity (i.e., 55% or less live-hatched larvae). Station 9, with microlayer collected from a thick visible slick in Sequim Bay, resulted in an approximate 12% (nonsignificant) reduction in live-larval hatch.

TABLE 3. Percentage of Fertilized Eggs Hatching to Live Larvae

Location	Site	Sta	Depth	Sampling Period								
				b			c			d		
				Laboratory			Laboratory			In Situ		
				March 3 to 6			March 10 to 12					
				N	Mean	SE	N	Mean	SE	N	Mean	SE
Sequim Bay	Central	8	M	10	86	5	5	96	4	4	90	4
			B	10	81	3						
	Slick	9	M	5	74	5						
Central Sound	Pt. Pully	7	M	5	88	4	5	89	5			
Elliott Bay	Inner	1	M	5	0*	0	5	0*	0			
			B	5	82	7	5	92	4			
	East	2	M	5	53*	10	5	85	6			
	Outer	3	M	5	63	7	5	80	2			
	Hylebos	4	M	10	55*	8	5	84	9	2	38	
			B	10	73	6	5	93	3			
Commencement Bay	East	5	M	5	80	11						
	West	11	M				5	78	9			
	Outer East	6	M	5	78	9						
	Outer West	10	M				5	0*	0			
Port Angeles Harbor		13	M							4*	4	5

M = Microlayer, B = Bulk, SE = Standard Error

* = Significant reduction in percent live larvae

With the number of eggs and replicates tested in the bioassay, we were able to detect significant (95% confidence) toxicity with a 30% or greater reduction in live-larval hatch compared to the reference sites. Three other samples from the microlayer of the urban bays (Stations 3, 4, and 11) showed reductions in larval hatch of greater than 10% compared to Sequim Bay

(Station 8) (Figures 3a,b). By using greater numbers of eggs and replicates, the detectability of the tests could probably be increased, and samples such as these might also be demonstrated to have significant toxicity.

IN SITU BIOASSAYS

The first in situ bioassay resulted in 90% and 4% live-larval hatch in Sequim Bay seawater (rural bay) and Port Angeles Harbor (urban bay), respectively (Table 3). The second in situ test was interrupted by a strong storm condition the day before the samples were retrieved. Samples from Sequim Bay and Elliott Bay were completely lost. The sample from Hylebos Waterway had only 38% live larvae (Table 3), all of which showed morphological abnormalities, primarily kyphosis (bent spine).

DILUTION EXPOSURES AND CYTOTOXICITY

In the dilution test, the percent of live-larval hatch generally decreased with increasing percentages of toxic microlayer. For example, in the March 5 sample from inner Elliott Bay, a 1 to 1,000 dilution of the microlayer reduced larval hatch by about 50% compared to Sequim Bay seawater (Figure 4).

EMBRYO ANAPHASE ABERRATIONS

In cells of developing sole embryos, the percentage of mitotic cells with chromosomal anaphase aberrations ranged from 1 to 5% in Station 8 seawater to 23% in undiluted urban bay (Station 1) microlayer (Table 4). Embryos exposed to reference seawater that was diluted to 20 ppt showed no significant increase in abnormalities; however, those exposed to the same seawater reconstituted to 30 ppt with ASTM sea salts showed a very high incidence (54.7%) of abnormalities. Because microlayer samples were adjusted to 30 ppt before bioassay exposures, the finding that metals in the sea salt may increase abnormalities complicates the interpretation of these results. Also, embryos were preserved in 4% rather than 10% formalin, which may have reduced the resolution of damaged anaphase cells. In some samples, embryos had either died before reaching the blastodisc stage, or no mitosis was evident. Also, abnormalities were sometimes greater in diluted than in undiluted microlayers. These

(a)

(b)

FIGURE 3. Percent Live-Larval Hatch of Sand Sole Eggs Exposed During Embryogenesis to Samples Collected from Numbered Field Stations (see Figure 2). [B = bulkwater sample, all other samples were microlayer. Top = sampling period B (March 3 to 6); bottom = sampling period C (March 10 to 12). Horizontal scale of station numbers indicates results of ANOVA and ranking by Newman-Keul's multiple range test. * = significant reduction in live-larval hatch.]

