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Outer Continental Shelf Environmental Assessment Program

Final Reports of Principal Investigators

Volume 29 January 1985

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Ocean Service
Office of Oceanography and Marine Assessment
Ocean Assessments Division
Alaska Office

U.S. DEPARTMENT OF THE INTERIOR
Minerals Management Service

"Outer Continental Shelf Environmental Assessment Program Final Reports of Principal Investigators" ("OCSEAP Final Reports") continues the series entitled "Environmental Assessment of the Alaskan Continental Shelf Final Reports of Principal Investigators."

It is suggested that sections of this publication be cited as follows:

Malins, D. C., S.-L. Chan, R. C. Clark, Jr., and U. Varanasi. 1985. The nature and biological effects of weathered petroleum. U.S. Dep. Commer., NOAA, OCSEAP Final Rep. 29: 231-285.

OCSEAP Final Reports are published by the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Office of Oceanography and Marine Assessment, Ocean Assessments Division, Alaska Office, Anchorage, and printed by the General Services Administration Printers in Juneau, Alaska.

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Anchorage, AK 99513

OUTER CONTINENTAL SHELF
ENVIRONMENTAL ASSESSMENT PROGRAM

FINAL REPORTS OF PRINCIPAL INVESTIGATORS

VOLUME 29

JANUARY 1985

U.S. DEPARTMENT OF COMMERCE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NATIONAL OCEAN SERVICE
OFFICE OF OCEANOGRAPHY AND MARINE ASSESSMENT
OCEAN ASSESSMENTS DIVISION
ALASKA OFFICE

ANCHORAGE, ALASKA

The facts, conclusions, and issues appearing in these reports are based on research results of the Outer Continental Shelf Environmental Assessment Program (OCSEAP), which is managed by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and primarily funded by the Minerals Management Service, U.S. Department of the Interior, through interagency agreement.

Outer Continental Shelf Environmental Assessment Program

Final Reports of Principal Investigators

VOLUME 29 JANUARY 1985

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SUBLETHAL EFFECTS OF PETROLEUM HYDROCARBONS AND TRACE METALS, INCLUDING BIOTRANSFORMATIONS, AS REFLECTED BY MORPHOLOGICAL, CHEMICAL, PHYSIOLOGICAL, AND BEHAVIORAL INDICES

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Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 73

June 1982

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1. SUMMARY OF OBJECTIVES, CONCLUSIONS, AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

1.1 Summary of Objectives

The overall objectives of this program were to assess the potential effects of petroleum and petroleum-related activities on marine organisms indigenous to Alaskan waters. The principal objectives addressed were to:

(a) Determine the uptake of polycyclic aromatic hydrocarbons by salmonids and pleuronectids exposed to these compounds in sediment, water or via their diet.

(b) Study the metabolism of aromatic hydrocarbons by fish and evaluate the potential of their metabolites for interacting with DNA and other cellular constituents.

(c) Evaluate the uptake, disposition and toxicity of petroleum hydrocarbons in larval and adult invertebrates.

(d) Determine the activities of enzymes (aryl hydrocarbon monooxygenases) that metabolize aromatic hydrocarbons in a variety of aquatic species.

(e) Study the factors (e.g., temperature, routes and length of exposure) that influence the uptake, metabolism and disposition of petroleum hydrocarbons in marine fishes.

(f) Study the uptake and depuration of toxic trace metals by salmon and flatfish.

(g) Determine the effects of petroleum hydrocarbons and chemical dispersants on disease resistance and host defense mechanisms of marine fish and shellfish.

(h) Evaluate pathological effects that can result from exposing adult and juvenile flatfish to sediments contaminated with petroleum.

(i) Assess the cytopathological changes in marine fish resulting from exposure to petroleum hydrocarbons.

(j) Evaluate the avoidance, homing, and predator-prey behavior of salmon exposed to water-borne hydrocarbons, and the avoidance of oil-contaminated sediment by juvenile flatfish.

(k) Determine the effect of ingested and water-borne crude oil on reproductive success and/or the early developmental stages of salmonids, flatfish, smelt, and invertebrates.

(l) Assess the effects of water-borne hydrocarbons on the chemosensory-mediated behavior of invertebrates.

1.2 Summary of Conclusions

The conclusions of this program are summarized according to discipline; chemistry, pathology, and behavior and physiology.

1.2.1 Chemistry

Marine fish and invertebrates accumulate a broad spectrum of aromatic hydrocarbons when exposed to these compounds in water, sediment, or via force feeding or intraperitoneal injection. In fish, the mode of exposure to aromatic hydrocarbons can markedly influence the extent of accumulation of parent hydrocarbons and their metabolites as well as the types of metabolites formed. Also, increasing the number of benzenoid rings or the degree of alkyl substitution of aromatic hydrocarbons results in increased accumulation of these compounds by fish.

Measurement of aryl hydrocarbon monooxygenase activities show that most invertebrates and vertebrates investigated were capable of metabolizing aromatic hydrocarbons.

Regardless of species, mode of exposure or structure of hydrocarbon, results show that metabolic products are retained in the tissues of animals for a longer time than are the parent hydrocarbons.

Fish brain appears to accumulate mainly parent aromatic hydrocarbons (e.g., naphthalene), whereas other sites, such as liver and bile, contained primarily conjugated and nonconjugated metabolites of aromatic hydrocarbons.

Lowering the environmental temperature increased both concentrations and resident times of naphthalene in the tissues of fish perorally exposed to naphthalene. Lowering the temperature also decreased the bioconversion of naphthalene and altered the proportions of individual metabolites in the tissues.

The metabolite profiles of naphthalene in both adult and larval shrimp were similar to those reported for fish, and demonstrate that the early developmental stages of shrimp have the enzyme systems necessary for converting naphthalene to both conjugated and nonconjugated metabolites. In addition, exposure to low levels (1-100 ppb) of naphthalene in seawater impaired fertilization and early embryonic development of mollusc larvae, and survival of crustacea larvae.

The liver enzymes of pleuronectids convert benzo(a)pyrene into reactive intermediates (such as epoxides) that bind to DNA and proteins. Such interactions are known to damage critical cellular constituents in mammals.

Studies with both aromatic hydrocarbons and metals demonstrate that the skin and epidermal mucus of fish are involved in both the uptake and discharge of these compounds.

Salmonid and pleuronectid fish accumulated water-borne lead and cadmium; the metals persisted in many organs and tissues for weeks after the exposure was terminated. Cadmium was preferentially bound by low molecular weight proteins in liver cytosol; high accumulation of lead was found in the brains of fish.

Food chain transfer of aromatic hydrocarbons was demonstrated. Sea urchins feeding on 2,6-dimethylnaphthalene-exposed algae accumulate and metabolize this hydrocarbon.

1.2.2 Pathology

Juvenile and adult flatfish and adult spot shrimp exposed to oil-contaminated sediment did not show an altered resistance to bacterial infection. Juvenile salmon exposed to seawater-accommodated crude oil for 2 weeks, as well as salmon and trout perorally exposed to crude oil for up to 10 months, also showed no demonstrable changes in disease resistance or evidence of immune dysfunction. However, preliminary tests suggested an adverse effect of a petroleum dispersant (Corexit 9527) on disease resistance of salmon.

Three species of flatfish exposed to oil-contaminated sediments for up to 4 months differed substantially in the degree to which they accumulated parent aromatic hydrocarbons. Pathological changes occurred in the livers of all flatfish species tested, but similar abnormalities were frequently observed in controls. In addition, the physicochemical characteristics of sediment greatly influenced its retention of petroleum. Retention of aromatic hydrocarbons was more than 10-fold greater in high-silt than in high-sand sediment.

Ultrastructural changes in the liver and lens tissues of adult trout occurred after high doses of petroleum hydrocarbons were administered perorally for 2-12 months. Also, both salmon and flatfish exposed to waterborne hydrocarbons exhibited gill lesions characterized by loss of surface cells.

Exposing embryonic smelt and flatfish to low ppb concentrations of seawater-accommodated crude oil resulted in high mortality at hatching. The eye and brain of exposed smelt embryos appeared to be target organs and, in the later phases of embryonic development, exhibited extensive necrocytosis. In flatfish there was evidence of disruption in both epithelial cell mitochondria and in the olfactory epithelium.

1.2.3 Behavior and Physiology

At low (15-150) ppb concentrations of waterborne hydrocarbons, spot shrimp overt feeding behavior and the sea urchin pedicellarial defense response were reduced by half. At these hydrocarbon concentrations nudibranchs failed to locate mating conspecifics and suffered impaired reproduction and embryological abnormalities. In addition, less than 10% of the smelt eggs exposed throughout embryogenesis hatched; of the larvae that hatched, only 10% survived. Flatfish embryos exposed to

80 ppb developed into normal larvae, but embryos exposed to more than 130 ppb hatched into dead or grossly abnormal larvae.

At waterborne hydrocarbon concentrations of 150-500 ppb there was a significantly increased consumption of exposed sea urchins by starfish predators and of exposed salmon fry by salmon predators. Salmon predation decreased sharply after the predators were exposed to oil for 3 or more days. Exposing salmon eggs throughout embryo and alevin development at these hydrocarbon concentrations resulted in a 400% increase (compared to controls) in mortality; exposure as either embryos or alevins alone increased mortality by 100-150% over that of controls.

Exposing adult salmon to 1-2 ppm of aromatic hydrocarbons caused a 3 day delay in return from offshore in seawater to their "home" stream; concentrations of 2-3 ppm inhibited upstream spawning migration. However, short term exposure to a 4 ppm concentration did not discernably affect adult salmon olfactory perception. Also, exposing adult salmon to 40 ppm of freshwater-accommodated crude oil did not alter their homing capability in freshwater or their rate of return.

Juvenile flatfish did not consistently avoid oil-sediment mixtures containing 8,000-10,000 ppm total hydrocarbons, and maturing trout fed large amounts of crude oil (1,000 ppm added to food) for 7 months did not show statistically significant changes in their hatching success.

1.3 Implications with Respect to OCS Oil and Gas Development

Research findings from this program have clear implications with respect to petroleum effects on aquatic species and consequently to OCS oil and gas development. Most of the studies were designed as laboratory experiments with emphasis on exposures of aquatic organisms in flowing-seawater tanks. Controlled studies with experimental designs of the type reported here are indispensable parts of a total program directed at understanding effects of petroleum on the marine environment. The degree to which laboratory results can be directly applied to natural events remains a considerable problem. However, lacking the opportunity for testing target species directly under natural conditions, models, such as those used in present studies, and representative test situations, must be applied.

1.3.1 Chemistry

The results of studies exposing a variety of fish species to ppb concentrations of metals and aromatic hydrocarbons imply that low levels of both types of compounds arising from petroleum operations could result in substantial metal and hydrocarbon accumulation in fish. This is particularly notable for flatfish, which show a striking ability to accumulate both types of pollutants. Also, the tendency of fish to accumulate considerable amounts of the metabolic products of aromatic hydrocarbons [e.g., metabolites of benzene, naphthalenes, anthracene,

benzo(a)pyrene] is a cause for concern because of the toxicity ascribed to certain metabolites in other animal experiments. These studies have clearly established that aromatic hydrocarbons are converted to a variety of oxidized products by marine organisms and that the metabolites tend to be retained in tissues for a longer time than the parent hydrocarbons. Thus, in assessing marine pollution, considerable bias may arise from determining only the concentrations of parent hydrocarbons in marine animals.

Results show that polycyclic aromatic hydrocarbons, such as benzo(a)-pyrene, can persist in sediment and are thus available for continual uptake by demersal fish. Benzo(a)pyrene is rapidly and extensively metabolized by flatfish into a number of mutagenic and carcinogenic compounds. The extent of metabolism and retention times of metabolites by flatfish are considerably greater for benzo(a)pyrene than for naphthalene. Although benzo(a)pyrene is a minor component of crude oil, these factors raise serious concerns regarding benzo(a)pyrene and other high molecular weight polycyclic aromatic hydrocarbons in the marine environment.

Findings also show that lowering the water temperature resulted in an increased retention of petroleum hydrocarbons and their metabolites in the tissues of exposed salmon and flatfish. These results suggest that fish in colder regions may accumulate particularly heavy burdens of potentially damaging xenobiotics from prolonged petroleum exposure. This finding is of major importance when considering the environmental effects of arctic and subarctic petroleum operations.

The low concentrations (1 ppb) of aromatic hydrocarbons that produce adverse effects on the fertilization and early embryonic development of molluscs indicate the incompatibility of aromatic petroleum hydrocarbons and gametes of these species in the water. This is of considerable importance because the gametes of many commercially important species of molluscs are exuded directly into the water where fertilization takes place.

It was shown that dimethylnaphthalene can be accumulated by algae and transferred to sea urchins feeding on the algae. Moreover, sea urchins and spot shrimp were shown to be capable of metabolizing aromatic hydrocarbons and retaining both metabolites and the parent compound, which raises serious concern about the transfer of potentially toxic metabolites through the food web.

1.3.2 Pathology

Exposing flatfish and spot shrimp to crude oil-contaminated sediments, and feeding crude oil to salmonid species, produced no demonstrable alterations in disease resistance. Preliminary testing suggested that chemical dispersants may reduce disease resistance. However, additional research to verify and expand this observation would be necessary before implications could be made.

Exposing juvenile and adult flatfish to oil-contaminated sediment resulted in pathological changes which were considered reversible. But whether flatfish exposed to similarly contaminated sediments could, under natural conditions, successfully compete for food, reproduce, escape predators, and perform other vital functions remains unknown. However, the cytopathological changes observed in surf smelt embryos (e.g., necrosis of eye and brain tissue) were severe. It was concluded these changes would clearly affect development and survival.

1.3.3 Behavior and Physiology

Behavioral studies indicate that salmon are likely to avoid acutely toxic concentrations of petroleum hydrocarbons, and migrating salmon which encounter subavoidance levels would be unlikely to suffer a detrimental effect on the physiological processes involving homing capability. An adverse effect on hatching success or survival of offspring as a result of crude oil ingestion is also unlikely. It should be noted, however, that the effect of oil exposure on other important behavioral and physiological aspects of reproduction, such as redd building, mate selection, and egg laying, were not investigated. For intertidally spawned salmon eggs, ppb concentrations of weathered crude oil resulted in a high mortality of embryos and alevins, but only when exposure encompassed a considerable portion of the early developmental stage.

Studies on predator-prey reactions indicate that salmon fry exposed to ppb concentrations of petroleum hydrocarbons for 24 hr are much more susceptible to predation than non-exposed fry. Conversely, salmon predators exposed to similar ppb hydrocarbon concentrations did not statistically significantly reduce prey consumption for at least 3 days. Thus, salmonid fry may be vulnerable to predation immediately after an oil spill, while a continued exposure may impair adult feeding.

It was concluded from these studies, however, that the overall probability of salmon encountering concentrations of petroleum capable of eliciting severely adverse effects is slight; only in unusual circumstances would substantial damage to Pacific salmonids be anticipated.

In contrast, low ppb concentrations of waterborne weathered crude oil resulted in a high mortality of flatfish and smelt embryos and larvae. Oil-exposed eggs were often ruptured, with subsequent fragmentation of the chorions and disintegration of the embryos, and affected larvae were unable to swim normally. Low hydrocarbon concentrations also had a pronounced effect on invertebrate chemosensory mediated behaviors, such as feeding, defense, and reproduction.

Therefore, from the studies concerned with the early developmental stages of fish and the chemosensory mediated behavior of invertebrates, the petroleum concentrations necessary to produce deleterious effects observed could realistically be expected to occur in the marine environment. Due to the subtle nature of behavioral changes, and the likelihood that dead eggs and abnormal larvae would sink out of the water column, it

is doubtful that these effects could be detected during field evaluation of oil contamination. The result would be, therefore, that pollutant effects would be substantially underestimated. Thus, the most useful application of this data would be through systems modeling.

Experiments concerning the effect of oiled sediment on flatfish behavior indicate that high levels of crude oil incorporated in the sediment were apparently accepted by juvenile flatfish without noticeable behavioral effects. Although there is little direct evidence from these studies that oil-contaminated sediment is detrimental to the health of juvenile and adult flatfish, it is still a reasonable assumption that long-term residence by these fish in a heavily oil-contaminated environment is not compatible with survival.

2. INTRODUCTION

The responses of marine organisms to environmental contaminants are reflected in a number of changes detectable at organismic, as well as at tissular, cellular, subcellular, and molecular levels. The general purpose of this study was to detect these petroleum-related changes in marine species and to evaluate their implications for the survival and health of the animals.

When petroleum is transported in, or obtained from, coastal or offshore areas, petroleum hydrocarbons and associated trace metals inevitably escape into the marine environment. These materials, at various levels, can produce critical damage to marine resources. Damage by crude oil components takes many forms (Blumer, M., Testimony before Subcommittee on Air and Water Pollution, Senate Comm. on Public Works, Machias, Maine, 8 Sept. 1970).

1. Direct kill of organisms through coating and asphyxiation, through contact poisoning, or through exposure to water-soluble toxic components of oil at some distance in space and time from the accident.

2. Destruction of the generally more sensitive juvenile forms of organisms.

3. Incorporation of sublethal amounts of oil and oil products into organisms resulting in reduced resistance to infection and other stresses and in failure to reproduce.

4. Destruction of the food sources of higher species.

5. Exposure to long-term poisons, e.g., carcinogens.

6. Low level interruption of any of the numerous events necessary for the feeding, migration, and propagation of marine species and for the survival of those species which stand higher in the marine food web.

7. Contamination of marine food resources, making them unfit for human consumption.

Studies by OCSEAP RU 73 were largely concerned with the indirect, long-term effects of petroleum such as those detailed in items 2, 3, 5, and 6. These effects are much more difficult to detect and evaluate than those related to acute exposures, but may over a period of time have even more serious consequences for marine biota.

3. BACKGROUND

3.1 Chemistry

With increased exploration, production, and transportation of petroleum, and the inevitability of accidental oil release, petroleum hydrocarbons have become common contaminants of the marine environments. At the time our OCSEAP research was initiated, most studies concerning the uptake and biochemical effects of oil on aquatic organisms focused on accumulation of parent hydrocarbons in whole organisms, and, to a lesser extent, in specific tissues (Lee et al. 1972, Anderson 1975, Varanasi and Malins 1977).

However, in the mid-1970's, an increasing interest developed in the enzyme systems of aquatic organisms that convert aromatic hydrocarbons to their electrophilic metabolites (Payne 1976, Pedersen et al. 1976, Philpot et al. 1976, Gruger et al. 1977). The hepatic tissues of many aquatic organisms contain enzymes, such as aryl hydrocarbon monooxygenases (AHM), capable of metabolizing aromatic hydrocarbons (Malins 1977, Varanasi and Malins 1977, Bend and James 1978). Some aromatic hydrocarbon metabolites have been shown to be mutagenic and carcinogenic in mammals (Sims and Grover 1974). The early reviews clearly point out that petroleum hydrocarbons can induce or enhance AHM activity in aquatic species. Evidence is rapidly accumulating to suggest that all vertebrate marine organisms possess the AHM system; there are conflicting reports on its presence in invertebrates. In contrast, studies on the uptake and disposition of polycyclic aromatic hydrocarbons (PAH) were few and virtually no information was available concerning the extent of PAH metabolism or profiles of PAH metabolites in either marine fish or invertebrates.

This report describes results of studies conducted to assess the uptake, metabolism, and disposition of various hydrocarbons in marine organisms, with special emphasis on tissue concentrations of hydrocarbons and their metabolites and the types of metabolites formed *in vivo*. Studies conducted by other researchers during the course of our investigations are referred to in Section 7.1.

3.2 Pathology

Effects of Petroleum on Disease Resistance

Considerable evidence indicates that petroleum hydrocarbons and associated trace metals affect host defense mechanisms in various mammals (Kripke and Weiss 1970, Koller 1973, Koller and Kovacic 1974, Stjernsward 1974, Cook et al. 1975, Koller et al. 1975, Koller and Roan 1980), and birds (Vengris and Mare 1974). In addition, a few studies suggest an immunosuppressive potential in fish (Robohm and Nitkowski 1974, O'Neill 1981). Because disease is the result of a complex interaction among the host, the pathogen, and the environment, any environmental perturbation which compromises host defense can precipitate an outbreak of disease; particularly those diseases caused by the many opportunistic bacterial pathogens.

This report presents the results of experiments undertaken to assess the effects of various exposures of crude oil on the disease resistance of commercially important species of the Northeastern Pacific Ocean, including salmonid and flatfish species, and a crustacean, the spot shrimp (Pandalus platyceros). A preliminary investigation of the effects of chemical dispersants on disease resistance is also presented.

Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment

The considerable amount of literature which reports histopathological changes in marine fish as a result of exposure to petroleum hydrocarbons (see Malins 1982 for comprehensive review) primarily reflects exposures to waterborne hydrocarbons. These reported laboratory studies can, at best, only suggest the effects of oil exposures on bottom-dwelling fish coming in contact with contaminated sediment.

Only a few studies are available on the pathological effects of exposure to oil-contaminated sediment. In one of these, a field study, two species of flounder (Pseudopleuronectes americanus and Limanda ferruginea) were collected from control stations and from stations close to the ARGO MERCHANT oil spill; no correlation was established between petroleum from the spill and observed morphological damage (Sawyer 1978).

Early studies of the AMOCO CADIZ oil spill off the Coast of France, however, suggested a definite link between the spilled petroleum and gross pathological alterations, such as fin erosion in plaice (Pleuronectes platessa) from Aber Benoit and Aber Wrac'h (Miossec 1981). A later survey of plaice from Aber Benoit and Aber Wrac'h between 1979 and 1980 revealed fin and tail necrosis, extensive gill lesions, abdominal muscle and gastric gland degeneration, and increased concentrations of hepatic macrophage centers (Haensly et al. 1982). A comparison of these findings to those from a reference area (Baie de Douarnenez and the ports of Loctudy and Ile Tudy) suggested a likely association between spilled oil and the observed biological alterations in plaice.

The studies presented in this report were designed to evaluate the possible relationships between biological anomalies and crude oil-contaminated sediment under controlled laboratory conditions.

Cytopathology

Several reviews have discussed the results of histological and ultrastructural studies of aquatic organisms exposed to environmental contaminants (Hawkes 1977, Hodgins et al. 1977, Gardner 1978, Hawkes 1980, Malins 1982). This section concentrates on studies of embryonic and larval fish, an area that warrants special attention because even low levels of petroleum seem to have a particularly deleterious effect on the early life stages. Eggs, embryos, and larvae are very susceptible to external environmental influences, for, in contrast to the adult organism, the early development stages have few, if any metabolic "resting points," a greater surface-volume ratio, fewer cells, and undeveloped or poorly developed defense and homeostatic mechanisms (LeGore 1974). Petroleum compounds capable of interacting with nucleic acids (see chemistry section of this report), or of interfering with cell migration, communication, or metabolic activity can alter normal development.

Our studies of early developmental stages describe the effects of the seawater-soluble fraction of crude oil on smelt embryos, particularly cytopathological changes in the brain and eye, and to a lesser extent, on the timing of these changes. We also examined the effects of waterborne crude oil on the morphology of flatfish larvae.

3.3 Behavior and Physiology

Chemical agents released by animals, and chemical signals in the environment itself, can influence a variety of activities: symbiosis (Ache and Davenport 1972); homing (Cook 1969); reproduction (Atema and Engstrom 1971, Kittredge et al. 1971, Ryan 1966); site selection and larval settlement (Crisp 1974); evaluation of local habitat (van Weel and Christofferson 1966, Laverack 1974); and detection of both predators and prey (Phillips 1978). The importance of chemical sensing in aquatic organisms has been long recognized, but only during the past decade has there been extensive research on the chemical communication of aquatic species and the effects that man-induced contaminants may have in interfering with this communication. There is clear evidence that oil products interfere with chemosensory-mediated behavior (Atema et al. 1973), and that aromatic hydrocarbons in particular are probably the most active petroleum components in this regard (Kittredge et al. 1974, Takahashi and Kittredge 1973). Behavioral disruptions at exposure concentrations in the low parts-per-billion (ppb) range have been noted among marine organisms as diverse as bacteria, algae, and invertebrates (Johnson 1977, Jacobson and Boylan 1973). Although disruption of invertebrate behavior may occur at low ppb hydrocarbon concentrations, vertebrate behavioral reponses and changes in activity patterns during

hydrocarbon exposure have been observed at only high ppb or low parts-per-million (ppm) concentrations (Pattern 1977).

Field observations have suggested that mobile marine organisms do not avoid areas of petroleum contamination. Cross et al. (1978) reported dead fish and crustacea subsequent to the AMOCO CADIZ incident, and MacLeod et al. (1978) cited the presence of Bunker C oil in the stomach of codfish taken near the site of the ARGO MERCHANT spill. This project is the first to report on the behavior of adult salmon exposed to waterborne hydrocarbons or flatfish exposed to oil-contaminated sediment.

Infertile gametes and teratogenic effects on progeny were demonstrated for trout exposed to DDT (Burdick et al. 1964, Macek 1968), and in flathead sole (Hippoglossoides elassodon) fed a single dose of benzo(a)pyrene (BaP) (Hose et al. 1981). The studies of trout reproduction discussed in this report represent the first known investigation of the effects on the reproductive processes of fish from long-term dietary exposure to crude oil components.

In reviews of acute toxicity and sublethal biological effects of petroleum on arctic and subarctic marine fishes, Craddock (1977) and Patten (1977) presented evidence of lethargy, loss of appetite, and alterations in schooling behavior associated with exposure to various seawater-soluble fractions of petroleum. However, no studies were reported on the influence of petroleum on predator-prey behavior which has been described as a sensitive indicator of perturbed environmental conditions (Goodyear 1972, Hatfield and Anderson 1972, Sylvester 1972, Coutant et al. 1974, Yocum and Edsall 1974, Sullivan et al. 1978, Woltering et al. 1978). The purpose of the present studies was to determine the influence of crude oil in seawater on salmonid predator-prey interactions. Coho salmon (Oncorhynchus kisutch) were chosen as predators since this species has been identified as a primary predator of juvenile salmonids in seawater (Parker 1971).

4. STUDY AREA

Most experiments were performed in the laboratories at either the Northwest and Alaska Fisheries Center (NWAFC) in Seattle, or at the NWAFC's saltwater field station at Mukilteo, Washington. Field experiments were conducted in the Puget Sound area.

Organisms used in experiments are representative of temperate, arctic, and subarctic species, and with few exceptions were either collected from Puget Sound or were indigenous anadromous fishes of the Puget Sound drainage.

5. METHODS

5.1 Chemistry

5.1.1 Accumulation and Biotransformation of Specific Aromatic Hydrocarbons in Salmonids

Fingerling coho salmon (ca 20 g; purchased from DomSea Farms, Bainbridge Island, WA) maintained in freshwater, were injected intraperitoneally (i.p.) with 2.5 µCi of ^{14}C-labeled benzene (sp. act. 25 mCi/mmole), naphthalene (NPH) (sp. act. 5mCi/mmole), or anthracene (sp. act. 23 mCi/mmole) dissolved in 0.05 ml of ethanol. The fish injected with benzene were sampled (3 fish per time point) 6 and 24 hr after injection. Anthracene and NPH-exposed fish (3 fish per time point) were sampled 24, 72, and 144 hr after the injection. Brain, liver, gallbladder, heart, muscle (flesh), and residual carcass were analyzed for parent hydrocarbons and metabolic products; tissues were added to 2-5 ml of 90% formic acid overlaid with 5-10 ml of hexane at room temperature. After 12-24 hr, a saturated solution of sodium hydroxide was added until the solution was strongly alkaline (pH>12). Hydrocarbons remained in hexane and metabolites in the aqueous phase. (For further details see Roubal et al. 1977a.)

5.1.2 Accumulation of Petroleum Hydrocarbons by Fish Exposed to Seawater Soluble Fraction (SWSF) of Prudhoe Bay Crude Oil (PBCO)

Coho salmon (11-19g; purchased from DomSea Farms, Bainbridge Island, WA) and starry flounder (Platichthys stellatus) (32-186g; captured from Puget Sound) were exposed to a SWSF of PBCO in seawater[a], at 10°C, under continuous flow-through bioassay conditions. The apparatus used is depicted in Figure 1. The concentration of total soluble hydrocarbons in flowing seawater delivered from the solubilizer was 5 ppm as measured by capillary gas chromatography (GC). Hydrocarbons were analyzed by gas chromatography-mass spectrometry (GC/MS). SWSF delivered from the solubilizer was diluted with seawater to produce a final hydrocarbon content of 0.9 ± 0.1 ppm (Fig. 1). Coho salmon were exposed to the 0.9 ± 0.1 ppm SWSF for a 6-week period, followed by 6 weeks of holding exposed fish in oil-free seawater to evaluate depuration.

Muscle tissue of salmon was analyzed for concentrations of SWSF hydrocarbons starting one week after the beginning of the exposure period. Excised tissues were thoroughly rinsed with 3.5% saline and aliquots were digested at room temperatures in 4 N sodium hydroxide. The digests were analyzed for hydrocarbons using capillary GC. Similar analyses were made on samples of starry flounder muscle gills and liver from 10 fish. (For further details see Roubal et al. 1978.)

5.1.3 Metabolism of NPH by Coho Salmon

Four coho salmon (160 ± 35g; purchased from DomSea Farms, Bainbridge Island, WA) maintained at 10° ± 0.5°C were force-fed 74.6 uCi of ^{3}H-NPH

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a Seawater salinity was 27-30°/oo in all chemistry studies.

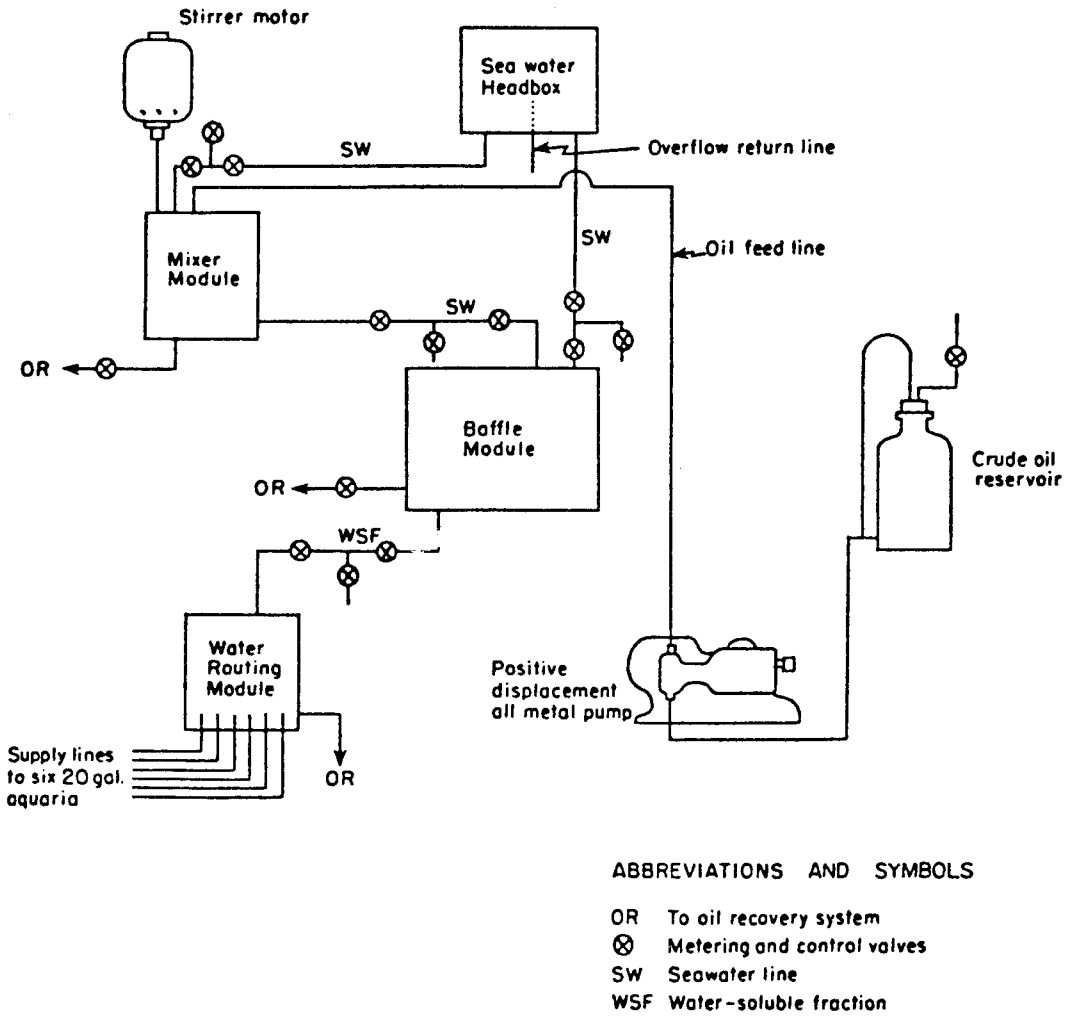

FIGURE 1. Schematic of oil-in-seawater solubilizer system used to produce the seawater soluble fraction (SWSF) of crude oil. (From Roubal et al. 1977b)

(sp. act. 85 mCi/mmole) dissolved in salmon oil. After 16 hr, the livers and gall bladders were removed and pooled for analysis. Tissues were homogenized in distilled water, saturated with sodium chloride, and proteins were precipitated with acetone. Metabolites were extracted into ethyl acetate, the extract dried under nitrogen, and the residue was redissolved in methanol. Metabolite extracts were chromatographed by high-pressure liquid chromatography (HPLC). (For further details see Collier et al. 1978.)

5.1.4 Naphthalene and Its Metabolites in Fish Skin and Mucus

Rainbow trout (Salmo gairdneri) (150 ± 50g; purchased from Trout Lodge, Puyallup, WA), held at 8°C were either force-fed 74.6 µCi of [1,4,5,8,-^{3}H] NPH (sp. act. 83.3 mCi/mmole) dissolved in 250 µl of salmon oil or injected i.p. with 40 µl of oil containing 94.6 µCi of ^{3}H-NPH. Starry flounder (100 ± 20g; captured from Puget Sound) held at 12°C were force-fed a gelatin capsule containing 87 µCi of ^{3}H-NPH (sp. act. 198 mCi/mmole) dissolved in salmon oil. Concentrations of NPH and total metabolites in skin and mucus were determined by digestion of the sample at room temperature in hexane and 4 N NaOH. Concentration of the NPH was determined from radioactivity in the hexane layer, and concentration of total metabolites was determined from the aqueous layer. Concentrations of NPH and its metabolites in the skin were compared with those in the liver. (For further details see Varanasi et al. 1978 and Collier 1978.)

5.1.5 Accumulation and Biotransformation of NPH by Flatfish

Sexually immature starry flounder and rock sole (both species: 82 ± 30g) were captured near the mouth of the Columbia River and at Point Pully in Puget Sound, respectively, and were maintained at experimental temperatures of 12 ± 1°C in flowing seawater for a period of 2 wk prior to treatment. Fish were fed daily to satiation on a mixture of earthworms and euphausiids.

Force-feeding study. Test fish of both species were force-fed a gelatin capsule (No. 5) containing 56 µCi of [1-^{3}H]-NPH (sp. act. 198 mCi/mmole) dissolved in 25 µl of salmon oil. The fish were then placed in aquaria supplied with flowing seawater at 12°C. Three to six fish were analyzed at 24, 48, and 168 hr after the initiation of exposure. Rock sole were analyzed also at 6 wk. Fish were not fed during the first week after the initiation of NPH exposure.

Injection Study. Starry flounder were injected i.p., with 25 ul of salmon oil containing 56 µCi of the [1-^{3}H]-NPH (sp. act. 198 mCi/mmole). The fish were held at 12°C, and sampled at 24 and 168 hr after injection.

Sample collection. Epidermal mucus and skin were collected, and samples of muscle, liver, brain, gills, blood, kidney, stomach, intestine, and bile were also collected.

Analytical methods. Radioactivity associated with both NPH and total metabolites in each tissue (~100 mg) was determined by digestion in hexane-sodium hydroxide. Dry weight of each tissue was obtained by freeze-drying and the values expressed as percent of wet weight of tissue. Lipid content of liver, muscle, and skin of starry flounder and rock sole (Lepidopsetta bilineata) were also determined.

Data were statistically analyzed using Student's t-test. Also, rates of decline of NPH and metabolite concentrations in tissues were obtained by assuming a lognormal distribution and describing the decay of concentration by $y = ax^{-b}$.

NPH metabolites were isolated from liver, muscle, skin, and bile of the exposed rock sole and starry flounder; the samples were homogenized in methanol and then extracted twice with hexane to remove NPH, followed by extraction with a mixture of boiling methylene chloride:2-propanol:water (75:25:2, v/v/v) and twice with boiling ethanol:diethyl ether (50:50, v/v) to remove metabolites.

Individual classes of metabolites were separated via thin-layer chromatography (TLC). Nonconjugated metabolites were separated using a solvent system of p-dioxane:benzene:acetic acid (25:90:4, v/v/v). Conjugated metabolites were separated by TLC using a solvent system consisting of the upper phase of 1-butanol:concentrated ammonium hydroxide:water (80:20:100, v/v/v). Nonradioactive standards added to each sample allowed visualization of individual classes of metabolites after staining with color producing reagents.

After the determination of the position of the various metabolites the adsorbant was scraped from the chromatograms in 5 mm bands and radioactivity in each band was determined. (For further details see Varanasi et al. 1979, 1981.)

5.1.6 Effect of Temperature on Disposition of NPH and Its Metabolites in Fish

(a) Coho salmon (150 ± 50 g; purchased from DomSea Farms, Bainbridge Island, WA) maintained at 4 and 10°C were force-fed 5.55 μCi NPH (sp. act. 5.1 mCi/mmole) to determine the effect of temperature on the amount of [1-^{14}C] NPH incorporated into selected organs. After 8 and 16 hr the fish were sampled and the brain, liver, kidney, gallbladder and gut were removed along with samples of blood, dark muscle and light muscle. To analyze for NPH and total metabolites in the fish, the formic acid digestion/hexane extraction method was used (see Section 5.1.1).

(b) Sexually immature starry flounder from the same group of fish used in Section 5.1.5 were held at 4 ± 1°C in flowing seawater for 2 wk before the experiment was conducted. Fish were fed daily (to satiation) a mixture of earthworms and euphausiids.

The fish were force-fed 56 μCi of [1-^{3}H]-NPH (sp. act. 198 mCi/mmole) dissolved in 25 μl of salmon oil, but were not fed thereafter. Six fish were sampled at 24 and 168 hr after force-feeding.

NPH and its metabolites were analyzed by the same procedures outlined in Section 5.1.5. (For further details see Varanasi et al. 1981a.)

5.1.7 Uptake and Metabolism of Sediment-Associated Aromatic Hydrocarbons by Flatfish

English sole (62 ± 22 g; captured from Puget Sound) were held in flowing seawater (12.0 ± 0.5°C) and fed a diet of minced clams for two weeks. The feeding was stopped three days prior to the initiation of experiments.

Oil-contaminated sediment (1%, v/v) was prepared as described by McCain et al. (1978) except that 3.6 mCi ^{3}H-BaP (sp. act. 0.83 mCi/mmole) and 0.14 mCi ^{14}C-NPH (sp. act. 167 mCi/mmole) were dissolved in PBCO prior to mixing with the sediment. The oil-contaminated sediment was placed in a 17-l glass aquaria to a depth of 5-6 cm where it was allowed to stand in flowing seawater (20 l/day) for 24 hr (day 1).

Six fish were placed in the experimental tank for a 24-hr exposure on day 2, three fish were sampled on day 3, and the remaining 3 fish were placed on clean sediment in flowing seawater for 24 hr. Immediately after the first six fish were removed on day 3, five additional fish were placed in the experimental tank. These fish were exposed to the oiled sediment for 168 hr before sampling on day 10.

Samples of sediment and sediment-associated water (SAW) were taken from 2 cm below the sediment/water interface. The wide end of a glass pipette was vertically inserted into the sediment while the tip of the pipette was covered. After positioning the pipette, the tip was uncovered to allow sediment and SAW to rise within the pipette. The sample was carefully transferred to a vial and the SAW was decanted off after the suspended particles had settled.

Samples of wet sediment, unfiltered and filtered (0.45 μ, millipore) SAW, and samples of gill, skin, muscle, blood, liver, bile, stomach, and intestine were analyzed for total radioactivity (^{3}H and ^{14}C).

Ethyl acetate extracts of sediment, SAW, and liver and bile--before and after enzymatic hydrolysis with β-glucuronidase or sulfatase--were analyzed by TLC for parent BaP and its metabolites. Four solvent systems were employed for TLC analyses: Solvent system A (toluene: ethanol, 9:1, v/v) was used for separation of nonconjugated BaP metabolites; solvent system B (Plate was developed up to 6 cm in ethyl acetate and then redeveloped in the same direction with toluene:ethanol, 100:3, v/v) was used for separation of nonconjugated NPH metabolites, solvent system C (upper phase of concentrated ammonium hydroxide:water:n-butanol, 10:50:40, v/v/v) was used for separation of conjugated NPH metabolites,

and solvent system D (hexane:diethyl ether, 95:5, v/v) was used for separation of nonconjugated BaP metabolites from BaP and liver lipids. Assessments of NPH and total NPH metabolites in sediment, SAW, bile, and liver were also made by a solvent partitioning method using hexane and sodium hydroxide. In addition, ethyl acetate-soluble metabolites from bile before and after enzymatic hydrolyses were analyzed by HPLC.

Protein from the aqueous phase of liver homogenates was pelleted by centrifugation, followed by extraction with acetone and diethyl ether, dryed, and then solubilized to determine radioactivity that was not extractable. (For further details see Varanasi and Gmur 1981.)

5.1.8 BaP Metabolism by English sole

English sole (Parophrys vetulus) (74 ± 17g; captured from Puget Sound), held at 12°C in flowing seawater, were force-fed ^{3}H-BaP (2 mg/kg body wt) and liver, muscle and bile were analyzed for BaP and its metabolites by methods described above (Section 5.1.7). Three fish were sampled at 8, 16, 24, 48 and 168 hr after force feeding BaP. (For further details see Varanasi and Gmur 1981b.)

5.1.9 Binding of BaP Intermediates to DNA Catalyzed by Liver Enzymes of Fish

Starry flounder (131 ± 42g; captured near the mouth of the Columbia River) and English sole (105 ± 33g; captured from Puget Sound) were injected intraperitoneally with 10 mg/kg of BaP, 3-methylcholanthrene (MC), or PBCO, in corn oil. Control fish in these studies were untreated, because there was no detectable difference in the binding of metabolically activated ^{3}H-BaP to DNA when liver enzymes from untreated or corn oil-treated fish were used. The fish were sampled 24 hr after injection and supernatants of liver homogenates (10,000 x g) were prepared. Rat liver supernatants were prepared in the same manner. The influence of a number of parameters, such as substrate concentration, temperature, reaction time, and concentrations of cofactors were tested to obtain optimum conditions for in vitro binding assays. The standard reaction mixture contained: 2 mg of DNA added in 2.5 ml of 0.02 M phosphate buffer (pH 7.4); 0.75 mg NADPH added in 0.1 ml of 0.1 M EDTA (pH 7.4); and 0.2 ml of the 10,000 x g supernatant (5 mg protein). The reaction was started by adding 5 nmoles of BaP in 50 µl of ethanol. The mixture was incubated in the dark for 15 min at 25°C when fish liver supernatant was used, and at 37°C when rat liver supernatant was used. DNA was isolated from the reaction mixture by extraction with phenol saturated with phosphate buffer followed by ethanol precipitation.

BaP metabolites were formed by incubating liver supernatants with ^{3}H-BaP under the conditions described above, without the addition of DNA. The mixture was extracted with ethyl acetate (2 x 6 ml) and radioactivity in aqueous and organic phases was determined. Separation and quantification of ethyl acetate-soluble metabolites were carried out using both TLC and HPLC. (For further details see Varanasi and Gmur 1980 and Varanasi et al. 1980.)

5.1.10 AHM Activities in Different Species

Fish, crabs, and snails were collected during NOAA cruise No. MF-77-1 of the Miller Freeman. Livers from fish, visceral organs from crab, and whole snail were frozen and held at -60°C during transit (from Alaska to Seattle) and in the laboratory prior to analyses. The specimens were collected from January 25 to February 10, 1977, in areas northeast of Kodiak, Alaska.

Analyses of AHM activities were also carried out on livers of Pacific cod (Gadus macrocephalus) which were found to have pseudobranchial tumors. The fish were collected during a Miller Freeman cruise in Alaska, and were part of another OCSEAP project (Research Unit 332).

Specific activities of AHM were measured using ^{3}H-BaP as the substrate for the AHM. The procedures employed were a modification of those by DePierre et al. (1975). The temperature and pH of the AHM assay mixtures were optimized for the fish (i.e., 25°C and pH 7.5) and assays were performed with NADPH (tetrasodium salt; Sigma Chemical Co.) rather than with a NADPH-generating system.

A typical assay reaction in 2.1 ml contained 0.67 mM NADPH, 1.4 mM $MgCl_2$, 50 ul of enzyme source (25 mg protein/ml), 20 ul of an acetone solution of 3.2 mM tritiated benzo(a)pyrene (0.96 µCi), and 60 mM Tris HCl buffer (pH 7.5). The enzyme source was a 20% (wt/vol) homogenate of tissue (e.g., liver) in cold 0.25 M sucrose solution that was separated as a supernatant fraction from cellular debris by centrifugation at 9,000xg (or 10,000xg) for 20 min. Duplicate reaction mixtures (in open culture tubes under subdued light) were shaken for 10 min at 25° before initiation of reactions by the addition of the ^{3}H-BaP. Incubation time was 20 min. During work up, two hexane extractions were employed, in contrast to a single extraction according to DePierre et al. (1975). This procedure resulted in better agreement among assays and less variation in blanks.

Additional analyses of hepatic AHM activities were conducted with the use of NPH as the substrate (Nilsson et al. 1976). Protein contents of the enzyme sources were determined by the method of Lowry et al. (1951).