FIGURE 4. Example of Dilution Series Bioassay (Sample 1B, inner Elliott Bay.)

inconsistencies may have been accentuated by a block effect in the sampling design where all embryo samples were taken from the same dilution treatment beaker. More consistent results might be obtained if each embryo had been exposed separately to each dilution. Also, because each sample contains a complex mixture of organic and metal contaminants, dilutions may have complicated interactive effects on the chemical speciation in different samples.

Nevertheless, embryos exposed directly to microlayers collected from urban bay Stations 2, 4, 10, and 1 (which had an original salinity of 27 ppt) generally showed significantly greater incidences of chromosomal abnormalities than those exposed to microlayer or bulkwater samples from the reference bay (Station 8).

CYTOTOXICITY

The toxicity screening test using rainbow trout cell cultures was performed using extracts from ten microlayer samples at three dilutions. Compared to cell growth in the control group, cultures treated with microlayer extracts

TABLE 4. Anaphase Aberrations in Developing Embryos

	STATION#	SAMPLE TIME	SAMPLE ID. #	% SAMPLE DILUTIONS	# OF EMBRYOS EXAMINED	MEAN % ABNORMAL	SIGNIF-ICANCE
	1	B	850305	100	4	23.63	++
				10	4	9.77	++
				1	5	15.02	++
				0.1	5	5.98	-
		C	850311	100		12.05	++
				10	5	6.14	-
				1	4	DE	++
				0.1	5	8.05	-
	2	B	850305	100	5	7.42	-
				10	5	10.1	+
				1	5	7.86	+
				0.1	5	8.34	+
TREATMENTS	4	B	850306	100	5	4.12	-
				10	4	16.75	++
				1	3	NM	++
				0.1	3	16.27	++
	10	C	850312	100	5	DE	++
				10	6	10.56	+
				1	2	4.65	-
				0.1	5	13.24	++
	B	C	850310	100	5	7.44	-
CONTROLS	B-BW	FRESH	850322	0	2	1	-
	CONTROL-BW	FRESH	-----	0	13	4.58+3.9	
SALINITY EFFECTS	B-BW	SA	850327	0	5	54.66	++
	B-BW	S	850327	0	5	7.8	-

BW= BULKWATER, ALL OTHERS MICROLAYER

DE = DEAD EMBRYOS

NM = NO MITOSIS OBSERVED

NS = NOT SIGNIFICANT

SA = SALINTY ADJUSTED

S = SALINITY OF 20 PPT

++=P<.01

+ =P<.05

- = NOT SIGNIFICANT

generally showed less cell growth, and dilutions of microlayer were somewhat less inhibitory than the undiluted extracts. Cell growth was greatest in microlayer extracts from Sequim Bay (Stations 8 and 9) or Port Angeles Harbor (Station 13) and the Central Basin (Station 7). All samples in which cell growth was less than 70% of the control value were from Elliot Bay (Stations 1 and 2), Commencement Bay (Stations 4 and 10), or near West Point (Station 12) (Table 5). The relatively shallow slope of the dose response curves indicates that we may be seeing responses only in the initial phase of a typical sigmoid toxicity curve (i.e., mitotic activity was inhibited, but cell death had not yet occurred). A more marked decrease in cell growth followed by overt cell death would be expected at higher doses of microlayer extract. Thus, higher doses would probably yield a more well-defined response and would have allowed the definition of an EC_{50} level of microlayer.

IN VITRO ANAPHASE ABERRATIONS

Extracts from 10 microlayer samples at two dilutions were evaluated for their ability to induce chromosomal anaphase aberrations in dividing trout cells. Significant increases in aberrations were found in 6 of 10 undiluted extract treatments including those from the reference samples from Central Basin (Station 7) and Sequim Bay (Station 8) (Table 6). Those showing no significant increases in aberrations were from Elliot Bay (Stations 2 and 3) and Commencement Bay (Station 4). Based on a comparison to our other results, we do not believe that this test provided a reliable indication of the presence of genotoxic compounds. The extraction procedure or some other methodology may account for the unsuitability of this test under the conditions used here.

CHEMISTRY

Samples showing statistically significant or, in some cases, even marginal toxicity, as well as those from reference stations in Central Sound and Sequim Bay, were selected for chemical analyses. In addition, microlayer and bulk water samples collected above the discharge from the sewage outfall near the West Point sewage treatment plant (Station 12), Puget Sound were also analyzed.