5.1.11 Uptake, Metabolism and Toxicity of Hydrocarbons in Invertebrates

(a) Sperm and eggs from artificially spawned mussels, Mytilus edulis, (collected from Puget Sound) were placed in 400 ml of seawater containing ^{3}H-NPH (sp. act. 198 mCi/mmole) at concentrations of 100, 10, and 1 ppm and in control seawater at 11 ± 1°C. Each test and control condition was run in triplicate. Samples of eggs and/or larvae were removed from each container at 0.5, 1, 2, 3, 6, 12, and 24 hr, and were preserved in 5% buffered formalin solution. Water samples were taken at each time period for determinations of the NPH concentration. Specimens were counted and the developmental stages identified.

(b) Sperm and eggs of oysters, Crassostrea gigas, (collected from Puget Sound) were separately exposed to a seawater solution of ^{3}H-NPH (sp. act. 198 mCi/mmole) at two concentrations (10 and 1 ppb)

for 15 min after which the complementing gametes were added. In addition, non-exposed gametes of both sexes were introduced into seawater containing NPH at concentrations of 10, 1, and 0.1 ppb and one control in uncontaminated seawater. Larval samples were taken at intervals of up to 48 hr and were preserved in 5% buffered formalin. Water samples were taken at each time point to determine the concentration of NPH. The eggs and/or larvae in each sample were counted and the developmental stages identified.

(c) One-year old spot shrimp (collected from Puget Sound) were exposed in flow-through aquaria for 7 days to the SWSF of PBCO at an average concentration of 110 ppb as determined by GC. The SWSF was obtained from the solubilizer (Fig. 1). The animals were washed, extracted, and the extracts were analyzed for accumulated hydrocarbons by GC/MS. Abdomens were separated from thoracic segments and analyzed separately. (For further details see Sanborn and Malins 1980.)

(d) Newly metamorphosed larval stages of spot shrimp and Dungeness crab, Cancer magister, (collected from Puget Sound) were exposed in flowing seawater (10 ± 1°C) to 8-12 ppb of [1-^{14}C] NPH (sp. act. 5 mCi/mmole) or [1-^{14}C] NPH complexed with bovine serum albumin (BSA). The spot shrimp and Dungeness crab were hatched in the laboratory from ovigerous females. The shrimp larvae hatched each day were held in separate holding tanks and fed brine shrimp. Exposure periods varied from 12 to 24 hr and depuration studies were carried out for periods of up to 132 hr. Larvae were examined for both [1-^{14}C] NPH and its metabolites. Total radioactivity in the animals was determined, and total metabolites of [1-^{14}C] NPH were determined by employing formic acid/hexane extractions (see Section 5.1.1). (For further details see Sanborn and Malins 1978.)

(e) Adult spot shrimp were placed in an 80 ppb seawater (10 ± 1°C) solution of ^{3}H- (sp. act. 198 mCi/mmole) and ^{14}C- (sp. act. 3.67 mCi/mole) labeled NPH; the ^{3}H/^{14}C ratio was 48:1. The concentration was maintained under flow-through conditions. The animals were removed after 10 hr, washed, the thorax and abdomen separated, and the tissues analyzed by HPLC. (For further details see Sanborn and Malins 1980.)

(f) Stage I spot shrimp larvae that had been hatched in the laboratory were exposed to an 18 ppb seawater solution of ^{3}H-NPH (sp. act. 198 mCi/mmole) at 10°-12°C. After 10 hr the larvae were washed, weighed, extracted and analyzed for NPH metabolites by HPLC.

5.1.12 Food Chain Transfer of 2,6-Dimethylnaphthalene (2,6-DMN) to Sea Urchins via Algae

The marine algae, Fucus distichus, and the green sea urchin (Strongylocentrotus droebachiensis) were collected locally. The Fucus was exposed to 2,6-DMN as follows: Two hundred grams of the wet algae was rinsed in seawater and then introduced into an 68 l aquarium containing seawater and 10 mCi of [2,6-^{3}H]-DMN (sp. act. 2 Ci/mmole) in 0.5 ml of ethanol. Samples of the seaweed were removed at 5 hr intervals over a period of 35 hr, rinsed well, divided into aliquots, then frozen at -20°C until analyzed for incorporated tritium.

A fresh batch of the seaweed was harvested and added to an aquarium containing the ^{3}H-2,6-DMN. After 25 hr, the time of maximum tritium-incorporation, the exposed Fucus was removed, rinsed well in seawater, and then placed in an aquarium containing 6 sea urchins (170 ± 4g). A new batch of the 2,6-DMN-treated Fucus was added 24 hr later to the sea urchin aquarium, after removal of unconsumed Fucus. This sequence of presenting exposed Fucus to the sea urchins at 24 hr intervals was repeated throughout the 14 day experiment.

After three days of feeding on the 2,6-DMN-treated Fucus, 3 sea urchins were removed, rinsed, and frozen at -20°C until analyzed. The remaining 3 sea urchins were removed after 14 days of feeding on the treated Fucus and handled similarly.

The exoskeleton of sea urchins was pulverized and extracted with hot methanol and filtered. Aliquots were analyzed for tritium by scintillation counting. Half-gram portions of the powdered exoskeleton residue remaining after methanol extraction were also digested in tissue solubilizer and then assayed for tritium.

Fucus samples and aliquots of homogenized gonadal and digestive tract were analyzed for 2,6-DMN by digestion in hexane-sodium hydroxide. Metabolites of 2,6-DMN were analyzed by extracting soft tissues with hot methanol-diethyl ether, followed by TLC of the extracts. Sulfate fractions were removed from TLC plates and extracted with hot methanol. After removal, the sulfate fractions were treated with aryl sulfatase at 37°C in buffer (pH 5.0, 0.2 M acetate). The digests were extracted with ethyl acetate and analyzed by TLC. The 3- and 4-hydroxy derivatives of 2,6-DMN, isolated as a single band, were resolved on a TLC plate developed 5 times with toluene. (For further details see Malins and Roubal 1982.)

5.1.13 Biological Fate of Metals in Fish

Exposure conditions for metals studies

Water-immersion Studies. Coho salmon (200 ± 20 g; obtained from National Marine Fisheries Service, Manchester, WA) and starry flounder (30 ± 15 g; captured from near the mouth of the Columbia River) were held at experimental temperatures of 4° or 10°C for a period of two weeks, and then exposed to either seawater-borne lead-210 or cadmium-109 (150 ppb) under partial flow-through conditions.

The fish were exposed to either lead or cadmium for a period of two weeks at each temperature then three or four fish per exposure group were sampled. At the end of the two-week period, remaining fish were placed in control seawater for depuration and sampled after 7 and 37 days. A group of control fish of similar weight was kept under identical conditions in control seawater. Concentrations of lead and cadmium in control seawater were less than 5 and 2 ppb, respectively.

Injection Studies. Coho salmon (80 ± 5 g) were injected intravenously (i.v.) with 32 ± 2 µg of metals as either lead nitrate mixed with ^{210}Pb or cadmium chloride mixed with ^{109}Cd dissolved in 250 µl of tris buffer (pH 7.2). A similar experiment was carried out with fish which were exposed to 150 ppb of nonradioactive cadmium or lead for 2 wk prior to i.v. injection of radiolabeled metals to determine if prior exposure to metals caused any alterations in radioactivity associated with metal binding proteins.

Analytical methods for metals studies.

Samples of mucus, skin (with scales), scales, skin, blood, gills, liver, and kidney were obtained and concentrations of ^{210}Pb or ^{109}Cd were determined in these tissues by liquid scintillation spectrometry. Concentrations of total lead or cadmium in these tissues, expressed on a wet weight basis, were obtained from ratios of ^{210}Pb/Pb and ^{109}Cd/Cd in stock solutions used in the exposures. Concentrations of nonradioactive lead and cadmium in seawater and fish tissues were determined by Laucks Analytical Laboratories (Seattle, Washington).

Samples of liver and kidney of fish from the injection study were homogenized and cytosol obtained. Three samples were chromatographed on a column packed with Sephadex G-75 superfine. The radioactivity in the eluted fractions was determined to assess the distribution of metals bound to various protein fractions. The protein concentration in each eluant was determined by a modified method of Lowry et al. (1951). (For further details see Varanasi and Markey 1978 and Reichert et al. 1979.)

5.2 Pathology

5.2.1 Effects of Petroleum on Disease Resistance

Acquisition, Handling, and Exposure of Test Animals. Test animals for disease resistance studies were obtained from the following sources: juvenile coho salmon were from the Issaquah Hatchery of the Washington Department of Fisheries, from the Willard National Fish Hatchery, Cook, Washington and from Sashin Creek, Little Port Walter, Alaska. The Sashin Creek fish were received as eyed eggs and reared at the Northwest and Alaska Fisheries Center (NWAFC), Seattle, Washington. Juvenile rainbow trout were from a stock maintained at the NWAFC and were the progeny of fish originally obtained from the Spokane Hatchery of the Washington Department of Game. Adult flatfish were captured by otter trawl and juvenile flatfish collected by beach seine in Puget Sound; adult spot shrimp were collected by pot fishing in Puget Sound.

All experimental animals were held under laboratory conditions for at least 2 wk before the start of testing. The salmonid fish were fed an Oregon moist pellet (OMP) diet at 2% of their body weight, and the flatfish were fed a mixture of minced clams and krill. Spot shrimp were maintained on a diet of fish offal.

Several different methods were used to expose fish and crustacea to petroleum hydrocarbons. Salmonids were exposed both via the diet, by mixing OMP with PBCO as described by Hodgins et al. (1977), and via flow-through exposure to the SWSF of PBCO (Roubal et al. 1977b). Flatfish and spot shrimp were exposed to PBCO or Cook Inlet crude oil (CICO) by maintenance on oil-contaminated sediment (McCain et al. 1978). Sediment, water, and selected tissues from test animals were analyzed for total extractable petroleum hydrocarbons (TEPH) by the methods described by Malins et al. (1980).

In vivo Assays of Immunocompetence.

(a) Throughout these studies oil-exposed animals were compared to controls for their ability to survive a laboratory challenge by the marine fish pathogen Vibrio anguillarum. Several different bacterial isolates were used, the taxonomic identity of each was confirmed by conventional cultural and biochemical tests, and by deoxyribonucleic acid hybridization.

As needed, bacteria were cultivated overnight in either Trypticase soy broth (BBL) or brain heart infusion (Difco) on a reciprocal shaker at room temperature (ca 23°C). Both media were supplemented with an additional 10 mg/l NaCl. Ten-fold serial dilutions were prepared in 0.15 M NaCl and the numbers of viable bacteria were estimated by standard spread-plate technique. For challenge, fish were transferred from the exposure facilities at the Mukilteo Field Station to the Disease Isolation Laboratory at the NWAFC. Groups of 5 to 20 test animals were placed in individual 38 l aquaria containing aerated seawater (salinity 26-30 $^{o}/_{oo}$) or freshwater maintained at the exposure temperature (10-15°C). Fish were challenged with selected test concentrations of bacteria by either i.p. injection (flatfish) or by subcutaneous injection at the posterior insertion of the dorsal fin (salmonids). Shrimp were challenged by injection of bacteria at the suture separating the thorax and abdomen on the dorsal surface. Aquaria were checked daily for mortalities for a minimum of 10 d after challenge. Tissues from dead fish and shrimp were cultured for bacteria; only in those cases in which V. anguillarum was reisolated in pure culture were the deaths attributed to the bacterial challenge.

LD_{50} values (i.e., the number of bacteria that kill 50% of the animals) and their 95% confidence intervals (C.I.) were calculated by logit analysis (Cox 1970). The LD_{50} values were statistically compared using a method similar to that described by Litchfield and Wilcoxon (1949). The 95% C.I. for the difference between any two LD_{50} values was calculated and the hypothesis that the two values were equal was rejected (α=0.05) if this C.I. did not contain the point zero. In those cases in which inadequate partial kills prevented calculation of an LD_{50} by the logit procedure, the method of Reed and Muench (1938) was used. In addition, statistical comparisons between the percent mortality occurring in oil-exposed and control groups challenged with the same number of bacteria were made using Chi-square analyses.

(b) An additional test was conducted to assess the effect of oil exposure on the adaptive immune response of fish. Groups of PBCO-exposed and control rainbow trout were vaccinated with a heat-killed vaccine prepared from V. anguillarum and subsequently challenged with varying concentrations of the living organisms.

In vitro Assays of Immunocompetence.

(a) Numbers of antibody-forming cells in anterior kidney and splenic tissues of PBCO-exposed and control rainbow trout were determined by a modified Jerne plaque assay (Chiller et al. 1969). Fish were immunized against trinitrophenol conjugated with lipopolysaccharide (LPS) from Escherichia coli B. Plaque formation was assayed on a lawn of sheep red blood cells (SRBC) which were coated with LPS, in a soft agarose matrix. Rainbow trout sera, frozen and thawed one time, were used as a source of complement.

(b) Serum agglutinating antibody levels were compared between oil-exposed and control fish by the microdilution technique (Microtiter, Cooke Engineering Company). Fish were immunized against V. anguillarum and 21 d later 2-fold serial dilutions of serum were tested for specific agglutinins. Fish were held at 15°C to facilitate antibody formation.

(c) For polyclonal lymphoid cell activation assays, peripheral blood leukocytes from oil-exposed and control fish were incubated with purified protein derivative (PPD) prepared from the tubercle bacillus. Activity was quantified by measurement of plaque-forming ability on lawns of SRBC's in agarose.

(d) The degree of mitogenic stimulation was compared between PBCO-exposed and control fish under assay conditions previously described in detail by Etlinger et al. (1976). Briefly, leukocyte cultures prepared from splenic tissue were assayed for lymphocyte proliferation following incubation in the presence of the plant-derived mitogenic substance concanavalin A (Con A). Stimulation was quantified by measurement of the incorporation of ^{3}H-thymidine in the proliferating cellular DNA.

(e) Early in the course of these in vitro investigations of immunocompetence it was noted that the oil-exposed rainbow trout had reduced spleen sizes compared to those of the controls. Since antibody-forming leukocytes have been previously identified as one of the principal cellular components of the spleen (Chiller et al. 1969), this condition was further examined. Spleen-weight to body-weight ratios were measured and cellular composition with respect to total numbers of erythrocytes and leukocytes-thrombocytes were determined. These same hematological parameters were also determined for anterior kidney tissue homogenates and peripheral blood.

Effects of Corexit 9527 on Disease Resistance. Juvenile coho salmon were exposed for 30 min at 15 ± 1°C to 30 ppm (v/v) Corexit 9527 in seawater containing various concentrations of V. anguillarum. Control fish were similarly exposed in seawater containing either bacteria or

Corexit only. Fish were then transferred to individual 38 l aquaria containing aerated fresh seawater and mortality was monitored for 10 d. All dead fish were examined by bacterial culture and death was considered to be due to V. anguillarum only when the bacterium was reisolated in pure culture.

5.2.2 Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment

Acquisition and Handling of Test Animals. English sole, rock sole, or starry flounder were captured with otter trawl or beach seine from either the mouth of the Columbia River or in Puget Sound. Fish were transported to Mukilteo, Washington and held for 7-14 days prior to use. All fish were determined to be actively feeding before experiments were initiated. Two types of sediment (either high-silt or high-sand content) contaminated with 0.2, 0.5, or 1.0% (v/v) PBCO were tested; both juvenile and adult stages were examined and exposures ranged from 2 wk to 4 mo. The general approach for all experiments was similar and is outlined below.

Experimental Design. Fish were randomly assigned to a test or control group, weighed, measured, and cold-branded (Fujihara and Nakatani 1967) for individual identification. Prior to each test, samples for hematology and histopathology were collected from 6 to 10 control fish. Hematological tests included hematocrit, hemoglobin, and total red blood cell and leucocyte counts using standard techniques as described by Blaxhall and Daisley (1973). Tissue samples (gill, skin, fin, intestine, kidney, liver, and spleen) were placed in phosphate-buffered formalin. In addition, samples of liver, skin and muscle were placed either in glass vials or aluminum foil and stored at -20°C for subsequent chemical analysis. Sediment samples (400 g) for analysis of TEPH were collected and stored at -20°C.

At intervals of 1 to 4 wk, depending on the length of the experiment, sediment samples and samples of 3 to 6 test and control fish were collected using the same procedures. The remaining fish were weighed and measured and returned to the aquaria.

Fish were fed to satiation, twice daily five days per week, a mixture of clams, euphausids, and live earthworms. Tanks were monitored daily for mortality. Water flow in the 200 l test and control aquaria was maintained at 3 l/min; average water temperature, 8.5°C; average salinity, 27 $^{o}/_{oo}$; light was maintained on a 12L:12D schedule.

Sediment was collected at 2 sites near Sequim, Washington. The high-sand type (approximately 99% of particles >0.07 mm diameter) was from Port Williams; the high-silt type (48% of particles <0.07 mm), from a lagoon adjacent to the Battelle Northwest Laboratory. PBCO and sediment were mixed together for 30 min in fiberglass-lined cement mixer at concentrations of 0.2, 0.5, or 1.0% v/v.

Both control and test apparatuses were made of fiberglass-lined plywood containers set inside larger fiberglass tanks (Fig. 2). The design allowed water circulation through the sediment. Sediment alone or sediment mixed with oil was layered at a thickness of 5 cm in the containers and the tanks flushed with sea water for 24 hr prior to use.

Histological Procedures. For light microscopic examination, preserved tissues were embedded in paraffin and sectioned at 5 µm following the procedures outlined by Preece (1972). As necessary, gills and bones were decalcified using a commercial decalcification solution (Scientific Products, Redmond, WA) prior to processing. Paraffin sections were routinely stained with Mayer's hematoxylin and eosin-phloxine (Luna 1968). As stained tissues from each fish were examined microscopically, the presence of parasites and descriptions of all observed lesions were recorded. For further characterization of the components of specific lesions, additional sections were stained with May-Grunwald Giemsa for RNA and DNA; Masson's trichrome for collagen; Periodic acid-Schiff (PAS) for glycogen, mucin and basement membrane; Brown and Brenn's Gram stain for bacteria; Congo red for amyloid; Laqueur's method for alcoholic hyalin; Ziehl-Neelsen method for acid-fast bacteria; Gomori's iron reaction for iron pigments; or with the Armed Forces Institute of Pathology method for lipofuscin and ceroid (Thompson 1966, Luna 1968, Preece 1972).

All sectioned tissues were examined using a blind system. Each fish was assigned a number when necropsied, then prior to microscopic examination the fish was assigned a random identification number. Histopathologists examining tissue sections had available only information on the species, length, weight, sex, and the presence of grossly visible lesions. All slides from an individual specimen were first screened for abnormal tissues, and those sections with obvious lesions were segregated for more extensive examination.

Chemical Analysis. Hydrocarbon analyses of sediment and fish tissue were performed using a modification of procedures described by MacLeod et al. (1977) which involved gravimetric determination of total extractable hydrocarbons and GC for alkanes and arenes. By subtracting the concentration of total extractable materials in the control sediment from the level of extractable hydrocarbons in the oil-contaminated sediment, the value of total extractable petroleum hydrocarbons (TEPH) was determined. Some data presented are defined as selected aromatic hydrocarbons (SAH), which is a sum of the major individual aromatic hydrocarbons.

5.2.3 Cytopathology

A variety of life stages of flatfish, salmonids, and smelt were exposed to crude oil. The design and exposure regimes for each experiment is described in Section 5.3.2 and 5.3.3 e and f.

All tissues were fixed prior to microscopic examination in 0.75% glutaraldehyde, 3% formalin, 0.5% acrolein in 0.1 M sodium cacodylate

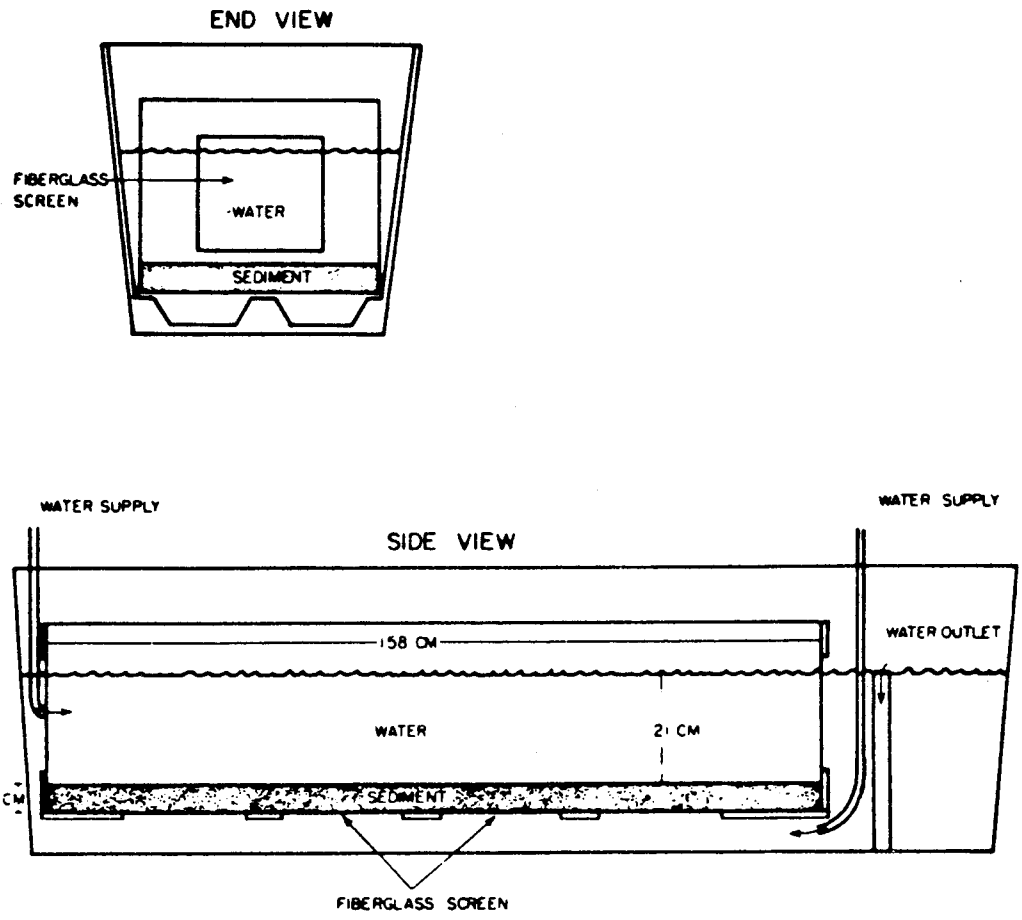

FIGURE 2. Diagram of aquaria used in experiments involving exposure of flatfish to oil-contamined sediments. (From McCain et al. 1978)

buffer with 0.02% $CaCl_2.H_2O$, and 5.5% sucrose (Hawkes 1974). The tissues designated for light microscopy (LM) or for transmission electron microscopy (TEM) were post-fixed for 1 1/2 hours in 1% osmium tetroxide in the same buffer, dehydrated in an ethanol series, and embedded in plastic. For LM, sections were cut at either 1.0 or 0.5 µm. The 1.0 um sections were stained with Richardson's mixture or with a PAS reagent (Nevalainen et al. 1972); the 0.5 um sections were stained with toluidine blue or a trichrome (MacKay and Mead 1970). For TEM, sections were cut with a diamond knife and stained with lead citrate and uranyl acetate and examined with a Philips EM-301 electron microscope. For scanning microscopy (SEM), samples were dehydrated after the initial fixation, critically point dried, coated with gold-palladium, and examined with an AMR-1000 scanning electron microscope.

5.3 Behavior and Physiology

5.3.1 Chemical Analysis of Water, Sediment and Tissue

Water and Sediment Analysis. Water samples for hydrocarbon analysis were collected in 100 or 300 ml screw cap glass bottles with Teflon(R)-lined lids. The samples were usually extracted on the day of collection; however, if they were to be processed later, 3 ml of concentrated hydrochloric acid was added to eliminate microbial activity and the samples were stored at 2°C.

Two methods of extraction and analysis were used. In initial experiments, a recovery standard was added to the 100-ml water samples and extracted using 5 ml of carbon disulfide. The organic phase was separated and evaporated to 0.8 ml, a GC internal standard added, and 8 µl of the extract was gas chromatographed on a 1-m long by 2-mm ID glass column packed with 0.2% carbowax 1500 on 59/80 mesh carbopack C (Supelco, Belleforte, PA). Nitrogen was used as a carrier gas with a flow rate of 46 ml/min and temperature programming was from 40 to 215°C at 15°C/min.

In later studies, water samples were collected in 300 ml bottles containing 12 ml of methylene chloride. Immediately after collection, the samples were shaken for 3 min to partition hydrocarbons into the methylene chloride and the samples were stored at 2°C. At the time of processing, recovery standards were added and the water samples were extracted three times with a total of 24 ml of methylene chloride. The extract was dried with sodium sulfate and reduced to 0.8 ml in a concentrator tube. GC internal standards were added and the extracts analyzed by glass capillary-gas chromatography (characteristics described by MacLeod et al. 1977). The limit of detection for individual aromatic compounds in the water sample was 1 ppb.

For both GC methods employed, identities of hydrocarbon components were confirmed by mass spectral analysis (for description of equipment and characteristics see Malins et al. 1980), and hydrocarbon concentration was determined from the total area under the chromatogram. All data were corrected for extraction efficiency except where expressly stated.

For specific analysis of benzene, 1 ml of benzene-seawater mixture was placed in a 2 ml GC vial, capped, and allowed to stand for 18 to 20 hr. One ml of air was then removed from the vial headspace and injected directly into a GC. These results were compared with results of benzene standards prepared by injection of µl amounts of benzene-methanol solutions into GC vials containing 1 ml of seawater, which were then processed in the same manner.[1]

Extraction and analysis of petroleum hydrocarbons in sediment followed a procedure described by Brown et al. (1979).

Tissue Analysis. Analytical procedures for tissue analysis on trout followed methods of Warner (1976) utilizing alkaline digestion, solvent extraction, and silica gel chromatography. To reduce losses of volatile compounds of PBCO, the alkaline digestion procedure was modified by adding 6 ml of 4 N NaOH to the 10 g of sample, and the sample digested at 30°C for a minimum of 16 hr.

Silica gel chromatography Fraction 3 from the modified Warner method, containing triaromatic compounds, was concentrated to 2.0 ml and analyzed using an Aminco-Bowman spectrofluorometer (American Instrument Company, Silver Spring, Maryland). Dilute solutions of PBCO (0.1 µg/ml to 10.0 µg/ml in methylene chloride:petroleum ether [20:80 v/v] were used as standards for the spectrofluorometric quantitation of the samples. The maximum excitation wavelength and maximum emission wavelength for PBCO were found to be 262 nm and 364 nm, respectively.

Chemical analysis of surf smelt embryos, salmon liver, and salmon brain tissues for petroleum hydrocarbons was conducted using methods described by Malins et al. (1980).

5.3.2 Preparation of Oil-Water Mixtures

Four flow-through systems introduced petroleum hydrocarbons into water:

(a) The system developed by Roubal et al. (1977b) provided a SWSF of crude oil composed almost entirely of monocyclic aromatic hydrocarbons (Roubal et al. 1978). For several studies a mixture of hydrocarbons modeled on the SWSF of fresh PBCO was formulated, and contained 95% (by weight) of those hydrocarbons detected in the SWSF (Table 1).

(b) A method of introducing the "model" hydrocarbon mixture into water was devised using a water jet eductor (Schutte and Koerting, Cornwells Heights, PA, type 264, 1/2 in, capacity ratio of 0.36). Water was passed through the eductor at a flow rate of 16 l/min, and a pressure of 5.5 kg/cm^2. The hydrocarbon mixture and individual

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[1] An alternative method for analysis of benzene, especially when benzene was a component of the total SWSF, was to use carbon disulfide as an extracting solvent. Carbon disulfide is not detected by GC and thus does not mask the GC response to benzene.

TABLE 1. Composition of mixture of monocyclic aromatic hydrocarbons modeling the SWSF of PBCO.

Hydrocarbon	% by weight in mixture
toluene	57.0
o-xylene	16.2
benzene	7.9
1,2,4-trimethylbenzene	7.3
p-xylene	4.8
m-xylene	4.8
ethylbenzene	2 0

components were introduced into the vacuum port of the eductor by a calibrated, continuous flow, syringe pump; the reduced pressure in the eductor port vaporized the hydrocarbon mixture prior to its mixing with water. The effectiveness of the eductor in solubilizing the mixture of monocyclic hydrocarbons in the water was determiend by GC analysis of replicate water samples taken at 20 min intervals with known injection rates of the model mixture set on the calibrated syringe pump. Since the solubilizing system was found to precisely introduce hydrocarbons into water (Fig. 3), the concentrations of hydrocarbons present during testing were often based on calculated values.

(c) The SWSF of crude oil was also produced by a flow-through apparatus similar to that described by Nunes and Benville (1978). Crude oil was continuously pumped (1 ml/min) onto the surface of seawater in a 40-cm-diameter glass carboy. A flow of 4 l/min of seawater at a constant head penetrated a dispersion plate and dripped through the oil layer. The resultant SWSF was continuously removed from the bottom of the carboy and delivered to exposure tanks.

(d) Weathered oil was prepared in a wave machine that subjected fresh PBCO or CICO to mixing with seawater by wave action, exposure to sunlight, and loss of volatile components through evaporative processes (Fig. 4). A paddle hinged at the bottom was attached to an electric motor which produced a steady wave periodicity of 48/min. At the opposite end from the paddle an artificial beach was added to dampen wave action and simulate water-accommodated oil passing down through the gravel of a chum salmon spawning redd. Coarse gravel (80%, 1 to 5 cm in diameter, remainder fine) was spread 25 cm deep over a perforated

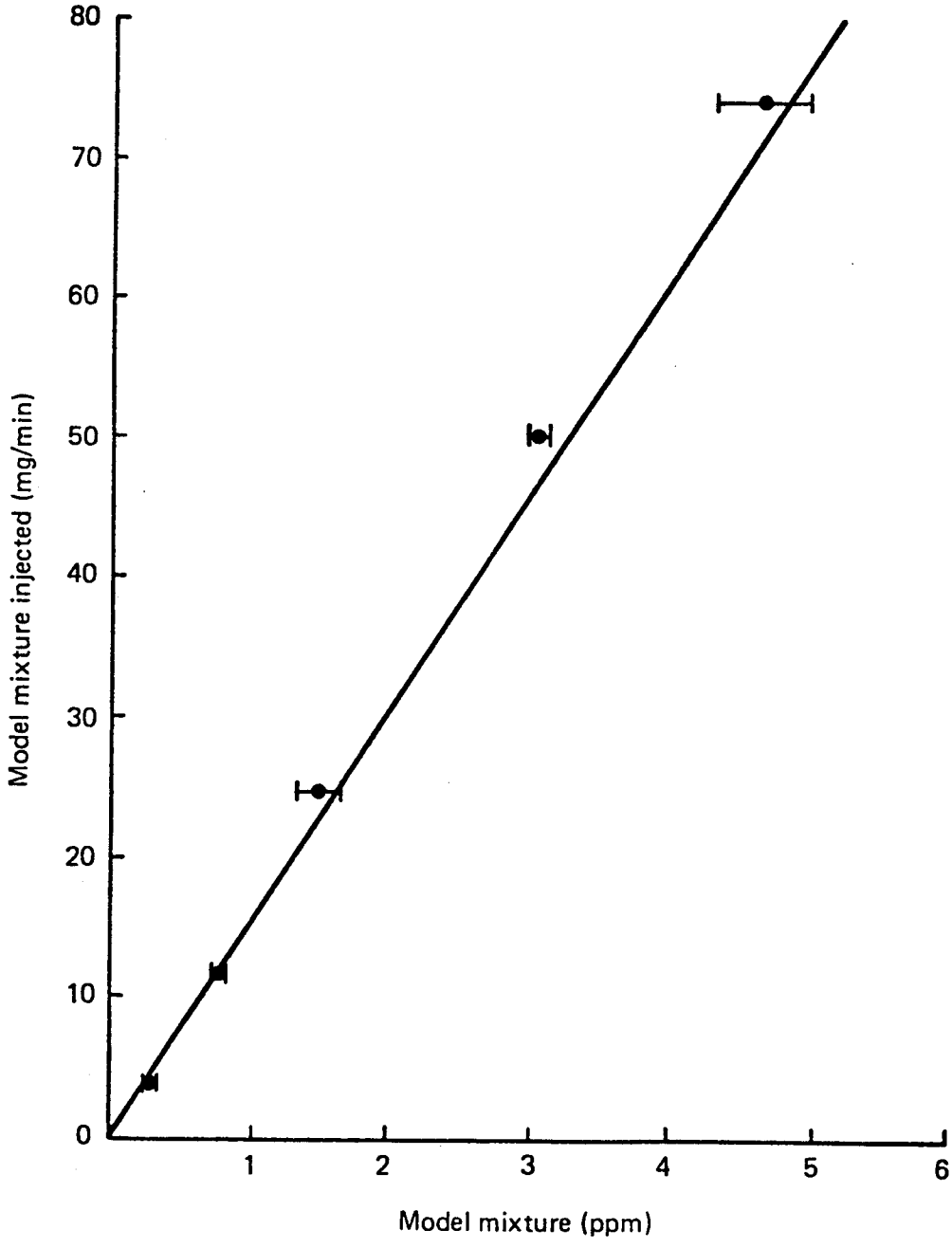

FIGURE 3. Amount of aromatic hydrocarbon mixture injected into the solubilizing system and ppm of mixture present in the water as calculated from water flow (line) and by GC analysis (circles, average GC value). Horizontal bars represent standard error of GC values. (From Maynard and Weber 1981)

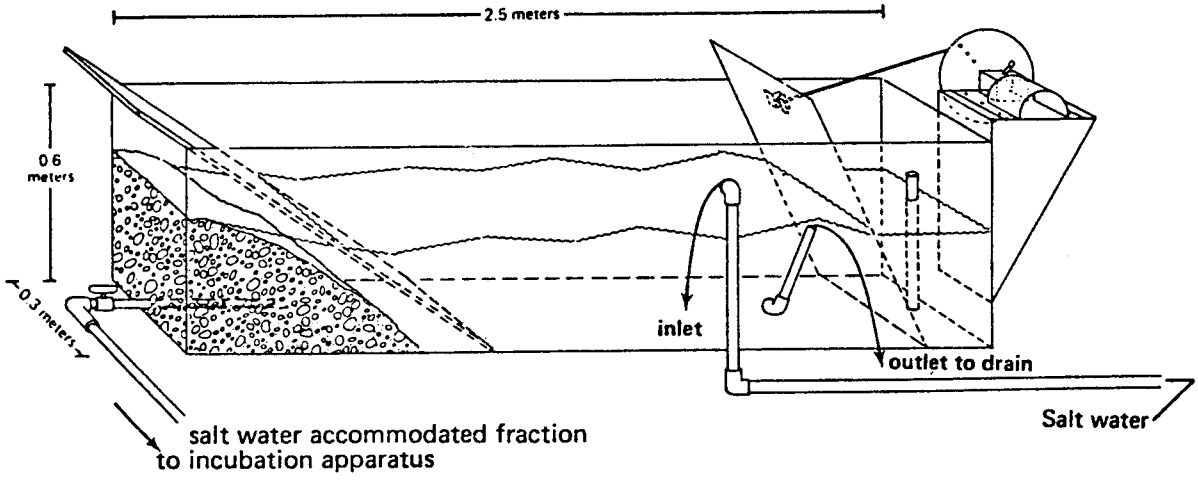

FIGURE 4. Apparatus for weathering of crude oil.

pipe. Over this gravel beach was placed a removable baffle which isolated the gravel from the oil during the weathering process, thus preventing oil from coming in contact with the "beach" before weathering was completed.

Six-hundred and eighty ml of fresh PBCO or CICO were layered on the water surface (91 $\mu l/cm^2$) in the wave machine resulting in an initial oil concentration of 4,000 ppm. During the weathering process, there was a continuous flow of seawater (27-30 ‰ salinity) through the wave machine with a water replacement time of once per hour. Water overflow was removed 25 cm beneath the water's surface via an adjustable standpipe in order to maintain the surface oil slick. After 30-48 hr of flow-through operation and wave action, the oil was defined as weathered.[1] For some experiments the salinity of the incoming water was then reduced (to 16-24 ‰) and the baffle removed, allowing the

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[1] Weathering of PBCO and CICO is defined here as a loss of volatile components generally those hydrocarbons with boiling points less than 210°C and representative of the naphtha fraction (See Table 53, Section 6.3.1[e]). Presumably the principal processes in this loss are a combination of dissolution and evaporation. The term seawater-accommodated fraction (SWAF) denotes water removed from under the oil slick. In water samples collected, there was often a Tyndall effect indicating that along with the soluble petroleum hydrocarbon components there were some oil particles dispersed in the water column.

oil contaminated water to come in contact with the gravel. The SWAF was then drawn off through the gravel and delivered to the exposure apparatus. In other experiments the gravel in the wave machine was replaced with an inclined plane similar to the removable baffle shown in Figure 4, and the perforated pipe for SWAF delivery extended through the incline.

5.3.3 Vertebrate Studies

Behavior of Pacific salmon exposed to petroleum hydrocarbons

(a) Olfactory disruption in juvenile coho salmon. Two series of electrophysiological studies were conducted on juvenile coho salmon ($\bar{x}$ length 20 cm). The first series, using salmon reared in seawater, used standard experimental procedures and recording techniques to monitor the electroencephalographic (EEG) response from the olfactory bulb (Hara 1973, Bodznik 1975). Amino acid stimulants (L-serine, L-alanine, and L-methionine at 10^{-3}M) and individual aromatic hydrocarbons were dissolved in seawater filtered to 5 um. Extracts of PBCO were prepared by hand shaking 100 ul of oil with 50 ml of filtered saltwater for 5 min. After the mixture stood for 1 h, the SWAF was drawn off from beneath the slick. Concentrations of undiluted stock solutions of individual aromatic hydrocarbons and the SWAF were determined by GC.[1]

The second series, using coho reared and maintained in freshwater, used EEG to assess detection and disruption. Control water and water used to make L-serine solutions were obtained from the same source as that used in producing a water-"model" hydrocarbon mixture. The "model" hydrocarbon mixture (Table 1) was introduced into the water using a syringe pump and water jet eductor. Hydrocarbon solutions were placed in Teflon wash bottles and replenished at least every 30 min. To assess detection of aromatic hydrocarbons the olfactory bulb EEG response to different hydrocarbon concentrations introduced in the nares was obtained from a minimum of ten, 5-10 sec rinses with solutions containing 1.9, 2.8, or 3.7 ppm of the aromatic hydrocarbon mixture. Olfactory disruption as a result of aromatic hydrocarbon exposure was evaluated by recording the EEG response to 10^{-3} M L-serine solution both before and after rinsing the nares continuously for 5-10 sec, 10 min, or 20 min with a solution containing 4.0 ppm of the aromatic hydrocarbon mixture. The EEG responses were quantified by measuring the area under the integrated EEG signal for the first 2 through 6 sec following stimulation of the nares. Relative changes in amplitude of the EEG response was analyzed using an unpaired t-test for detection experiments and a matched pair t-test for disruption experiments.

(b) Migratory and Homing Behavior in Adult Salmon. One field study evaluated whether salmon avoid their home stream when petroleum hydrocarbons are present in the water. Two others were designed to

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[1] A single 20 ml aliquot of each stock solution was extracted with 5 ml of carbon disulfide and processed using proceedures described in the first part of Section 5.3.1.

determine if short-term exposure to petroleum components had an effect on a salmon's homing capability.

Avoidance reaction. The study site was located on Chambers Creek, southern Puget Sound, Washington. One kilometer from the mouth of the creek is a tidewater dam having a central spillway, ladders on each side and trapping facilities at the upstream head of each ladder. The average discharge of the creek during the period of study (October-November, 1978) was 1.8 m^3/sec with approximately 12% of the water passing through the west fish ladder, 28% passing through the east ladder, and the remainder passing over the spillway. Water temperature was 9-10°C. The mixture of monocyclic aromatic hydrocarbons introduced into the water contained the following percentages of individual components by volume: benzene, 7.9%; toluene, 57.0%; ethyl benzene, 7.0%; m- and p-xylene, 23.3%; and o-xylene, 4.8%. This mixture closely approximated the water-soluble fraction of PBCO in its relative proportions of aromatic hydrocarbons (Table 1).

To generate the water-hydrocarbon mixture, water from the dam impoundment was pumped through a water-jet eductor at a flow rate of 12 l/min and a pressure of 6.7 kg/cm^2. The hydrocarbon mixture was introduced into the vacuum port of the eductor at flow rates of 3 to 105 ml/min. The eductor vacuum served as a pump; the hydrocarbon flow was regulated by a calibrated metering valve. From the eductor the water-hydrocarbon mixture was piped to the top of the west fish ladder (the east ladder was left untouched) where a diffuser pipe, with its outlet 0.6 m below the water surface, provided dispersion of the water-hydrocarbon mixture across the width of the ladder with further mixing taking place in the water turbulence in the ladder.

Prior to an avoidance test, the salmon in the traps at the head of each ladder were counted and placed upstream; water depth in the top step of each ladder was measured and the flow was calculated using the Francis, sharp-crested, weir formula (King 1954). The duration of each test varied from 4.5 to 22 hr, depending upon the numbers of migrating fish, stream discharge and tidal fluctuation.

At the end of each test, the amount of hydrocarbons used was measured; duplicate water samples for chemical analysis were taken from the top of the ladder into which the hydrocarbons were introduced, and from the middle of the ladder as tidal height permitted (a water sample was also collected from the top of the opposite ladder serving as control); and the numbers of fish in each trap were counted by species and placed upstream.

Disruption of homing capability. Two studies concerning the effect of petroleum exposure on homing capability were similar in experimental design (capture-exposure-transport-release) but differed in species used, petroleum components, and geographical location.

In the autumn of 1976 adult male chinook salmon were collected from the homing pond of the School of Fisheries, University of Washington, Seattle (Fig. 5). The fish were divided into control and experimental

groups, lightly anesthetized with tricaine methanesulfonate, identified with individually coded spaghetti tags inserted under the dorsal fin, and each group was placed in a 2,400-l flow-through circular holding tank supplied with 16 l/min of fresh water. Water delivered to the test (oil exposure) tank was first passed through a sealed 38-l glass mixing chamber containing glass baffles to extend the duration of oil-water contact. PBCO was metered into the mixing chamber by a calibrated, continuous-flow, syringe pump. The fresh water-accommodated fraction (FWAF) was introduced at the bottom of the test tank and drained at the surface via a central stand pipe, thus producing a distribution of crude oil throughout the water column. The calculated concentrations of PBCO introduced in the water ranged from 0.5 to 40.6 ppm; an oil film and a Tyndall effect were evident during all trials. Concentrated HCl was added to the water samples collected for hydrocarbon analysis; the samples were then sealed and allowed to sit for 24 hr. Any oil film on the surface was removed prior to extraction. Thus, the FWAF concentrations determined by GC are considerably less than the calculated amount of crude oil introduced, and more closely reflect that fraction which is actually freshwater-soluble.[1]

After 14-18 hr of oil exposure, the fish were transported 7 km downstream and released. Three times a week all the fish in the University of Washington homing pond were examined and tagged salmon were recovered. A third group of 18 chinook salmon had their nares occluded with vaseline-saturated cotton. The olfactory-occluded fish were treated identically to controls and were released in equal numbers along with control and oil-exposed salmon during the first three tests.

A second series of experiments was conducted in the autumn of 1977. Returning salmon were trapped at the head of a tidewater fish ladder located on Tulalip Creek in northern Puget Sound. The trapped jack coho salmon (males which mature and return to spawn after one ocean growing season) were divided into control and oil-exposed groups, tagged using the same procedure as described for chinook salmon, and each group was placed in a 600 l holding tank with a water inflow of 16 l/min. Water was pumped into the tanks from the bottom of the fish ladder, and the salinity varied with tidal fluctuations. Treated fish were exposed to a mixture of monocyclic aromatic hydrocarbons representative of the SWSF of PBCO (Table 1). This hydrocarbon mixture was injected into the water using a syringe pump and water jet eductor. The water-hydrocarbon mixture was introduced into the bottom of the circular test tank, and drained at the surface via a central standpipe.

The concentration of aromatic hydrocarbons in the water was calculated from the measured amounts of aromatic hydrocarbon mixture injected per minute and water flow rate. In addition, water samples for GC analysis were taken from test and control tanks at the middle and just prior to termination of the exposure period. After 8-22 hr

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[1] Some oil which was accommodated at the time of sampling probably equilibrated with the water in the sample bottle during storage; thus, the reported GC concentrations are considered maximum values.

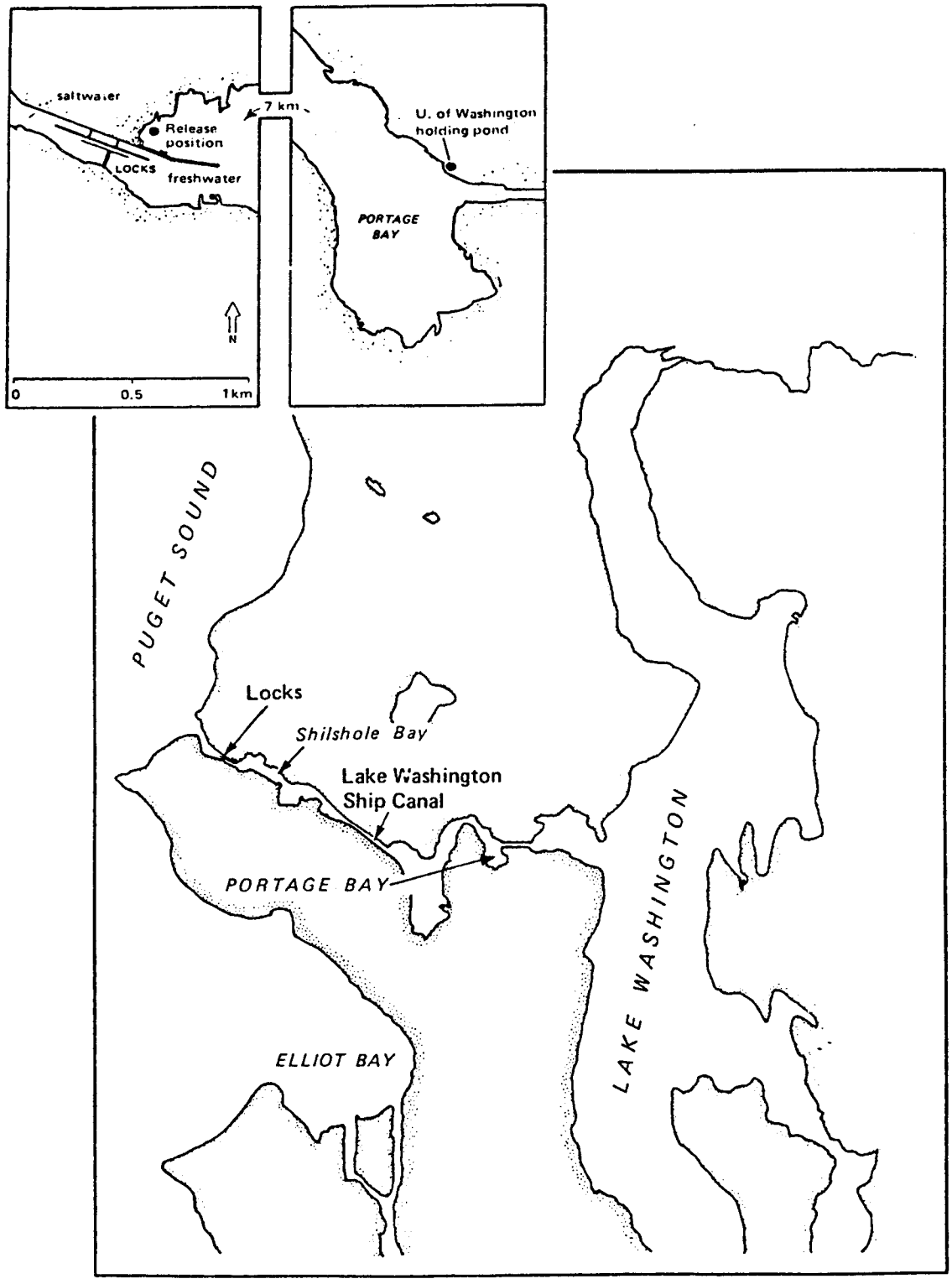

FIGURE 5. Location of adult chinook salmon study and detail of release and recovery sites.

exposure, both control and treated fish were transported and released at one of two marine sites located 1.6 or 4.7 km from the "home" stream. The Tulalip Creek trap and fish ladder were monitored daily and the tagged fish recovered.