TABLE 5. Growth of Trout Cells (RGT-2) Exposed to Microlayer Extracts

STATION	SAMPLE TIME	SAMPLE ID. #	MICRO- LAYER EXTRACT UG/ML	MEAN TOTAL # OF CELLS	% IN- CREASE	% OF CONTROL
1	B	850305	91	69748	77	60
			45	72240	83	62
			18	82032	108	70
2	B	850305	89	78436	99	67
			45	79196	101	68
			18	92004	133	79
3	B	850305	82	85140	116	73
			41	84684	115	73
			16	85384	117	73
4	B	850306	90	69476	76	60
			45	77608	97	67
			18	82472	109	71
7	C	850711	87	83568	112	72
			43	92696	135	80
			17	105476	168	91
8	B	850300	87	96376	145	93
			43	95792	143	82
			17	105636	168	91
9	B	850303	84	99992	154	86
			42	89452	127	77
			17	89956	128	77
10	C	850312	149	61756	57	53
			75	73992	88	63
			30	82344	112	72
12	D	850509	96	78628	100	67
			48	78716	100	68
			19	82044	108	70
13	A	850212	94	97704	148	84
			47	102244	159	88
			19	100904	156	87
DMSO	--	--	--	116523 SD=12005	--	--
UNTREATED	--	--	--	89782	--	--
0-TIME COUNTS	--	--	--	39403	--	--

TABLE 6. Anaphase Aberrations in In Vitro Trout Cells (RTG-2)

STATION	SAMPLE TIME	SAMPLE ID. #	MICROLAYER EXTRACT UG/ML	MEAN PERCENT ABERRATIONS	SIGNIFICANCE
1	B	850305	45	20	+
			91	23	++
2	B	850305	44	13	-
			99	16	-
3	B	850305	41	19	+
			82	17	-
4	B	850306	45	15	-
			90	13	-
7	C	850311	43	21	++
			87	18	+
8	B	850303	43	20	+
			97	21	++
9	B	850303	42	13	-
			84	20	++
10	C	850312	75	13	-
			149	11	-
12	D	850509	48	16	-
			96	21	++
13	A	850212	47	17	-
			94	18	+
DMSO	-	---	5	15.5	---
(UL/ML)	-	---	5	SD=3.5	---
UNTREAT-	-	---	0	15.4	---
ED	-	---	0	SD=1.1	---

++ P< 0.01
+ P< 0.05
- NOT SIGNIFICAT

High concentrations of PAHs were found in many of the urban bay samples with total PAH (naphthalene through benzoperylene) concentrations exceeding 54,000 ng/L (Table 7). No detectable (total of all PAHs <500 ng/L) PAHs were found in bulk water samples from any sites. One microlayer sample from Central Sound (Point Pully) contained measurable levels of some of the lighter fraction hydrocarbons (benzenes and naphthalenes). These hydrocarbons were also detected in the thick visible slick sample from Sequim Bay. No heavier fraction PAHs were found at these reference stations. Both samples were collected in thick visible slicks. We are uncertain whether they represent natural biogenic organic compounds collected in the slick or a type of gasoline or fuel oil contamination. In any case, this was in contrast to many of the urban bay samples that contained high concentrations of compounds such as fluoranthene, pyrene, chrysene, and benzo(a)pyrene (BaP) (Table 7). The microlayer sample off West Point (12D) had a high total PAH concentration based primarily on phenanthrene, fluoranthene, and pyrene (see Table 7). High concentrations of dimethyl-ethylbenzenes were also present.

Concentrations of pesticides were detected in eight microlayer samples. Highest total pesticides were in Elliot Bay (3B) and Commencement Bay (4B). In addition to the urban bay microlayers, some pesticides were also detected in Sequim Bay and Central Sound (Table 8).

High concentrations of PCBs measured as Aroclor (1254) were found in four microlayer samples, all from the urban bay areas. Polychlorinated biphenyl concentrations in these samples were approximately 40 to 129 times greater than the Environmental Protection Agency (EPA) Water Quality Criteria of 30 ng/L (see Table 8).

Nine microlayer samples were analyzed for volatile chlorinated organics (EPA 601) and purgable aromatics (EPA 602). Only very low levels of a few compounds were detected in a few samples.