(c) Predator-Prey Behavior. Two series of experiments were designed: the first tested oil-exposed prey; the second, oil-exposed predators. In both series the SWSF of CICO was prepared using a solubilizer similar to that described by Nunes and Benville (1978). Water samples were collected twice weekly from the exposure tanks and analyzed for total hydrocarbon content by GC. For both studies the salinity was 26.5 $\pm$ 1.3 ‰ ($\bar{x}$ $\pm$ sd) and temperature 13.4 $\pm$ 0.7°C.

For the oil-exposed prey experiments, chum salmon (O. keta) fry were obtained from the Washington State Department of Fisheries Hatchery at Hoodsport, Washington. The predators were 2 yr old coho salmon from the Department of Fisheries Hatchery at Issaquah. They were obtained as fertile eggs, hatched and reared in freshwater at the NWAFC, Seattle, to an age of 1 year and then in seawater at the Mukilteo Facility to an age of 2-plus years and an average length of 35.0 cm.

Three to 4 days before an experiment, test and control fry were distinctively marked by cold-branding (Fujihara and Nakatani 1967). The coho salmon predators were trained to feed on chum fry for at least one month before testing, and were always starved 48 hr before being used.

Test fry were exposed to the SWSF of CICO for periods of 24, 48, 72, and 96 hr while holding control fry under similar conditions for identical lengths of time. Ten test and 10 control fry were then introduced simultaneously into an observation tank containing 3 coho predators. After approximately one-half of the fry were consumed, the predation was halted and the numbers of test and control prey remaining were determined. Fifty percent predation usually took place in a few minutes but was never allowed to extend beyond 2 hr.

For the oil-exposed predator studies, fish were obtained from the following sources: adult coho salmon from a stock reared at the NWAFC and rainbow trout fry from Trout Lodge, Tacoma, Washington. The trout fry were maintained (fed to satiation) on a diet of OMP. The coho predators were maintained on OMP until four months prior to the experiments, then fed exclusively on live chum salmon or rainbow trout fry. Although natural predation by coho salmon on Salmo gairdneri in seawater is unlikely, we concluded after preliminary trials that this combination provided a viable experimental design for a laboratory evaluation.

Two 170 l rectangular fiberglass tanks were used as the exposure and control tanks; circular 950 l fiberglass tanks were used for the predation tests. Seawater inflow rates to the circular tanks provided a 95% exchange within 24 hr.

The coho salmon predators were randomly divided into two groups of 21 fish each. The group designated as the test group was exposed to the SWSF of CICO. All test fish were exposed (together) to the oil for a period of 17 days, with predator evaluations occurring after 3, 10, and 17 days of exposure. Each of the test groups was further divided into subgroups of 3 fish each for the predator evaluations, so that there were 7 replicates for each group at each of the 3 evaluation periods. Each fish was cold-branded (Fujihara and Naktani 1967) for identification and maintained in the same subgroup throughout the study, with the following exceptions: three mortalities occurred in the control group (one fish each from Subgroups 1, 3, and 6), just prior to the final testing period. The 2 surviving fish from Subgroup 1 were redistributed to Subgroups 3 and 6 to maintain the proper population of 3, which were essential to stimulate feeding behavior. The predators were not fed between evaluation periods.

Predators were transferred from the control or exposure tanks to the predator evaluation tanks at 4 pm on the day prior to testing. At 9 am on the day of testing 10 rainbow trout fry were transferred without acclimation into a tank containing one of the 7 predator subgroups (3 adult coho salmon); the number of prey surviving after 10 minutes was recorded. Surviving prey were discarded. Predators were returned to their respective control or oil-exposure tanks immediately after the predator-prey evaluations. At the termination of the experiment, the brains and livers of 3 control (Subgroup 4) and 6 oil-exposed fish (Subgroups 1 and 6) were analyzed for hydrocarbon content.

(d) Avoidance Behavior of Flatfish Exposed to Oil-Contaminated Sediment

English sole (0-1 yr, 45-120 mm length) were tested in the choice apparatus shown in Figure 6. The choice apparatus was a 61 x 152 cm box with a water depth of 20 cm controlled by standpipes at each end. The box contained two identical 3,400 cm^2 trays, each filled with 15 l of sediment to a depth of 3.5 cm. A perforated pipe running the width of the box and located 2 cm above the water surface provided seawater at 12 l/min; half of the flow was directed toward each outlet. Studies in which dye was introduced in the water column showed that this water flow configuration provided negligible mixing of water between sides of the test apparatus. When the trays contained sediment, the water volume of the test apparatus was 113 l with a water replacement flow of 6 times/hr. Seawater temperature averaged 10°C, and salinity averaged 29 $^{o}/_{oo}$. Light intensity at the water surface was uniform (200 lux) and maintained on a 10L:14D schedule.

High-sand content sediment was collected from a beach near Sequim, Washington (Port Williams) - an area known to have low levels of petroleum contamination in previous assays, and for which sediment characteristics (particle size, organic carbon, and metal content) have been determined (McCain et al. 1978). Juvenile English sole were collected in Puget Sound with a beach seine.

Control tests were conducted to determine the movement of flatfish in the choice apparatus when both trays contained uncontaminated sediment. The uncontaminated sediment was rinsed with flowing seawater in the test apparatus for 4 to 24 hr and then 20 juvenile English sole were released on the sediment of one tray; sides for release were alternated between tests. After 19 to 21 hr in the test apparatus, the fish were fed diced clams introduced simultaneously on each tray for either 15 min or to satiation, whichever occurred first. Three hours later the water was turned off and a screen inserted between the two sediment-containing trays. The standpipes were removed and the water level lowered to 5 cm depth. The fish in each side were removed with a dip net, counted, measured, visually checked for stomach fullness, and either the test was terminated or the fish were returned to the side from which they were taken and the process repeated the next day.

Prior to testing flatfish avoidance of oil, the sediment in one tray (used in previous control test) was removed and mixed with either 375 or 750 ml of PBCO in a cement mixer for 30 min to give an initial oil concentration of either 2.5 or 5% (v/v). After rinsing the oiled sediment for 4 to 24 hr, 20 juvenile English sole were released on the tray containing uncontaminated sediment. The same feeding and counting procedure was followed as described for control tests. In addition, at the termination of each test, or at time of counting if the test was continued, a sample of sediment, sediment-associated water, and above-sediment water were collected from each side for GC analysis, and/or for gravimetric determination of TEPH. The sediment sample consisted of a 150 g composite obtained from the surface of each tray by inserting a 50 g capacity corer 2.0 cm deep. For sampling of sediment-associated water, an open ended glass tube (1.7 x 25 cm) was vertically inserted 2 cm into the sediment while holding the upper end of the tube closed. After positioning, the upper end was opened and 50 ml of water rising within the tube was removed with a syringe. Prior to chemical analysis of the sediment-associated water for petroleum hydrocarbons, the samples were centrifuged at 2000 g for 10 min to remove suspended particulates. Above sediment water was taken by submerging and filling a 315 ml glass sample bottle containing 3 ml of concentrated hydrochloric acid.

The biological data were analyzed using two by two contingency table analysis.

(e) Effect of Petroleum Hydrocarbons on Development of Embryos and Larvae

The experimental design for exposure of early development stages of salmon, flatfish, and smelt to petroleum was dependent largely upon the fishes' spawning characteristics and the conditions under which embryogenesis occurs. The beginning of each of the following 3 subsections briefly describes the fishes' natural spawning behavior.

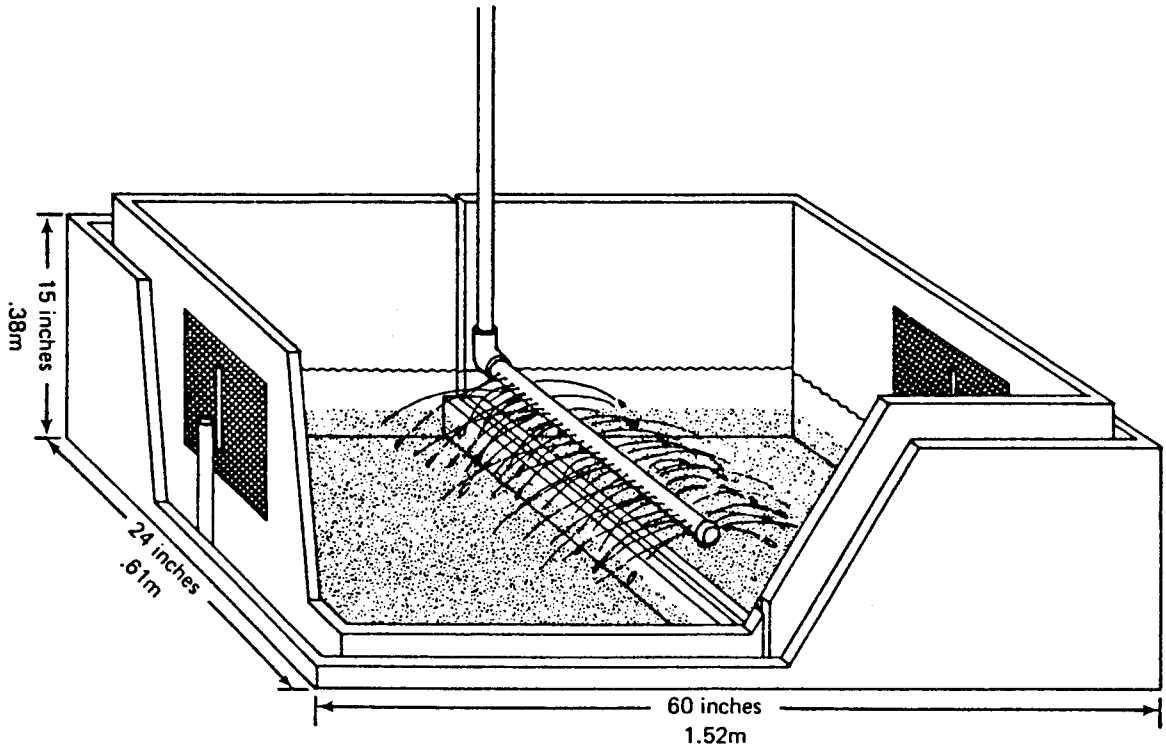

FIGURE 6. Choice apparatus used in testing avoidance of juvenile English sole to oil-contaminated sediment.

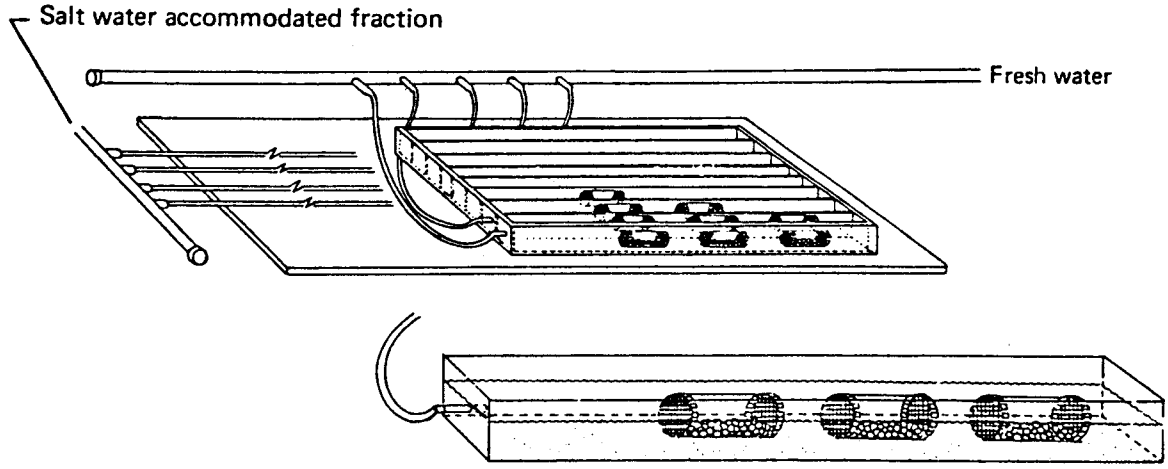

FIGURE 7. Chum salmon egg incubation apparatus with delivery of brackish SWAF of weathered PBCU from the wave generator (Fig. 4) for 3 hr/day and delivery of fresh water for 21 hr/day.

(1) Chum Salmon. Chum salmon frequently spawn in tidal areas at at the mouths of streams (Neave 1966, Bakkala 1970). Eggs are deposited in redds and then covered with gravel as a result of upstream redd digging and stream flow. At least one high tide a day inundates the redds with salt and brackish water; the length of exposure to water of high salinity depends upon redd location. After approximately 50-90 days (development rate is a function of water temperature) the eggs hatch; however, the salmon alevins remain in the gravel for another 30-50 days before emerging and migrating.

Chum salmon eggs were obtained from the U.S. Fish and Wildlife Service National Fish Hatchery at Quilcene, Washington. Immediately after fertilization, the eggs were transported to the Mukilteo laboratory. Subsequent sampling from control groups indicated that 97.5% of the salmon eggs were fertilized. One day after fertilization, approximately 70 chum salmon eggs were placed in each of 56 (30 x 75 cm) glass cylinders, and both ends covered with Teflon netting. A glass tray was divided longitudinally into eight troughs and pea gravel was layered 2.5 cm deep on the bottom. Seven cylinders of eggs were placed horizontally into each trough (Fig. 7).

Eggs were exposed 3 hr/day to oil-contaminated brackish water (16-24 °/oo salinity, and 4.5-10.2 °C ambient temperature) at a flow rate of 400 ml/min per trough. For 21 hr/day the eggs received fresh dechlorinated water (5.5 to 10.5°C ambient temperature) at the same flow rate for a water replacement of 8 times per hr. Exposure to oil-contaminated water occurred 4 days per week for 16 consecutive weeks. On the other 3 days eggs and alevins received uncontaminated brackish water for 3 hr/day. The oil exposure conditions (by group) designated for each of the 8 troughs, are expressed diagrammatically in Figure 8.

(2) Flatfish. Sand sole and English sole are pleuronectid flatfish with pelagic eggs and larvae. Flatfish eggs are released on or near the bottom; after about one day, depending upon depth of spawning, the eggs rise to float near the water's surface (Ketchen 1956, Alderdice and Forrester 1971). Eggs hatch after approximately one week and the larvae emerge relatively undeveloped.

Gravid English sole and sand sole were obtained by trawling in Puget Sound. Eggs were stripped from ripe females and fertilized immediately with sperm from a ripe male on board the trawling vessel. Fertilization success and subsequent viability in flatfish eggs was high (approximately 90%) as indicated by cell cap formation. Twenty-four hrs after fertilization (early cell-cap stage) approximately 350 to 700 flatfish eggs were introduced into 1,000 and 2,000 ml separatory funnels containing 900 and 1800 ml, respectively, of uncontaminated seawater or the SWAF of weathered PBCO obtained from the wave machine. The funnels were attached to an air supply through the bottom and the water bubbled slowly, thus creating a current in the funnel which kept the eggs in suspension. Funnels were submerged in a water bath with 10°C flowing seawater.

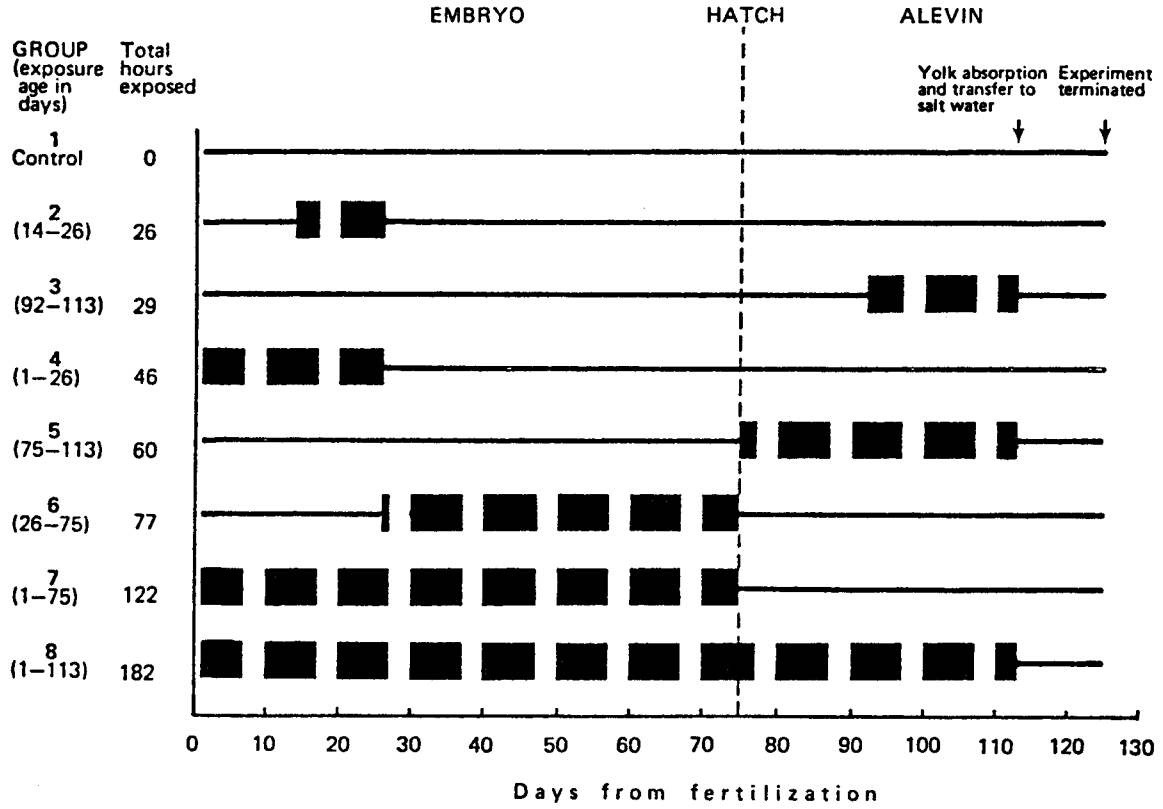

FIGURE 8. Exposure groups of chum salmon embryos and alevins showing duration and stage of exposure. Solid bars represent weekly exposure periods of 3 hr/day, 4 day/wk.

(3) Surf Smelt. Surf smelt are found along the Pacific coast from California to the Aleutian Islands. They spawn intertidally on coarse sand-pea gravel beaches, the adhesive eggs being submerged intermittently at high tide until the embryos hatch as well-developed larvae. Duration of embryonic development (8 to 30 days) depends upon both water and air temperature (Yap-Chiongco 1941, Loosanoff 1937, Pentilla 1978), and hatching is promoted by wave agitation at completion of embryogenesis (Misitano 1977).

Surf smelt eggs were collected from either the intertidal area of Hood Canal, Puget Sound, or from spawning females from Hood Canal. All surf smelt eggs were visually checked at the initiation of each experiment and only those with viable embryos were used in the tests. Four to six days after fertilization, 400 to 500 eggs were placed in each of four square-sided baskets (7 x 14 x 14 cm with 570-µm mesh Teflon netting on the bottom) containing 2.5 cm of fine gravel (2-8 mm diameter) (Fig. 9). The baskets were submerged 3 hr/day throughout the incubation period in the SWAF of weathered CICO with a flow rate of 750 ml/min to each basket. The SWAF from the wave generator was introduced into a diluter and three concentrations used in exposure:

(1) undiluted (100%) SWAF from the wave machine, (2) 1/2 diluted (50%) SWAF, and (3) 3/4 diluted (25%) SWAF. Exposure of surf smelt eggs to the SWAF of CICO was repeated once in the month of November and once in December of 1979, at ambient water temperatures of 8.8 to 11.5°C and air temperatures ranging from -2.2 to 16.7°C.

(4) Water Samples for Hydrocarbons. During the 16 wk exposure period of chum salmon embryos and alevins to the SWAF of weathered PBCO, water samples were collected, 4 days/week after 30, 54, 78 and 102 hr of weathering and analyzed for total hydrocarbons. At 102 hr the gravel and wave machine were cleaned, the cleaned gravel replaced, fresh oil added, and the sampling schedule repeated.

Flatfish embryos were exposed to the SWAF of PBCO which was first weathered for 48 hr in the wave machine. (During oil weathering the seawater temperature was 7.8 to 9°C, and salinity was 27 to $30^{o}/_{oo}$.) Water samples for chemical analysis were taken at the initiation of the tests, after 3 days of exposure, and again after hatching (day 8). Water samples were collected by transferring the contents of the incubation funnel into a beaker. The water was then siphoned from the beaker through a screened cylinder to prevent passage of embryos and/or larvae. At midincubation the SWAF was replaced with a portion of the original SWAF which had been refrigerated at 2°C in a sealed glass bottle with a Teflon-lined lid.

In experiments concerned with the early developmental stages of surf smelt, CICO was continuously weathered throughout each 3 wk test. Water samples for chemical analysis were collected daily from the trough containing undiluted SWAF, and 35 samples were analyzed for total hydrocarbons.

In the above experiments, the concentration of extractable material in control water averaged 2.2 $\pm$ 1.1 ($\bar{x} \pm$ SD, N=10) ppb; none of this extracted material was identified as being of petroleum origin. The hydrocarbon concentrations were not corrected for extraction efficiency which averaged 67% + 9 ($\bar{x} \pm$ SD, N=34), 80% $\pm$ 6 ($\bar{x} \pm$ SD, N=16), and 84% + 6 ($\bar{x} \pm$ SD, N=35) for data associated with experiments on salmon, flatfish, and smelt, respectively.

Data were analyzed using chi-square tests, robust locally weighted regression (Cleveland 1979), and predictive sample reuse (Geisser and Eddy 1979). The latter analysis is designed to determine within- and between-group differences, and to select the correct model $\geq$95% of the time.

(f) Effect of Crude Oil on Salmonid Reproductive Success

Fish used were 3-year-old rainbow trout of Cape Cod strain, obtained in June, 1975 from the Washington State Department of Game Hatchery in Spokane. At the beginning of the study the fish measured 41 to 53 cm in fork length and weighed 1.0 to 1.8 kg. The fish were randomly placed in approximately equal numbers in one or the other of two adjacent circular fiberglas tanks (1.8 m diameter) continuously supplied with dechlorinated water at 30 l/min; water depth was 0.8 m. Water temperature was 11 + 1°C and artificial light was maintained at a natural light: dark cycle.

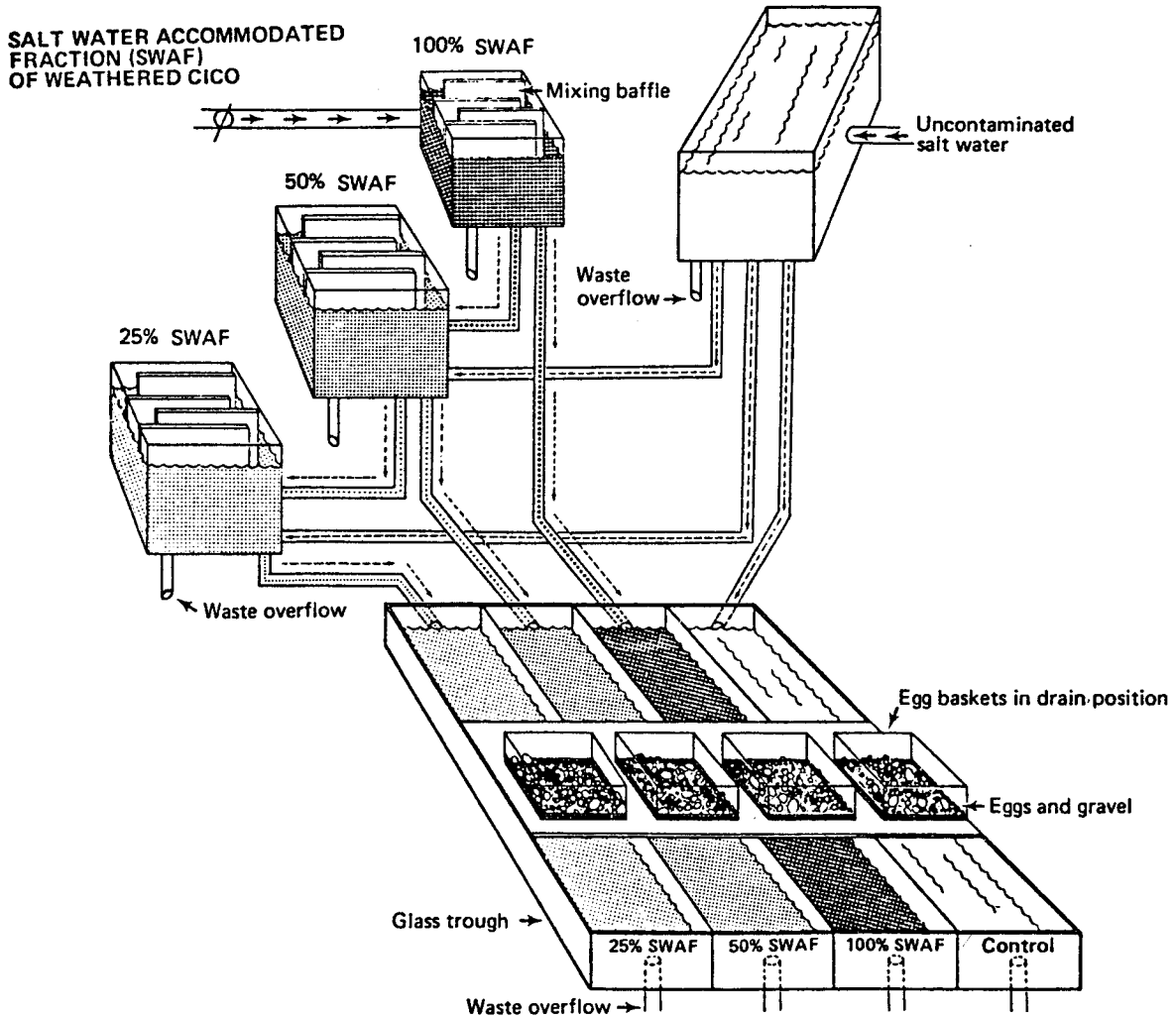

FIGURE 9. Surf smelt embryo exposure apparatus with delivery of the SWAF of weathered CICO from the wave generator (Fig. 4).

Petroleum-coated food (1 part oil:1,000 parts food, by weight) was routinely prepared in the following manner: Two kg of 1/4-inch diameter Oregon moist pellets were placed in a 4 liter glass beaker. Two g of PBCO were mixed with 148 ml of FREON(R) TF solvent (trichlorotrifluoroethane) and poured over the food. The food and oil were thoroughly mixed and the food was spread over porcelain-covered metal trays for 90 min of air drying in a fume hood. The food was then weighed into daily aliquots, sealed in plastic bags, and frozen until used. Food for control experiments were prepared identically except that the crude oil was omitted. Fish were fed these diets at an approximate rate of 2% (wet weight of food) of body weight each workday between July, 1975 and August, 1976.

In late November 1975, all fish were examined for degree of maturity. Subsequent biweekly and then weekly examinations were made. As females ripened they were spawned and eggs were fertilized using standard trout-

culture methods (Leitritz 1959). Ripe males were consistently available for the duration of the spawning period from January through February 1976. All eggs from control fish were divided into equal aliquots and one aliquot was fertilized with sperm from an oil-exposed test male and the other with sperm from one control male. Ten of the oil-exposed females were similarly treated; eggs from the remaining oil-exposed females were fertilized with sperm from oil-exposed males only. A total of 31 test and 10 control crosses were made. Eggs were incubated at 7° to 9°C. Mortality data were collected through the yolk-sac absorption stage and statistically analyzed using the Mann-Whitney modification of Wilcoxon's sum of ranks test (Langley 1971).

At the time of spawning, samples of adult tissues and eggs were collected and frozen for later analysis of petroleum hydrocarbons.

5.3.4 Invertebrate Studies

Effect of Petroleum Hydrocarbons on Sea Urchin Defense Behavior

Seawater-soluble fractions of PBCO were prepared according to the flow-through method of Roubal et al. (1977b). Solutions containing single aromatic hydrocarbons were prepared by a modified method described by Nunes and Benville (1978). In the latter, filtered seawater dripped into a glass reservoir at a constant head from a perforated bucket. Hydrocarbons were introduced continuously near the top of the glass reservoir via a calibrated, repeating, syringe pump and the seawater-hydrocarbon mixture was drawn off at the bottom of the reservoir and delivered to a 2-l glass exposure beaker (for details see Johnson 1979). The flow rate of the seawater-hydrocarbon mixture through the exposure beaker was 200 ml/min; average salinity was 28 $^{o}/_{oo}$; average water temperature was 10°C.

The reported SWSF hydrocarbon concentrations produced by PBCO are the summed concentrations of the monocyclic aromatic compounds. A gas chromatogram representative of the SWSF of PBCO is given in Figure 10, and represents the type of data used in determining the concentration of hydrocarbons present in exposure water for all experiments with invertebrates. The concentration of each component was found to be similar to that reported in detail by Roubal et al. (1978) with the monocyclic aromatic hydrocarbons accounting for 95% of the total SWSF; the remainder consisted of naphthalenes and cyclohexanes.

Urchins are preyed upon by starfish, including the sunflower starfish (Pycnopodia helianthoides, Mauzey et al. 1968), and when presented with a chemical stimulus from their starfish predator, the sea urchins exhibit a defensive response that is consistent and repeatable. The starfish can move faster than the urchin, but the urchin, if overtaken, possesses a compliment of globiferous pedicellariae that are activated by a water soluble exudate from the starfish (Phillips 1978). These globiferous pedicellaria are distributed liberally over the aboral surface of urchins, and a typical 3 cm diameter urchin may possess 100 or more. Each pedicellaria articulates on a peduncle and has three jaws, each tipped with a hollow tooth capable of delivering

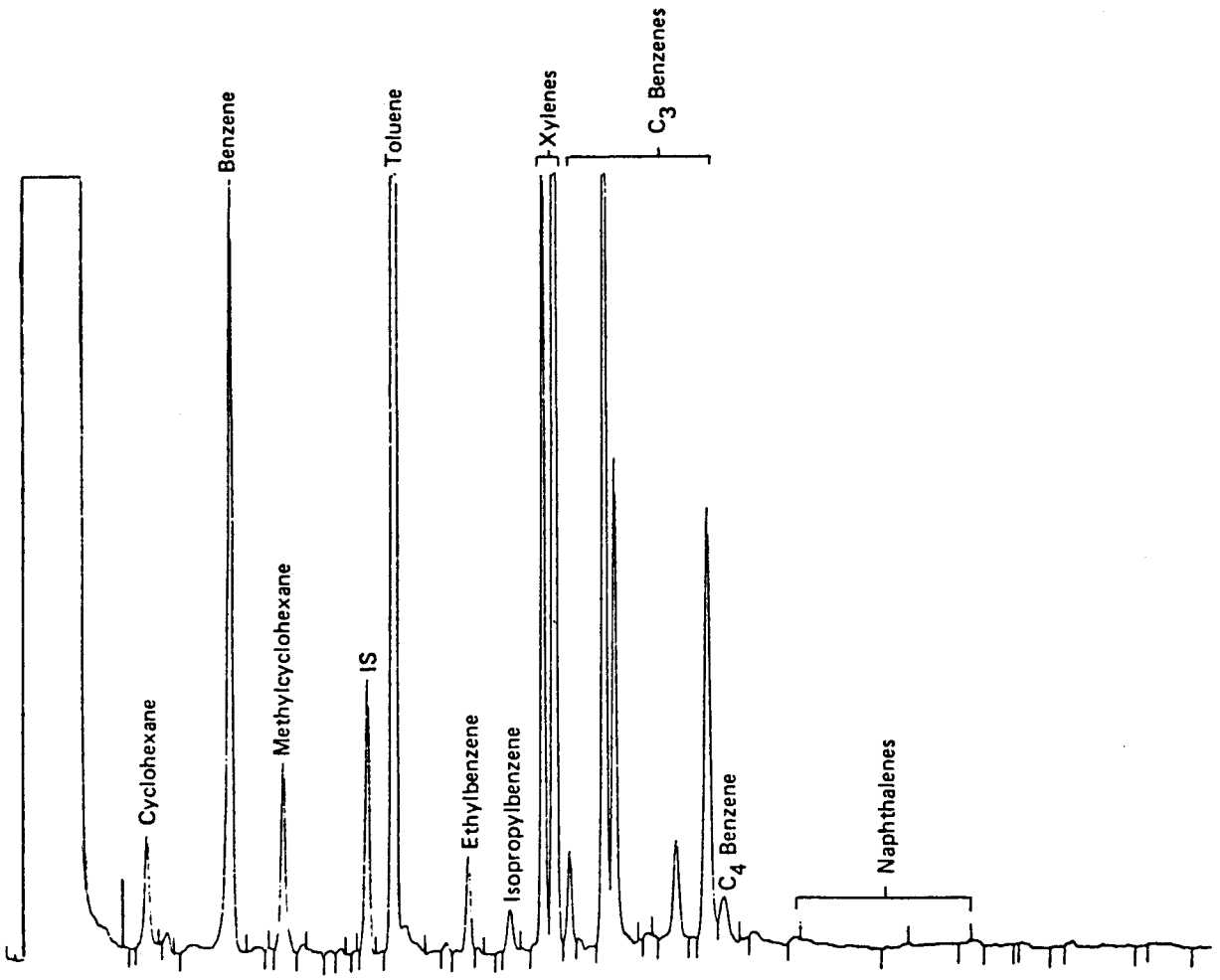

FIGURE 10. Gas chromatogram of the SWSF of PBCO.

venom (Campbell 1976). When stimulated with starfish exudate the pedicellariae rise to a position perpendicular to the test (exoskeleton) and the jaws open to a 180° angle. If a pedicellaria were to contact a tube food of Pycnopodia, the jaws would close, inject venom, and produce a rapid (though not necessarily total) withdrawal of the starfish.

Starfish exudate was prepared by placing a Pycnopodia in its equal volume of 5-µm-prefiltered seawater for 1 hr. The resulting exudate was refiltered (to 5 um), tested for efficacy in activating pedicellariae, then frozen in aliquots. Before each test a thawed aliquot was kept at ambient water temperature, which ranged seasonally from 7-14°C.

Prior to behavioral testing, sea urchins (2.5 to 3.5 cm test diameter) were exposed to hydrocarbons by one of two methods. Most exposures consisted of holding 5 urchins for 24 hr in 2-l flow-through exposure

beakers containing the SWSF of PBCO or single aromatic hydrocarbons, and then placing them individually in 100-ml beakers containing 40 ml of the same hydrocarbon solution. The urchins were then left undisturbed for approximately 10 min before stimulation with starfish exudate. The second method was to expose the urchins individually in the 100-ml beakers for only 10 min before testing. Following exposure, 1 ml of starfish exudate was slowly administered with a syringe in a circular motion over each urchin. The defense reponse assay consisted of counting the number of pedicellariae that opened to 180°.

If 75 or more pedicellariae responded to starfish stimulus, the response was considered total (100%); if less than 75 opened, a partial response was recorded (0-99%). For each test, the average response of at least 5 hydrocarbon treated urchins was divided by the average response of at least 5 controls to yield a response index for each treatment. This method of analysis takes into consideration variations in response of controls for each group tested.

Reproductive Behavior of Dorid Nudibranchs Exposed to Petroleum Hydrocarbons

(a) Chemosensory disruption. Mature dorid nudibranchs were collected from the seawater system at the Mukilteo Biological Station. The adult nudibranchs were exposed to the SWSF of PBCO for 24 hr in 2-l flow-through aquaria with a seawater flow of 250 ml/min and a temperature and salinity of 13.6 ± 0.6°C ($\bar{x}$ + range) and 27.0 ± 2°/oo, respectively. The SWSF was produced by a method described by Roubal et al. (1977b); to obtain different hydrocarbon concentrations in each aquarium the SWSF flows were diluted by mixing with seawater. Two water samples were collected from each aquarium for GC analysis of hydrocarbon concentration.

Following SWSF exposure, the nudibranchs were assayed in "untreated" seawater for chemotatic behavioral response.[1] Individual nudibranchs were placed in the bottom arm of a "Y" choice chamber and allowed to move into either the "stimulus" arm or the non-stimulus ("blank") arm. (For details of testing apparatus see Malins et al. 1978 or Johnson 1979.) The "stimulus" arm contained four reproductive nudibranchs. The "blank" arm was of identical construction, but did not contain nudibranchs. Clean seawater filtered to 5 µm supplied each arm of the choice chamber at a flow rate of 60 ml/min. The "stimulus" and "blank" chambers were alternated and thoroughly washed between tests to remove mucus. A positive response denoted movement toward either chamber; "no choice" meant the nudibranchs did not make a definite movement into either arm within 10 min from the start of the test.

The percentage moving toward the "stimulus" chamber for the SWSF exposed group of nudibranchs was statistically compared with the control percentage using an arc sin transformation test for equality of two percentages (Sokal and Rohlf 1969).

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[1] The reproductive behavior of the nudibranch is thought to be initiated by a sex pheromone as has been implicated in mediating reproductive aggregations in other gastropods (Dinter 1975, Audesirk 1977).

(b) Embryological development. The exposure system was identical to that used above in testing chemosensory disruption of dorid nudibranchs. There was, however, a difference in determining the petroleum hydrocarbon concentration in each aquarium. Over the 18-day exposure period four water samples for GC analysis were taken from the oil solubilizer head box. Hydrocarbon concentration in each aquarium was calculated from the average SWSF concentration in the head box and the amount of SWSF diluted with untreated seawater.

Four groups of 20 mature nudibranchs of approximately equal size (total weight 2.1 g for each group) were placed in 4 separate 2-l flow-through aquaria. The first egg masses laid by the dorid nudibranchs were collected and placed in holding containers within each exposure aquaria; replicate egg aliquots of 2-3 mm^3 from the center of each egg mass were taken daily 5 days per week until hatching was complete or the experiment terminated. The eggs were fixed in buffered preservative (Hawkes 1974, with the addition of 17 g of synthetic seawater per liter of buffer to attain 800 mOsm). In addition, eggs laid each following day were collected, weighed, and then discarded.

Embryonic development was studied using light microscopy. Eggs in aliquot samples were counted and each normal egg assigned to one of the following 7 categories: One-cell; two-cell; blastula; gastrula; early velum; late velum; and shell.[1] Abnormal eggs were categorized as: moderate abnormality (a recognizable embryo but malformed, or with extra cellular material in the capsule) or severe abnormality (no recognizable embryo and often no capsule present). Photomicrographs of the normal and abnormal categories listed above are given in reports by Malins et al. (1978) and/or Mumaw (1978).

Feeding Behavior of Shrimp Exposed to Petroleum Hydrocarbons

Spot shrimp were caught in commercial shrimp pots at depths of 100 to 120 m in Puget Sound. Following capture the shrimp were maintained in 1200-l flow-through tanks at ambient seawater temperature of 10 to 12°C and a salinity of 27 to 30 °/oo. Three days prior to testing, individual shrimp were moved to 3-l glass exposure tanks, each with a flow rate of 300 ml/min. The shrimp were not fed for 3 days prior to exposure or during the 6 day exposure period. The number of shrimp tested and the number of observations made using 4 SWSF concentrations of PBCO are given in Table 2.

To obtain different concentrations of hydrocarbons in exposures, the SWSF from an oil solubilizer (Roubal et al. 1977b) was diluted just prior to its entry into the test chambers while maintaining a total flow of 300 ml/min (Fig. 11). The shrimp chambers were enclosed in black plastic and observations on feeding behavior were made through one-way mirrors. Each data point consisted of three, 3-min observations of background activity, response to seawater control, and response to a 1:10 dilution of artificial squid extract (Mackie 1973). Seawater

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[1] Developmental stages used here are described in detail in several reports involving nudibranch development (McGowan and Pratt 1954, Perron and Turner 1977, Thompson 1960, 1962, 1967).

TABLE 2. Number of shrimp tested and number of tests conducted on feeding behavior in relation to concentrations of the SWSF of PBCO.

Hydrocarbon concentration[a]		Number of shrimp tested	Number of tests
ppb ($\bar{x} \pm$ sd)	N		
Control		6	36
11 ($\pm$1)	2	3	18
18 ($\pm$3)	4	9	54
40 ($\pm$12)	2	4	24
575 ($\pm$485)	7	3	7

a. Based on GC data of water taken from shrimp test chamber.

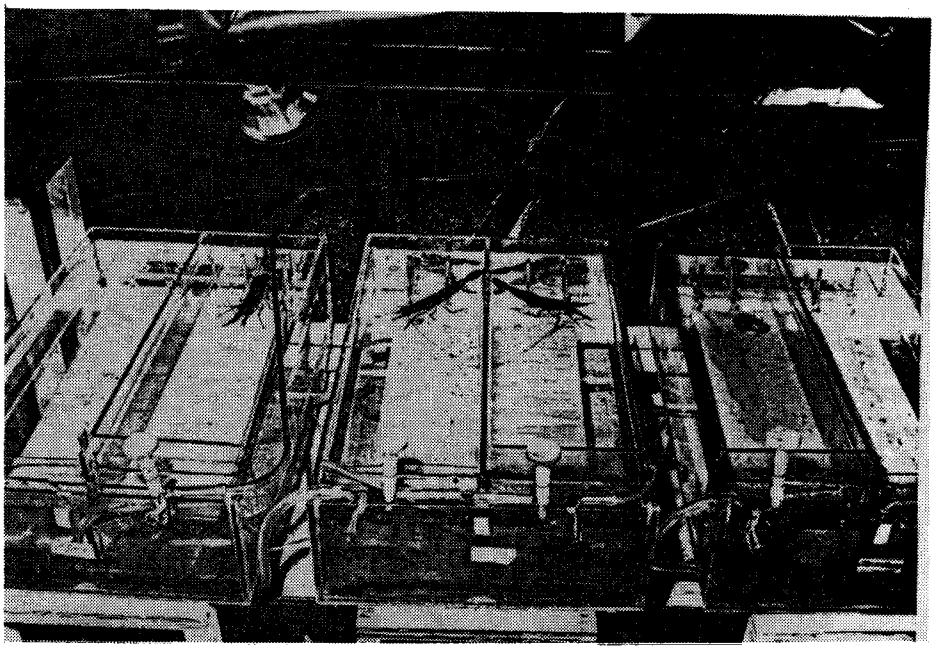

FIGURE 11. Glass chambers for testing effect of the SWAF of PBCO on the feeding response of spot shrimp. Valves and mixing box in foreground of each chamber are for adjusting flow rate and SWAF concentration.

control and squid extract stimulants were introduced at the upstream end of the test chamber at a flow rate of 10 ml/min.

Two behavioral indices were selected for evaluating feeding responses to a given stimulant[1]: (1) Perepod probing--Probing the substrate with the first perepods was recorded as the number of probing bouts during a test; this activity is indicative of higher order searching behavior. (2) Contact with stimulant inlet--Defined as grasping or actively picking at the inlet with chelae or mouth parts; this behavior was considered representative of maximum feeding response.

6. RESULTS

6.1 Chemistry

6.1.1 Uptake and Biotransformation of Specific Hydrocarbons in Salmonids

Coho salmon injected with radiolabeled benzene, NPH, and anthracene differentially retained these hydrocarbons in liver, brain and muscle as indicated by the data in Figure 12, Table 3. In the tissues examined, the percent of administered dose at all time periods was the lowest in fish exposed to benzene and the highest in those exposed to anthracene (Table 3).

The data in Table 3 also show that the administered hydrocarbons were metabolized by coho salmon and metabolic products were present in the liver and gall bladder. However, in the brain most of the recoverable radioactivity was in the form of the parent hydrocarbons 24 hr (>98%) after injection.

To delineate the nature of metabolic products, solvent extracts of various tissues were analyzed by TLC. The results revealed a wide spectrum of metabolic products in various proportions in the brain, liver, gall bladder, heart, and flesh of fish at 24 hr after administration of NPH. Naphthol, a dihydrodiol derivative of NPH and their conjugates (e.g., glucuronides, sulfates and mercapturic acids) were detected.

6.1.2 Accumulation of Petroleum Hydrocarbons by Fish Exposed to SWSF of PBCO

The hydrocarbon composition of SWSF of PBCO is given in Table 4 which shows the presence of mono and diaromatic hydrocarbons. Data are presented in Table 5 for the accumulation of hydrocarbons in coho salmon exposed to a SWSF of PBCO equal to 0.8 ppm of aromatic hydrocarbons in flow-through seawater. After 1 wk of exposure, no hydrocarbons representative of the SWSF were detected in muscle tissue. Exposures from 2 to 6 wk, however, resulted in the accumulations of significant

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[1] Three additional behaviors were also recorded but not presented here; antennular flicks, antennular cleaning, and forward movement. For each of these behaviors there was often a response to seawater control stimulus which resulted in inconclusive data. For details of these observations and results see Malins et al. (1977) and/or Miller (1980).

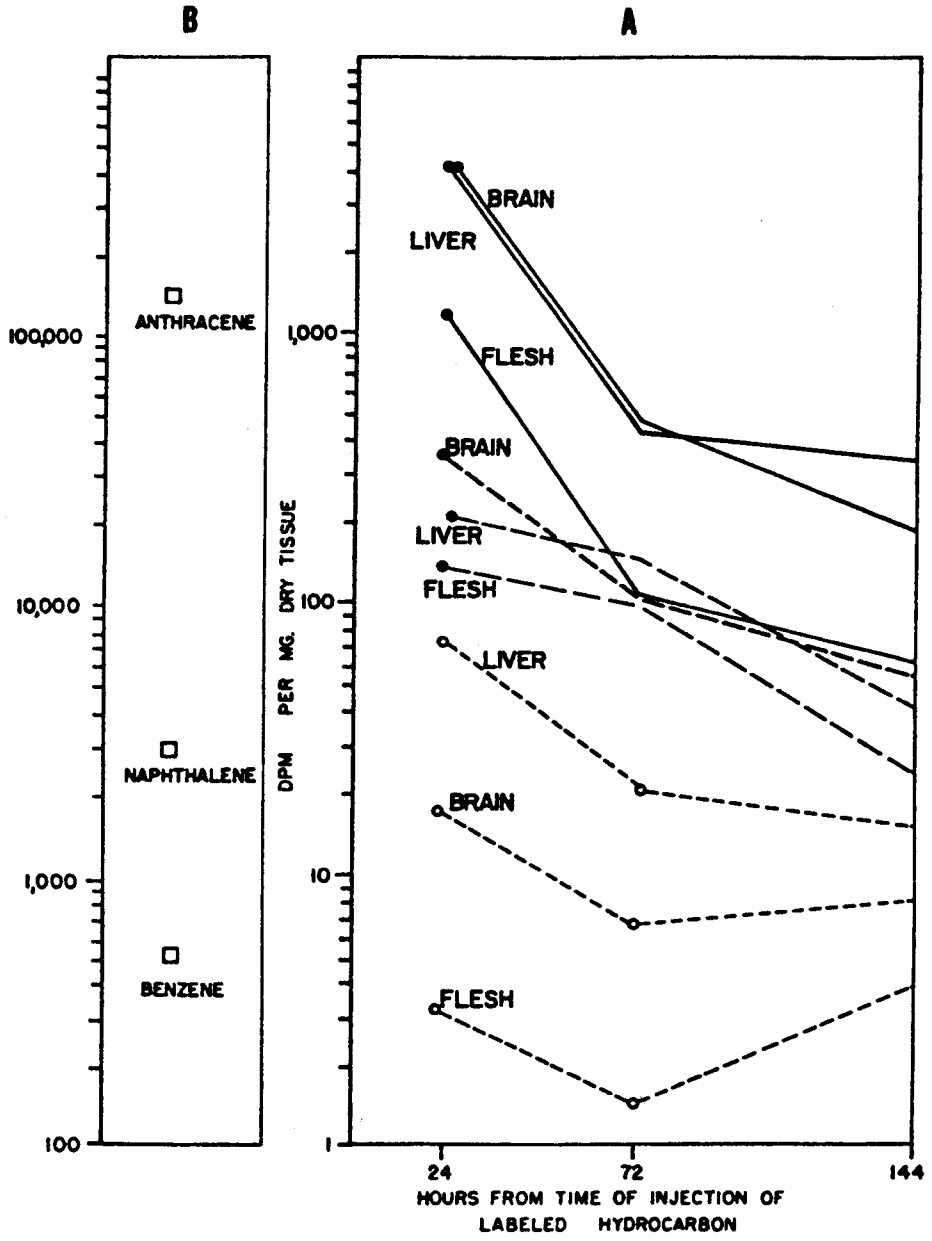


FIGURE 12. Radioactivity in tissues of coho salmon receiving ^{14}C-labeled hydrocarbons (2.5 uCi) by intraperitoneal injection. A, anthracene radioactivity (———), naphthalene radioactivity (— — — —) and benzene radioactivity (-----); B, 24-hr values for radioactivity in the gall bladder of injected fish. Total carbon-14 in organs as % of administered dose is given in Table 3. (From Roubal et al. 1977a)

Table 3 *Distribution of hydrocarbons, total aromatic metabolites and total carbon-14 in organs of coho salmon receiving hydrocarbons by intraperitoneal injection*

Hydrocarbon	Time after injection (hr)	Tissue[a]																			
		Brain					Liver				Gall bladder				Flesh				Carcass			
		pCi radioactivity (x1000) for whole organ	Total carbon-14 in organ (% administered dose)	Un[b] (%)	Met (%)		pCi radioactivity (x1000) for whole organ	Total carbon-14 in organ (% administered dose)	Un (%)	Met (%)	pCi radioactivity (x1000) for whole organ	Total carbon-14 in organ (% administered dose)	Un (%)	Met (%)	pCi radioactivity (x1000) for whole organ	Total carbon-14 in organ (% administered dose)	Un (%)	Met (%)	pCi radioactivity (x100) for whole organ	Total carbon-14 in organ (% administered dose)	Un (%)	Met (%)
Benzene	6	0.14	0.0028	98	2		1.2	0.024	68	32	0.3	0.006	37	63	3.3	0.066	100	0	310	6.2	100	0
	24	0.018	0.00036	100	0		0.38	0.00076	58	42	0.07	0.0014	29	71	0.3	0.006	100	0	11	0.22	100	0
Naphthalene	24	3.0	0.060	98	2		4.6	0.092	87	13	13	0.26	28	72	83	1.70	96	4	770	15.4	99	1
	72	0.71	0.142	76	24		2.1	0.042	55	45	–[c]	–	–	–	42	0.84	86	14	170	3.4	89	11
	144	0.49	0.010	80	20		0.91	0.018	59	41	–	–	–	–	20	0.40	87	13	260	5.2	92	8
Anthracene	24	39	0.78	98	2		60	1.20	36	64	400	8.0	10	90	580	11.6	87	13	1700	34	97	3
	72	2.7	0.054	96	4		8.8	0.18	42	58	–	–	–	–	68	1.36	99	1	2500	50	86	14
	144	1.4	0.028	91	9		7.5	0.15	31	69	–	–	–	–	39	0.78	54	46	1000	20	60	40

[a] Heart data: Although heart was analyzed, levels of radioactivity in benzene-injected fish were too low to be statistically significant and were not reported. Twenty-four hr after injection of naphthalene, 97% of the radioactivity was represented by naphthalene, and the total radioactivity was 1000 pCi; later measurements were not taken. Ninety-six percent of the recovered radioactivity in the heart of anthracene-injected fish was attributed to anthracene after 24 hr; the total radioactivity was 4000 pCi. Measurements at later times were not taken

[b] Percentages shown are percentages of the total tissue radioactivity represented by parent hydrocarbons (unmetabolized; Un) and their total aromatic metabolites (Met)

[c] Analysis not performed

(From Roubal et al. 1977a)

Table 4 Hydrocarbon content of flow-through exposure water

Hydrocarbons	Concentration[a] (ppm) (Mean ± std. deviation)[b]
Cyclohexane	0.02 ± 0.002
Benzene	0.04 ± 0.006
Toluene	0.4 ± 0.05
Ethylbenzene	0.005 ± 0.002
m-Xylene	0.2 ± 0.003
o- and *p*-Xylenes	0.07 ± 0.03
C_3-Substituted benzenes	0.03 ± 0.005
C_4-Substituted benzenes	0.01 ± 0.003
C_5-Substituted benzenes	trace[c]
Naphthalene	0.003 ± 0.002
1-Methylnaphthalene	0.003 ± 0.001
2-Methylnaphthalene	0.003 ± 0.002
C_2-Substituted naphthalenes	0.01
C_3-Substituted naphthalenes	0.005

[a] Values corrected for losses during workup

[b] Content of the hydrocarbons, cyclohexane through naphthalene was determined seven times during exposure. Values reported for the methylnaphthalenes are averages for two analyses. Single values are reported for C_2- and C_3-substituted naphthalenes. Hydrocarbon content of flow-through water (≃ 0.8 ppm at the beginning of solubilizer operation) was only slightly greater (≃ 1.0 ppm) after five weeks of continuous operation

[c] ≃ 0.001 ppm was the lower limit of hydrocarbon detection

(From Roubal et al. 1978)

amounts of substituted and unsubstituted benzenes and naphthalenes (Table 5). After 5 wk of exposure, which was the time of maximum hydrocarbon accumulation in salmon, the bioconcentration factors (concentration of hydrocarbons in tissue/concentration of hydrocarbons in water) in muscle for C_3-substituted benzenes, NPH, 1- MN, 2-MN, C_2-substituted NPH, and C_3-substituted NPH were 50, 80, 190, 130, 85, and 140, respectively. The C_4- and C_5-substituted benzene fraction of SWSF was the most prominent fraction in muscle throughout the exposures. After 5 wk of exposure, the latter amounted to 5.5 ppm, or a bioconcentration factor of 550 for C_4- and C_5-substituted benzenes in muscle tissue. When fish were exposed for 6 wk and transferred to clean seawater for 1 wk, the aromatic hydrocarbons were not found in muscle.

Tables 5 and 6 present data on hydrocarbons accumulated in muscle, liver, and gills of starry flounder exposed to a SWSF of PBCO. In contrast, to salmon, starry flounder was found to have considerable concentrations of aromatic hydrocarbons in tissues after 1 wk exposure. Bioconcentration of hydrocarbons in flounder muscle was greater after 1 wk than after 2 wk of exposure. The C_4- and C_5-substituted benzene fraction of SWSF was concentrated 10 times more in muscle of flounder than in salmon muscle after 2 wk of exposure.

Table 5 Hydrocarbons in muscle tissue of coho salmon *(O. kisutch)* and starry flounder *(P. stellatus)* exposed to the water-soluble fraction of Prudhoe Bay crude oil using flow-through exposure[a]

	Coho salmon								
	Weeks of exposure								
	2		3		5		6		1 Week depuration (6 weeks exposure)
Hydrocarbons	Bioconcentration[b]	ppm dry tissue[c]	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	
C_3-Substituted benzenes	1.1	0.31 SS[e]	2.4	0.66 SS	2	0.55 SS	1	0.27 SS	NF[f]
C_4-Substituted benzenes	10	0.30 ± 0.12	30	0.90 ± 0.12	50	1.5 ± 0.09	10	0.40 ± 0.18	NF
C_4-C_5-Substituted benzenes	150	1.5 ± 0.61	170	1.7 ± 0.50	550	5.5 ± 1.0	200	2.0 ± 1.5	NF
Naphthalene	20	0.07 ± 0.03	50	0.14 ± 0.07	80	0.24 ± 0.06	40	0.12 ± 0.06	NF
2-Methylnaphthalene	30	0.10 ± 0.05	100	0.31 ± 0.01	190	0.56 ± 0.14	70	0.20 ± 0.10	NF
1-Methylnaphthalene	30	0.10 ± 0.03	70	0.22 ± 0.00	130	0.40 ± 0.08	50	0.16 ± 0.08	NF
C_2-Substituted naphthalenes	30	0.31 ± 0.30	40	0.36 ± 0.00	85	0.90 ± 0.24	40	0.44 ± 0.30	NF
C_3-Substituted naphthalenes	50	0.23 ± 0.09	30	0.15 ± 0.02	140	0.70 ± 0.22	80	0.40 ± 0.40	NF

	Starry flounder							
	Weeks of depuration (2 weeks exposure)							
	1		2		1		2	
C_3-Substituted benzenes	20	5.5 ± 2.0	4	1.0 ± 0.30	1	0.27 ± 0.06		NF
C_4-Substituted benzenes	500	15 ± 5.7	70	2.2 ± 1.2	6	0.18 ± 0.03	10	0.30 ± 0.02
C_4-C_5-Substituted benzenes	9300	93 ± 34	1700	17 ± 6.2	980	9.8 ± 0.40	2600	26 ± 1.6
Naphthalene	700	2.1 ± 1.5	240	0.72 ± 0.30	100	0.30 ± 0.02	270	0.80 ± 0.04
2-Methylnaphthalene	2800	8.3 ± 3.6	470	1.4 ± 0.60	110	0.33 ± 0.03	200	0.60 ± 0.02
1-Methylnaphthalene	2000	6.1 ± 4.3	330	1.1 ± 0.50	113	0.34 ± 0.01	270	0.82 ± 0.04
C_2-Substituted naphthalenes	2400	24 ± 9.7	540	5.4 ± 2.3	270	2.7 ± 0.80	700	7.0 ± 1.6
C_3-Substituted naphthalenes	3400	17 SS	1000	5.0 ± 2.0	420	2.1 ± 0.00	1600	8.0 ± 0.10

[a] 0.9 ± 0.1 ppm (total hydrocarbons) in flow-through water. See Table 4

[b] Bioconcentration = ppm hydrocarbon in dry weight tissue/ppm hydrocarbon in water

[c] Mean ± Standard error of mean for two 10- to 15-g composite samples each prepared from separate groups of coho salmon (2 fish/group)

[d] Mean ± Standard error of mean for two 10- to 15-g composite samples each prepared from separate groups of starry flounder (5 fish/group)

[e] SS = Single sample value

[f] NF = Not found; below limits of detection (≤0.05 ppm)

(From Roubal et al. 1978)

After 2 wk of maintaining exposed starry flounder in clean seawater, the concentration of several hydrocarbons of the SWSF in muscle tissue was still elevated, e.g., 26 ppm for the C_4- and C_5-substituted benzene fraction and 8.0 ppm for the C_3-substituted NPH fraction. In the gills and liver of starry flounder, levels for all hydrocarbons of the SWSF was either near to, or below, the limits of detection (i.e.<9 ng/g dry wt.) after the fish were in clean oil-free water for 2 weeks.

6.1.3 Metabolism of NPH by Coho Salmon

Both liver and gall bladder of coho salmon force-fed NPH contained compounds which were identified as the glucuronide, sulfate, dihydrodiol, glucoside and 1-hydroxy derivatives of NPH (Fig. 13; Table 7). Substantially higher concentrations of each of these metabolites were

Table 6 Hydrocarbons in liver and gills of starry flounder *(P. stellatus)* exposed to the water-soluble fraction of Prudhoe Bay crude oil using flow-through exposure[a]

Hydrocarbons	Liver								Gills							
	Weeks of exposure				Weeks of depuration (2-weeks exposure)				Weeks of exposure				Weeks of depuration (2-weeks exposure)			
	1		2		1		2		1		2		1		2	
	Bioconcentration[b]	ppm dry tissue[c]	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue	Bioconcentration	ppm dry tissue
C_1-Substituted benzenes	6	1.6	10	2.6	1	0.23	1	0.2	7	1.8	4	1.1	1.1	1.1		NF[d]
C_2-Substituted benzenes	200	6.0	320	9.6	10	0.38	6	0.19	170	5.0	100	3.1	110	3.4		NF
C_4-C_5-Substituted benzenes	3,600	36	11,000	110	2,900	29		ND[e]	3,000	30	2,100	21	2,300	23		ND
Naphthalene	500	1.5	1,100	3.3	300	0.90		ND	150	0.44	250	0.75	160	0.47	100	0.31
2-Methylnaphthalene	1,000	3.0	2,000	6.2	300	0.90		NF	770	2.3	400	1.2	170	0.50		NF
1-Methylnaphthalene	800	2.4	1,600	5.0	300	1.0		NF	600	1.8	310	0.94	170	0.50		NF
C_2-Substituted naphthalenes	1,000	10	2,800	28	900	9.0	25	0.25	800	8.0	320	3.2	400	3.9	20	0.22
C_3-Substituted naphthalenes	1,100	5.3	4,400	22	2,000	10		NF	1,500	7.6	700	3.5	860	4.3		NF

[a] 0.9 ± 0.1 ppm (total hydrocarbons) in flow-through water. See Table 4
[b] Bioconcentration = ppm hydrocarbon in dry weight tissue/ppm hydrocarbon in water
[c] Single values for 5-g composite samples prepared from 10 fish
[d] NF = Not found; below limits of detection (≤0.10 ppm)
[e] ND = Not determined

(From Roubal et al. 1978)

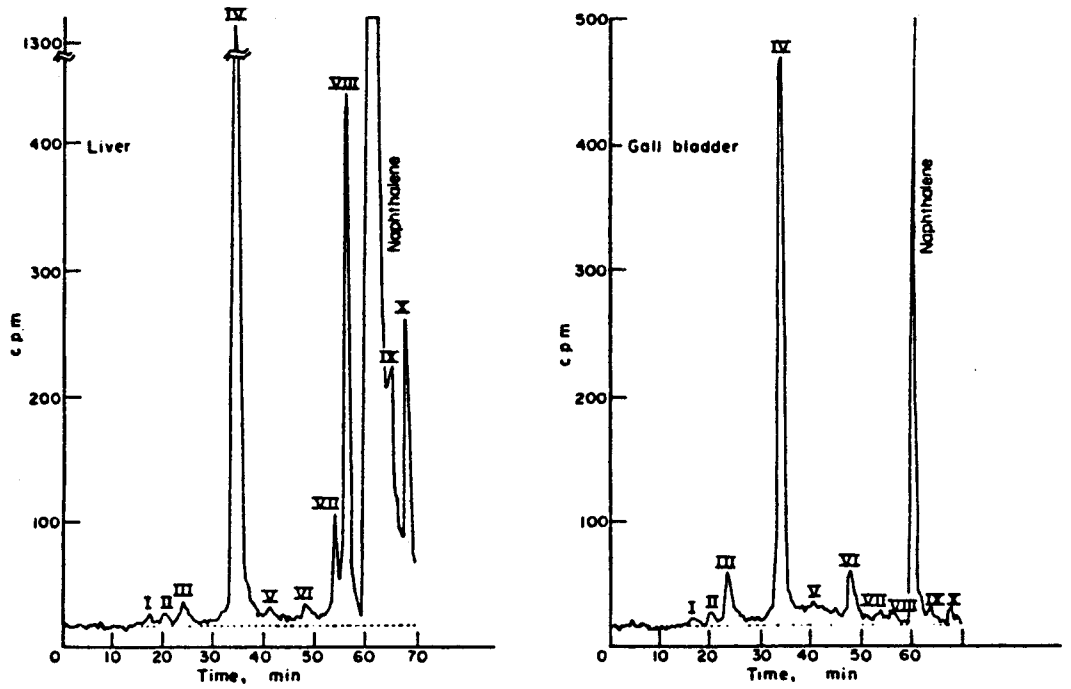


FIGURE 13. HPLC profiles of derivatives of naphthalene in liver and gall bladder of coho salmon 16 hr after force-feeding of ^{3}H-naphthalene. Profiles represent single analysis of pooled tissues from 4 fish. Identification and quantification of peaks are given in Table 7: (---) represents background level of 16 cpm; dpm = cpm/0.46. (From Collier et al. 1978)

found in the gall bladder as opposed to the liver. However, the liver had a higher organ burden of dihydrodiol than did the gall bladder. These data represent the first use of high pressure liquid chromatography (HPLC) for analyzing aromatic hydrocarbon metabolites in fish.

6.1.4 NPH and Its Metabolites in Fish Skin and Mucus

Skin. Skin of rainbow trout contained maximum concentrations of NPH (322 ppb) and of its metabolites (17 ppb) 24 hr after the fish were force fed ^{3}H-NPH. The concentrations of both NPH and its metabolites subsequently declined to 10.3 and 2.5 ppb, respectively, at 168 hr (Table 8). The data on changes in concentrations of NPH and its metabolites with time in the skin were statistically treated and both curves fit a lognormal probability distribution function (Table 8). Statistical treatment of the data revealed that the rates of change in concentrations of NPH and its metabolites in the skin of the force-fed fish were significantly different ($P<0.05$). Up to 24 hr after the treatment, NPH concentration increased more rapidly than that of the metabolites (Table 8). Subsequently, NPH concentration declined more rapidly so that

Table 7 Metabolites present in liver and gall bladder of coho salmon 16 hr after feeding of ^{3}H-naphthalene

Peak	Compound	Liver		Gall bladder	
		pmoles/g% Dry wt	Admin dose ($\times 10^{-3}$)	pmoles/g% Dry wt	Admin dose ($\times 10^{-3}$)
I*		0.38	0.022	2.9	0.026
II	Glucuronide	2.0	0.11	20	0.18
III	Sulfate	1.7	0.097	21	0.19
IV	Dihydrodiol	110	6.1	200	1.7
V	Glucoside	0.88	0.050	4.6	0.040
VI	1-Naphthol	2.0	0.11	30	0.27
VII*		3.9	0.22	3.7	0.033
VIII*		17	0.94	3.4	0.030
IX*		15	0.83	3.2	0.028
X*		11	0.61	3.5	0.031

* Uncorrected for extraction efficiency.

(From Collier et al. 1978)

at 168 hr, NPH metabolites represented a larger portion (17.6%) of the total radioactivity in the skin than at 24 hr (4.4%) (Table 9).

To assess the importance of fish skin in the disposition of hydrocarbons, concentrations of NPH and its metabolites in skin were compared with corresponding values for liver, which is an active site for hydrocarbon metabolism. At 16 hr after force-feeding, the concentration of NPH in skin was 46% of the liver concentration. The ratio (tritium in skin/tritium in liver) for NPH remained more or less constant thereafter. This ratio for the metabolites remained about the same throughout the experiment. The maximum value of percent-administered dose in the skin was 0.87 at 24 hr. The skin contained only 0.03% of the administered dose at 168 hr after the treatment (Table 9).

Skin of starry flounder contained 68 ppb of NPH and 25 ppb of its metabolites at 24 hr after the fish were force fed ^{3}H-NPH (Table 10). At 24 hr, 0.4% of the administered dose was present in skin and at 168 hr, 0.02% remained. When comparing these data with those obtained for rainbow trout, it should be noted that starry flounder were kept at 12°C and rainbow trout were maintained at 8°C. The difference in temperature may have an important effect on the levels of total radioactivity found in skin. At 168 hr, 78% and 17.6%, respectively, of the total radioactivities in the skin of starry flounder and rainbow trout (Table 9) were attributable to the metabolites.

Rainbow trout held at 8°C were injected i.p. with ^{3}H-NPH; 4 hr after injection the skin contained 114 ppb of NPH and 6.2 ppb of the metabolites at (Table 11). The maximum concentation of NPH, 244 ppb, was reached at 16 hr following the injection, and subsequently declined to 19.1 ppb at 168 hr. The concentration of metabolites continued to increase in the skin reaching a maximum value of 14.4 ppb at 48 hr and then decreasing to 3.8 ppb at 168 hr.

TABLE 8

CONCENTRATIONS OF NAPHTHALENE AND ITS METABOLITES IN SKIN AND EPIDERMAL MUCUS OF RAINBOW TROUT (*Salmo gairdneri*) EXPOSED TO NAPHTHALENE VIA FORCE FEEDING[a,b]

Time elapsed after treatment (hr)	Naphthalene		Metabolites[c]	
	(dpm/mg)	(ppb)	(dpm/mg)	(ppb)
Skin				
4	51.3 ± 18.3 (10)	35.0	6.1 ± 1.8 (12)	4.7
16	249.8 ± 29.8 (12)	172.9	20.0 ± 2.0 (16)	15.6
24	465.2 ± 46.1 (7)	321.9	21.3 ± 1.8 (7)	16.6
48	109.1 ± 18.7 (13)	75.5	8.0 ± 1.4 (13)	6.2
168	14.9 ± 1.1 (11)	10.3	3.2 ± 0.6 (9)	2.5
Mucus				
4	2.9 ± 1.0 (4)	2.0[0.01]	89.8 ± 7.6 (3)	69.9 [0.46]
16	18.7 ± 3.1 (4)	12.9 [0.09]	107.0 ± 23.4 (4)	83.3 [0.55]
24	64.4 ± 53.9 (2)	44.6 [0.29]	167.0 ± 7.6 (4)	130.0 [0.89]
48	65.1 ± 33.2 (4)	45.0 [0.30]	151.0 ± 39.9 (4)	117.6 [0.78]
168	42.2 ± 30.2 (4)	29.2 [0.19]	51.3 ± 31.4 (4)	41.2 [0.27]

[a] Fish were force fed 74.6 μCi of 1,4,5,8-[^{3}H]naphthalene and samples were taken from four or five fish at each time interval. Concentration of both naphthalene and metabolites in water was less than 0.01 ppb at all times.

[b] Mean ± SE; values in parentheses represent number of individual measurements including duplicate measurements on samples from the same fish. All concentrations are given on dry weight basis. Because epidermal mucus contained as much as 99.34% water, concentrations in brackets are also given on wet weight basis.

[c] Concentration of metabolites was calculated using molecular weight of naphthol. The data fitted log normal distribution and the pdf is given by:

$$f(x;\alpha,\mu,\sigma) = \frac{1}{(x-\mu)\sigma\sqrt{2\pi}} \exp - \frac{1}{2\sigma^2}[\ln(x-\alpha)-\mu]^2,$$

where α is a location parameter, μ is a scale parameter, and σ is a shape parameter. For example, the data for skin in this expeiment yielded parametric values of: $\alpha = 5$, $\mu = 4.064$, and $\sigma = 1.738$ (naphthalene); $\alpha = 1$, $\mu = 1.766$, and $\sigma = 1.371$ (metabolites).

(From Varanasi et al. 1978)

These data (Table 11) also fit a lognormal distribution probability function; the rates of change in concentration of NPH and its metabolites in the skin of injected fish were significantly different ($P<0.001$). As in the force-feeding study, both the rates of increase as well as subsequent rate of decline of NPH concentration in the skin of the injected fish were greater than the respective rates of increase and decline of metabolites. Hence, the proportion of the metabolites increased steadily and comprised as much as 15.1% of the total radioactivity in the skin of fish at 168 hr (Table 9).

Starry flounder held at 12°C were injected with ^{3}H-NPH, and skin contained 70 ppb of NPH and 15 ppb of the metabolites at 24 hr after

TABLE 9

VARIOUS PARAMETERS SHOWING PATTERNS OF ACCUMULATION AND RELEASE OF NAPHTHALENE AND ITS METABOLITES IN SKIN OF RAINBOW TROUT (*S. gairdneri*) EXPOSED TO NAPHTHALENE

Mode of exposure[a]	Time (hr)	Total radioactivity in skin (μCi)	Relative percentage[b]		Administered dose in total skin (%)	[^{3}H in skin/^{3}H in liver][c]	
			Naphthalene	Metabolites		Naphthalene	Metabolites
Force feeding	4	0.06	89.4	10.6	0.08	0.05 ± 0.01 (4)	0.09 ± 0.02 (4)
	16	0.31	92.6	7.4	0.41	0.46 ± 0.12 (4)	0.09 ± 0.02 (4)
	24	0.65	95.6	4.4	0.87	0.52 ± 0.10 (3)	0.17 ± 0.06 (3)
	48	0.16	93.2	6.8	0.21	0.51 ± 0.10 (4)	0.20 ± 0.08 (4)
	168	0.03	82.4	17.6	0.03	0.51 ± 0.12 (4)	0.10 ± 0.01 (3)
Injection	4	0.20	95.4	4.6	0.21	0.13 ± 0.05 (3)	0.10 ± 0.03 (3)
	16	0.43	97.2	2.8	0.45	0.38 ± 0.08 (3)	0.25 ± 0.15 (3)
	24	0.38	95.4	4.6	0.40	0.34 ± 0.12 (3)	0.26 ± 0.03 (3)
	48	0.22	90.0	10.0	0.23	0.14 ± 0.04 (2)	0.29 ± 0.10 (3)
	168	0.04	84.9	15.1	0.04	0.19 ± 0.06 (2)	0.12 ± 0.03 (2)

[a] Details of exposure conditions are given in the footnotes to Tables 8 and 11.

[b] Relative percent of naphthalene and metabolites are based on disintegrations per minute per milligram values given in Tables 8 and 11.

[c] Values for radioactivity (disintegrations per minute per milligram dry weight) associated with naphthalene and metabolite fractions of each skin sample were divided by corresponding values for the liver of the same fish. Numbers represent mean ± SE of three or four ratios. Values in parentheses indicate number of individual fish.

(From Varanasi et al. 1978)

TABLE 10. Concentrations of naphthalene (NPH) and its metabolites in skin and epidermal mucus of starry flounder (P. stellatus) exposed to NPH via force-feeding.[a]

Time elapsed after treatment (hr)	Skin		Mucus	
	NPH ppb	Metabolites ppb	NPH ppb	Metabolites ppb
4	30±15[b]	24±3	10±4	41±10
24	68±40	25±7	14±7	30± 8
48	15± 3	20±4	3±1	12± 3
168	2± 1	7±1	1±0.5	4± 1

[a] Fish were fed 56 μg of ^{3}H-NPH at 12±1°C.
[b] $\bar{X}$±S.D.

TABLE 11

CONCENTRATIONS OF NAPHTHALENE AND ITS METABOLITES IN SKIN AND EPIDERMAL MUCUS OF RAINBOW TROUT (*Salmo gairdneri*) EXPOSED TO NAPHTHALENE VIA INTRAPERITONEAL INJECTION[a,b]

Time elapsed after treatment (hr)	Naphthalene		Metabolites[c]	
	(dpm/mg)	(ppb)	(dpm/mg)	(ppb)
Skin				
4	164.5 ± 98.5 (3)	113.8	7.9 ± 0.5 (3)	6.2
16	352.6 ± 2.7 (3)	244.0	9.9 ± 1.4 (3)	7.7
24	306.4 ± 151.0 (3)	212.0	14.7 ± 0.2 (3)	11.4
48	167.8 ± 28.9 (2)	116.1	18.5 ± 3.6 (3)	14.4
168	27.6 ± 13.5 (3)	19.1	4.9 ± 0.4 (3)	3.8
Mucus				
4	18.2 ± 9.3 (3)	12.6	173.7 ± 49.6 (2)	135.2
16	4.8 ± 4.8 (3)	5.4	90.8 ± 20.6 (3)	70.7
24	2.3 ± 2.3 (3)	1.6	110.3 ± 11.0 (3)	85.8
48	nd (3)[d]	nd	270.5 ± 85.9 (3)	210.5
168	nd (3)	nd	nd (2)	nd

[a] 1,4,5,8-[^{3}H]naphthalene (94.6 μCi) was injected ip in each fish and samples were taken at designated time intervals from a total of three fish. The concentration of both naphthalene and metabolites in water was less than 0.01 ppb at all times.
[b] Mean ± SE; values in parentheses represent number of individual measurements. All measurements are given on dry weight basis.
[c] Concentration of metabolites was calculated using molecular weight of naphthol.
[d] None detected.

(From Varanasi et al. 1978)

TABLE 12. Concentrations of naphthalene (NPH) and its metabolites in skin and epidermal mucus of starry flounder (P. stellatus) exposed to NPH via intraperitoneal injection.[a]

Time elapsed after treatment (hr)	SKIN		MUCUS	
	NPH ppb	Metabolites ppb	NPH ppb	Metabolites ppb
24	70±35[b]	15±3	1.2± 1	60±41
168	11± 3	4±0.4	ND[c]	ND

[a] Fish were injected with 75 µg of ^{3}H-NPH at 12°±1°C.
[b] $\bar{x}$±S.D.
[c] ND = not detected.

exposure and 11 ppb of NPH and 4 ppb of the metabolites at 168 hr (Table 12).

Mucus. Results in Table 8 show that at each sampling time, epidermal mucus from rainbow trout fed NPH contained considerably larger concentrations of metabolites than parent hydrocarbon. Epidermal mucus is generated when epithelial mucin released by the mucus cells comes in contact with surrounding water; therefore, concentrations of aromatic compounds calculated on a dry weight basis would more closely approximate the actual concentrations of these compounds in epithelial mucin. However, because epidermal mucus of rainbow trout contained more than 99% water, concentrations of NPH and its metabolites are also given on a wet weight basis in Table 8. Concentrations of both NPH and its metabolites in the mucus initially increased and reached maximum values 24 hr after the force feeding. The data fit lognormal distribution, and using a one-tailed F-test of homogeneity of variance, it was calculated that rates of changes in concentration of NPH and metabolites in the mucus of the force-fed fish were not significantly different (Table 8).

Epidermal mucus of starry flounder contained 14 ppb (dry weight) of NPH and 30 ppb of the metabolites 24 hr after the fish were fed NPH. During the entire exposure period, the mucus contained higher concentrations of the metabolites compared to NPH (Table 10).

Epidermal mucus of the rainbow trout in the injection study contained larger concentrations of metabolites than NPH at each sampling period. NPH was not detected in the test fish after 24 hr. Metabolites were present in mucus for the first 48 hr, but were not detected at 168 hr (Table 11). The data in Tables 8 and 11 reveal that there was no correlation between either relative proportions or rates of change of concentrations of NPH and metabolites in skin and mucus of the test fish.

6.1.5 Accumulation and Biotransformation of NPH by Flatfish

Force-feeding Study (Starry Flounder) at 12°C. Tissues of starry flounder contained considerable concentrations of both NPH and its metabolic products 24 hr after force-feeding NPH (Fig. 14). Maxima in concentrations of both the hydrocarbon and its metabolic products occurred during the first 48 hr,then began to decline in all tissues except bile (Fig. 14). Statistical treatment of these data shows that the rates of decline of NPH concentrations in liver, skin, and blood were significantly greater than the respective rates of decline in metabolite concentrations (Fig. 14). At 168 hr, all sites examined, except brain, contained considerably more radioactivity associated with metabolic products than with NPH (Fig. 14; Table 13); more than 70% of the total radioactivity in blood and liver was associated with the metabolic products. Throughout the experiment, radioactivity in bile of starry flounder was largely due to metabolites.

Force-feeding Study (Rock Sole) at 12°C. Comparison of the data in Figures 14 and 15 revealed that the radioactivity in all tissues of rock sole was considerably greater than the radioactivity in the corresponding tissues of starry flounder at 24 hr. Moreover, values for NPH and metabolite concentrations in tissues of rock sole were significantly ($P<0.01$) higher than the corresponding values for starry flounder at 168 hr. Liver, skin, and muscle of starry flounder contained 11.6, 3.0, and 1.7 g of lipid, respectively, per 100 g of wet tissue; whereas, liver, skin, and muscle of rock sole contained 4.3, 1.5, and 1.0 g of lipid, respectively, per 100 g of wet tissue.

Differences between rates of decline in NPH and the metabolite concentrations in liver, blood, and skin of rock sole were also measured. As with starry flounder, the rate of decline in NPH concentration in each tissue was greater than the rate of decline in metabolite concentration (Fig. 15). It was also determined that the extent of decline of NPH in each of the tissues was greater for starry flounder than for rock sole. From 24 to 168 hr, there was 12.4- and 2.3-fold decrease, respectively, in NPH and metabolite concentrations in the livers of rock sole; corresponding values for starry flounder were 63.0 and 2.6.

Stomach and intestine of rock sole contained more radioactivity than those of starry flounder at both 24 and 168 hr. At 24 hr, stomach and intestine of the rock sole contained 0.3 and 6.0%, respectively, of the administered dose, whereas the corresponding values for starry flounder were 0.2 and 1.5.

Because considerable radioactivity was present in the rock sole after 1 wk, some fish were sampled at 6 wk after the treatment. Gills and kidney contained considerable concentrations of metabolic products, whereas bile and liver contained smaller concentrations, and in the other tissues examined radioactivity was barely detectable (Fig. 15).

Injection Study (Starry Flounder) at 12°C. At 24 hr after an i.p. injection of ^{3}H-NPH, livers of starry flounder contained considerably higher concentrations of NPH than did livers of fish receiving the same dose of NPH via force-feeding (Figs. 14 and 16). At 168 hr, livers of fish in the injection study contained 16 times as much NPH and 1.2 times as much metabolites as livers of the force-fed fish. For a period of 24 to 168 hr after the treatment, the decrease in the NPH concentration in the liver of fish in the injection study was 9.3-fold and in the feeding study it was 63-fold; the decline in the metabolite concentrations were 1.1 and 2.6, respectively (Figs. 14 and 16).

6.1.6 Effect of Environmental Temperature on Disposition of NPH and Its Metabolites in Fish

Studies to determine the effect of temperature on the amount of ^{14}C-NPH incorporated into selected organs of coho salmon are presented in Table 14. The results of this work show that 16 hr after force feeding, fish maintained at 4°C have statistically greater concentrations of NPH than fish maintained at 10°C ($P<0.05$). For example, in the brain there was 2 times as much NPH at 4°C as at 10°C, while in the liver at 4°C there was 6 times as much as at 10°C.

Starry flounder held at 4°C had 1.6 to 15 times greater tissue concentrations of NPH at 24 hr post exposure than in corresponding tissues of fish held at 12°C (Table 15). The largest differences were observed for liver, stomach, and intestine. At 24 hr, large amounts of NPH (>30% of the administered dose) were present in the gastrointestinal (GI) tracts of the fish at 4°C, whereas less than 3% of the dose was retained in the GI tracts of fish at 12°C. Tissue concentrations of NPH at 4°C and 12°C were much lower at 168 hr than at 24 hr; however, the decline in NPH concentrations from 24 to 168 hr was much greater at 12°C than that at 4°C resulting in even larger differences between NPH concentrations in most tissues of the two groups of fish at 168 hr (Table 15).

FIGURE 14. Concentrations of naphthalene and its metabolites in tissues of starry flounder, at 24, 48, and 168 hr after the feeding of 56 uCi of 1-^{3}H-naphthalene. Values are expressed as mean ± s.e. (for number of fish tested at each time period, see Table 13). Values of dry weight of tissues are given below as percent of the wet weight of tissue: stomach (22%), blood (14%), liver (25%), skin (33%), brain (18%), muscle (21%), gills (17%), kidney (18%), bile (12%), intestine (22%), and epidermal mucus (2.8%). Rates of decline in concentrations of naphthalene (N) and total metabolites (M) were calculated using the equation $y = ax^{-b}$. Values for regression coefficient b were for liver b(N) = 1.845, b(M) = 0.404; for blood b(N) = 1.671, b(M) = 0.837; and for skin b(N) = 1.499, b(M) = 0.616. (From Varanasi et al. 1979) →

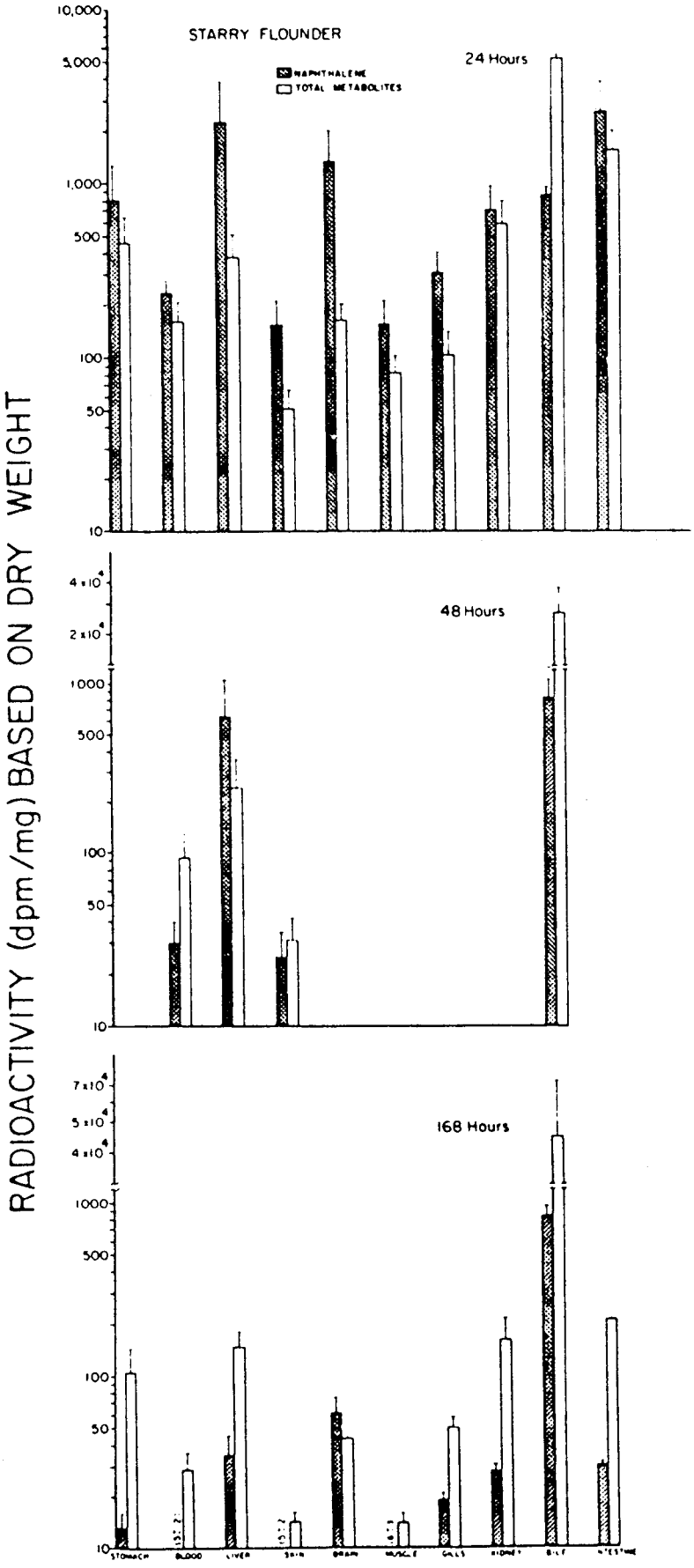

STARRY FLOUNDER
24 Hours
NAPHTHALENE
TOTAL METABOLITES
48 Hours
168 Hours
RADIOACTIVITY (dpm/mg) BASED ON DRY WEIGHT
10,000
5,000
1,000
500
100
50
10
4 x 10⁴
2 x 10⁴
1000
500
100
50
10
7 x 10⁴
5 x 10⁴
4 x 10⁴
1000
500
100
50
10
STOMACH
BLOOD
LIVER
SKIN
BRAIN
MUSCLE
GILLS
KIDNEY
BILE
INTESTINE

Table 13 Total Radioactivity (Expressed as % Administered Dose) and Distribution of Naphthalene and its Metabolites in Pleuronectids Exposed to ^{3}H-Naphthalene

Treatment	Species and # of samples	Time after treatment (hr)	Stomach			Liver			Skin			Brain			Muscle			Bile			Intestine		
			% dose	N[a]	M[a]	% dose	N	M	% dose	N	M	% dose	N	M	% dose	N	M	% dose	N	M	% dose	N	M
Force-feeding	R.S.[b] 6	24	0.30[c] ±0.06	55	45	1.8 ±1.5	84	16	1.0 ±0.6	73	27	0.06 ±0.004	85	15	3.4 ±0.5	68	32	1.7 ±0.6	5	95	6.0 ±3.2	63	37
	6	48	—[d]	—	—	0.4 ±0.3	62	38	0.5 ±0.4	57	43	—	—	—	—	—	—	2.2 ±1.6	2	98	—	—	—
	6	168	0.03 ±0.01	29	71	0.12 ±0.05	49	51	0.08 ±0.02	50	50	0.01 ±0.001	74	26	0.3 ±0.04	57	43	1.4 ±0.8	1	99	0.2 ±0.05	65	35
Force-feeding	S.F. 4	24	0.20 ±0.10	64	36	0.7 ±0.5	85	15	0.4 ±0.2	75	25	0.04 ±0.02	89	11	1.3 ±0.4	65	35	0.2 ±0.2	14	86	1.5 ±0.8	62	38
	4	48	—	—	—	0.3 ±0.5	76	24	0.11 ±0.04	45	55	—	—	—	—	—	—	0.9 ±0.4	3	97	—	—	—
	4	168	0.05 ±0.02	5	95	0.02 ±0.01	19	81	0.02 ±0.01	26	74	0.003 ±0.001	59	41	0.09 ±0.02	27	73	0.9 ±0.4	2	98	0.19 ±0.06	9	91
ip injection	S.F. 3	24	—	—	—	3.6 ±0.4	97	3	0.38 ±0.12	84	16	—	—	—	—	—	—	0.49 ±0.04	7	93	—	—	—
	3	168	—	—	—	0.4 ±0.1	76	24	0.06 ±0.01	79	21	—	—	—	—	—	—	2.3 ±0.09	1	99	—	—	—

[a] Relative % of N = naphthalene and M = total metabolites.
[b] R.S. = Rock sole; S.F. = Starry flounder.
[c] Mean ± S.E.
[d] Not done.