Bulk water samples were analyzed from Port Angeles Harbor (Station 13), West Point (Station 12), and Sequim Bay (Station 8), and none contained any detectable aromatic hydrocarbon contaminants.

TABLE 7. Concentrations (ng/L) of Aromatic Hydrocarbons in Microlayer Samples

Sampling Station*	13A	10C	7C	1B	4B	2B	3B	9B	12 D
Secbutylbenzene	**	9921	**	**	**	**	**	5827	**
1-methyl-4-isopropylbenzene	1630	**	**	**	**	891	**	4129	**
Indan	1240	53500	39747	**	**	**	**	6810	**
Indene	**	**	1224	**	**	**	**	4816	**
1-3-dimethyl-5-ethylbenzene	**	68169	52178	**	**	**	**	**	**
1-2-diethylbenzene	**	**	**	**	**	1610	**	5855	9403
1,2,3,5-tetramethylbenzene	1610	7088	4422	**	**	**	**	3690	6939
Naphthalene	700	7162	2673	5099	784	2688	1316	2754	6096
Benzothiophene	**	**	**	**	**	**	**	3253	13861
2-methylnaphthalene	360	15693	3484	1839	**	1030	**	3086	1437
1-methylnaphthalene	**	7781	857	**	**	**	**	1609	**
Biphenyl	**	21364	2423	1607	1652	943	**	3119	8087
2-6-dimethylnaphthalene	260	12580	2182	1261	**	1170	**	2861	**
Acenaphthylene	**	**	**	**	**	**	**	**	**
Acenaphthene	**	**	**	4445	**	1661	**	**	**
2,3,6-trimethylnaphthalene	**	**	**	**	1673	**	**	**	**
Fluorene	2490	**	**	5383	**	1495	**	**	**
Dibenzothiophene	45300	**	**	**	**	**	**	**	**
Phenanthrene	270	18876	**	**	9703	12702	3684	**	8383
Anthracene	**	**	**	**	**	2937	**	**	**
1-methylphenanthrene	5900	**	**	2292	**	**	**	**	**
Fluoranthene	19020	388370	**	40899	36591	14498	3403	**	6414
Pyrene	2724	245680	**	33243	35710	12299	3010	**	9736
Benz(A)anthracene	85670	5340	**	14604	9396	5751	524	**	**
Chrysene	42670	14137	**	24041	31552	10206	1404	**	**
Benzo(b)fluoranthene	58360	107420	**	11097	20106	4700	**	**	**
Benzo(k)fluoranthene	56030	7145	**	8626	10507	3654	**	**	**
Benzo(e)pyrene	2811	181692	**	5548	9039	2541	**	**	**
Benzo(a)pyrene	64570	107600	**	6125	3141	2849	**	**	**
Perylene	85310	**	**	**	1980	**	**	**	**
Indeno(1,2,3-c,d)pyrene	24270	**	**	**	**	**	**	**	**
Dibenz(a,h)anthracene	67800	**	**	**	**	**	**	**	**
Benzo(g,h,i)perylene	26570	**	**	**	**	**	**	**	**
TOTAL Naphthalene thru Benzo(g,h,i)perylene	616384	1122679	11621	166111	169854	83103	13341	16682	54014

*See Figure 2. A, B, C, and D refer to dates, see Methods section. No aromatic hydrocarbons detected in microlayer samples 4C, 11C, 1C, 2C, 3C, 8C, 7B, and 8B or in bulk water from stations 8, 12, or 13.

**Not detected. Detection limit is about 500 ng/l for total aromatic hydrocarbons.

TABLE 8. Concentrations (ng/L) of Pesticides and PCB in Microlayer Samples^(*)

Compound	Station								
	1B	3B	4B	7B	7C	8C	9B	10C	11C
Alpha BHC (Lindane)		.4667							
Beta BHC		.533						<.077	
Gamma BHC		<.007							
Delta BHC		.0433	.137						
Aldrin		<.007	1.76			<.080	.272	<.077	
Heptachlor		<.007						<.077	
Heptachlor Epoxide		.007	.129			<.080			
Endosulfan I		.217		<.064		<.080		.115	
Unresolved p,p'-DDE & Dieldrin		.2		<.064		<.080		<.077	
Unresolved Endrin & Endosulfan II		<.666		<.064		<.800		<.077	
p,p'-DDD		.666		<.645		<.800		<.077	
Endrin Aldehyde		.566	.333	.097	.13	.12		.115	.154
Endosulfan Sulfate		<.666						.115	
p,p'-DDT		.12	1.69					.192	

PCB (Aroclor 1254)	1201	1941	3894					2429	

*No detectable pesticides or PCB in other samples.