(From Varanasi et al. 1979)

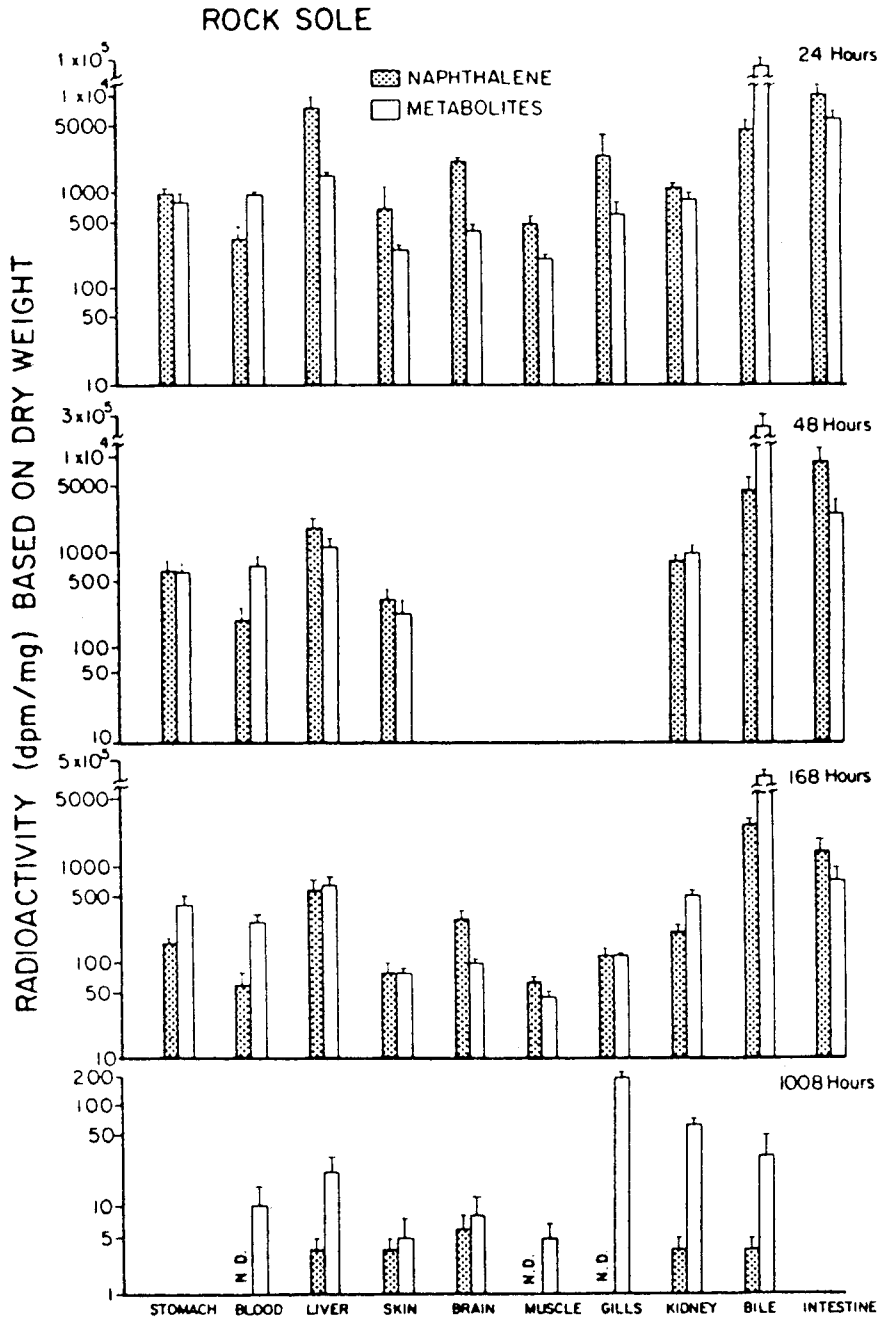


FIGURE 15. Concentrations of naphthalene and its metabolites in tissues of rock sole at 24, 48, 168, and 1008 hr after the feeding of 56 uCi of 1-^{3}H-napthalene. Other pertinent details are given in Figure 14 and Table 13. Regression coefficient for rates of decline of naphthalene N and total metabolites M was for liver, b(N) = 1.999, b(M) = 0.530, for skin, b(N) =0.771, b(M) = 0.558; and for blood, b(N) = 0.812, b(M) = 0.601. N.D. = not detected. (From Varanasi et al. 1979)

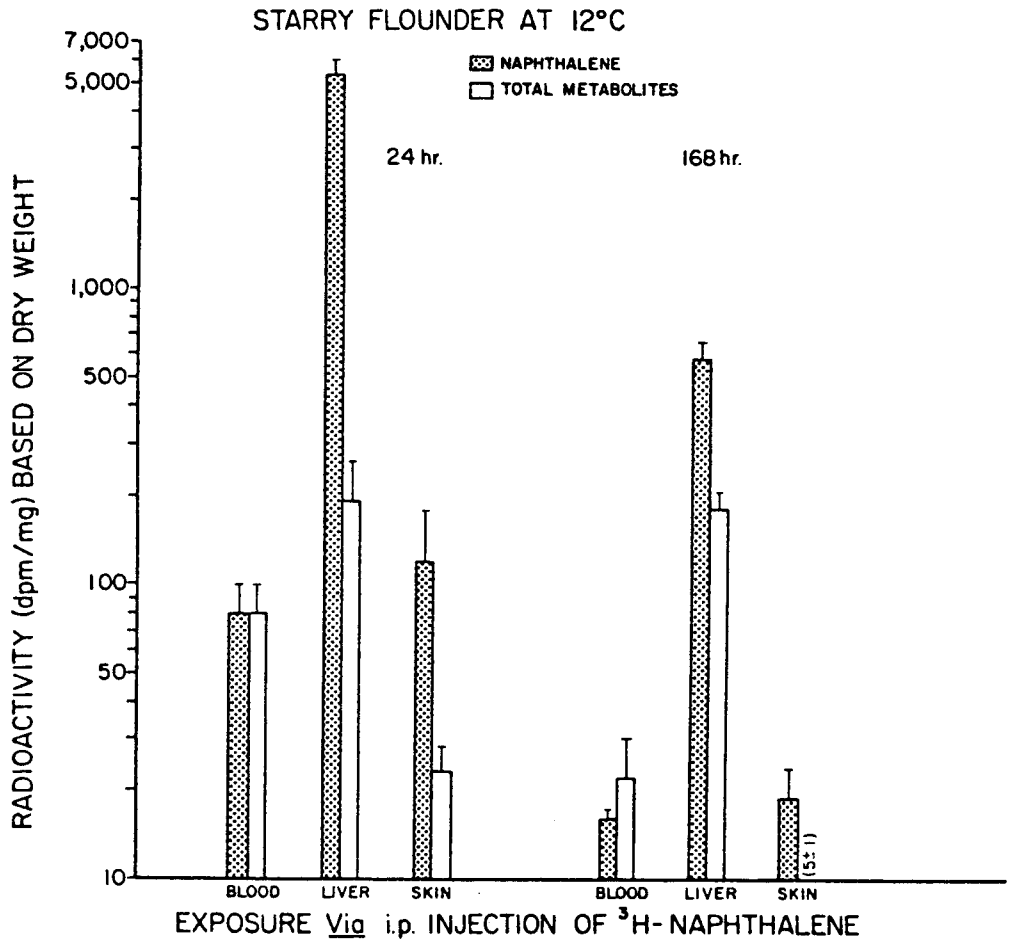


FIGURE 16. Concentrations of naphthalene and its metabolites in tissues of starry flounder 24 and 168 hr after intraperitoneal injection of 56 μCi of ^{3}H-naphthalene. Other pertinent details are given in Figure 14 and Table 13. (From Varanasi et al. 1979)

Concentrations of metabolites in tissues of fish 24 hr after exposure were not substantially larger at the lower temperature (Table 15). Ratios of values for NPH concentrations in most tissues of fish at 4° vs. 12°C increased markedly from 24 to 168 hr; such a marked increase was not observed for the metabolite concentrations. After one week, metabolite concentrations in most tissues of fish at 4°C were not much greater than those at 12°C (Table 15); exceptions were the blood and muscle of fish at 4°C which contained substantially higher concentrations of metabolites than blood and muscle of fish at 12°C.

Table 14 Distribution of naphthalene in organs of coho salmon after force-feeding of 1-^{14}C-naphthalene

Organ	8 hr				16 hr			
	4°C*		10°C†		4°C*		10°C*	
	ng/g Dry wt	% Admin. dose	ng/g Dry wt	% Admin. dose	ng/g Dry wt	% Admin. dose	ng/g Dry wt	% Admin. dose
Brain	880 ± 290	0.022 ± 0.0087	630 ± 160	0.017 ± 0.0042	1400 ± 95	0.039 ± 0.0017	640 ± 120	0.017 ± 0.0035
Liver	3000 ± 1200	0.64 ± 0.37	1000 ± 300	0.21 ± 0.049	2300 ± 250	0.46 ± 0.035	400 ± 200	0.10 ± 0.052
Kidney	510 ± 99	0.042 ± 0.0012	540 ± 190	0.060 ± 0.018	930 ± 120	0.092 ± 0.019	230 ± 110	0.046 ± 0.022
Gall bladder	950 ± 410	0.025 ± 0.010	420 ± 40	0.014 ± 0.0021	2200 ± 990	0.061 ± 0.044	1100 ± 580	0.035 ± 0.017
Dark muscle	1300 ± 520	0.52 ± 0.23	860 ± 200	0.39 ± 0.11	2600 ± 1200	1.2 ± 0.57	1700 ± 790	0.90 ± 0.43
Light muscle	120 ± 48	0.65 ± 0.33	85 ± 11	0.49 ± 0.035	230 ± 57	1.3 ± 0.39	77 ± 40	0.72 ± 0.35
Blood	140 ± 33	0.036 ± 0.0098	97 ± 8.0	0.067 ± 0.0021	200 ± 39	0.13 ± 0.035	48 ± 21	0.041 ± 0.016
Gut + contents	NA‡	67 ± 9.9	NA	74 ± 17	NA	56 ± 9.6	NA	57 ± 28

* Average values for 3 fish ±S.E.M.
† Average values for 2 fish ±S.E.M.
‡ NA = not applicable—dry weight not determined.

(From Collier et al. 1978)

Table 15 Naphthalene and its metabolites in naphthalene-fed starry flounder at 4°C

Tissue	Naphthalene pmoles/mg dry wt.	$[C_4/C_{12}]$[a]	% admin. dose[b]	Metabolites pmoles/mg dry wt.	$[C_4/C_{12}]$[c]	% admin. dose
	24 hr after feeding ^{3}H-naphthalene[d]					
Liver	35.7 ± 10.8[e]	[7.8][f]	6.7 ± 1.6	1.2 ± 0.4	[1.4]	0.22 ± 0.04
Muscle	1.9 ± 0.6	[5.6][f]	5.1 ± 1.3	0.4 ± 0.1	[2.1]	1.05 ± 0.02
Bile	6.2 ± 2.0	[3.3]	0.09 ± 0.01	24.7 ± 6.8	[2.2]	0.39 ± 0.05
Stomach	17.7 ± 8.9	[9.8][f]	1.30 ± 0.60	2.9 ± 1.4	[2.9]	0.22 ± 0.09
Intestine	86.1 ± 31.2	[15][f]	30.80 ± 12.7	9.5 ± 1.4	[2.7]	3.07 ± 0.48
Skin	1.2 ± 0.4	[3.6][f]	1.02 ± 0.3	0.2 ± 0.1	[1.6]	0.16 ± 0.03
Brain	6.5 ± 2.0	[2.2]	0.08 ± 0.03	0.4 ± 0.1	[1.1]	0.005 ± 0.001
Blood	1.1 ± 0.4	[1.9]	—	0.9 ± 0.2	[2.5]	—
Kidney	2.5 ± 0.6	[1.6]	—	1.7 ± 0.5	[1.3]	—
Gills	2.5 ± 0.6	[3.7][f]	—	0.7 ± 0.2	[3.0][f]	—
Mucus	0.4 ± 0.1	[5.1]	—	0.3 ± 0.1	[2.1]	—
	168 hr after feeding ^{3}H-naphthalene					
Liver	2.75 ± 0.82	[34][f]	0.57 ± 0.16	0.52 ± 0.18	[1.6]	0.09 ± 0.03
Muscle	0.34 ± 0.11	[26][f]	1.30 ± 0.58	0.12 ± 0.04	[3.6][f]	0.33 ± 0.10
Bile	2.42 ± 0.97	[1.3]	0.04 ± 0.01	74.5 ± 35.3	[0.7]	1.21 ± 0.47
Stomach	0.42 ± 0.11	[4.2][f]	0.03 ± 0.004	0.28 ± 0.08	[0.1]	0.02 ± 0.003
Intestine	0.57 ± 0.16	[3.4][f]	0.14 ± 0.03	0.53 ± 0.17	[0.3]	0.14 ± 0.04
Skin	0.12 ± 0.02	[10][f]	1.30 ± 0.03	0.06 ± 0.02	[2.0]	0.06 ± 0.01
Brain	0.77 ± 0.29	[5.6][f]	0.010 ± 0.004	0.11 ± 0.04	[1.1]	0.0014 ± 0.0006
Blood	0.10 ± 0.03	[9.6][f]	—	0.36 ± 0.09	[5.6][f]	—
Kidney	0.29 ± 0.07	[4.8][f]	—	0.55 ± 0.18	[1.6]	—
Gills	0.28 ± 0.09	[7.0][f]	—	0.21 ± 0.06	[1.9]	—

[a] Ratio of concentration of naphthalene in tissues of fish exposed at 4° and 12°C
[b] % administered dose was calculated using individual concentration value and total weight of each organ: each value for % admin. dose is mean ± S.E. (6 fish)
[c] Ratio of concentration of metabolites in tissues of fish exposed at 4° and 12°C
[d] Fish were fed 56 μCi (198 mCi/mMole) of ^{3}H-1-naphthalene
[e] Average value for six fish ± S.E.
[f] Concentrations at 4° and 12°C were significantly ($P < 0.05$) different from each other

(From Varanasi et al. 1981a)

Profiles of NPH Metabolites

The chromatograms in Figure 17 show profiles of metabolites in liver, skin, and bile from a rock sole at 12°C. In livers of both rock sole (Table 16; Fig. 17) and flounder (Table 17), 1,2-dihydro-1,2-dihydroxy NPH (dihydrodiol) was the major metabolite (40%) at 24 hr after feeding of the NPH at 12°C. Considerable proportions of conjugates (e.g., glucuronides, mercapturic acids and sulfate/glucosides) were also present in liver of rock sole and starry flounder (Fig. 17; Table 18). Profiles of metabolites in the skin of starry flounder were similar to that in the skin of rock sole (Table 16) and were characterized by the presence of large concentrations of the dihydrodiol.

Analyses of the metabolites in the liver of starry flounder exposed to the NPH via an i.p. injection at 12°C show that at 24 hr, liver contained primarily non-conjugated metabolites of NPH (76.7%) of which the dihydrodiol (41.5%) and naphthols (21.3%) were the major components (Table 18). Comparison of data from Tables 17 and 18 reveals that,

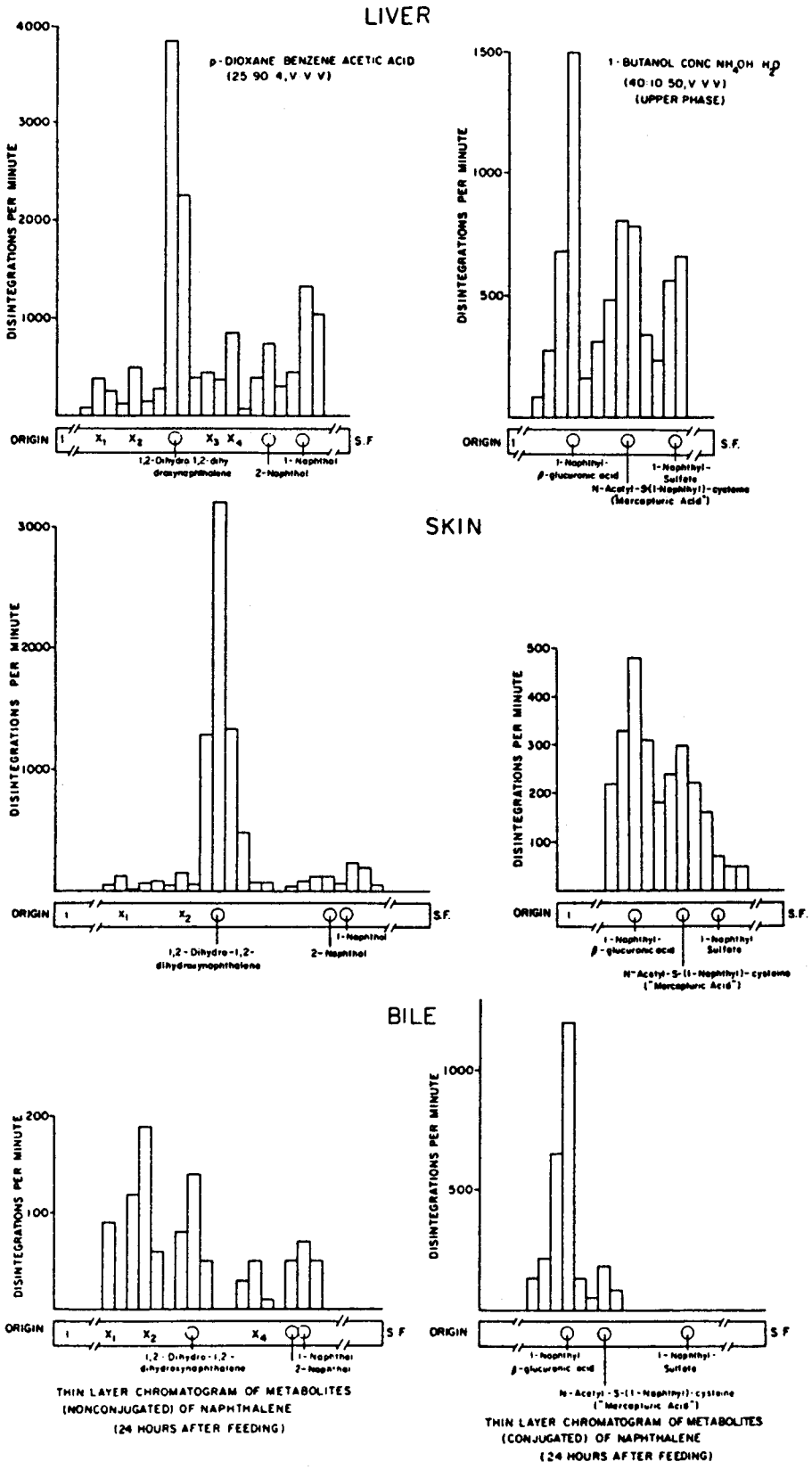


FIGURE 17. (A) Profiles of metabolites in liver of a rock sole.
(B) Profiles of metabolites in skin of a rock sole.
(C) Profiles of metabolites in bile of a rock sole.
(From Varanasi et al. 1979)

Table 16 Metabolites in rock sole

Metabolites	Liver[a] % of total[b] metabolites	Liver[a] pmoles/mg dry wt.	Bile % of total[b] metabolites	Bile pmoles/mg dry wt.	Skin % of total[b] metabolites	Skin pmoles/mg dry wt.
24 hr after feeding of ^{3}H-naphthalene						
Total conjugates	31.9 ± 2.7[c]	0.88 ± 0.21	92.8 ± 0.4	172.6 ± 0.8	18.2 ± 9.4	0.08 ± 0.06
Total non-conjugates	68.1 ± 2.7[c]	2.08 ± 0.01	7.2 ± 0.4	13.4 ± 0.8	81.8 ± 9.4	0.30 ± 0.10
Glucuronides	12.8 ± 0.6	0.37 ± 0.06	80.7 ± 0.9	150.1 ± 1.6	8.6 ± 4.1	0.04 ± 0.03
Mercapturic acids	14.2 ± 3.1	0.41 ± 0.16	11.0 ± 0.7	19.7 ± 0.2	5.6 ± 1.6	0.02 ± 0.01
Sulfate/glucosides	4.1 ± 1.5[c]	0.11 ± 0.01	1.1 ± 0.2	2.0 ± 0.3	4.1 ± 4.0	0.02 ± 0.03
Dihydrodiol (1,2-isomer)	38.7 ± 5.0[c]	1.19 ± 0.15	3.5 ± 0.2	6.5 ± 0.4	59.7 ± 2.9	0.23 ± 0.09
Naphthols (1- & 2-)	16.1 ± 4.4	0.51 ± 0.13	0.8 ± 0.1	1.5 ± 0.2	10.7 ± 2.7	0.04 ± 0.01
Uncharacterized (X_1-X_4)	13.4 ± 3.0	0.34 ± 0.03	2.9 ± 0.5	5.4 ± 1.0	11.5 ± 5.9[d]	0.04 ± 0.01
168 hr after feeding of ^{3}H-naphthalene						
Total conjugates	54.5 ± 2.6	0.65 ± 0.06	96.2 ± 0.1	714.3 ± 1.1		
Total non-conjugates	45.5 ± 2.6	0.56 ± 0.10	3.8 ± 0.1	28.2 ± 1.1		
Glucuronides	27.2 ± 6.9	0.32 ± 0.06	85.9 ± 0.9	637.8 ± 6.4		
Mercapturic acids	13.3 ± 2.4	0.16 ± 0.05	8.5 ± 0.8	63.2 ± 5.9		
Sulfate/glucosides	14.0 ± 2.9	0.18 ± 0.07	1.8 ± 0.1	13.4 ± 0.5		
Dihydrodiol	6.8 ± 5.4	0.09 ± 0.08	1.4 ± 0.2	10.0 ± 1.1		
Naphthols	29.3 ± 9.3	0.34 ± 0.09	0.7 ± 0.1	5.6 ± 0.5		
Uncharacterized (X_1-X_4)	9.5 ± 6.3	0.13 ± 0.10	1.7 ± 0.1	12.6 ± 0.4		

[a] Each value is mean ± s.d. of four samples

[b] Based on total extracted metabolites, an average of 8, 10, and <0.5% of total radioactivity respectively, remained in liver, skin, and bile

[c] Significantly ($P < 0.001$) different from corresponding value at 168 hr

[d] Only X_1 and X_2 were present in skin

(From Varanasi et al. 1979)

whereas profiles of metabolites in the livers of starry flounder from the feeding and injection studies were quite different, biliary metabolites were similar for fish in both studies, and did not change significantly with time. Regardless of species or mode of exposure, there was an increase in the proportion of conjugated metabolites in liver and muscle from 24 to 168 hr after NPH-exposure.

At both 4° and 12°C the dihydrodiol was the major metabolite (39.7-80.9% of total extractable metabolites) present in liver and muscle of starry flounder at 24 hr after feeding of NPH (Table 19). At 24 hr, naphthols represented from 1.5 to 10.7% of the total extracted metabolites in liver, muscle or bile of fish at 4° and 12°C (Table 19). The peak X_4 in chromatograms (Figs. 17 and 18) was tentatively identified as 1,2-naphthoquinone. Glucuronides, sulfates/glucosides and mercapturic acids were also present in liver, muscle, and bile from the NPH-exposed starry flounder (Table 19) at both 4° and 12°C. At 24 hr, no marked differences were observed in the proportions of most metabolite classes in liver of fish at 4° and 12°C.

Table 17 Metabolites in starry flounder[a]

Metabolites	24 hr after feeding of ^{3}H-naphthalene					
	Liver		Bile		Skin	
	% of total metabolites	pmoles/mg dry wt.	% of total metabolites	pmoles/mg dry wt.	% of total metabolites	pmoles/mg dry wt.
Total conjugates	43.6 ± 9.9[b]	0.54 ± 0.39	91.8 ± 0.3	4.83 ± 0.01	24.0 ± 1.6	0.04 ± 0.01
Total non-conjugates	56.4 ± 9.9[b]	0.62 ± 0.21	8.2 ± 0.3	0.44 ± 0.01	76.0 ± 1.6	0.12 ± 0.01
Glucuronides	32.2 ± 7.2	0.40 ± 0.30	81.6 ± 0.1	4.29 ± 0.01	12.5 ± 0.4	0.02 ± 0.01
Mercapturic acids	9.5 ± 3.6	0.12 ± 0.10	8.9 ± 0.1	0.47 ± 0.01	9.7 ± 2.1	0.01 ± 0.01
Sulfate/glucosides	1.9 ± 0.1[b]	0.01 ± 0.03	1.4 ± 0.1	0.07 ± 0.01	1.7 ± 2.5	0.01 ± 0.01
Dihydrodiol	39.7 ± 13.9[b]	0.41 ± 0.04	4.0 ± 0.1	0.21 ± 0.01	44.1 ± 1.3	0.07 ± 0.01
Naphthols (1- & 2-)	10.7 ± 3.9	0.14 ± 0.12	1.5 ± 0.1	0.08 ± 0.01	11.7 ± 0.4	0.02 ± 0.01
Uncharacterized (X_1-X_4)	6.0 ± 0.7	0.07 ± 0.05	2.6 ± 0.3	0.14 ± 0.02	20.3 ± 3.3	0.03 ± 0.01
	168 hr after feeding of ^{3}H-naphthalene					
Total conjugates	62.3 ± 8.8	0.15 ± 0.03	91.5 ± 0.3	42.6 ± 0.1		
Total non-conjugates	37.7 ± 8.8	0.10 ± 0.05	8.5 ± 0.3	4.0 ± 0.1		
Glucuronides	16.6 ± 1.6	0.04 ± 0.01	81.7 ± 0.3	38.2 ± 0.1		
Mercapturic acids	10.9 ± 6.6	0.03 ± 0.03	8.8 ± 0.1	4.1 ± 0.1		
Sulfate/glucosides	34.8 ± 14.8	0.08 ± 0.02	0.9 ± 0.1	0.4 ± 0.1		
Dihydrodiol	12.4 ± 11.7	0.03 ± 0.05	3.0 ± 0.1	1.4 ± 0.1		
Naphthols	11.5 ± 3.9	0.03 ± 0.02	2.9 ± 0.2	1.4 ± 0.1		
Uncharacterized (X_1-X_4)	14.0 ± 7.1	0.03 ± 0.01	2.6 ± 0.1	0.1 ± 0.1		

[a] See Figure 14 and Table 16 for details

[b] Signficantly ($P < 0.05$) different from corresponding values at 168 hr

(From Varanasi et al. 1979)

From 24 to 168 hr, there was a significant ($p<0.05$) decrease in the proportion of the dihydrodiol fraction and an increase in sulfate/glucoside fraction in both liver and muscle of fish at 4° and 12°C (Table 19). Moreover, the proportion of the glucuronide fraction also increased with time in liver, muscle, and bile of fish at 4°C. Individual metabolite classes in liver and muscle of fish at 4° and 12°C did not vary directly with the concentrations of total metabolites; ratios of concentrations of total metabolites (Table 15) at 4° and 12°C for the liver and muscle at 168 hr were 1.6 and 3.6, respectively, whereas ratios for the dihydrodiol at this time were 4.5 and 8.3, respectively (Table 19).

Metabolites in bile from both groups of fish (4° or 12°C) at 24 and 168 hr were characterized by high percentages (>85%) of the conjugates, of which glucuronides were the major components (Table 19). Analyses of bile from fish at 8 hr after exposure at 12°C also showed the glucuronides to be the major components (Table 19, Footnote e). Very small proportions (<10%) of the nonconjugates (dihydrodiol and naphthols) were present in bile of starry flounder from 8 to 168 hr.

Table 18 Metabolites in starry flounder exposed to ^{3}H-naphthalene via ip injection[a]

Metabolites	Liver		Bile	
	% of total metabolites	pmoles/mg dry wt.	% of total metabolites	pmoles/mg dry wt.
24 hr after injection				
Total conjugates	23.2 ± 1.7[b]	0.099 ± 0.007	96.9 ± 0.7	21.70 ± 0.16
Total non-conjugates	76.7 ± 1.7[b]	0.328 ± 0.007	3.1 ± 0.7	0.70 ± 0.16
Glucuronides	8.3 ± 0.7	0.036 ± 0.003	88.7 ± 0.1	19.87 ± 0.03
Mercapturic acids	N.D.	N.D.	7.0 ± 0.1	1.57 ± 0.01
Sulfate/glucosides	15.0 ± 0.7	0.064 ± 0.003	1.2 ± 0.1	0.26 ± 0.02
Dihydrodiol	41.5 ± 1.4[b]	0.178 ± 0.006	1.8 ± 0.3	0.42 ± 0.07
Naphthols (1- & 2-)	21.3 ± 0.8	0.091 ± 0.003	0.1 ± 0.2	0.01 ± 0.01
Uncharacterized (X_1-X_4)	13.8 ± 0.5	0.059 ± 0.002	1.2 ± 0.4	0.28 ± 0.10
168 hr after injection				
Total conjugates	33.8 ± 1.5	0.13 ± 0.01	97.5 ± 0.4	108.9 ± 0.5
Total non-conjugates	66.2 ± 1.5	0.25 ± 0.01	2.5 ± 0.4	2.8 ± 0.5
Glucuronides	9.7 ± 0.3	0.04 ± 0.01	89.5 ± 0.5	100.0 ± 0.6
Mercapturic acids	N.D.	N.D.	6.7 ± 0.4	7.5 ± 0.5
Sulfate/glucosides	24.1 ± 0.3	0.09 ± 0.01	1.3 ± 0.1	1.4 ± 0.1
Dihydrodiol	29.1 ± 8.3	0.11 ± 0.03	0.8 ± 0.1	0.9 ± 0.1
Naphthols	20.7 ± 5.2	0.08 ± 0.02	0.6 ± 0.2	0.7 ± 0.2
Uncharacterized (X_1-X_4)	16.5 ± 4.6	0.06 ± 0.02	1.0 ± 0.3	1.2 ± 0.3

[a] See footnotes under Figure 16 and Table 16 for details

[b] Significantly ($P < 0.05$) different than corresponding values at 168 hr

(From Varanasi et al. 1979)

Enzymatic hydrolysis of glucuronide and sulfate fractions isolated from bile revealed that these derivatives were formed primarily by conjugation with the dihydrodiol of NPH (Table 20; Fig. 19). Smaller amounts of naphthols (1- and 2-isomers) were also present in the hydrolysis products from the glucuronide fraction. Patterns of hydrolysis products of biliary glucuronides from fish at 4° and 12°C were similar at 24 hr after exposure (Table 20).

6.1.7 Uptake and Metabolism of Sediment-Associated NPH and BaP by Flatfish

The concentrations of BaP in sediment did not change significantly over 10 days (Table 21); however, a trend of declining NPH concentrations was evident. Chromatographic analyses of BaP-derived radioactivity (^{3}H) from sediment and SAW revealed that BaP remained largely (>93%) in the form of parent hydrocarbon throughout the experiment.

Table 19 Comparison of metabolite profiles in starry flounder exposed to dietary naphthalene at 4° and 12°C

Metabolites[a]	Liver			Muscle			Bile	
	% of total metabolites[b]			% of total metabolites[b]			% of total metabolites[b]	
	4°C	12°C[d]	$[C_4/C_{12}]$[c]	4°C	12°C	$[C_4/C_{12}]$[c]	4°C	12°C[d,e]
	24 hr after feeding of ^{3}H-naphthalene							
Total conjugates	27.1 ± 1.8	43.6 ± 9.9	[0.5]	11.5 ± 0.3	9.4 ± 0.04	[2.7]	86.5 ± 1.6	91.8 ± 0.3
Total non-conjugates	72.9 ± 1.8[e]	56.4 ± 9.9[e]	[1.1]	88.5 ± 0.3[e]	90.6 ± 0.04[e]	[2.1]	13.5 ± 1.6	8.2 ± 0.3
Glucuronides	15.9 ± 0.4[f]	32.2 ± 7.2	[0.4]	4.6 ± 0.4[f]	5.4 ± 0.9[f]	[1.9]	73.9 ± 0.1[f]	81.6 ± 0.1
Mercapturic acids	11.2 ± 0.4	9.5 ± 3.6	[0.9]	3.2 ± 0.5	2.6 ± 1.1	[2.8]	10.9 ± 0.2	8.9 ± 0.1
Sulfate/glucosides	N.D.	1.9 ± 0.1[f]	—	3.6 ± 0.9[f]	1.5 ± 0.2[f]	[5.4]	1.8 ± 0.3	1.4 ± 0.1
Dihydrodiol (1,2-isomer)	48.4 ± 0.2[f]	39.7 ± 13.9[f]	[1.1]	77.3 ± 0.7[f]	80.9 ± 0.5[f]	[2.1]	6.5 ± 1.4	4.0 ± 0.1
Naphthols (1- & 2-)	8.8 ± 0.9	10.7 ± 3.9	[0.6]	1.8 ± 0.1	2.4 ± 0.01[f]	[1.6]	2.8 ± 0.6	1.5 ± 0.1
Uncharacterized (X_1 – X_4)	15.7 ± 0.6	6.0 ± 0.7	[2.1]	9.4 ± 0.2	7.2 ± 0.9	[2.8]	4.2 ± 0.5	2.6 ± 0.3
	168 hr after feeding of ^{3}H-naphthalene							
Total conjugates	54.1 ± 1.3	62.3 ± 8.8	[1.7]	28.9 ± 0.5	46.1 ± 0.3	[2.6]	94.6 ± 0.2	91.5 ± 0.3
Total non-conjugates	45.9 ± 1.3	37.7 ± 8.8	[2.1]	71.1 ± 0.5	53.9 ± 0.3	[5.5]	5.5 ± 0.2	8.5 ± 0.3
Glucuronides	32.3 ± 0.3	16.6 ± 1.6	[3.8]	13.1 ± 1.3	23.7 ± 1.4	[2.3]	85.1 ± 0.2	81.7 ± 0.3
Mercapturic acids	11.7 ± 0.2	10.9 ± 6.6	[1.8]	7.2 ± 0.2	8.0 ± 0.9	[3.8]	7.7 ± 0.2	8.8 ± 0.1
Sulfate/glucosides	10.1 ± 0.1	34.8 ± 14.8	[0.6]	8.7 ± 2.1	14:4 ± 0.5	[2.5]	1.8 ± 0.1	0.9 ± 0.1
Dihydrodiol (1,2-isomer)	29.7 ± 0.5	12.4 ± 11.7	[4.5]	47.4 ± 0.4	23.9 ± 0.9	[8.3]	2.5 ± 0.1	3.0 ± 0.1
Naphthols (1- & 2-)	6.2 ± 0.2	11.5 ± 3.9	[0.9]	6.9 ± 1.2	21.6 ± 0.6	[1.3]	0.6 ± 0.1	2.9 ± 0.2
Uncharacterized (X_1 – X_4)	10.0 ± 1.1	14.0 ± 7.1	[5.1]	16.7 ± 1.1	8.4 ± 0.4	[8.2]	2.3 ± 0.3	2.6 ± 0.1

[a] Characterized by R_f values of TLC standards. Each value is mean ± S.D. of three samples of pooled homogenates prepared from 4 to 6 fish

[b] Based on total extracted metabolites; an average of 8, 13, and <0.5% of total radioactivity respectively, remained in liver, muscle, and bile from fish taken at 24 hr

[c] Ratio of concentration (pmole/mg dry wt) of each class of metabolite at 4°C to that at 12°C.

[d] Taken from Varanasi *et al.* (1979)

[e] Biliary metabolites were also analyzed at 8 hr after the exposure; major component (>75%) was glucuronide fraction. Free dihydrodiol and naphthols constituted less than 10% of the total extracted metabolites

[f] Significantly ($p < 0.05$) different from the corresponding value at 168 hr

(From Varanasi et al. 1981a)

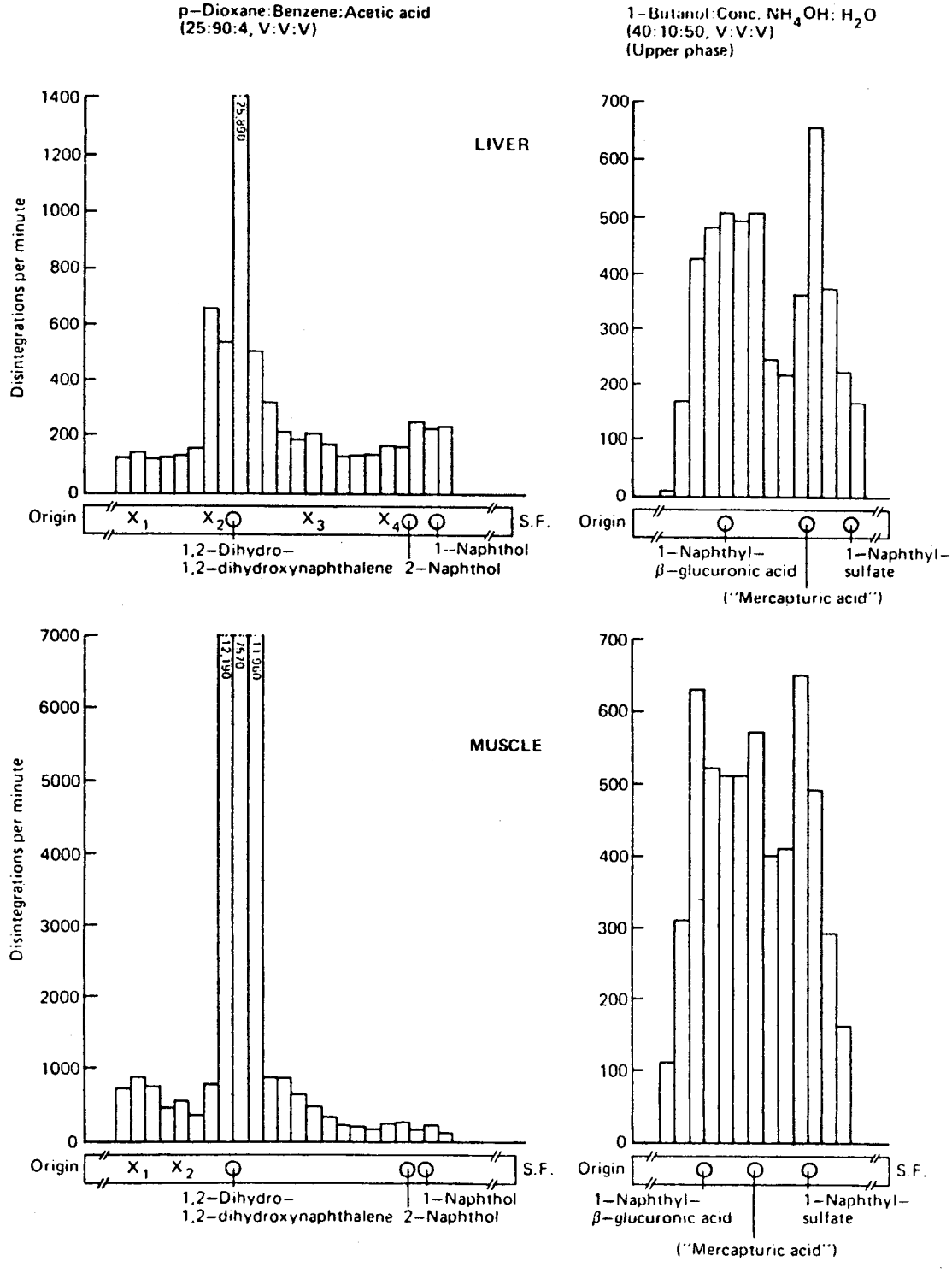


FIGURE 18. Metabolite profiles in liver and muscle of naphthalene-fed starry flounder at 4°C. (From Varanasi et al. 1981a)

Table 20 Ethyl acetate-soluble hydrolysis products of conjugated metabolites[a] isolated from bile of naphthalene-fed starry flounder

Temp (°C)	Time after feeding (hr)	Conjugates	Hydrolysis products[a] dihydrodiol[b] (%)	1-naphthol[b] (%)	2-naphthol[b] (%)	% of conjugate fraction unhydrolyzed
12	8	glucuronides	62	8	30	45
12	24	glucuronides	71	7	22	28
12	168	glucuronides	89	4	7	28
4	24	glucuronides	74	6	20	56
12	168	sulfates/ glucosides	78	5	17	45

[a] Metabolite fractions were incubated with β-glucuronidase from *Helix pomatia* which contained significant amounts of aryl sulfatase for 48 hr at 37°C. (pH = 5.0)
[b] characterized by R_f values of TLC standards

(From Varanasi et al. 1981a)

Analyses of NPH-derived (^{14}C) radioactivity in sediment also showed that no more than 7% of the total radioactivity was present as total metabolites in sediment throughout the exposure (Table 21). For the first 3 days, the radioactivity (^{14}C) in the SAW was primarily due to NPH. However, samples of the water from days 4 to 10 contained on the average 16% of the total radioactivity (^{14}C) in the form of NPH metabolites (Table 21).

Radioactivity derived from both BaP and NPH was detected in tissues and body fluids of English sole within 24 h after the fish were placed on the sediment containing ^{3}H-BaP, ^{14}C-NPH and 1% PBCO (Table 22). From 24 to 168 hr of exposure, BaP-derived radioactivity increased significantly ($p<0.05$) in liver (5 fold), and bile (16 fold), whereas NPH-derived radioactivity decreased for all tissues and fluids except for gill and bile. An increase of NPH-derived radioactivity occurred in the bile (Table 22).

Following 24 hr of exposure to oil-contaminated sediment, fish placed (for 24 hr) on "clean" sediment showed a significant decrease ($p<0.05$) in NPH-derived radioactivity from most tissues (Table 22). Except for a small decline in muscle, no significant change occurred in the levels of BaP-derived radioactivity.

Chromatographic analyses of BaP and its metabolites in liver (Fig. 20) and bile of fish at 24 hr revealed that 2% or less of the total radioactivity in these samples was due to unconverted BaP (Table 23). TLC analyses of bile, both before and after enzymatic hydrolysis, showed the presence of nonconjugated BaP metabolites (quinones, phenols, diols, and the more polar compounds as well as glucuronide (51%) and sulfate (15%) conjugates (Table 23).

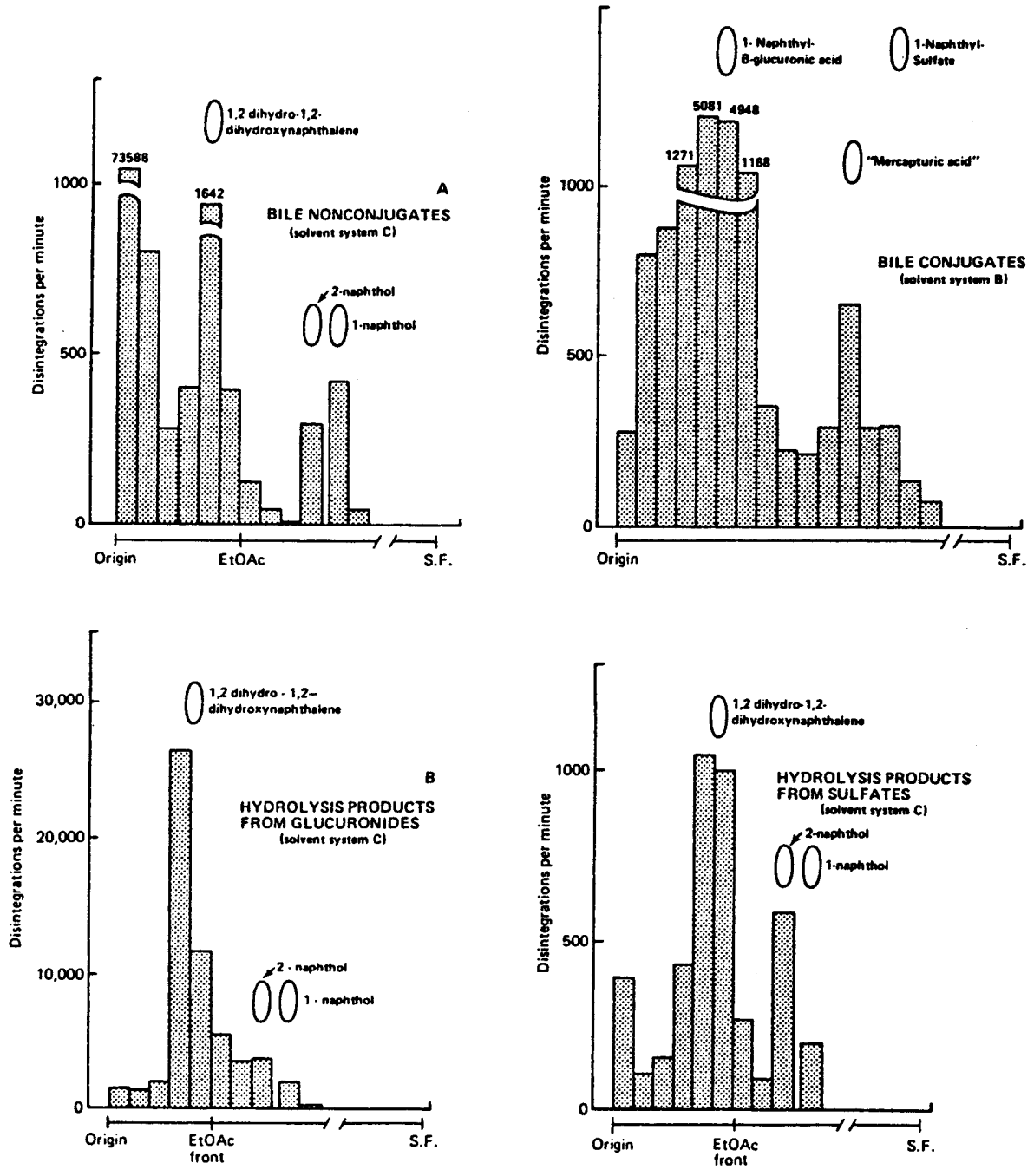


FIGURE 19. (A) Thin-layer chromatograms of biliary naphthalene metabolites from naphthalene-fed starry flounder at 12°C (168 hr after feeding). (B) Thin-layer chromatograms of hydrolysis products of glucuronide and sulfate/glucoside fractions isolated from thin-layer chromatograms of (A). (From Varanasi et al. 1981)

TABLE 21

Concentrations of B[*a*]P- and NPH-derived radioactivity in sediment, sediment-associated water (SAW) and the water column.[a]

		Sediment		Sediment-associated water (SAW)				Water column			
		B[*a*]P	NPH	B[*a*]P		NPH		B[*a*]P		NPH	
				unfiltered	filtered	unfiltered	filtered	unfiltered	filtered	unfiltered	filtered
Day	Event			pmol hydrocarbon equivalents/g of sample							
1	Sediment was placed in aquarium	2200 ± 400(97)[b]	14000 ± 3000(94)	–[c]	–	–	–	–	–	–	–
2	Begin 24 h exposure	1700 ± 300(96)	13000 ± 2000(93)	190	10.6	960	238(93)	15	0.7	110	20
3	Remove 24 h exposed fish for sampling and depuration experiment; place 168 h exposure fish on oil-contaminated sediment	1300 ± 100	7200 ± 600	250	7.4(97)	420	124(94)	4	0.3	72	38
4	Continue 168 h exposure of fish to oil-contaminated sediment	2500 ± 600(96)	3200 ± 700(94)	190	7.0(96)	–	212(84)	3	–	130	–
7		990 ± 150(93)	960 ± 140(94)	–	6.0(96)	470	361(86)	–	–	–	–
9		1400 ± 200	600 ± 100(96)	130	6.1	–	–	2	0.1	–	36
10	Remove fish for sampling	2300 ± 340(96)	2500 ± 400(97)	–	14.2	–	317(81)	–	–	–	–

[a]Sediment and SAW samples were taken from 2 cm below the sediment/water interface as described in the text. SAW was decanted off from the sediment and analyzed before and after filtration. Three portions of sediment were analyzed to get average amount of tritium and carbon-14 radioactivity in each sample. Concentration values (mean ± SD) for sediment are based on dry weight. Samples of water at the air/water interface (water column) were also analyzed after filtration.

[b]Values in parentheses are percentages of total radioactivity present as the parent hydrocarbon.

[c]Not done.

(From Varanasi and Gmur 1981a)

TABLE 22

Hydrocarbon-derived radioactivity[a] in tissues of English sole exposed to [^{3}H]B[*a*]P and [^{14}C]NPH in sediment containing 1% Prudhoe Bay crude oil.

	Gill	Blood	Liver	Skin	Muscle	Bile	Stomach	Intestine
24-h Exposure								
B[*a*]P	0.390	0.39	0.35	0.140	0.044	8.3	0.22	0.38
	± 0.049	± 0.11	± 0.13	± 0.067	± 0.001	± 3.1	± 0.14	± 0.04
NPH	14.4	7.8	66	3.3	8.9	330	19.2	20.4
	± 4.5	± 1.8	± 11	± 1.5	± 2.3	± 123	± 4.9	± 5.8
168-h Exposure								
B[*a*]P	0.55[b]	0.67	1.60[b]	0.140	0.036[b]	130[b]	0.30	0.45
	± 0.10	± 0.38	± 0.06	± 0.016	± 0.003	± 15	± 0.18	± 0.17
NPH	55	3.3[b]	23[b]	1.5	1.2[b]	980[b]	5.2[b]	6.1[b]
	± 42	± 0.9	± 8	± 1.0	± 0.4	± 300	± 0.9	± 2.8
24-h Depuration after 24-h exposure								
B[*a*]P	0.51	0.460	0.66	0.100	0.032[b]	58	0.138	0.28
	± 0.10	± 0.052	± 0.21	± 0.011	± 0.004	± 32	± 0.015	± 0.12
NPH	5.3	2.5[b]	19[b]	2.9	2.3[b]	1600[b]	3.4[b]	6.3[b]
	± 1.0	± 0.6	± 9	± 2.3	± 0.9	± 600	± 1.0	± 0.6

[a]Values are expressed as nmoles of hydrocarbon equivalents per g of dry wt (mean ± SD).
[b]Significantly different ($P < 0.05$) from corresponding values at 24 h after the exposure.

(From Varanasi and Gmur 1981a)

Examination of the aqueous phase remaining after ethyl acetate extraction of liver showed that greater than one-third of the radioactivity in the aqueous phase was bound to cellular macromolecules in fish liver at both 24 and 168 hr after the BaP exposure (Table 23).

Analyses of NPH-derived radioactivity in the liver of fish at 24 and 168 hr revealed that 85% and 21%, respectively, of the total radioactivity was due to unconverted NPH (Table 24). Radioactivity in bile was due primarily to NPH-metabolites (>99%) of which glucuronides were the major component. Hydrolysis of the glucuronide fraction revealed the presence of a large proportion (70%) of the 1,2-dihydro 1,2-dihydroxynapthalene and a much smaller proportion (11%) of 1-naphthol (Table 24).

Chromatographic analyses revealed that a large proportion of polar metabolites having R_f values lower than BaP-9,10-dihydrodiol (Table 23) were conjugated with glucuronic acid. Smaller proportions of BaP-7,8-dihydrodiol, BaP-9,10-dihydrodiol and BaP-4,5-dihydrodiol were also conjugated with glucuronic acid. Analysis of this sample by HPLC (Fig. 21) confirmed the presence of the dihydrodiols and revealed several isomers of 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxy-BaP in the hydrolysis products after the glucuronidase treatment of the bile sample. Moreover, the HPLC demonstrated that a number of isomeric phenols were

present in bile (Fig. 21). Hydrolysis products after the treatment of the aqueous phase of bile with aryl sulfatase were qualitatively similar to those produced after the glucuronidase treatment (Table 23).

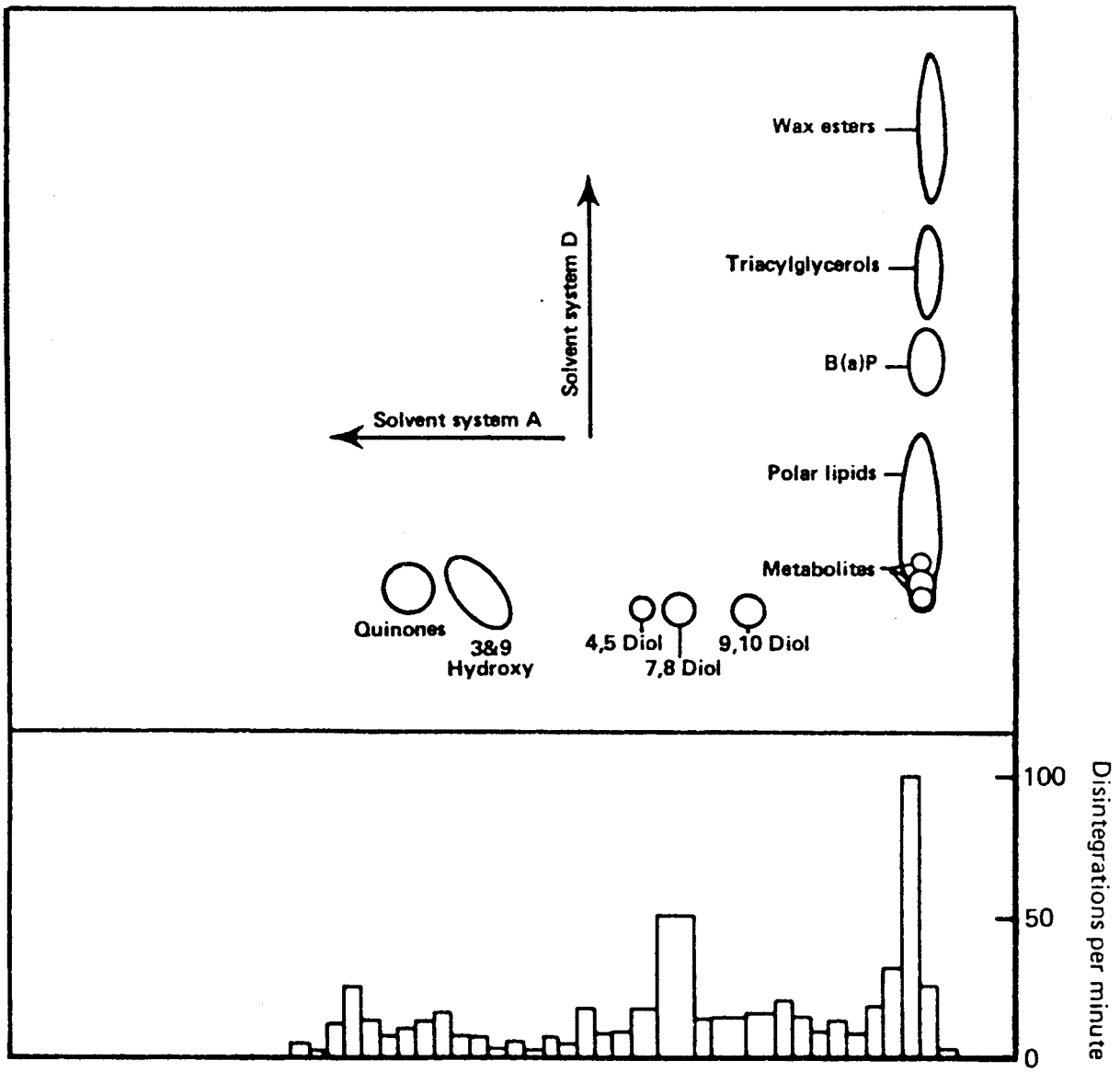


FIGURE 20. Two-dimensional TLC of liver extracts. Liver lipids and [^{3}H]B(a)P were separated from polar lipids and B(a)P metabolites by developing the plate in one direction with the solvent system D (hexane:diethyl ether, 95:5;v/v) with appropriate standards. The plate was then turned 90° and redeveloped in solvent system A (toluene:ethanol, 9:1,v/v). The resulting profile of B(a)P-derived radioactivity is from one fish from the 168-h exposure group. (From Varanasi and Gmur 1981a)

TABLE 23

Proportions of B[*a*]P and its metabolites in liver and bile of English sole exposed simultaneously to [^{3}H]B[*a*]P and [^{14}C]NPH in sediment.

	Liver		Bile		
	Before enzymatic hydrolysis		Before enzymatic hydrolysis	After treatment of aqueous phase with β-glucuronidase	After treatment of aqueous phase with aryl sulfatase
	24 h[a]	168 h[b]		24 h[b]	
% of total radioactivity					
Unconverted B[*a*]P	2	0.8 ± 0.1	2 ± 3	0.3 ± 0.3	1.1 ± 0.5
Ethyl acetate extractable metabolites	16	19 ± 2.6	14 ± 10	51 ± 3.6	15 ± 3.4
Radioactivity in aqueous phase	82[c]	80[c] ± 2.6	84 ± 12[d]	49 ± 3.6	84 ± 2.8
% of total metabolites in ethyl acetate					
Origin		40.8 ± 2.0	42.7 ± 2.9	12.5 ± 1.9	16.9 ± 4.6
Fraction P		20.6 ± 3.8	13.2 ± 1.6	27.9 ± 9.4	12.7 ± 5.8
9,10-diol		2.4 ± 0.8	2.9 ± 1.9	6.8 ± 4.8	5.9 ± 2.4
X		2.3 ± 0.6	9.7 ± 4.0	12.4 ± 2.3	5.7 ± 6.1
7,8-diol		5.9 ± 0.4	4.5 ± 0.3	12.2 ± 4.2	8.3 ± 1.6
4,5-diol		2.5 ± 0.4	3.6 ± 2.2	1.4 ± 2.4	6.5 ± 2.6
X'		n.d.	n.d.	5.6 ± 1.4	n.d.
Phenols		5.8 ± 1.5	1.9 ± 1.6	8.6 ± 1.0	10.2 ± 1.7
Quinones		11.6 ± 1.6	3.6 ± 1.8	2.1 ± 1.3	9.0 ± 2.5
Unclassified		31.0 ± 6.3	18.0 ± 4.6	10.3 ± 5.7	25.0 ± 6.9

[a]Three livers were pooled and one set of values obtained.

[b]Each value is the mean of three individual values ± SD.

[c]41 and 35 ± 2%, respectively, of the radioactivity in the aqueous phase at 24 and 168 h was unextractable.

[d]Treatment with glucuronidase and sulfatase released a total of 66% of the radioactivity into ethyl acetate. The remaining radioactivity is assumed to be due to glutathione conjugates.

(From Varanasi and Gmur 1981a)

6.1.8 BaP Metabolism by English Sole

Concentrations of BaP-derived radioactivity in muscle (1.4 ± 0.4 pmole/mg dry tissue) was substantially lower than that in liver (40 ± 1.5 pmole/mg) at 24 hr; however, the percent administered dose in the muscle (1.1 ± 0.5) was similar to that in the liver (1.4 ± 0.5); bile contained 2.6 ± 0.5% of the administered dose (Fig. 22). From 24 to 168 hr, concentrations of BaP derived radioactivity increased significantly in bile, decreased significantly in muscle, and did not change significantly in liver. (Fig. 22).

TABLE 24

Proportions of NPH and its metabolites in liver and bile of English sole exposed simultaneously to [^{14}C]NPH and [^{3}H]B[*a*]P in sediment.

	Liver		Bile[a]		
			Before enzymatic hydrolysis	After hydrolysis of glucuronide fraction[c]	After hydrolysis of sulfate/glucoside fraction[d]
	24 h[b]	168 h		168 h	
% of total radioactivity					
Unconverted NPH	85	21 ± 11	n.d.	n.d.	n.d.
Total metabolites	15[e]	79 ± 11[e]	100	100	100
% of total metabolites					
Glucuronides			88	n.d.	n.a.
Mercapturic acids			2	n.a.	n.a.
Sulfate/glucosides			3	n.a.	4.0
1,2 dihydrodiol			0.3	70.0	73.0
1-naphthol			n.d.	11.0	3.0
2-naphthol			n.d.	8.0	2.0
Unclassified			7	11.0	18.0

[a]Three samples of bile from fish exposed for 168 h were pooled to get sufficient radioactivity for quantitation of metabolite classes.
[b]There was not sufficient radioactivity in metabolite fraction to allow quantitation of individual classes.
[c]Glucuronide fraction was isolated by TLC of the bile extract and then hydrolyzed with β-glucuronidase.
[d]Sulfate/glucoside fraction was isolated and then treated with aryl sulfatase.
[e]This value includes one-third of the total radioactivity which was not extractable by solvents.
n.d. = not detected; n.a. = not applicable.

(From Varanasi and Gmur 1981a)

As with fish exposed to BaP in sediment, radioactivity in liver and muscle of these fish was primarily in the form of metabolites such as dihydrodiols, phenols and their sulfate and glucuronide conjugates (Table 25; Fig. 23). Less than 2% of the total radioactivity in these tissues (e.g. liver and muscle) was in the form of the parent hydrocarbon.

6.1.9 Binding of BaP Intermediates to DNA Catalyzed by Liver Enzymes of Fish

The results in Table 26 showed that the binding value for BaP to DNA was about 3 times greater for the untreated starry flounder than the corresponding value for English sole and rat. Moreover, pretreatment of English sole with PBCO resulted in an 18-fold increase in the binding value compared to the value for untreated fish; the increase in the binding in the case of PBCO-pretreated starry flounder was only 5-fold. The value for binding obtained with liver extracts from MC-pretreated starry flounder was about 10 times greater than that obtained

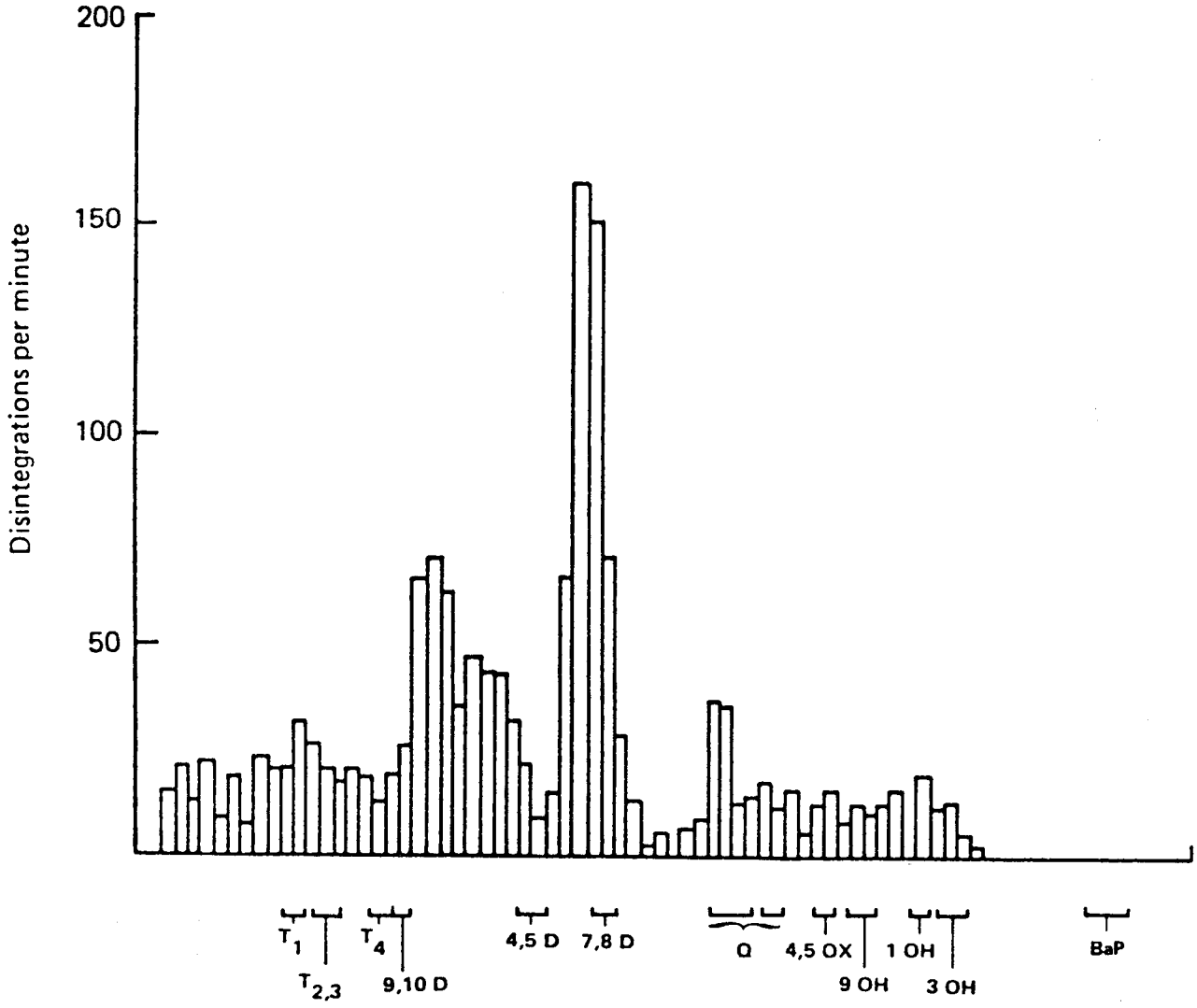


FIGURE 21. High performance liquid chromatography of ethyl acetate soluble metabolites released after the treatment of aqueous phase of bile from English sole with β-glucuronidase. Metabolites were separated by fractions collected at 15 sec. intervals. Abbreviations: T_1, $T_{2,3}$ and T_4 - tetrahydro tetrahydroxy BaP; 9,10 D-BP 9, 10- dihydrodiol; 4,5 D - BP 4,5-dihydrodiol; 7,8 D - BP 7,8 dihydrodiol; Q - quinones; 4,5 OX - BP 4,5 oxide; 1 OH, 3 OH and 9 OH-1-, 3- and 9-hydroxy BP. (From Varanasi and Gmur 1981b)

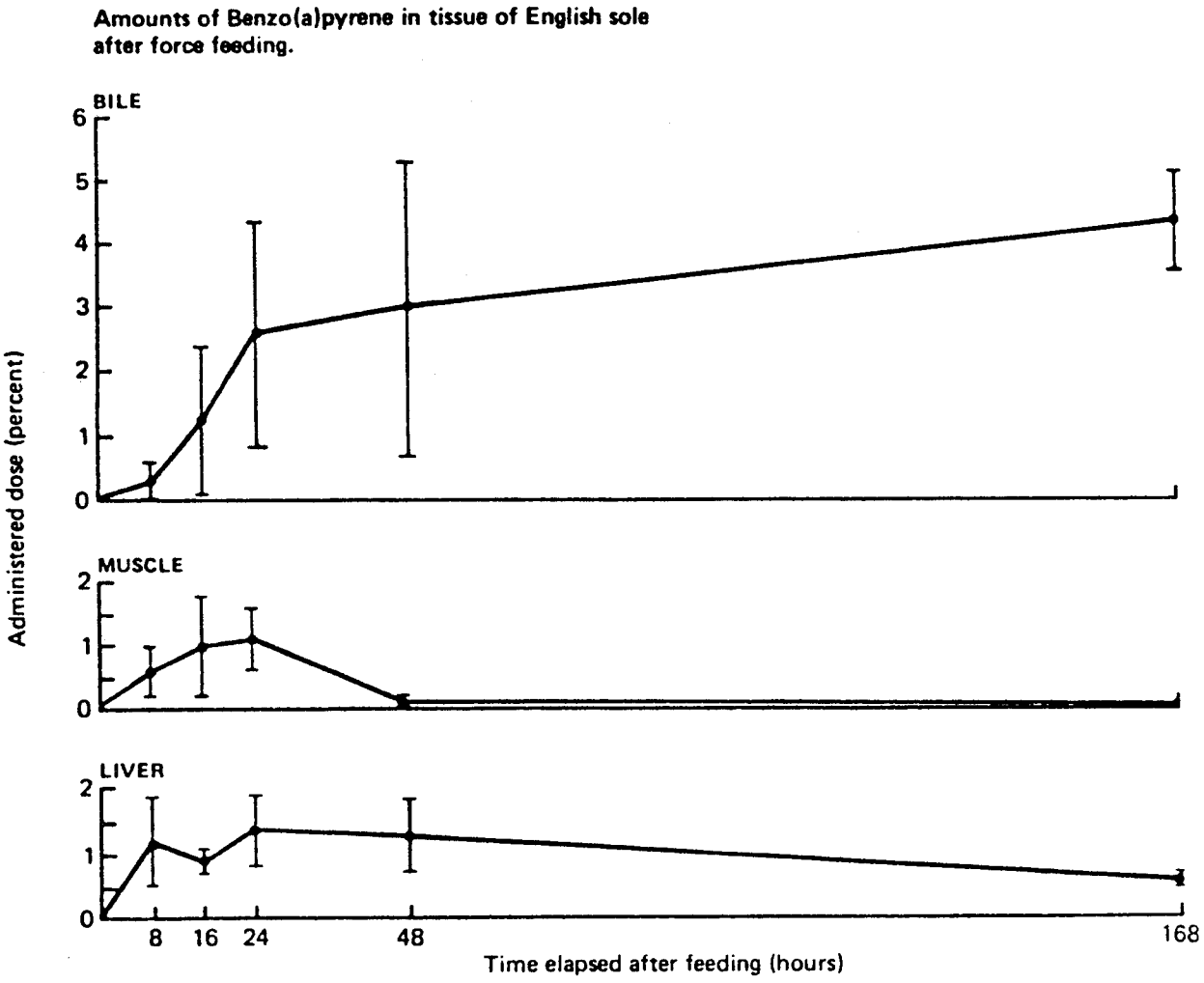


FIGURE 22. Benzo[a]pyrene-derived radioactivity in liver, muscle, and bile of English sole force-fed ^{3}H-BaP (2 mg/kg body weight). $\bar{X}\pm$S.D.

with the untreated fish; the binding of BaP to DNA was slightly greater when fish were pretreated with BaP than when they were pretreated with MC.

Figure 24 depicts HPLCs of ethyl acetate extractable metabolites formed by liver enzymes of MC-pretreated fish species and rat. The data revealed that for all three fish species 9,10-dihydro-9,10-dihydroxy-benzo(a)pyrene (BaP 9,10-dihydrodiol) and 7,8-dihydro-7,8-dihydroxy-benzo(a)pyrene (BaP 7,8-dihydrodiol) were the major metabolites; 3-hydroxy BaP was also present in considerable amounts. The metabolite profile for the MC-pretreated rat revealed the presence of a high proportion of phenols and quinones together with lower, but significant amounts of the non K-region dihydrodiols (BaP 7,8-dihydrodiol and BaP 9,10-dihydrodiol).

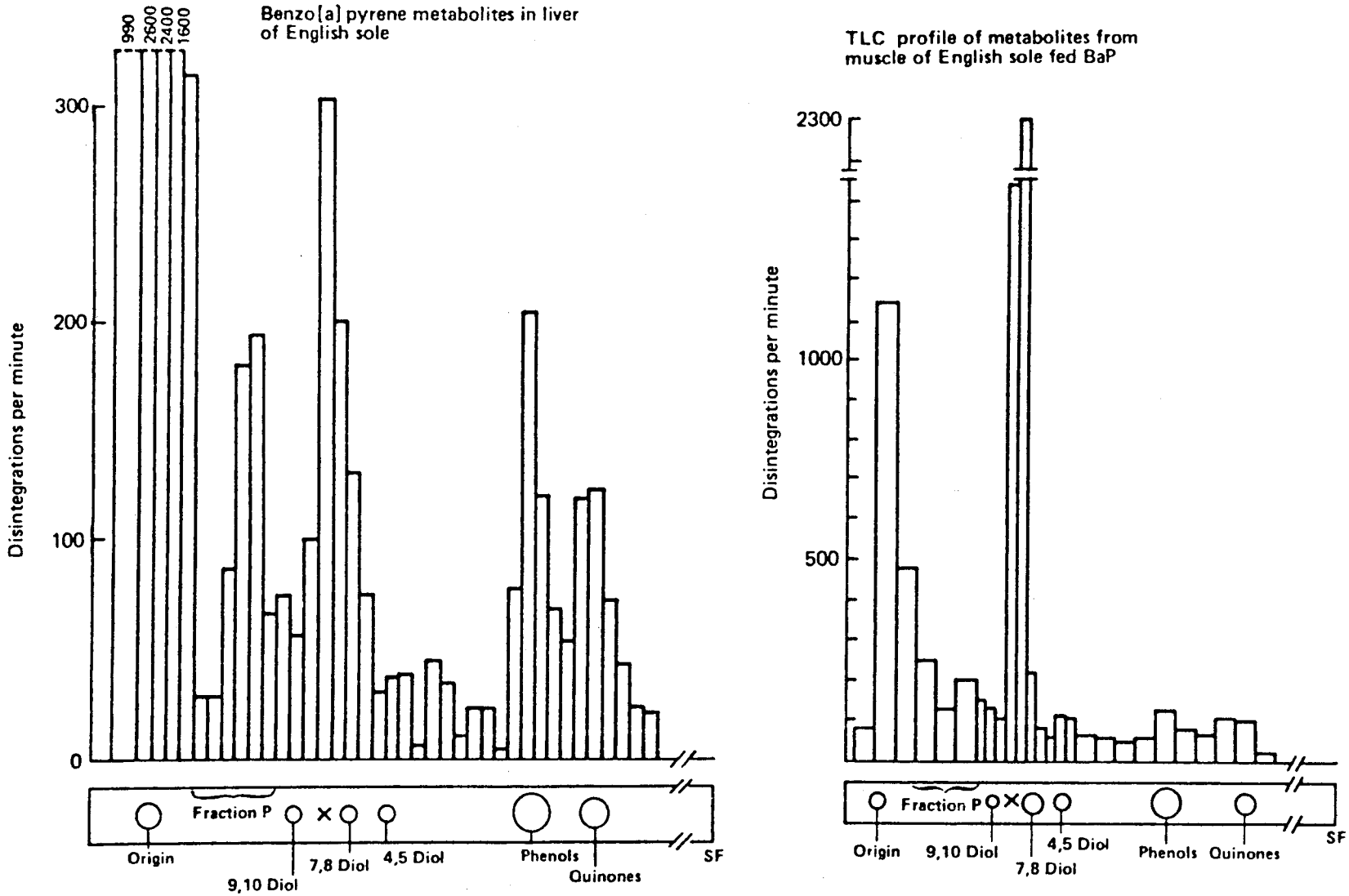


FIGURE 23. Thin-layer chromatograms of organic-solvent soluble BaP metabolites from liver and muscle of English sole force-fed ^{3}H-BaP (2 mg/kg body weight). Abbreviations are Fraction P, metabolites with R_f values between the origin and BaP 9,10-dihydrodiol; 9,10-diol, BaP 9,10-dihydrodiol; 7,8-diol, BaP 7,8-dihydrodiol; 4,5-diol, BaP 4,5-dihydrodiol.

TABLE 25. Distribution of BaP-derived radioactivity in liver and bile of English sole exposed to ^{3}H-BaP.[a]

	Liver	Bile
Unmetabolized BaP	1±0.1	<<1
Ethyl acetate-soluble metabolites	16±1	17±3
Glucuronides	83±1	46±4
Sulfates		11±4
Other aqueous-soluble metabolites		26±5

[a] Fish were force-fed ^{3}H-BaP (2mg/kg)dissolved in corn oil; liver and bile were analyzed 24 hr after the feeding. Each entry, expressed as percent of total radioactivity, is the mean ± S.D. of three values. (From Varanasi and Gmur 1981b)

6.1.10 Activities of Aryl Hydrocarbon Monooxygenases in Different Species

The activities of aryl hydrocarbon monooxygenases (AHM) are reported for marine species from Alaska waters, as follows: 15 samples of flathead sole (Hippoglossoides elassodon), 14 of arrowtooth flounder (Atharestes stomias), 24 of rock sole, 15 of pollock (Theragra chalcogramma), 4 of butter sole (Lepidopsetta isolepis), 19 of Pacific cod (Gadus macrocephalus), 4 of sea snail (Fusitriton sp.), and 8 of tanner crab (Chionoecetes sp.). The snails were found to have no detectable activity of AHM in whole body samples. The AHM activities of the species examined are presented in Table 27.

The specific activities of hepatic AHM for the fish ranged from 0.006 to 0.927 nmoles of BaP products per 20 min per mg of protein; values for the crab viscera AHM ranged from 0.005 to 1.03 nmoles/ 20 min/mg protein.

The activities of hepatic AHM for Pacific cod which had pseudobranchial tumors were indistinguishable from the activities found for the ordinary Pacific cod. The data for the tumor-bearing cod revealed AHM activity of 0.31 ± 0.16, while the normal cod provided AHM activity of 0.33 ± 0.26 Units/mg protein. There was no correlation between sex of fish and AHM activity.

TABLE 26 In Vitro Binding of Activated BaP to DNA Catalyzed by Liver Supernatants from Fish and Rat[a]

Species	System[b] (Supernatant)	pmole of BaP Equivalent/mg DNA/mg Protein[c]	% of Control Value
Starry flounder	Control	0.15	100
	MC	1.62	1,100
	BaP	1.70	1,100
	PBCO	0.74	500
	MC[d]	0.53	—[d]
English sole	Control	0.06	100
	PBCO	1.05	1,800
	MC	0.16	—[e]
Coho salmon	Control	0.02	100
	MC	0.97	4,900
	BaP	1.06	5,300
	MC[d]	0.30	—[e]
Rat	Control	0.06	100
	MC	0.69	1,200

[a]Maximum standard deviation between values from two separate experiments was 14 percent.

[b]Liver supernatants (10,000 x g) were obtained from either untreated (control) animals or those injected with 10 mg/kg of 3-methylcholanthrene (MC), benzo(a)pyrene BaP, or Prudhoe Bay crude oil (PBCO) when the water temperature for fish was 13°C.

[c]Liver supernatants (≈5 mg protein) from different animals were incubated in the dark with 5 nmole of BaP, 2 mg of salmon sperm DNA, and cofactors for 15 minutes at 25°C (for fish) and 37°C (for rat). Each value is an average of two experiments and three replicate measurements using pooled liver extracts from five animals. Binding values for incubation without NADPH were less than 0.001 and are subtracted from the values reported.

[d]Liver supernatants were obtained from MC-pretreated fish when the water temperature was 8°C.

[e]No control fish were sampled at 8°C.

(From Varanasi et al. 1980)

6.1.11 Uptake, Metabolism and Toxicity of Hydrocarbons in Invertebrates

(a) The changes that are induced by NPH were studied using the gametes from artificially spawned adults. Mussel gametes were combined in separate solutions containing 1, 10, and 100 ppb NPH. The survival of the resulting larvae, after 24 hr, was 27, 32, and 2%, respectively, for the three exposures compared to 69% survival for controls. In the 100 ppb solution, 24% of the exposed eggs failed to fertilize

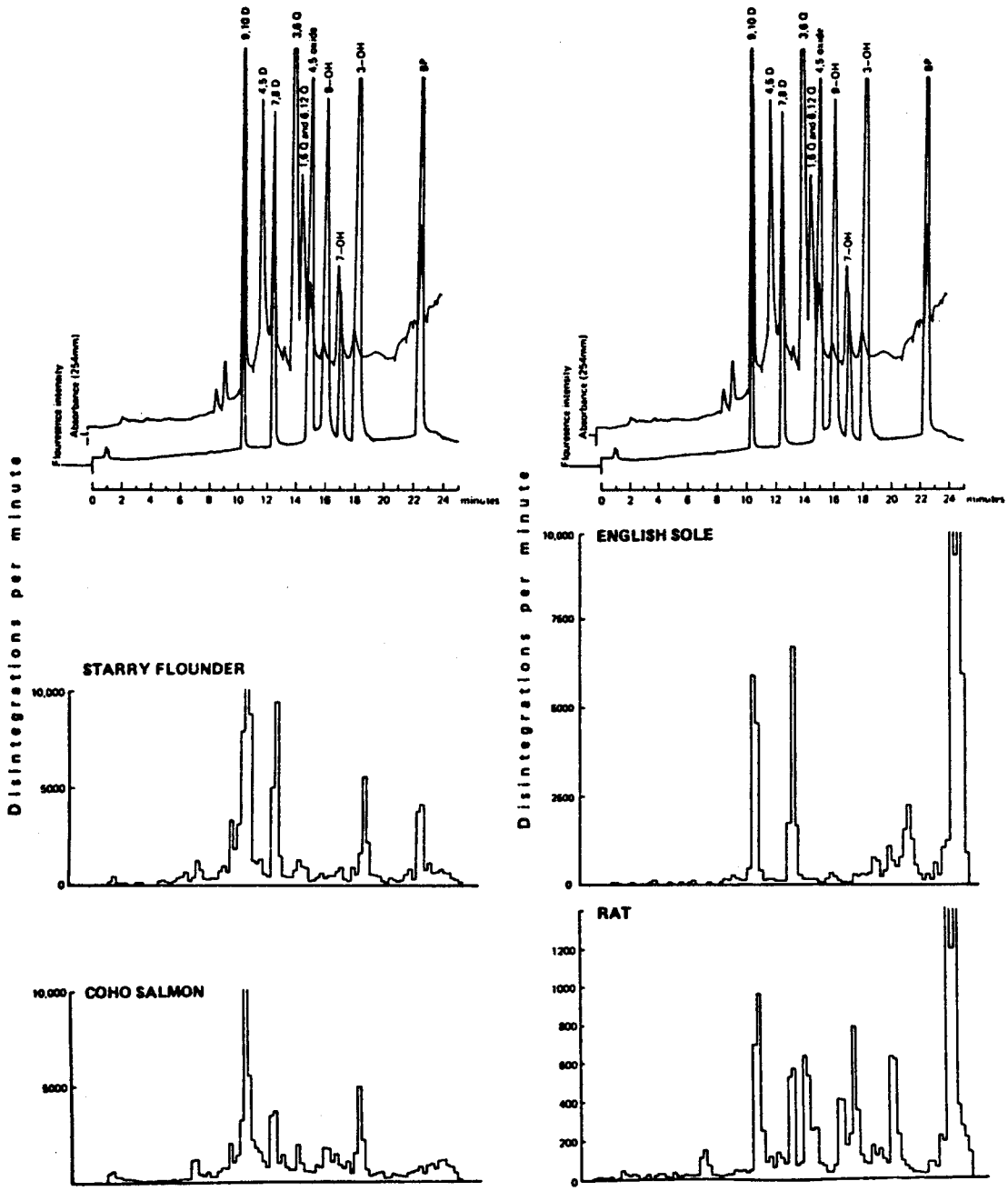


FIGURE 24. HPLC of reference BaP metabolites and ethyl acetate-extractable metabolites of ^{3}H-BaP produced by liver supernatants (10,000 x g) of MC pretreated starry flounder, coho salmon, English sole, and rat. Reference compounds and metabolites were detected by both UV and fluorescence spectrometry. Metabolites formed by incubating 5 nmole ^{3}H-BaP with liver supernatants (5 mg protein) of fish (at 25°) and rat at (37°) for 15 minutes were separated by fractions collected at 15-second intervals. Abbreviations are 9,10-D, BaP 9,10-dihydrodiol; 4,5-D, BaP 4,5-dihydrodiol; 7,8-D, BaP 7,8-dihydrodiol; 3,6-Q, BaP 3,6 quinone; 1,6-Q, and 6,12-Q, 1,6-BaP quinone and 6,12-BaP quinone; 9-OH, 9-OH-hydroxy BaP; 7-OH, 7-OH-hydroxy BaP; 3-OH, 3-hydroxy BaP; BaP, benzo(a)pyrene. (From Varanasi et al. 1980)

TABLE 27. Hepatic aryl hydrocarbon monooxygenases (AHM) activities for marine species from Alaska waters.[a]

Species	Sex	No.	Body Weight (g)	Body Length (cm)	Liver Weight (g)	AHM (Units per mg of protein)[b]
Flathead sole	M	7	136± 26	26.5±2.5	1.15±0.42	0.30±0.31
	F	8	232± 62	29.7±2.5	2.62±0.60	0.15±0.072
	M&F	15	187± 69	27.7±2.9	1.94±0.91	0.22±0.22
Arrowtooth flounder	M	9	185±129	27.2±4.5	1.50±0.85	0.063±0.037
	F	5	514±305	36.0±8.5	2.84±1.68	0.063±0.041
	M&F	14	303±256	30.3±7.3	1.98±1.33	0.063±0.038
Rock sole	M	7	240± 91	28.1±3.7	1.64±0.74	0.22±0.19
	F	17	399±237	31.3±5.3	2.88±1.80	0.21±0.20
	M&F	24	352±216	27.9±8.6	2.52±1.65	0.21±0.19
Pollock	M	8	324±227	33.0±7.6	3.38±1.27	0.19±0.20
	F	7	497±281	39.2±8.0	5.21±2.61	0.22±0.17
	M&F	15	405±260	35.9±8.1	4.24±2.15	0.21±0.18
Butter sole	F	4	244± 45	29.7±1.8	1.75±0.38	0.13±0.080
Pacific cod	M	9	980±404	44.4±5.4	3.31±1.05	0.43±0.26
	F	10	887±269	43.8±4.5	3.88±0.93	0.23±0.22
	M&F	19	931±333	44.1±4.8	3.61±1.00	0.33±0.26
(pseudobranchial tumors)	?	5	449±110	34.9±2.7	---	0.31±0.16[c]
Tanner crab (viscera)	F	8	---	---	---	0.25±0.35

[a] Each value represents $\bar{X}$±S.D.
[b] Using benzo(a)pyrene as substrate, one unit of AHM will convert 1.0 nanomole of benzo(a)pyrene to 1.0 nmole of oxidation products per 20 minutes at pH 7.5 at 25°C. Source of protein was the 9,000x g (or 10,000 x g) - 20 min. supernatant fraction of a 20% (wt/vol.) homogenate in cold 0.25M sucrose solution.
[c] Hepatic AHM from tumor-bearing Pacific cod.

compared to 3% failure in the controls. The highest concentration also produced abnormal embryological development in 25% of the animals after 1.5 hr of exposure, compared to 2% of the controls. After 24 hr all of the larvae exposed to 10 ppb water-borne NPH had reached the "straight hinge" stage. No animals at the other concentrations or the controls developed to straight hinge larvae within 24 hr.

Table 28 Hydrocarbon content of thoracic and abdominal segments of adult spot shrimp (*P. platyceros*) exposed to a water-soluble fraction of Prudhoe Bay crude oil.

Hydrocarbons	Water-soluble fraction† (ng/g)	Thorax§ (ng/g)	Bio-concn.	Abdomen§ (ng/g)	Bio-concn.
Cyclohexane	2·9	—‡		—‡	
Benzene	5·5	—‡		—‡	
Toluene	55	—‡		—‡	
Ethylbenzene	0·7	—‡		—‡	
Xylenes	37·1	320	9	350	9
C_3-substituted benzene	4·1	220	54	150	37
C_4/C_5-substituted benzenes	1·4	540	386	180	129
Subtotal		1080		680	
Naphthalene	0·4	10	25	20	50
1-Methylnaphthalene	0·4	100	250	40	100
2-Methylnaphthalene	0·4	110	275	60	150
C_2-substituted naphthalene	1·4	240	171	100	71
C_3-substituted naphthalene	0·7	190	271	20	29
Subtotal: C_1–C_3-substituted naphthalenes		640		220	
Total hydrocarbons	110	1730		920	

The shrimp were exposed for 7 days to a concn. of 100 p.p.b. of the water-soluble fraction of the oil.

† Calc. from representative analysis before dilution.
‡ Not quantifiable due to analytical procedure.
§ Concn. was determined as ng/g wet wt., detectable limit was 10 ng/g.

(From Sanborn and Malins 1980)

(b) The decrease in fertilization demonstrated in the mussel experiments and the effect of NPH on sperm and eggs were further explored using oyster gametes. The oyster sperm exposed to 10 ppb and 1 ppb of NPH and then combined with uncontaminated eggs resulted in 14 and 9% unfertilized eggs, respectively, while no effect was seen at 0.1 ppb NPH. However, only 5% of the eggs exposed to 10 ppb and 1 ppb NPH and then combined with uncontaminated sperm failed to fertilize. This compares to a 4% failure to fertilize when uncontaminated eggs or eggs exposed to 0.1 ppb NPH and uncontaminated sperm were combined.

(c) The data on accumulation of SWSF hydrocarbons in one-year-old spot shrimp show that detectable levels of low molecular weight aromatic hydrocarbons were readily accumulated in thoracic segments (Table 28) (probably associated with the hepatopancreas). The abdominal segments were found to contain significant concentrations of identified aromatic hydrocarbons. The data given in Table 28 represent hydrocarbon accumulations in experimental animals with respect to data obtained from a control group.

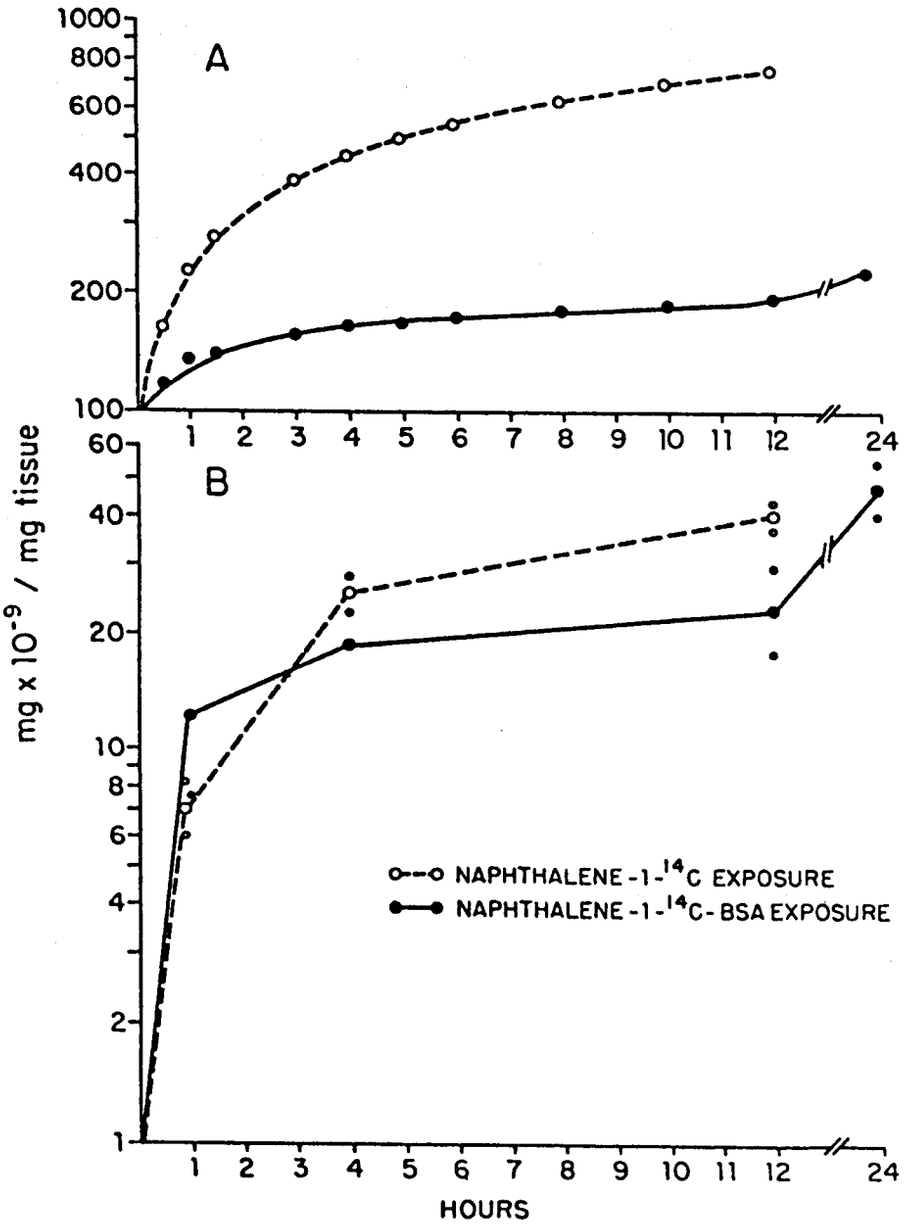


FIGURE 25. Accumulation of naphthalene and metabolic products (expressed as naphthol) on exposure of stage V spot shrimp (Pandalus platcyeros) to 8-12 ppb of water-borne [1-^{14}C]naphthalene and [1-^{14}C]naphthalene complexed with BSA. (A) Regression lines of concentrations of [1-^{14}C]naphthalene with sampling points indicated. (B) Median values of metabolic products with data ranges. (From Sanborn and Malins 1977)

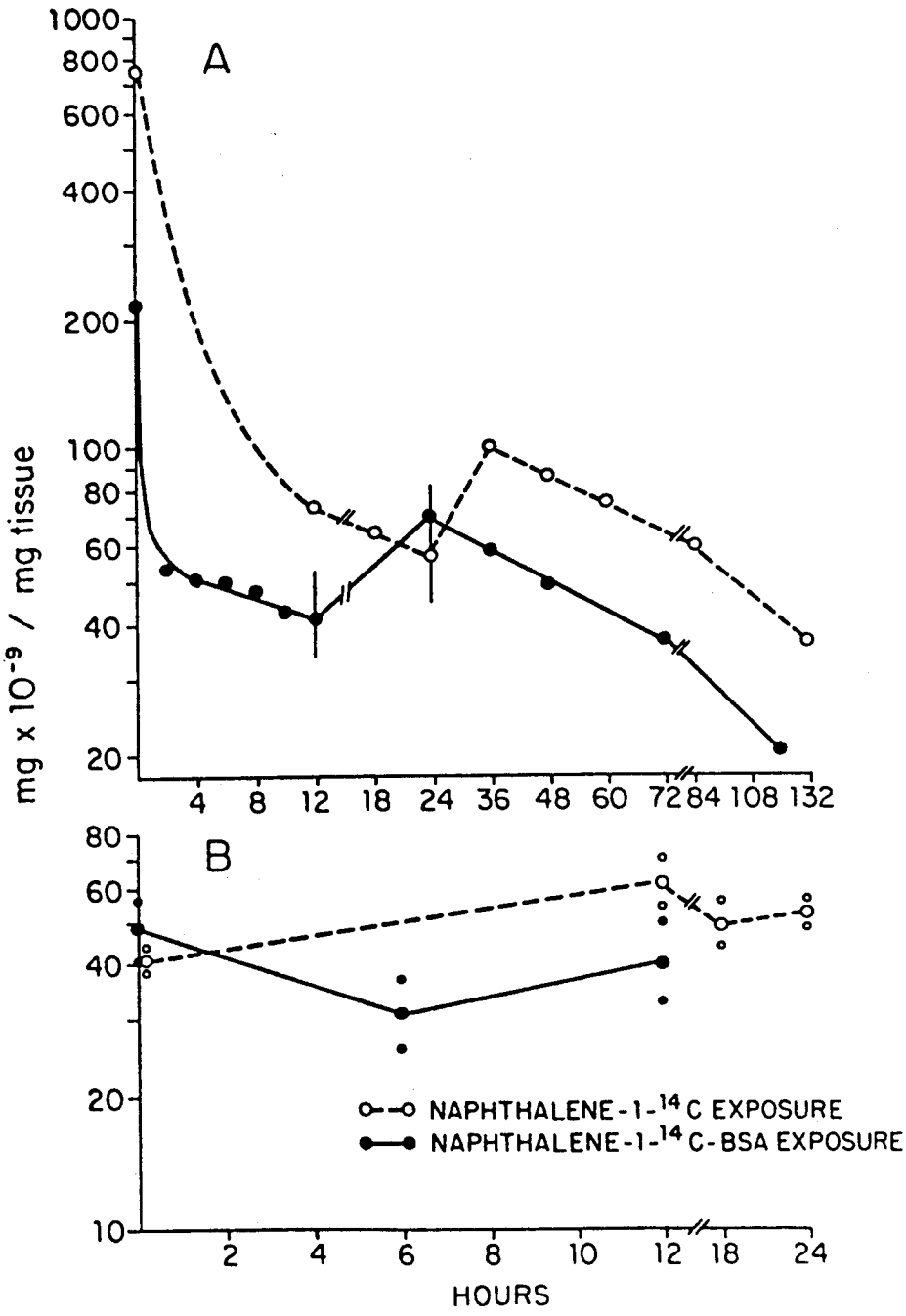


FIGURE 26. Depuration of naphthalene and metabolic products (expressed as naphthol) after exposure of stage V spot shrimp (Pandalus platyceros) to 8-12 ppb of water-borne [1-^{14}C]naphthalene (12 hr) and [1-^{14}C]naphthalene complexed to BSA (24 hr). (A) Regression lines of concentrations of [1-^{14}C]napthalene up to sampling points indicated by vertical lines; thereafter, not a regression line and points represent primarily metabolic products containing only small amounts of [1-^{14}C]naphthalene. (B) Median values of only metabolic products with data ranges. (From Sanborn and Malins 1977)

(d) The results of studies on shrimp and crab larvae indicated that 8 to 12 ppb of NPH-1-^{14}C and NPH-1-^{14}C bovine serum albumin (BSA) complex in flowing seawater caused 100% mortality in 24 to 36 hr in Dungeness crab zoea and in Stage 1 and Stage V spot shrimp larvae. Maximum accumulation of NPH in Stage V spot shrimp was nearly 4 times greater than in shrimp larvae exposed to the NPH-BSA complex (820 ppb vs 220 ppb) (Fig. 25). The percent of total radioactivity attributable to metabolic products (calculated as 1-naphthol) in larvae after 24 hr exposure to NPH was 9%. However, after 24 hr exposure to NPH complexed with BSA, 21% was present as metabolites. NPH was almost entirely released from tissues in 24 to 36 hr, whereas metabolic products were resistant to depuration (Fig. 26).