Eleven of the samples analyzed from the urban bay microlayers had high concentrations of several metals. Total metals in microlayers of Elliott Bay, Commencement Bay, off West Point, and Port Angeles sites were very high compared to normal values measured for Puget Sound bulk seawater. Total metals from Sequim Bay were 1 to 2 orders of magnitude lower, and concentrations in the Sequim Bay bulk water sample were lower still (Table 9).

TABLE 9. Metals Concentrations ($\mu\text{g/L}$) in Field Samples

LOCATION	STATION & DATE	CU			PB			ZN			AG			CD			HG			TOTAL METALS
		DISS.	PART.	TOT.	DISS.	PART.	TOT.	DISS.	PART.	TOT.	DISS.	PART.	TOT.	DISS.	PART.	TOT.	DISS.	PART.	TOT.	
ELLIOTT BAY	1B	31.24	149.00	180.24	1.43	64.83	66.26	247.00	188.69	435.69	.03	.04	.09	.28	.07	.33	.01	.03	.03	682.66
	2B	10.57	40.25	50.82	1.82	37.11	38.93	77.00	58.34	135.34	.04	.13	.16	.36	.06	.42	.01	.71	.72	226.38
	1C	259.34	2906.00	3213.34	.84	118.75	119.59	291.00	1126.09	1417.09	.11	.16	.26	.31	.08	.39	.01	.01	.01	4752.68
	2C	3.36	9.77	13.13	.15	13.58	13.73	4.99	15.24	20.23	.03	.08	.08	.24	.06	.30	.01	.01	.01	47.90
	3C	5.98	3.77	9.75	.30	6.98	7.28	4.18	6.06	10.24	.01	.02	.03	.42	.05	.47	.20	.21	.41	28.18
COMMENCEMENT BAY	4B	18.96	378.00	396.96	2.85	170.37	172.72	93.00	754.07	787.07	.03	.14	.18	.34	.10	.44	0.00	.06	.06	1357.45
	4C	45.72	586.00	631.72	1.79	643.54	654.93	111.00	324.83	435.83	.10	1.04	1.14	1.39	.20	1.59	.01	.06	.06	1725.27
	10C	4.26	21.67	26.13	.18	8.79	8.97	2.79	41.54	44.33	.01	.03	.04	.21	.04	.25	.01	.02	.03	79.75
	11C	2.91	.94	3.88	.18	1.21	1.39	.17	2.86	3.03	.01	.05	.06	.18	.02	.20	.01	.01	.02	6.55
CENTRAL SOUND	7B	4.68	11.10	15.78	.37	4.39	4.76	6.68	5.43	12.11	.02	.02	.04	1.67	.07	1.74	.05	.12	.17	34.60
	7C	4.16	33.21	37.37	.26	21.99	22.25	4.01	13.97	19.98	.04	.11	.15	.75	.15	.90	.02	.03	.05	60.70
SEDUIA BAY	9B	5.18	7.53	12.71	.50	6.03	6.53	29.00	17.52	46.82	0.00	.01	.01	.10	.02	.11	0.00	.01	.02	65.90
	9B	4.78	3.21	7.96	.14	2.78	2.89	40.00	10.45	58.45	0.00	0.00	.01	.10	.01	.11	0.00	.01	.01	69.43
	9B-BULK	.97	0.00	.97	.01	.01	.02	<5.0	.00	45.05	0.00	<0.0005	<0.0005	.06	0.00	.06	0.00	0.00	0.00	1.05
	9C	2.80	.30	3.10	.44	.28	.72	19.00	1.79	20.79	0.00	0.00	0.00	.42	0.00	.42			0.00	25.04
WASTE OUTFALL	120	14.02	129.00	143.02	.12	64.83	64.95	24.00	178.87	202.87	.01	.26	.26	.12	.06	.18	0.00	.09	.09	411.37
PORT ANGELES	13A	11.65	4.11	15.76	3.11	511.01	514.12	19.80	121.20	141.00	---	---	---	---	---	---	---	---	---	470.88