(e) The adult and Stage I larval spot shrimp exposed to 80 ppb and 18 ppb NPH, respectively, formed a number of conjugated and nonconjugated metabolites (Table 29). In adults, nonconjugated forms (quinones, naphthol, and dihydrodiol) represented 69% of the total metabolites. In the larval shrimp 39% of the metabolites were present as sulfate conjugates while 44% were present as naphthol.

Table 29 Conversion products formed by adult and larval spot shrimp (*P. platyceros*) exposed for 10 h to [^{3}H]- and [^{14}C]naphthalene.

Conversion product	% total conversion products		
	Adult		Larvae
	[^{3}H]Naphthalene	[^{14}C]Naphthalene	[^{3}H]Naphthalene
Naphthyl glucuronide	2	2	6(?)
Naphthyl sulphate	7	1	39
Unknown No. 1†	5	4	—
Unknown No. 2†	16	17	—
Naphthalene-1,2-dihydrodiol	17	20	4
Naphthyl glycoside	1	>1	—
Naphthoquinone†	36	32	7
α-Naphthol	16	24	44

† Not corrected for extraction efficiency.

(From Sanborn and Malins 1980)

6.1.12 Food Chain Transfer of 2,6-DMN to Sea Urchins via Algae

The maximum accumulation of 2,6-DMN in algae (Fucus distichus) occurred in 20-25 hr (Fig. 27). It was shown that greater than 99% of the tritium accumulated in Fucus after 25 hr was organic-solvent soluble; TLC of a Fucus extract showed no evidence of 2,6-DMN metabolism. Thus it was concluded that all of the tritium was associated with unmetabolized 2,6-DMN.

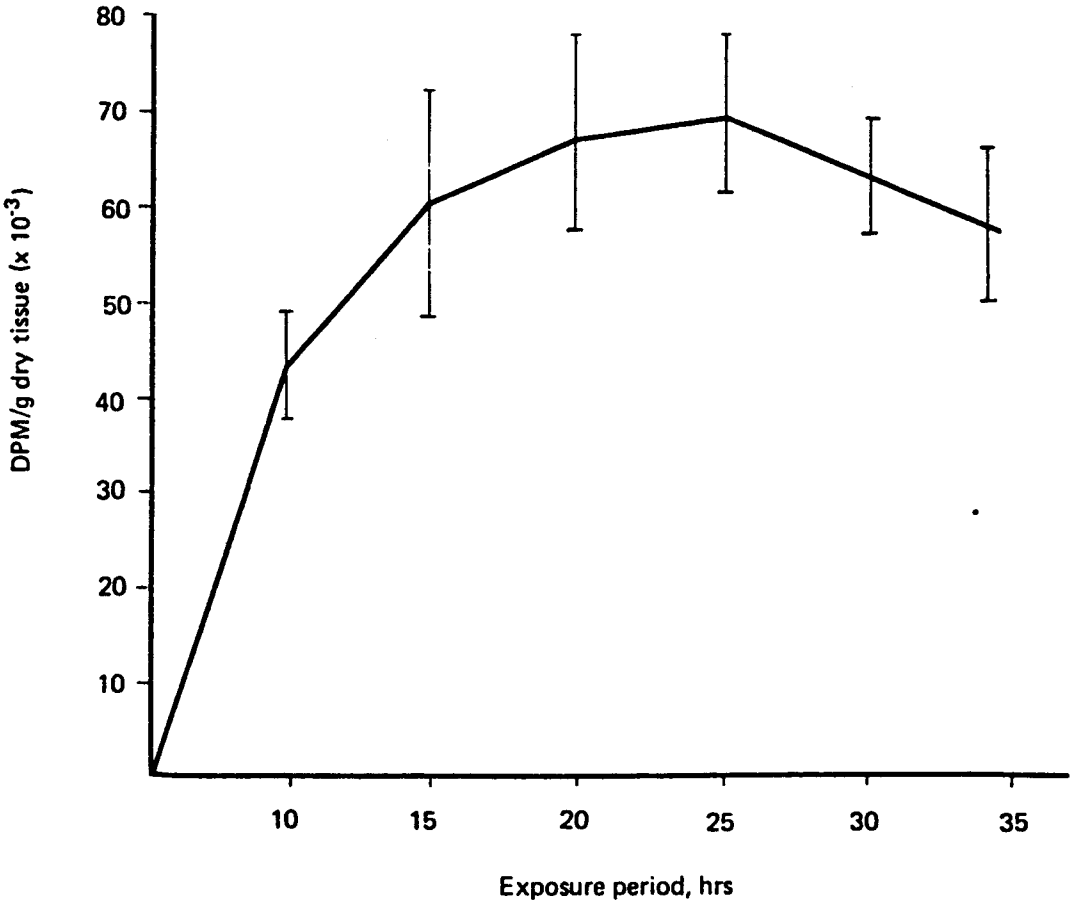


FIGURE 27. Accumulation of ^{3}H-2,6-dimethylnaphthalene in seaweed (F. distichus) exposed through seawater. Values are expressed as mean + SD of three aliquots from the same exposure experiment. (From Mathins and Koubal 1982)

The distribution of tritium in sea urchins (S. droebachiensis) after feeding on Fucus for 3 days was investigated (Fig. 28). Digestion of the methanol-extracted exoskeleton with tissue solubilizer, a process potentially destructive to metabolites, released 64% of the total radioactivity accumulated by the sea urchins. Other methods to extract radioactive compounds from the exoskeleton (e.g., treatment with organic solvents, dilute acid, EDTA, and a proteolytic enzyme) were not effective. The remaining extractable radioactivity was distributed among the soft tissues as shown in Figure 28.

A substantial amount of the 2,6-DMN-derived radioactivity isolated from the digestive tract and gonadal tissue from the 3 and 14 day experiments was in the form of conjugated metabolites (Fig. 29), primarily the sulfate. Hydrolysis of the sulfate fraction from the 3 day experiment with aryl sulfatase, followed by TLC showed fractions corresponding to the 3- (80%) and 4-hydroxy-2,6-DMN (20%).

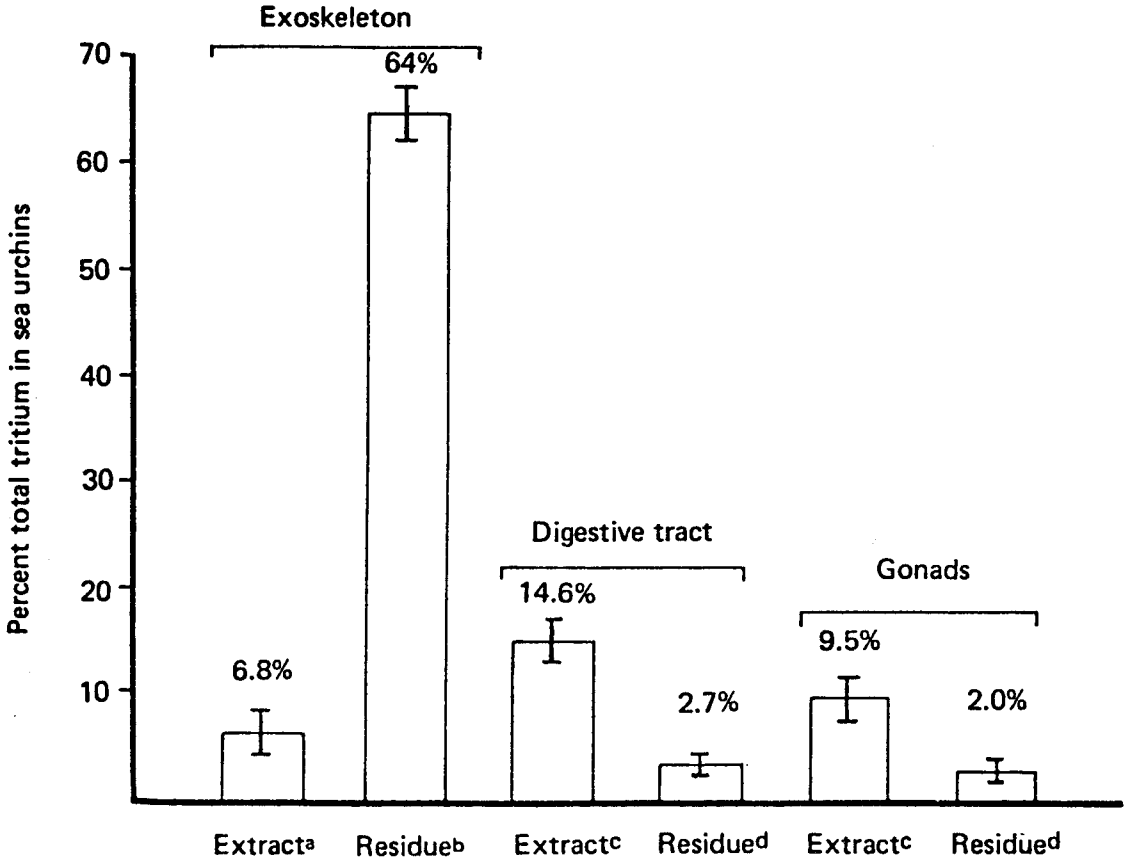


FIGURE 28. Tritium in sea urchins (S. droebachiensis) that consumed ^{3}H-2,6-dimethylnaphthalene for three days. Values are expressed as mean + SD. [a]Extracted with hot methanol. [b]Residue from hot methanol extract. Solubilized in Soluene Tissue Solubilizer and then analyzed by scintillation spectrometry. (From Malins and Roubal 1982)

TLC of soft tissue extracts after 3 and 14 days exposure gave no evidence for conjugated or nonconjugated methanol derivatives, and only minor amounts (<0.06 ng total/g dry tissue) of nonconjugated metabolites were found. Both 3,4-dihydro-3,4-dihydroxy-2,6-DMN and 3-hydroxy-2,6-DMN were detected in digestive and gonadal tissues after 14 days; however, after 3 days, only the former compound was found in the digestive tract. An unknown polar compound was detected in both digestive tract and gonadal tissues from animals exposed for 3 and 14 days.

6.1.13 Biological Fate of Metals

(a) Tissue Concentrations of Lead and Cadmium.

Both coho salmon and starry flounder exposed to ppb concentrations of lead and cadmium in seawater attained ppm concentrations of these

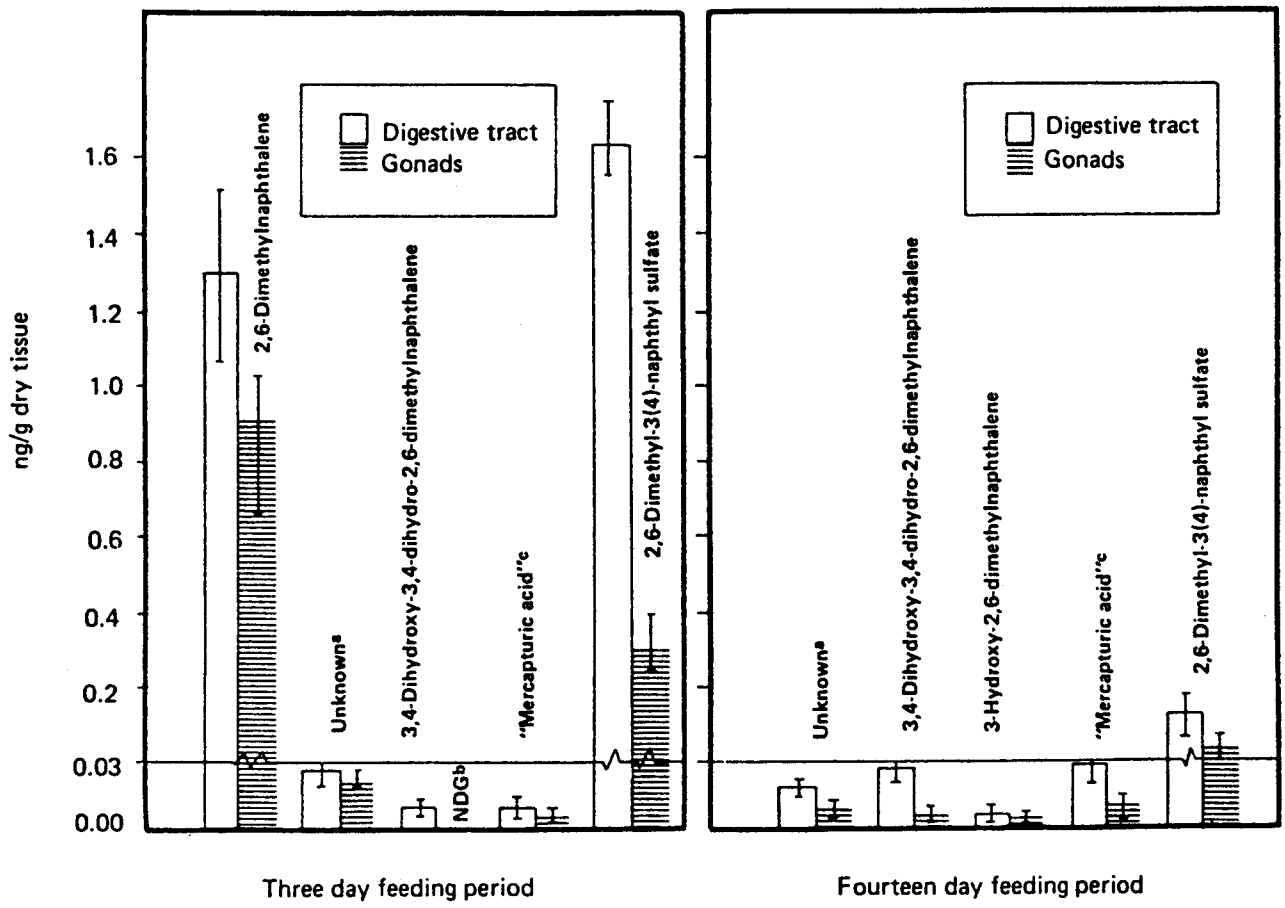


FIGURE 29. Aromatic compounds isolated from sea urchins (S. droebachiensis) that fed on Fucus distichus containing 2,6-dimethylnaphthalene. Values are expressed as mean ± SD. [a]Considered to be as 3-hydroxy-2,6-DMN for purposes of quantitation. [b]NDG; not detected in gonads. [c]R_f value corresponds to 1-naphthyl mercapturic acid. (From Malins and Roubal 1982)

metals in various tissues (bioconcentration values given in Tables 30 and 31 are based on wet weight of tissues). Data in Tables 30 and 31 show that high concentrations of lead accumulated in the brain of starry flounder, and that both species accumulated substantial concentrations of lead and cadmium in kidney, especially in the posterior section. Concentrations of metal in epidermal mucus of coho salmon were slightly greater than the levels in the surrounding water; however, metals (especially lead) were bioconcentrated to a much greater extent in starry flounder mucus.

The results in Table 30 and 31 show that the temperature of the surrounding water had a definite influence on the uptake and accumulation of metals in both fish species under investigation. Skin, liver, and kidney, of fish held at 10°C had considerably higher metal concentrations than the tissues of fish held at 4°C.

TABLE 30. Effect of temperature on bioconcentration[a] of lead and cadmium in tissues of saltwater-adapted coho salmon (Oncorhynchus kisutch) exposed to water-borne metals.[b]

Metal	Temp. °C	Mucus	Scales[c]	Liver	Brain	Kidney[d] a	Kidney[d] p
Pb	4	1.5	10.0	1.8	ND[e]	1.8	7.3
	10	1.8	24.9	3.0	ND	3.1	12.0
Cd	4	1.0	0.3	1.1	ND	0.7	0.9
	10	1.2	0.7	2.0	ND	1.2	2.5

[a] Bioconcentration=metal concentration in tissue (ng/g, wet wt)/metal concentration in seawater (ng/ml).
[b] Fish were exposed to 150 ppb of either lead or cadmium for two weeks.
[c] Scales with dermal and epidermal cells attached.
[d] a=anterior kidney; p=posterior kidney.
[e] ND=not detected.

(From Varanasi and Markey 1978, Reichert et al. 1979).

TABLE 31. Effect of temperature on bioconcentration[a] of lead and cadmium in tissues of starry flounder (Platichthys stellatus) exposed to water-borne metals.[b]

Metal	Temp. °C	Mucus	Skin[c]	Liver	Brain	Posterior kidney
Pb	4	4.2	1.2	3.9	1.3	5.7
	10	2.1	2.4	5.0	3.5	8.6
Cd	4	1.3	0.3	3.0	ND[d]	0.5
	10	1.3	1.1	10.5	ND	1.9

[a] Bioconcentration=Pb concentration in tissue (wet wt)/Pb concentration in water.
[b] Fish were exposed to 150 ppb of either lead or cadmium for two weeks.
[c] Skin with scales.
[d] ND=not detected.

(From Varanasi and Markey 1978, Reichert et al. 1979).

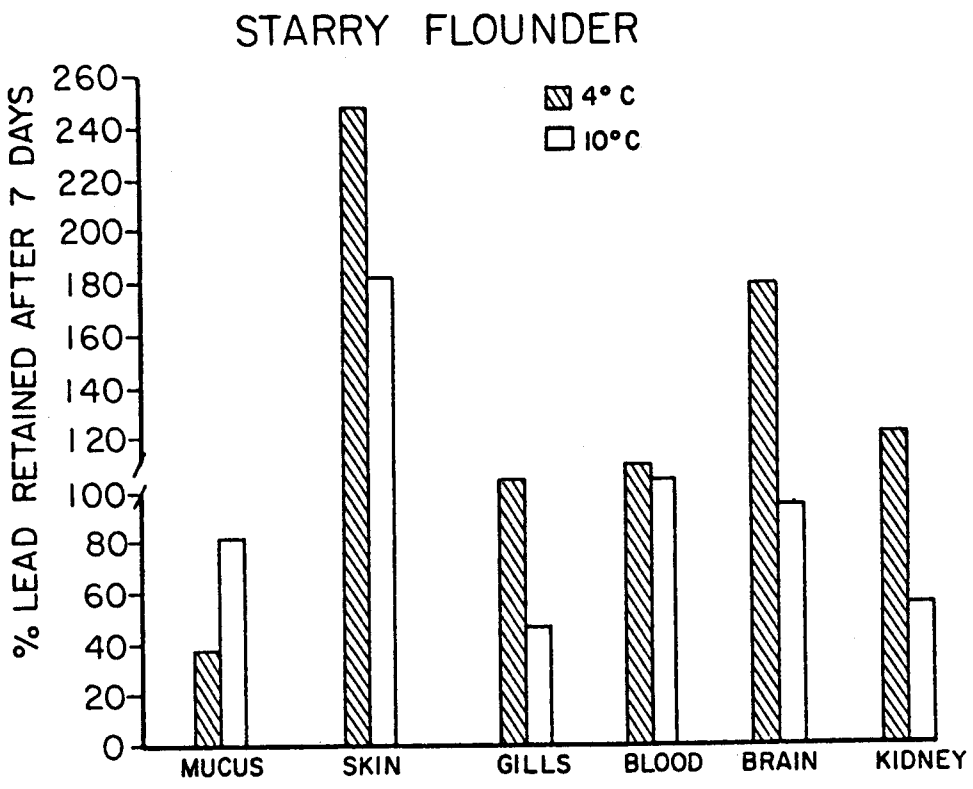


FIGURE 30. Effect of temperature on lead concentrations retained in the tissues of lead-exposed starry flounder after one week of depuration. (From Varanasi 1978)

Temperature of the surrounding water appears to have a distinct effect on retention of metals in tissues of starry flounder. For example, after 7 days of depuration, fish maintained at 10°C retained much lower concentrations of lead in tissues such as skin, gills, brain, and kidney compared to the lead concentrations in tissues of fish at 4°C (Fig. 30). No such temperature effect was observed for coho salmon.

When the metal-exposed coho salmon were placed for 7 days in seawater free of radioactive metals, lead concentrations increased in most tissues but decreased in blood, indicating transfer of lead from the blood to other tissues. For example, liver, scales, posterior kidney, and blood of lead-exposed coho salmon contained 109, 114, 110, and 74%, respectively, of the lead concentrations accumulated in these tissues at the end of the two-week exposure at 10°C. Conversely, cadmium concentrations decreased significantly in all tissues except kidney of cadmium-exposed coho salmon after 7 days of depuration. Liver, scales, posterior kidney, and blood of these fish contained 87, 53, 156, and 79%, respectively, of the original cadmium concentrations. In starry flounder, after a 7-day depuration, lead was also retained to a much greater extent than cadmium in these tissues.

After 37 days of depuration, substantial (>50%) concentrations of both lead and cadmium still persisted in most tissues of coho salmon. A notable finding was that metal concentrations in the posterior kidney of coho salmon continued to increase even after the fish were placed in seawater for a period of 5 wk. Also, more than 50% of the lead and cadmium accumulated in the scales of coho salmon was still present after five weeks of depuration. Depuration of starry flounder tissues was not studied for periods longer than 7 days, because after 7 days of depuration the mortality rate increased in both control and exposed fish.

(b) Distribution of Cadmium and Lead in Subcellular Fractions.

Samples of liver and kidney removed from the above coho salmon and starry flounder exposed to water-borne lead and cadmium were also used to investigate subcellular distribution of these metals. Cytosol fractions of liver and posterior kidney of cadmium-exposed starry flounder contained 72 and 80% of total tissue cadmium (^{109}Cd), respectively, and cytosol fractions of these tissues from coho salmon contained 62 and 48% of total tissue cadmium (^{109}Cd), respectively. Microsomal fractions of liver and kidney each contained 18% of the cadmium present in the tissue. Distribution of lead was somewhat different: liver of lead-exposed coho salmon contained almost equal proportions of lead in mitochondria, microsomes, and cytosol.

Intravenous injections of ^{109}Cd were employed to assess if cadmium in gills, liver, and kidney of cadmium-exposed fish was bound to metalloproteins (M.W. 10,000 daltons). Results with coho salmon show that similar to mammalian systems, a major fraction of the ^{109}Cd was bound to cadmium-binding proteins, CdBP ($\leq$8,900), in liver and kidney of coho salmon. Moreover, the percentage of the total ^{109}Cd in cytosol bound to CdBP was greater in those fish which had been exposed to 200 ppb Cd in seawater for two weeks, compared to controls in seawater only (Table 32). The studies with unexposed coho salmon indicate that CdBP concentrations in gills are low. Yet, within 24 hr after metal challenge there is an appreciable increase in the accumulation of CdBP-bound cadmium indicating induction of CdBP.

(c) Effect of Metal Exposure on Synthesis of Epidermal Mucus

To assess the effect of water-borne metals on mucus production, weight of mucus was determined for each test and control coho salmon. The results show that at 10°C, 150 ppb of either lead or cadmium in surrounding water induced significantly higher ($p<0.05$) mucus production in coho salmon (Table 33).

Our studies also show that when the fish were injected with either lead (^{210}Pb) or cadmium (^{109}Cd) salts substantial concentrations of lead (164 ppb, dry wt) and cadmium (744 ppb, dry wt) were present in the mucus for at least 2 days following the injection.

Table 32 Distribution of injected ^{109}Cd in coho salmon gill, liver, and kidney cytosol at 10°C

Organ	Time after injection (hr)	Percent of total Cd^{+2} of soluble fraction in: Coho[a] High M.W.[c] fraction	CdBP	Cd in cytosol[d]	Coho-M[b] High M.W. fraction	CdBP	Cd in cytosol
Liver	3	21[e,f]	79	85.3	11	89	82.5
	24	55	45	103.0	26	74	81.2
	48	16	84	200.2	7	93	97.7
Gills	3	91	9	21.7	45	55	29.6
	24	77	23	22.8	56	44	39.5
	48	76	24	57.3	51	49	18.8
Kidney	3	66	34	63.0	23	77	38.5
	24	71	29	83.4	39	61	70.0
	48	--[f]	--	--	22	78	88.9

[a] Fish not exposed to water-borne cadmium prior to injection.
[b] Fish exposed to 150 ppb water-borne cadmium for 2 weeks prior to injection.
[c] Molecular weight greater than 13,000 mol.wt.
[d] ng ^{109}Cd/mg cytosolic protein.
[e] Three fish were sampled for each time period and the organs were pooled.
[f] All values were normalized to 100% (more than 95% of the radioactivity was recovered).
[g] Fractions were lost.

(From Reichert et al. 1979)

Table 33 Average weight of epidermal mucus obtained from control and metal-exposed fish*

Sample	Metal	Mg mucus/g fish
Control†	—	7.2 ± 1.6 (10)‡
Test	Pb	12.7 ± 2.8 (10)§
Test	Cd	13.1 ± 2.9 (10)§

* Fish were exposed to 150 ppb of either lead or cadmium for a period of 2 weeks at 10°C.
† Control seawater contained < 5 ppb of lead and 2 ppb of cadmium.
‡ Values represent mean value ± S.D. and numbers in parentheses indicate number of individual fish.
§ Student *t*-test was performed on the data and values for the test were significantly ($P < 0.05$) different from the control value.

(From Varanasi and Markey 1978)

6.2 Pathology

6.2.1 Effects of Petroleum on Disease Resistance

Salmonids. In initial tests with coho salmon no alteration in disease resistance was observed from peroral exposure to PBCO. The LD_{50} dose of V. anguillarum for coho maintained on a diet containing 1,000 ppm PBCO for 34 days was not significantly different (P=0.05) from that of control coho fed a normal diet; LD_{50} values and their 95% C.I. were 1.1×10^3 (2.5×10^2 - 4.9×10^4) and 8.6×10^2 (1.3×10^2 - 5.7×10^3) bacteria for tests and controls, respectively. Similarly, no difference in mortality following bacterial challenge could be demonstrated between fish exposed to a SWSF of PBCO (ca 0.8 ppm) for 14 d and the controls. In this second experiment, however, the LD_{50} doses were less than 20 bacteria in both the oil-exposed and non-oil-exposed groups. The actual percent mortalities at the 20 bacteria dose level were 60% and 80%, respectively; these levels of mortality are not significantly different (P=0.05).

The results of additional tests with salmonids showed that rainbow trout perorally exposed to 10 or 1,000 ppm PBCO for 10 mo were not demonstrably immunosuppressed. As shown in Table 34 and Figure 31, trout exposed to PBCO for 10 mo and vaccinated against V. anguillarum survived bacterial challenge to the same degree as vaccinated, non-exposed controls; and in all cases the immunized fish survived at a higher rate than the nonimmunized controls.

The results of a preliminary assay with dispersant suggested, however, that simultaneous exposure of juvenile coho salmon to the oil-dispersant Corexit 9527 and V. anguillarum induced a greater rate of infection and subsequent mortality than that which occurred in fish similarly exposed to bacteria only (Table 35).

TABLE 34. LD_{50} values for oil-exposed and control rainbow trout immunized against Vibrio anguillarum and challenged with live bacteria.

Group	Concentration of PBCO in diet[a]	LD_{50} dose of V. anguillarum[b]
Immunized	1,000 ppm	9.2×10^4
	10 ppm	9.2×10^4
	None (control)	9.2×10^4
Nonimmunized	None (control)	2.3×10

[a] Fish were fed a PBCO-contaminated diet for 10 mo.
[b] LD_{50} values were calculated by the method of Reed and Muench (1938) using 5 bacterial concentrations with 10 fish/concentration.

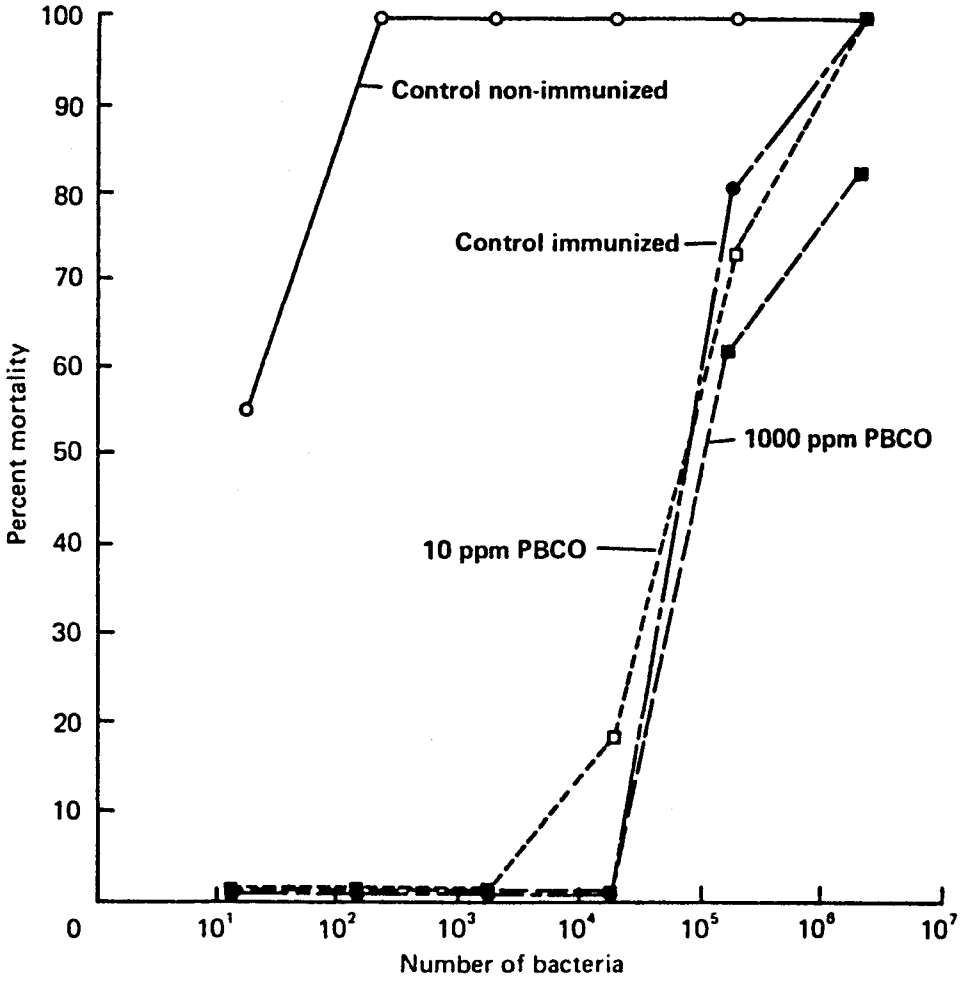


FIGURE 31. Effects of peroral exposure to PBCO for 10 mo on the immunocompetence of rainbow trout. Fish were vaccinated against Vibrio anguillarum by injection of heat-killed bacterial cells and challenged 21 days later with various concentrations of live bacteria. Each point represents percent mortality in a 10 fish group.

It was further demonstrated that exposure to PBCO for 10 mo did not markedly affect antibody levels or antibody formation. As shown in Table 36, the ability to synthesize antibodies, as reflected by agglutinin titer, appeared to decline slightly as a result of oil exposure; however, the difference was not statistically signficant (α=0.05). Moreover, the number of antibody-forming cells demonstrable in the spleens and anterior kidneys of trout exposed to 1,000 ppm PBCO did not differ substantially from those of controls. Numbers of plaque-forming cells per 10^6 lymphoid cells (PFC/10^6 lymphoid cells) in a 5-fish pool of kidney tissue from oil-exposed and control fish were 275 and 107, respectively. Numbers of PFC/10^6 lymphoid cells in splenic tissue were 193 and 218, respectively.

TABLE 35. Percent mortality among juvenile coho salmon exposed for 30 min to various concentrations of V. anguillarum and 30 ppm Corexit 9527, alone and in combination. Mortality was monitored for 10 days after exposure.

Treatment	Mortality % and (# dead/total #)
10^5 bacteria + Corexit	27 (8/30)[a]
10^5 bacteria only	3 (1/30)
10^4 bacteria + Corexit	17 (5/30)
10^4 bacteria only	3 (1/30)
Corexit only	0 (0/30)

[a] Significantly different than control of bacteria only (P=0.05)

Results of mitogenesis and polyclonal activation tests also suggested that long-term peroral exposure to PBCO did little to alter immunocompetence. Mitogenic stimulation indices (defined as the ratio of ^{3}H-thymidine incorporated by 10^6 Con A treated leukocytes/^{3}H-thymidine incorporated by 10^6 nontreated leukocytes) of splenic leukocytes from rainbow trout perorally exposed to 1,000 ppm PBCO for 10 mo and non-exposed controls were 15.0 $\pm$ 2.8 ($\bar{x}$ + SE) and 24.5 $\pm$ 5.0, respectively. Polyclonal lymphoid cell activition indices (expressed as the number of PFC/10^6 lymphoid cells) of peripheral blood leukocytes from rainbow trout similarly exposed to PBCO and controls were 17.3 $\pm$ 7.3 ($\bar{x}$ $\pm$ SE) and 14.6 $\pm$ 5.7, respectively.

One noteworthy finding of the rainbow trout studies was the observation of a significantly changed (reduced, P = 0.05) spleen/body weight in those fish maintained on a diet containing 1,000 ppm PBCO for 15 mo. The average ratio of spleen weight (in mg) to body weight (in g) for control fish was 0.69 while the ratio was 0.49 for the PBCO-exposed group. Subsequent testing indicated this reduction did not correlate with either a reduced number of erythrocytes or leukocytes-thrombocytes in the spleen or head kidney (Table 37), or in the peripheral blood (Table 38).

Flatfish. The results of disease resistance screening tests with adult starry flounder and adult rock sole exposed to PBCO are shown in Table 39. No significant differences (α=0.05) in mortality were observed in either starry flounder exposed for 2 or 6 wk to 1800 ppm PBCO in sediment or rock sole exposed for 2 wk to 2500 ppm PBCO in sediment and non-exposed controls when challenged with the same concentration of pathogenic bacteria.

TABLE 36. Effect of peroral exposure to PBCO for 10 mo on antibody formation in rainbow trout. Antibody formation was assessed by measuring agglutinating antibody titer to V. anguillarum bacterin 21 days after immunization. Results represent the geometric mean titers (log_2) of 10 fish from each exposure regime.

Concentration of PBCO added in diet	Mean antibody titer against V. anguillarum bacterin
1,000 ppm	4.3
10 ppm	5.2
None, immunized	5.7
None, non-immunized	<1

TABLE 37. Comparison of mean erythrocyte (rbc) and leukocyte-thrombocyte (wbc-t) counts from homogenates of spleen and anterior kidney of PBCO-exposed (10 ppm in diet for 15 mo) and control rainbow trout. Values are expressed as cells/mg of homogenate. ($\bar{X} \pm$ SD, N=10).

Group	Anterior kidney		Spleen	
	rbc	wbc-t	rbc	wbc-t
Oil-exposed	400 ± 134	600 ± 316	1400 ± 346	750 ± 209
Control	260 ± 100	530 ± 83	1100 ± 409	1000 ± 259

TABLE 38. Comparison of mean erythrocyte (rbc) and leukocyte-thrombocyte counts in the peripherial blood of rainbow-trout perorally exposed to 1,000 ppm PBCO for 15 mo, and controls.

	Exposure	
	1,000 ppm PBCO	Control
No. of rbc's[a]	1.2×10^6	1.4×10^6
No. of leukocytes-thrombocytes[a]	4.1×10^5	4.2×10^5

[a] Values expressed are cells per cm^3 and represent the average of 10 fish.

TABLE 39. Result of screening of flatfish for changes in disease resistance after exposure to PBCO-contaminated sediment.

Test species and exposure conditions	Number of bacteria	Percent Mortality[b] oil-exposed	control
Starry flounder exposed 2 wk on sediment contaminated with 1800 ppm PBCO[a]	$1.1x10^8$	80	60
	$1.1x10^7$	60	40
	$1.1x10^6$	0	0
	$1.1x10^5$	0	0
Starry flounder exposed 6 wk on sediment contaminated with 1800 PBCO	$1.3x10^8$	80	60
	$1.3x10^7$	60	40
	$1.3x10^6$	0	0
	$1.3x10^5$	0	0
Rock sole exposed 2 wk on sediment contaminated with 2500 ppm PBCO	$2.2x10^8$	80	80
	$2.2x10^7$	60	40
	$2.2x10^6$	0	0
	$2.2x10^5$	0	0

[a] Concentration of PBCO was determined at the start of exposure period.
[b] 5 fish per group

The results of testing of the effects of CICO on disease resistance of juvenile flatfish are shown in Table 40. English sole exposed to oil-contaminated sediment for up to 2 wk showed no significant difference (α=0.05) from controls in their ability to survive a laboratory bacterial challenge. The accompanying analyses of sediment, water, and liver tissue documented both the availability and uptake of petroleum hydrocarbons by the exposed fish.

Spot Shrimp. The results of tests in which adult spot shrimp were exposed for up to 4 wk to sediment contaminated with CICO are summarized in Table 41. LD_{50} values computed from mortality data following challenge with V. anguillarum indicated no significant differences (α=0.05) in disease resistance. Results of the analyses of sediment, water, and soft tissue for TEPH demonstrated that the petroleum hydrocarbons were both bioavailable and taken up by the exposed shrimp.

6.2.2 Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment

Five experiments were conducted in 1977 through 1979; parameters of the individual experiments are listed in Table 42.

TABLE 40. Results of disease resistance tests on CICO-exposed and non-exposed juvenile English sole and related petroleum hydrocarbon analyses.

Sediment Condition	Duration of Exposure (h)	LD_{50}[a] (95% confidence interval)	Total Petroleum Hydrocarbons (ppm)		
			Sediment	Water[b]	Liver Tissue[c]
Oil-contaminated	0	ND[d]	461.0	2,900	ND
Control	0	ND	0.3	0.002	ND
Oil-contaminated	24	6.2×10^5 (3.3×10^5-1.2×10^6)	295.0	0.180	4.2
Control	24	1.4×10^6 (7.3×10^5-2.6×10^6)	0.3	0.004	1.7
Oil-contaminated	168	2.9×10^6 (1.3×10^6-4.9×10^6)	282.0	0.037	8.0
Control	168	4.0×10^6 (1.5×10^6-6.8×10^6)	0.3	0.009	1.0

[a] LD_{50} values and their 95% confidence intervals were calculated by logit analysis using 5 bacterial concentrations.
[b] Water was collected 2 cm above the sediment-water interface.
[c] Values represent composite sample from 3 fish.
[d] ND=not determined.

TABLE 41. Results of disease resistance tests on CICO-exposed and non-exposed adult spot shrimp and related petroleum hydrocarbon analyses.

Sediment Condition	Duration of Exposure (h)	LD_{50}[a] (95% confidence interval)	Total petroleum hydrocarbons (ppm)		
			Sediment	Water[b]	Soft Tissue[c]
Oil-contaminated	0	ND[d]	1,015.0	0.690	ND
Control	0	ND	0.4	0.019	6.1
Oil-contaminated	24	2.4×10^6 (1.2×10^6-4.9×10^6)	714.0	0.077	40.4
Control	24	2.5×10^6 (1.1×10^6-5.4×10^6)	0.4	0.016	11.0
Oil-contaminated	168	2.5×10^7 (1.2×10^7-5.1×10^7)	475.0	0.002	16.3
Control	168	1.9×10^7 (1.0×10^7-6.4×10^7)	0.5	0.002	10.1
Oil-contaminated	672	2.8×10^7 (1.3×10^7-6.4×10^7)	234.0	0.023	11.8
Control	672	1.4×10^7 (7.8×10^6-2.4×10^7)	0.3	0.018	5.0

[a] LD_{50} values and their 95% confidence intervals were calculated by logit analysis using 5 bacterial contrations with 20 shrimp/concentration.
[b] Water was collected 2 cm above the sediment-water interface.
[c] Values represent a composite sample of abominal and thoracic tissues from 3 shrimp.
[d] ND, not determined.

TABLE 42. Experimental conditions employed in evaluating pathological changes in flatfish resulting from exposure to oil contaminated sediment.

Experiment Number	Month/ Year	Initial Oil Conc. % (vol/vol)	Flatfish Species	Age (years)	Number of fish	Sediment type	Duration (days)
1	2/77	0.2	English sole	1-3	35	high-sand	120
2	2/78	0.5	English sole Rock sole	1-2 1	41 41	high-silt	29
3	7/78	0.5	Starry flounder	0-1	50	high-sand	62
4	12/78	1.0	English sole	0-1	44	high-silt	126
5	9/79	1.0	English sole	0-1	50	high-sand	42

Concentrations of Sediment-Associated Petroleum Hydrocarbons

Sediment characteristics greatly influenced the retention of petroleum hydrocarbons. For example, in experiments 2 and 3 in which two different types of sediments received 0.5% PBCO, the high-sand type lost 77% of the TEPH during the first month; the high-silt type lost only 16% during the same period (Fig. 32). This difference between the rates of release of petroleum hydrocarbons was also reflected in the concentrations of TEPH in the interstitial water of the sediments (215 ppm in high-sand and 14 ppm in high-silt) 24 hr after they were mixed with oil and placed in aquaria with flowing seawater.