--- = NOT MEASURED
DISS. = DISSOLVED
PART. = PARTICULATE

DISCUSSION

CAUSES AND DISTRIBUTION OF LARVAL MORTALITY

Both the percent of embryos hatching to live larvae and the growth of trout cell cultures (as a percent of the controls) showed significant decreases in response to increasing microlayer concentrations of both PAHs and metals. A summary of the chemical and biological data is presented in Table 10. Toxicity tests on SSM that contain mixtures of many contaminants do not identify the component most responsible for the toxicity. In the case of metals, the concentrations of lead, copper, and zinc probably account for much of the toxicity, whereas silver, cadmium, and mercury probably do not make a significant contribution to the toxicity. For organics, individual PAHs were often present far in excess of the concentrations known to be toxic. For example, in samples that were significantly toxic (with the exception of 1C, which contained high metals) the concentrations of one compound alone, BaP, ranged from 2.8 to 107 $\mu\text{g/L}$. Morphological abnormalities in trout eggs (Hannah et al. 1982) and reduced hatching success in sand sole (Hose et al. 1982) occurred when eggs were exposed to only 0.21 and 0.1 $\mu\text{g/L}$ of BaP, respectively.

In the case of cell growth, a significant interaction also occurred between PAHs and pesticides. Regression models are as follows (where concentrations of total PAHs and pesticides are in ng/L and metals are in $\mu\text{g/L}$):

$$\begin{aligned}\ln \% \text{ live larvae} &= 4.43 - 0.000007 \text{ PAH} - 0.0006 \text{ metal} & (1) \\ R^2 &= 0.89, P = 0.0004\end{aligned}$$

$$\begin{aligned}\ln \text{ cell growth} &= 4.4 - 0.00048 \text{ metal} + 0.0000007 \text{ PAH pesticide} & (2) \\ R^2 &= 0.87, P = 0.02\end{aligned}$$

The density of flatfish eggs collected in the field did not show a significant relationship with SSM contaminant levels. The range of observed significance levels was $P = 0.21$ to 0.97 .

TABLE 10. Summary of Chemical and Biological Data

LOCATION	STATION & DATE	TOTAL+ METALS UG/L	NS/L			FLATFISH EGG CONC. (#/M ³)	TROUT CELL GROWTH % CONTROL	PER CENT LIVE LARVAE	NEUSTON ++ IN FIELD (#/M ³)	TOMS INDEX
			TOTAL+ PAH	PESTICIDES	PCB					
ELLIOTT BAY	1B	682	166111	ND	1201	0.71	60	0 *	34.1	26
	2B	226	83103	ND	ND	0.0	67	53 *	4.4	36
	3B	---	13341	2.89	1941	0.89	73	63	51.2	
	1C	4753	ND	ND	ND	0.37	---	0 *	17.8	44
	3C	28.2	ND	ND	ND	0.50	---	80	35.4	71
COMMENCEMENT BAY	4B	1357	169854	2.85	3894	0.0	60	55 *	32.4	39
	4C	79.8	ND	ND	ND	0.0	---	84	821.1	73
	10C	1725	1133679	0.54	2429	2.07	53	0 *	39.5	25
	11C	8.55	ND	0.15	ND	0.41	---	78	10.1	69
CENTRAL SOUND	7B	34.6	ND	0.097	ND	3.80	---	88	85.7	88
	7C	80.7	11621	0.13	ND	20.63	72	89	198	70
SEQUIM BAY	9B	66	16682	0.27	ND	98.99	86	74	403	51
	8B	69	ND	ND	ND	405.45	83	86	612	74
	8B-BULK	1.05	ND	ND	ND	0.0	---	81	610	82
	8C	25	ND	0.12	ND	20.89	---	96	238	76
WASTE OUTFALL	12D	411	54014	---	---	---	67	---	---	
PORT ANGELES	13A	471	643000	@	ND	---	84	36 *	---	

ND= NOT DETECTABLE

+ DOES NOT INCLUDE LESS THAN OR EQUAL TO VALUES IN TOTAL

* SIGNIFICANT TOXICITY WHEN ANALYZED BY NEWMAN-KEUL'S MULTIPLE RANGE TEST

--- = NOT MEASURED

@ PRESENT BUT NOT QUANTIFIED

++ = NEUSTON NOT INCLUDING FISH EGGS

Thus, these analyses strongly suggest that reductions in the live-larval hatch of sand sole embryos can result from exposure to either PAHs or metals at concentrations found in the sea surface in some areas of Puget Sound.

The total number of zooneuston, not including fish eggs, tends to be lower in the urban bays and higher in the Central Sound and Sequim Bay where densities are approximately 1 order of magnitude greater on the average (see

Table 1). Areas where total contaminant levels were high (Elliot Bay and Commencement Bay) generally had lower numbers of flatfish eggs present. However, the small number of data pairs does not provide a significant correlation coefficient.

The salinity of the microlayer samples was adjusted to 30 ppt before conducting the bioassays. In a few samples of low original salinity, the addition of sea salts may have increased the incidence of anaphase chromosomal aberrations (see sections titled Results, and Sole Embryo Anaphase Aberrations). However, apparent correlation occurred between the original sample salinity and subsequent toxicity in terms of percentage of live-larval hatch in the bioassays. For example, samples 1B and 1C, with a relatively high original salinity of 27 ppt, yielded 0% hatch, but a toxic sample (4B) with the lowest salinity (17 ppt) yielded 55% hatch.

Our results suggest that contaminants are most highly concentrated in visible slick areas. High concentrations of contaminants and subsequent toxicity occurred in samples from areas of high surface pressure (12 to 20 dynes/cm²) (i.e., in areas of visible surface slicks). Natural organic films are present everywhere on the sea surface. They form from biogenic organic exudates released by plankton in the water column that collect at the sea surface. In areas remote from contaminant sources (e.g., Station 9), these slicks may contain little or no contamination. However, in other areas, these natural films provide a substrate for the concentration of hydrophobic organic contaminants. Wind and current patterns collapse the films into thicker visible slicks. Such slicks are not restricted to urban bays but appear to move from place to place.

Toxicity can result from either metals, PAHs, or combinations of both. For example, according to the model in Equation (1), in areas where total SSM concentrations of both metals and PAHs reach 65 µg/L, larval hatch would be reduced to 50%. Further data of this type could lead to refinement of the model, including a consideration of the effects of individual metal or organic compound contaminants on this and other species with neustonic live stages. Such models would prove useful for defining areas of impact on fishery recruitment.

SOURCES OF SEA-SURFACE CONTAMINATION

The sources of sea-surface contamination in Puget Sound remain to be identified. Many of the urban bay sites sampled have been shown to have highly contaminated sediments (Long 1982; Riley et al. 1981). In Elliot Bay, a decreasing concentration of total PAH occurred from the inner to middle to the outer bay: 166, 83, and 13 $\mu\text{g/L}$, respectively. This gradient points to a probable source from the Duwamish River industrial area. Concentrations at these stations are great enough to reduce hatching success in sand sole Equation (1). A sample from inner Elliot Bay (1C) had no detectable hydrocarbons but had 0% live larvae in the bioassays. The metal analysis demonstrated a high concentration of metals, especially copper and zinc (Table 9) and toxicity in this sample was undoubtedly caused by the high metal content. Particles could be observed in this microlayer sample and were most likely derived from sand blasting activity in the industrial shipyards of Harbor Island.

Our previous study (Hardy et al. 1985b) suggested that much of the sea-surface metal enrichment originated either directly or indirectly from atmospheric deposition. This study also suggests the predominance of fossil fuel combustion products as the primary source of sea-surface contamination.

The ratio of methylphenanthrenes to phenanthrene (MP/P) in combustion mixtures is generally less than 1, whereas, unburned fossil PAH mixtures typically display a range of values from 2 to 6 (Prah1 et al. 1984). Our present data indicate that the toxic samples, with the exception of Port Angeles Harbor and Inner Elliot Bay, had MP/P ratios of less than 1 (see Table 7). Whether these contaminants are concentrated on the sea-surface from direct atmospheric deposition or secondarily from terrestrial runoff remains unclear.

Domestic sewage effluents represent another source of sea-surface contamination in Puget Sound. Mesocosm experiments (Word et al. 1985) indicate that about 10% of a typical subsurface sewage release will reach the water surface. A visible slick containing a high PAH concentration was located above the West Point Sewage outfall, but its organic composition was somewhat different than other contaminated microlayers (Table 7). High concentrations of silver indicate domestic sewage sources. The highest ratio of silver to total

metals (0.06 to 0.07%) was found in microlayer samples from off the West Point Outfall area and from Elliot Bay near Pier 54, both believed to be sewage enriched stations (see Table 9).

A TRIAD APPROACH

Our results confirm the utility of using a triad approach to measure environmental quality [i.e., the use of contaminant concentrations from the field, bioassay toxicity tests, and some measure of organism density or species richness of field communities (Chapman et al. 1984)]. We propose the following index, which may prove useful in assessing environmental quality, not only in the microlayer but also in sediments or water:

T = toxicity = mean fraction live in bioassay

O = organics concentration (ng/L) = $(1/1+\log(1+\text{tot PAH, PCB, and pesticides}))$

M = metals concentration ($\mu\text{g/L}$) = $(1/1+\log(1+\text{tot Pb, Cu, Zn, etc.}))$

S = species abundance or richness measure

e.g., Simpson's Index = $1 - \sum (n_i/N)^2$ or
 $S = \{1 - 1/[1+\log(1+\text{tot number of individuals})]\}$

$$\text{SUMMARY INDEX} = 100 \frac{(T + O + M + S)}{4}$$

This index (TOMS Index) is unique in that each of the terms varies between 1 (nonstressed) and 0 (heavily stressed). The summary index will always lie between 0 and 100 and thus allows a relative comparison between sites with very different characteristics. The index may prove to be more useful when individual terms (e.g., Pb or Cu) are scaled for their relative importance to pristine values. For example, one might use the ratio of metal concentrations to their EPA water quality criteria concentrations.

Applying this index to our limited data set yields values of 25 to 44 in the two urban bays and 70 to 87 in the two reference sites (excluding the thick natural slick in Sequim Bay) (Table 10). We suggest that this index be used for other data sets and, as experience is gained, an estimated break point for

a "degraded" environment may be defined. Certainly, from our data, it would appear that environments with an index of less than 50 should be considered greatly degraded. Such environments probably have either high concentrations of contaminants, low survival in bioassay tests, some change in community structure, or a combination of these factors.

CONCLUSIONS

This is the first study of the concentrations and effects of sea surface contaminants in Puget Sound. The results of the first year of study indicate the following:

- During the sole spawning season, urban bays in Puget Sound generally have lower concentrations of eggs present on the sea surface than rural bays or the central sound.
- In some areas of Puget Sound, SSMs (upper 50 μm) contain very high concentrations of anthropogenic contaminants.
- In about half of the samples tested, exposure of fertilized sole eggs to microlayer samples resulted in significantly decreased live-larval hatch and/or increased larval abnormalities.
- Both live-larval hatch of sand sole and growth of rainbow trout cell cultures decreased with increasing concentrations of metals and PAHs in the microlayer samples.
- The frequency of anaphase chromosomal aberrations was greater in developing sand sole embryos exposed to urban SSM samples containing high contaminant levels than in those exposed to rural microlayer samples where significant sea surface contamination was absent.

These conclusions raise considerable concern about the present state of sea-surface contamination in Puget Sound. Extremely high concentrations of contaminants were found in the SSM at some stations. Contamination was not limited to the immediate area of suspected sources, but was found at the outer edges of the urban bays as well. Contaminated surface films could be carried by wind and surface currents and deposited in intertidal beach areas.

To our knowledge, no other studies have examined the toxicity of sea-surface contaminants to neustonic eggs or larvae in Puget Sound or elsewhere. Consequently, our conclusions must be viewed as preliminary.

RECOMMENDATIONS

We recommend that further work be done in the following areas:

- Develop improved methodologies for sampling the sea-surface microlayer.
- Develop bioassay toxicity tests that can be used year-round and with other additional neustonic species.
- Determine the temporal and spatial extent of sea-surface contamination in Puget Sound and elsewhere.
- Characterize the relative importance of different sources of sea-surface contamination.
- Quantify the deposition of surface contaminants in intertidal areas and their effects on other species of fish and shellfish.

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