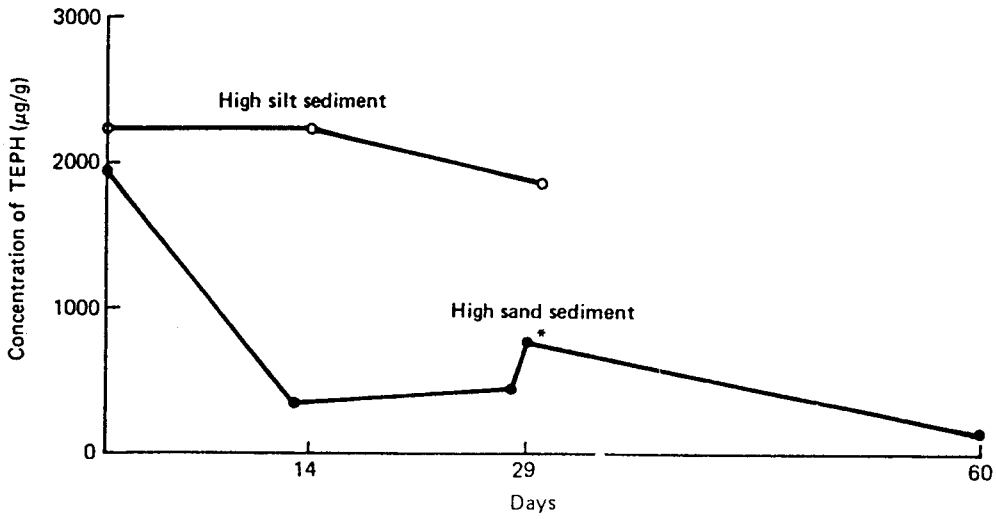


FIGURE 32. Concentrations of TEPH in PBCO-contaminated sediments from Experiment 2 (high-silt content sediment) and Experiment 3 (high-sand content sediment). *Indicates remixing of sediment. (From McCain and Malins 1982)

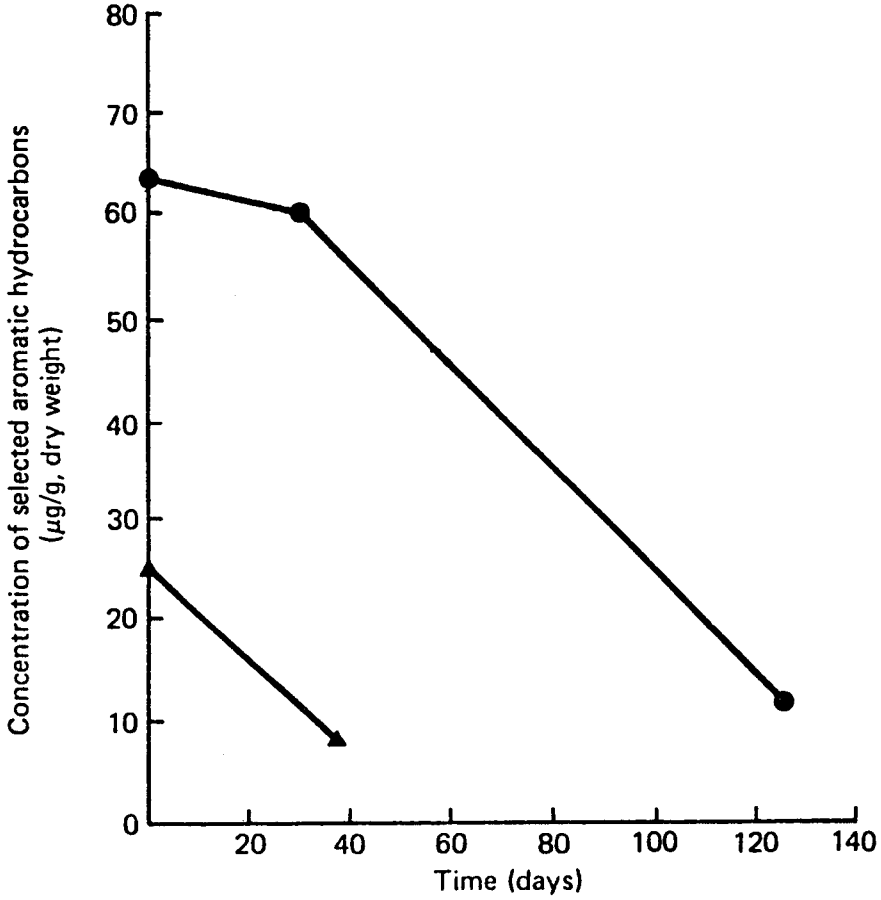


FIGURE 33. Concentrations of major aromatic hydrocarbons (see Figs. 34, 35) in PBCO contaminated sediments from Experiment 4 (high-silt content sediment = ●) and Experiment 5 (high-sand content sediment = ▲). Both experiments had initial PBCO concentrations of 1.0% (v/v).

Similar results were obtained in Experiments 4 and 5, in which the sediments received 1% PBCO (Fig. 33). The high-sand sediment lost 70% of the petroleum hydrocarbons during the first 38 days; the high-silt, only 4% after 30 days. By 126 days the high-silt sediment had lost an additional 77%. (The high-sand experiment was terminated after 42 days.)

The composition of the aromatic hydrocarbons (AHs) in the two sediment types from Experiments 4 and 5 differed slightly. Initially and at 30 days the relative concentration of the low molecular weight AHs in the high-silt sediment (Fig. 34) was higher than in the high-sand sediment at 38 days (Fig. 35). After 126 days all hydrocarbons measured in high-silt content sediment were greatly reduced and approximated the hydrocarbon concentrations found in high-sand content sediment after 38 days.

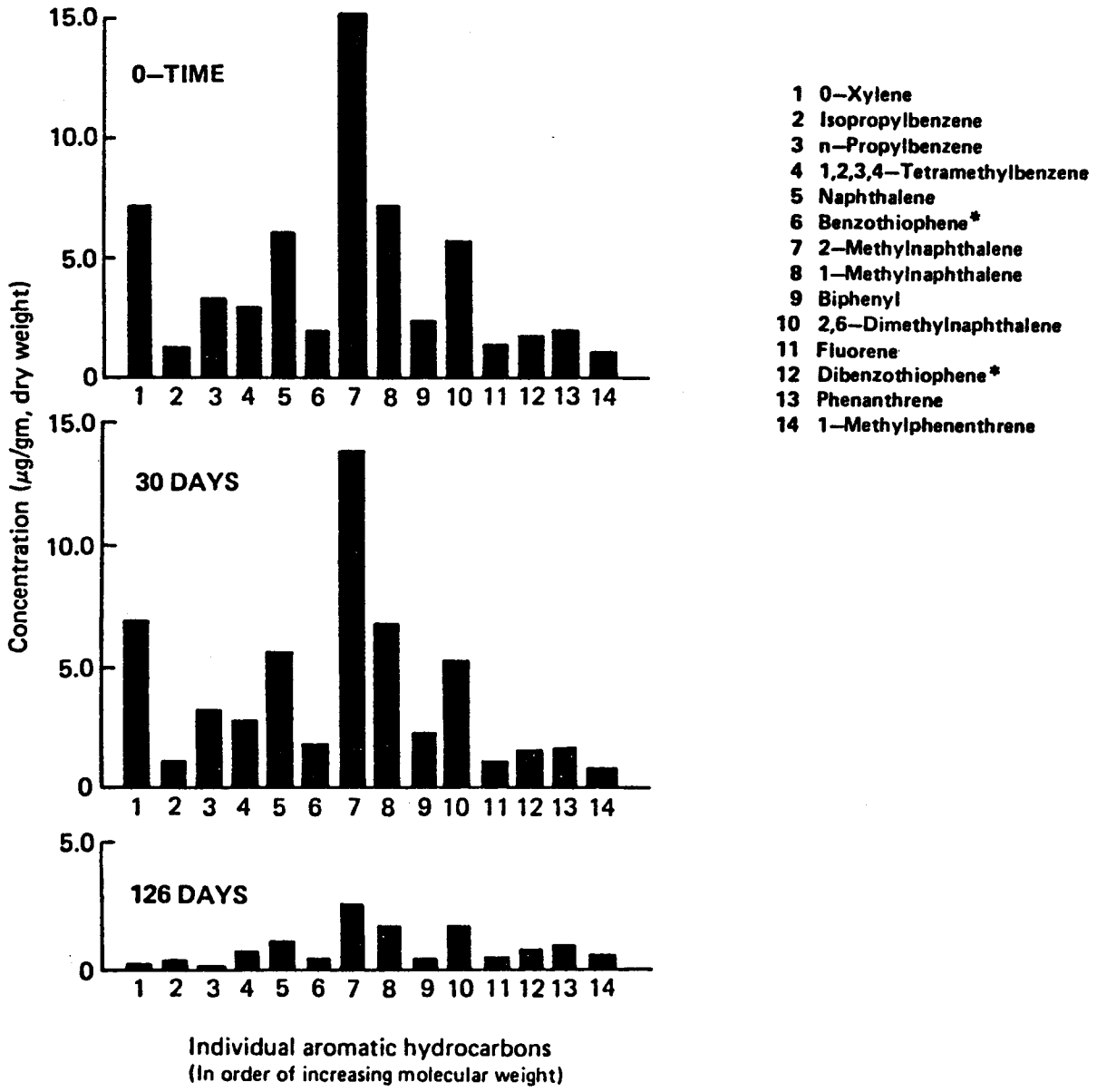


FIGURE 34. Concentrations of major aromatic hydrocarbons in PBCO-contaminated high-silt content sediment (Experiment 4) initially (0-time), and at 30 and 126 days. *Sulfur containing compound.

Tissue Uptake of Sediment-Associated Petroleum Hydrocarbons

In Experiment 1 (Table 42), using high-sand sediment and 0.2% PBCO, several aromatic hydrocarbons were detected in liver, skin and muscle of fish analysed after 11 days of exposure (Fig. 36). No AHs were found in the tissues of control fish. Test fish analyzed at 27 and 60 days had detectable levels of AHs only in liver tissue. 1-Methyl-naphthalene, 2-methylnaphthalene, and 1,2,3,4-tetramethylbenzene were

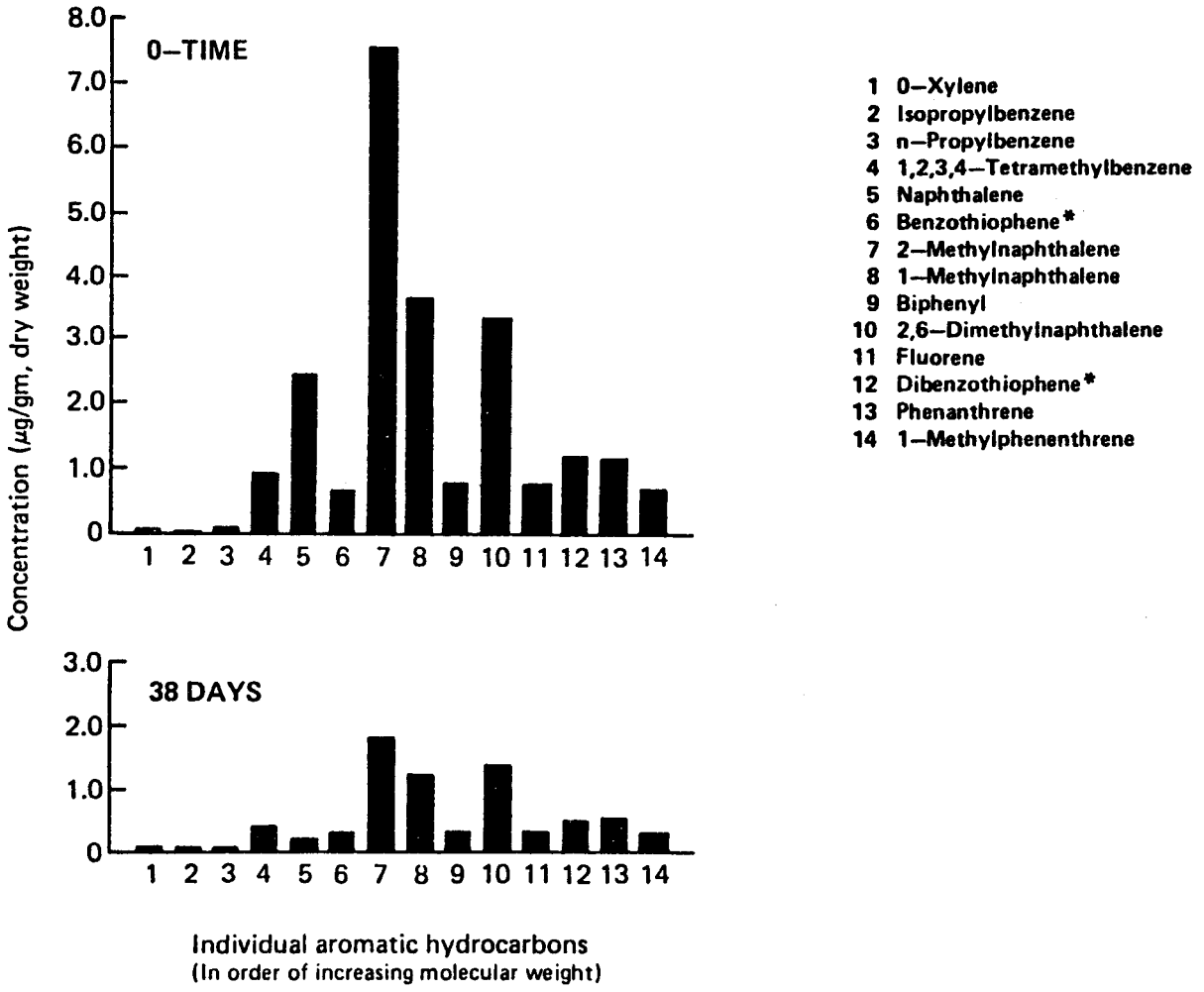


FIGURE 35. Concentrations of major aromatic hydrocarbons in PBCO-contamined high-sand content sediment (Experiment 5) initially (0-time), and at 38 days. *Sulfur containing compound.

the most abundant AHs detected in the livers during the first 4 wk of exposure. Detectable amounts of only the 2 latter hydrocarbons were found after 60 days.

Tissues of test fish from Experiment 1 also had substantial levels of several alkanes (C-11, 13, 17, 26, 28, and 31); concentrations of some of these compounds were as much as five-fold higher than tissues from control fish. At 27 and 60 days, however, the alkane concentrations were the same in both exposed and control groups.

The uptake of AHs by English sole and rock sole in Experiment 2 with high-silt sediment and 0.5% PBCO (Fig. 37) was less than the AH uptake from high-sand sediment with 0.5% PBCO (Fig. 36). The AHs were noticeably higher in tissues of rock sole than in tissues of English sole (Fig. 37).

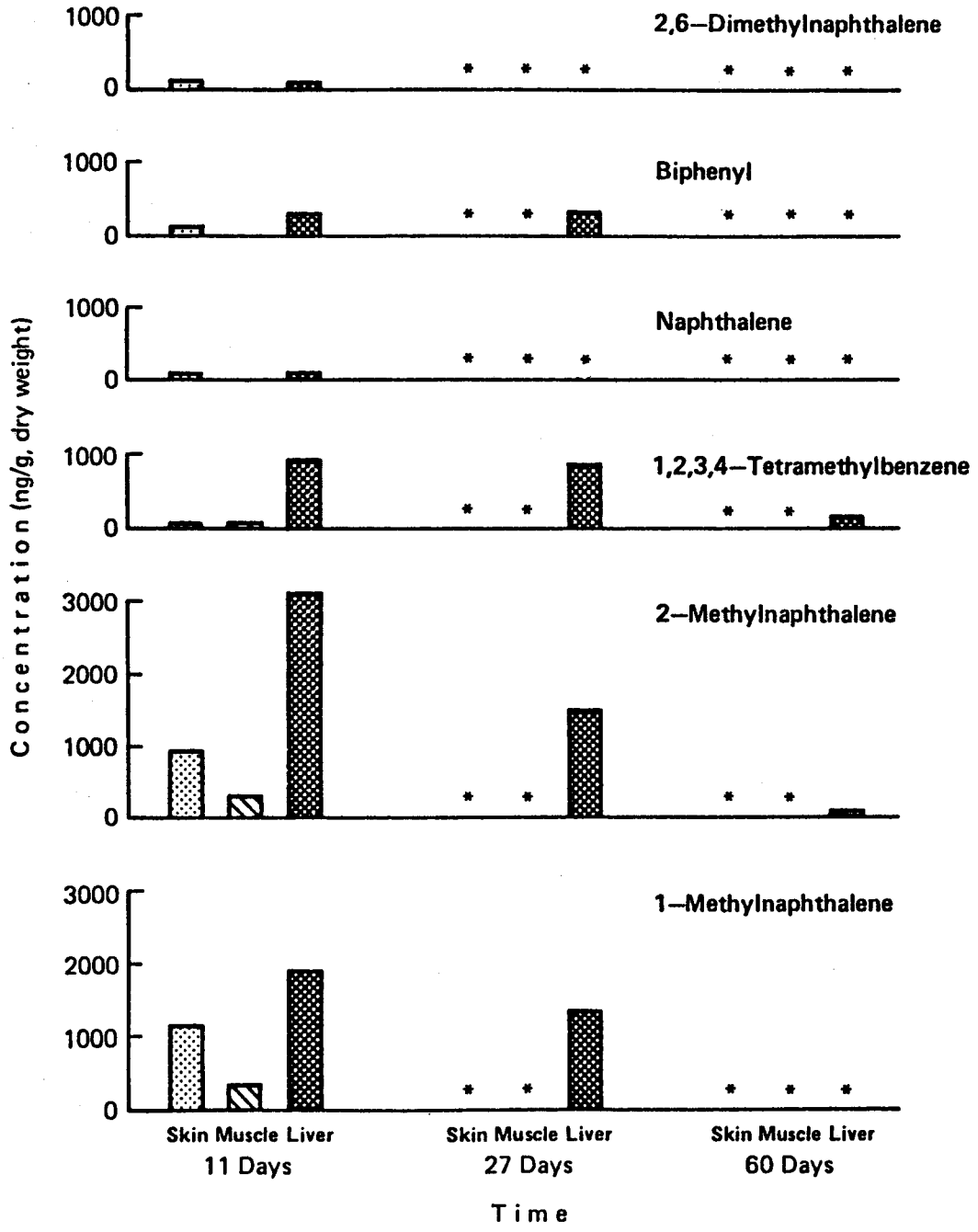


FIGURE 36. Concentrations of aromatic hydrocarbons in tissues of English sole exposed to PBCO-contaminated sediment for 11, 27, and 60 days. *Indicates not detected. (Derived from McCain et al. 1978)

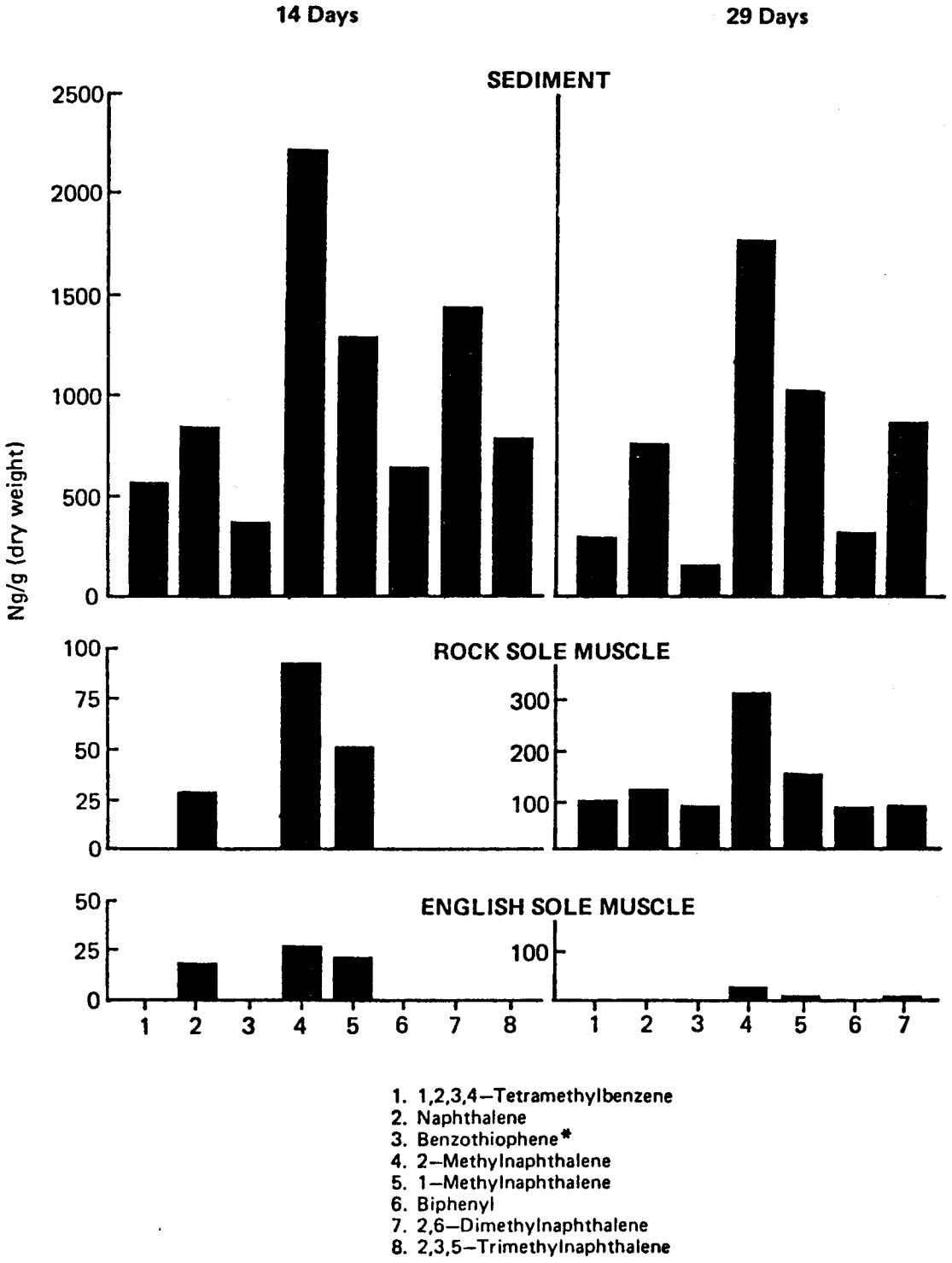


FIGURE 37. Concentrations of major aromatic hydrocarbons in PBCO-contaminated high-silt sediment and in the English sole and rock sole associated with the sediment at 14 and 29 days. Tissue levels are an average of 10 g muscle samples from 2 fish (Experiment 2). *Sulfur containing compound. (From McCain and Malins 1982)

Muscle tissue of English sole exposed for 91 and 126 days in Experiment 4 (high-silt, 1.0% PBCO) had no detectable AHs. However, the muscle tissue of the English sole exposed to 1.0% PBCO in high-sand sediment (Experiment 5) had detectable concentrations (26 and 41 ppb dry wt) of 1- and 2-methylnaphthalene after 38 days.

Biological Effects of Exposure to Oiled Sediments

Rock sole and starry flounder sustained high mortalities (30-50%) in both control and exposed groups after 30-60 days, whereas English sole had low mortalities in both of the 120 day tests (Experiments 1 and 4). As a result English sole were used to assess the biological effects of long-term exposure to oil-contaminated sediments.

In Experiment 1 both test and control fish lost weight during the first 2 mo, with the oil-exposed group losing more than the controls (Fig. 38). After this period, the control fish began to regain weight and at 4 mo, only 27% of the animals weighed less than at 2 wk. The oil-exposed fish were slower in their recovery from the initial weight loss, and at 4 mo 69% weighed less than they did initially. The numbers of fish gaining and not gaining weight at 4 mo were significantly different between the experimental and control groups (P=0.05). These results are apparently related to reduced feeding in the oil-exposed group, as indicated by consistently more uneaten food removed from the test aquarium than from the control aquarium.

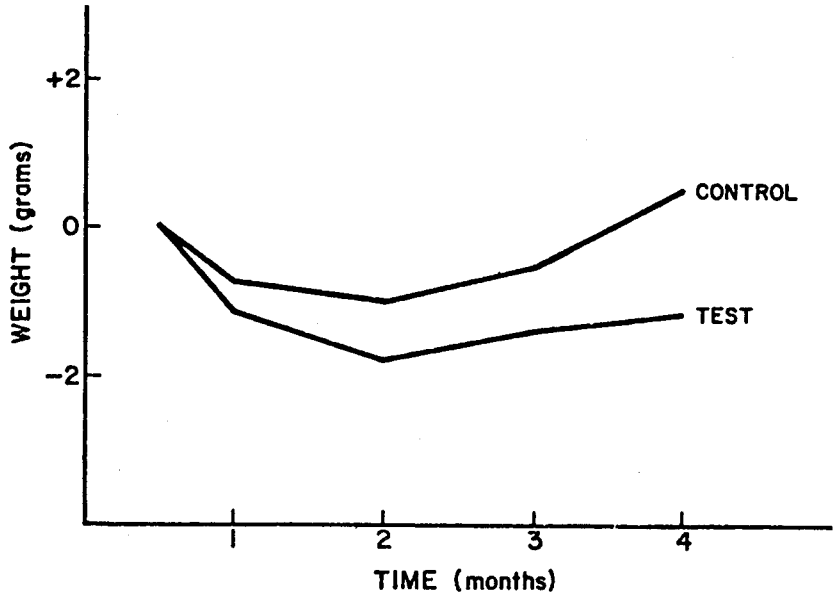


FIGURE 38. Weight changes in oil-exposed and control English sole over a period of 4 months (Experiment 1). Each observation at monthly intervals represents the average change (g) from weights at 2 wk for 16 test and 15 control fish. (From McCain et al. 1978)

During the first month of Experiment 4, there were slight but comparable weight losses in both test and control groups. Both groups recovered the lost weight, however, by the second month. Feeding responses were similar in both groups.

There was no consistent differences in any of the hematological parameters measured (hematocrit, hemoglobin, and total RBC; total and differential leukocyte counts) that could be related to oil exposure. Examination of spleen, kidney, intestine, fins, gills, or skin of English sole for histopathological changes revealed no differences between exposed and unexposed animals in either Experiment 1 or 4. In Experiment 1, however, more of the livers of oil-exposed fish had hepatocellular lipid vacuolization (HLV). HLV is manifested as a replacement of the cytoplasm by lipid vacuoles and was considered severe when 95% replacement occurred. This condition was found in its most severe form in half of the exposed animals during the first month of exposure; none of the controls of developed HLV. During the first month of Experiment 4 most test and control fish had severe HLV. After the first month this condition was rarely identified in either group.

6.2.3 Cytopathology

Effects of PBCO on Adult Flatfish and Salmonids.

Coho salmon and starry flounder exposed to 100 ± 90 ppb ($\bar{x}$, ± range) of the SWSF of PBCO for 5 days in a flow-through saltwater system (Roubal et al. 1977b) developed gill lesions resulting from the loss of the surface cells (Fig. 39). Immature mucous glands below the surface were exposed when the surface sloughed and their contents, in some instances, were exuded. The area of sloughing varied among gill filaments: 10 to 30 cells were lost in the smaller lesions and, in a few cases, the surface of the entire filament lost its outermost layer of cells.

Rainbow trout were fed PBCO in Oregon moist pellet (OMP) for five days per week (average of 120 mg PBCO/kg body wt/day). After 2 weeks of feeding, the hepatocytes of control fish contained large amounts of glycogen, whereas those of the oil-exposed fish had virtually none. Proliferation of the endoplasmic reticulum was evident in hepatocytes from oil-exposed fish only, and cochlear ribosomes, a common feature of cells rapidly synthesizing proteins (i.e., in embryos), were apparent in oil-exposed fish hepatocytes (Fig. 40).

In a 75 day feeding experiment with the same exposure regime, all fish gained weight and no mortalities were observed. At the termination of the experiment, the control fish had gained an average of 95.5% in body weight; the oil-fed fish, 70.5%. Glycogen deposits in the hepatocytes of test fish showed the same striking decrease as observed in the previous 14 day experiment. In addition, liver lipid reserves were reduced in the oil-fed fish.

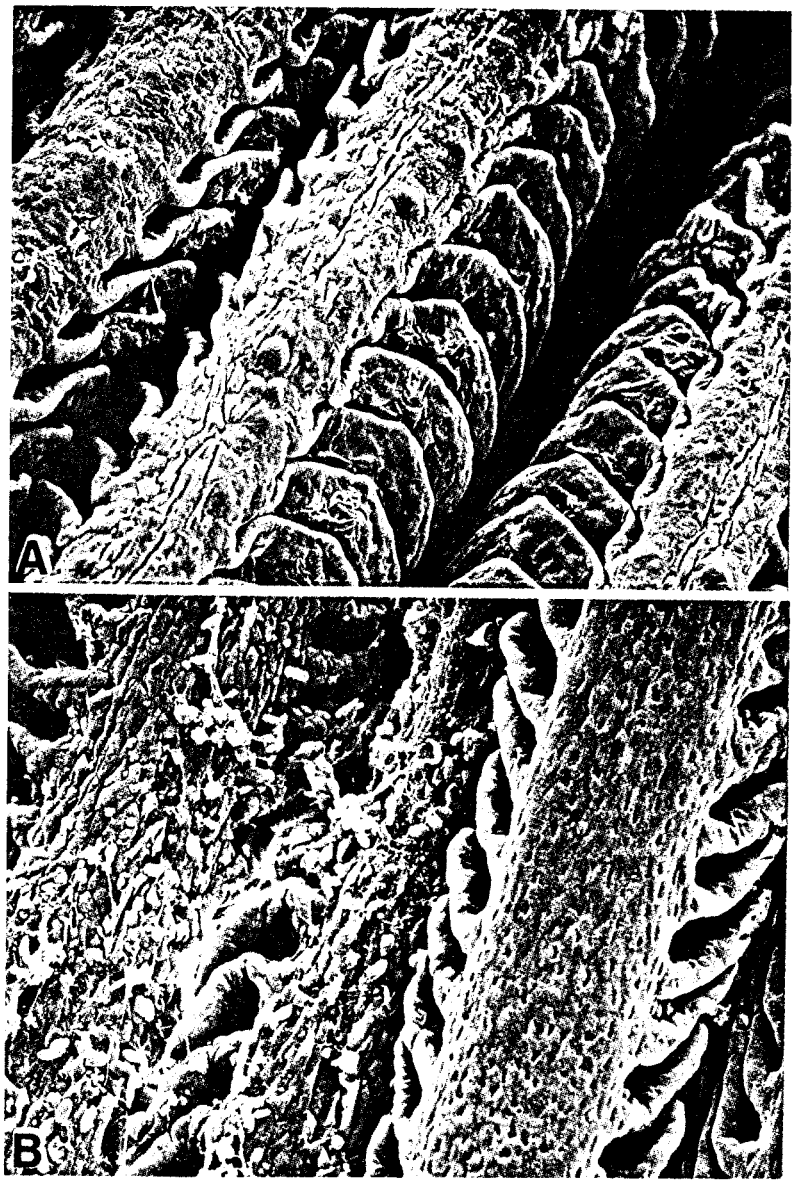


Figure 39A. SEM of gill from an untreated adult coho salmon. X 370. (From Hawkes 1977.)

Figure 39B. SEM of gill from an adult coho salmon exposed to 100 ppb of the SWSF of PDCO. Sloughed surface cells and an abundance of exuded mucus are present on two of the three gill filaments. X 370. (From Hawkes 1977.)

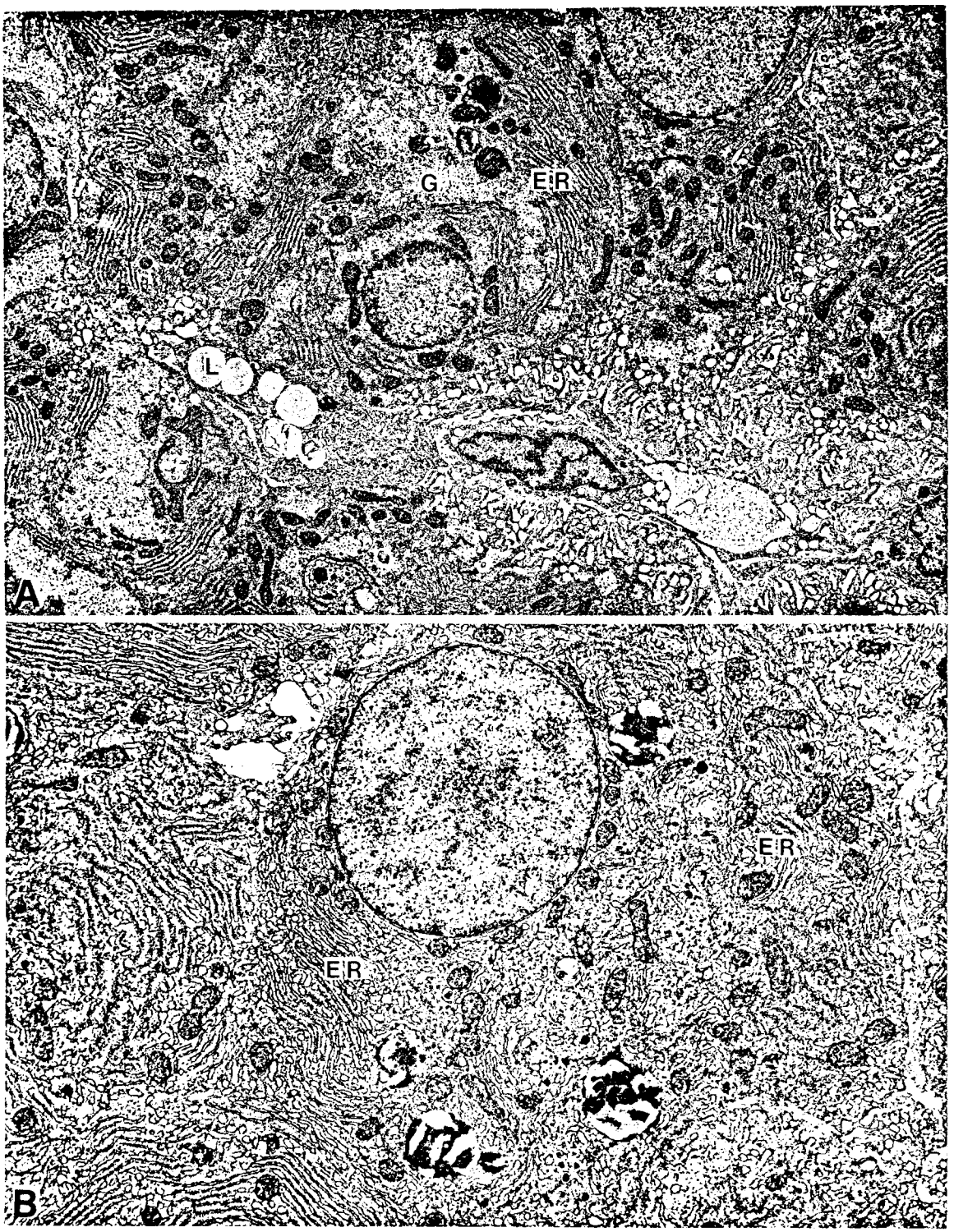


Figure 40A. TEM of untreated adult rainbow trout liver. There are abundant glycogen (G), lipid droplets (L), and several layers of endoplasmic reticulum (ER) at the periphery of the hepatocytes. X 6,400 (From Hawkes 1977.)

Figure 40B. TEM of liver section from an adult rainbow trout fed petroleum for 75 days. In addition to a lack of glycogen in the hepatocytes, the endoplasmic reticulum (ER) had proliferated, nearly filling the cytoplasm. X 7,500. (From Hawkes 1977.)

Rainbow trout fed a PBCO-contaminated diet (1000 ppm added to food) for 8 mo were sampled for microscopy at the time of spawning. Both TEM and LM analysis indicated an abnormal increase in collagen around the liver sinusoids. Trout from this same study continued to be fed the oil-contaminated diet for another 4 to 5 mo after spawning; these fish exhibited abnormally soft and enlarged eye lenses. Of trout fed PBCO for 12-13 mo the eye lens volume was double that of controls; 226 $\pm$ 81 mm^3 ($\bar{x} \pm$ SD) and 111 $\pm$ 19 mm^3, respectively.

The lens is composed of ribbon-like filaments which interdigitate and form a sphere. The filaments have simple projections on their broad surfaces which plug into pits on the adjacent fiber; in addition, there are complex interlocking series of protuberances on their thin side (Fig. 41A). In trout exposed to a crude oil contaminated diet for either 75 days or one year, the fiber structure changed: the surface became distorted and the interdigitating projections were irregular. In a longer experiment, rainbow trout were fed PBCO (120 mg/kg body wt/day) for 3 years; three of five petroleum-exposed fish developed cataracts, whereas all three control fish had normal lenses. In lenses with cataracts, the fiber surface was irregular and the lateral projections were severely deformed (Fig. 41B).

Effects of weathered PBCO on early development of sand sole.

SEM examination of 5 sand sole larvae exposed to 164 ppb of the SWSF of weathered PBCO during embryogenesis revealed 4 of 5 with severely reduced (both numbers and length) olfactory cilia (Fig. 42); the fifth appeared normal (comparable to controls). In fish with reduced olfactory cilia, the epidermal microridges, a feature of normal keratinocytes, were absent from the keratinocytes surrounding the olfactory epithelium. In addition, the keratinocytes of these four larvae were rounded and protruded from the skin surface, an indication of cellular hypertrophy.

TEM examination of sections from 8 sand sole larvae (4 exposed to 164 ppb of the SWSF as embryos, and 4 controls) revealed ultrastructural changes in the mitochondria of epidermal cells of oil-exposed larvae. Although the sample size was too small to definitively correlate observed changes to oil exposure, the abnormal mitochondria showed classic hydropic changes, a reduction in cristae and a reduction in the electron density of the matrix. Separation between the outer and inner mitochondrial membranes was quite pronounced and breaks in both membranes were observed. Other organelles such as the nuclei, Golgi assemblies, and microtubules were normal.

Effects of weathered CICO on early development of surf smelt.

Morphology of unexposed embryos. Sections of entire 21 or 27-day-old control embryos were examined with light microscopy and electron microscopy and all tissues appeared normal. In neurons of the brain the heterochromatin was evenly distributed in small clusters throughout the nuclei. The

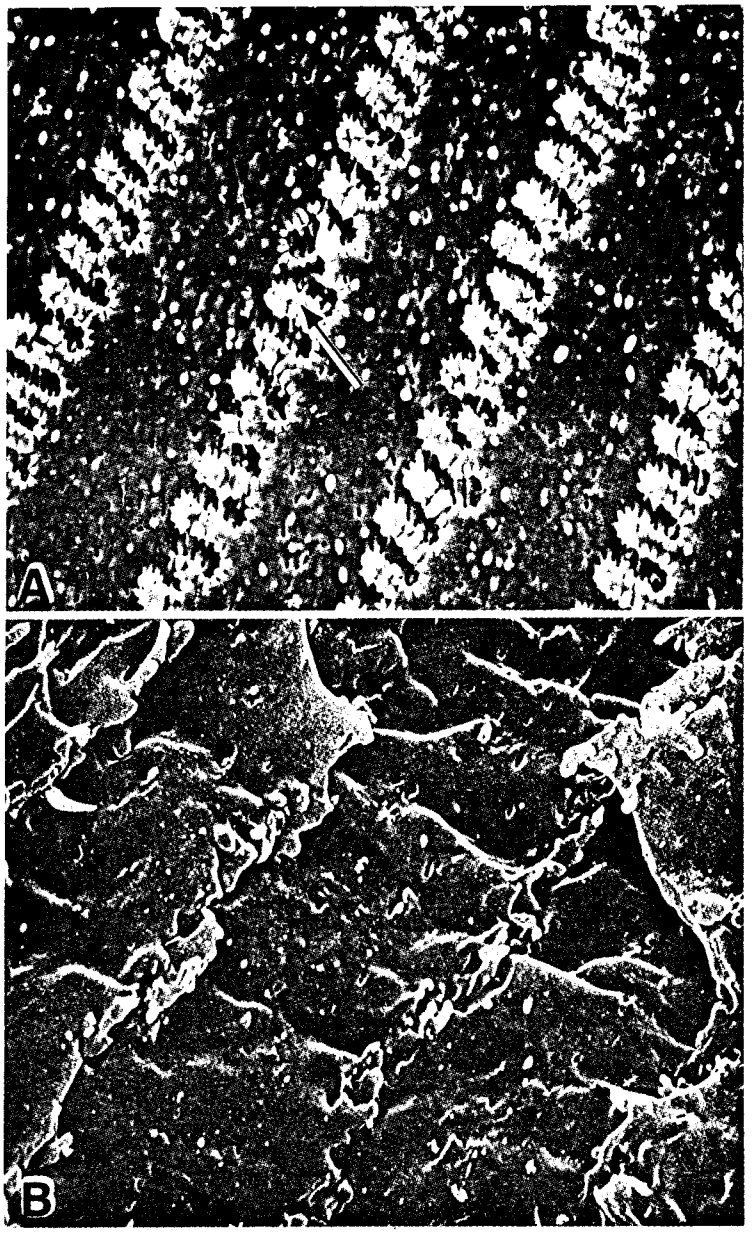


FIGURE 41A. SEM of normal lens fiber cells from adult rainbow trout. The lateral projections (arrow) suture the lens fibers together. X 2,900. (From Hawkes 1980)

FIGURE 41B. SEM of lens fiber cells from an adult trout perorally exposed to PBCO for three years. This fish had a cataract in both lenses. The lens fiber lateral projections are absent or grossly misshapen and the fiber surface is irregular. X 2,900. (From Hawkes 1980)

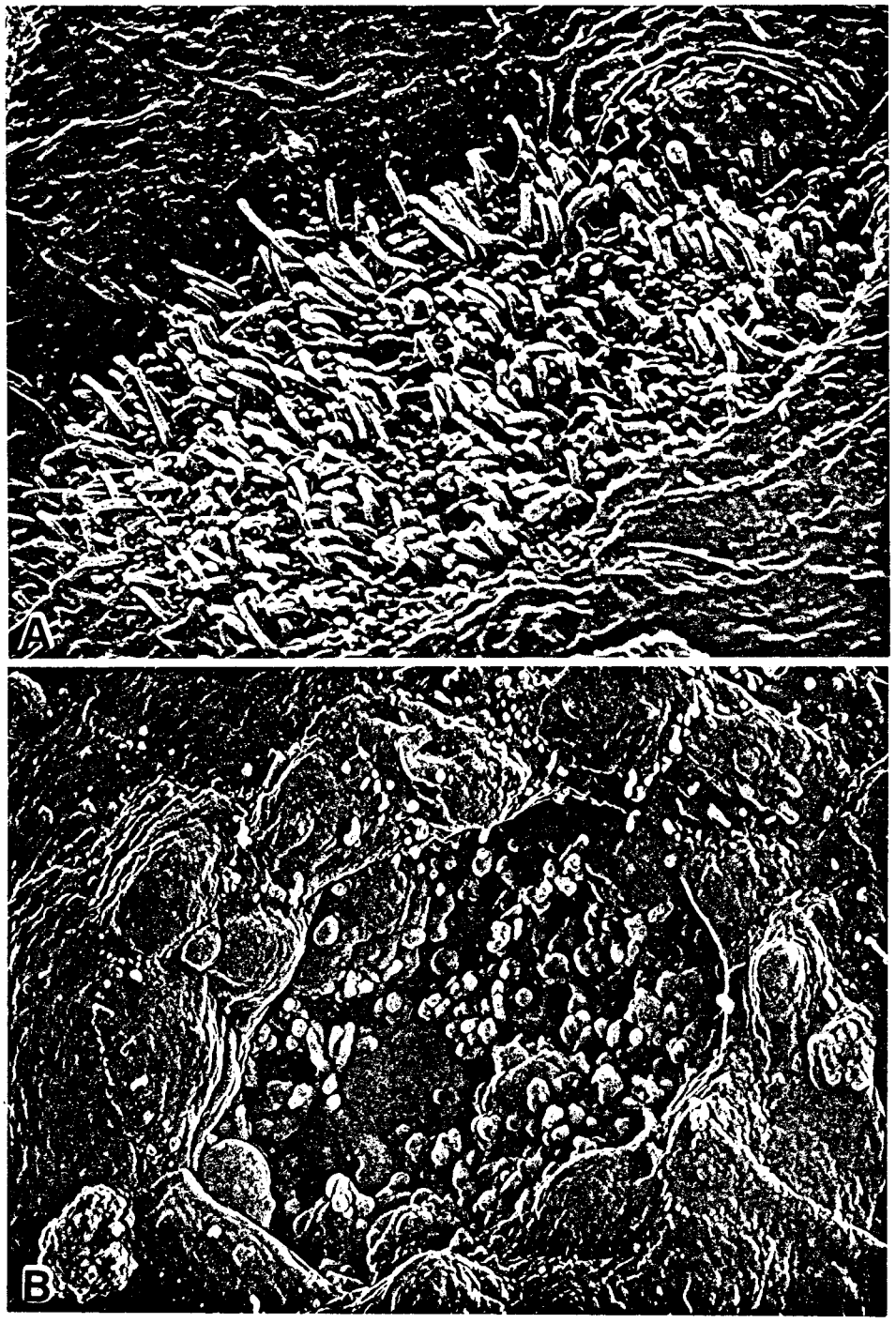


FIGURE 42A. SEM of normal olfactory epithelium from a sand sole larva. Chemosensory cilia about 2.5 um long protrude from the surface of the olfactory organ. X 3,000. (From Hawkes 1980)

FIGURE 42B. SEM of olfactory epithelium from a sand sole larva exposed to 164 ppb of the SWAF of CICO. The chemosensory cilia are reduced in size and numbers. X 3,000. (From Hawkes 1980)

cytoplasm of the neurons stained pale blue with Richardson's stain, and and the mitochondria, Golgi complexes, and polyribosomes appeared normal.

The retinas of both 21- and 27-day-old embryos had well-developed, melanized pigment epithelia with processes that extended between portions of the receptor cells. Only one type of receptor cell was observed in the embryos. This is typical of embryonic teleost cone cells (Ali 1959, Blaxter 1974), having a broad outer segment of tightly stacked membranes (saccules) containing the visual pigment, a connecting cilium, an inner segment with both ellipsoid and myoid regions, and a nuclear region at the innermost portion of the cell (Figs. 43A and 44A). At the outer boundary of the receptor cell, the processes of the pigment epithelium surrounded the tip of the cell, thus enclosing the area where the tightly stacked receptor membranes were located. The ellipsoid region was tightly packed with mitochondria and the myoid region was distinguished by numerous Golgi complexes, free ribosomes, and short profiles of rough endoplasmic reticulum. In addition to the cell nucleus, the inner portion of the receptor cells had synaptic complexes. Adjacent to the receptor cells were horizontal cells (Yamada and Ishikawa 1965, Kaneko 1970) of the neural zone of the retina. A region of embryonic neurons, 5-10 cells deep, lay between the horizontal cells and the internal plexiform layer. Adjacent to the internal plexiform layer was the ganglion cell layer followed by an optic nerve fiber layer which completed the inner retinal zones.

Morphology of CICO-exposed embryos. Neuronal damage was evident in the brain and eye in both age groups of embryos after exposure to 54 and 113 ppb CICO (Table 43); however, all other tissues appeared normal. In both the brain and eye, necrotic neurons appeared intensely blue-colored after application of Richardson's stain. These neurons also stained intensely with PAS reagents; no PAS reaction was evident in neuronal tissues of control embryos. Ultrastructurally, necrotic neurons were easily identified by their electron-dense nuclei and cytoplasm. In some nuclei, the heterochromatin was clumped in a central mass; in others, the heterochromatin was also condensed but distributed along the inside of the nuclear membrane. The cytoplasm of many of the necrotic cells was uniformly granular. However, some had clusters of autolysosomes which contained fragments of organelles such as mitochondria.

The retinal receptor cells of smelt embryos sampled 21 days after fertilization were normal, but the retinal receptors in 60 to 80% of the embryos sampled 6 days later had lesions (Table 43) localized in the ellipsoid and myoid regions of the inner segment. The most severe damage was in the myoid region (Figs. 43B and 44B). In contrast, the tightly stacked membranes of the outer segment were normal or only slightly disorganized, and the basal portion of the cell, which contains the nucleus, also appeared normal. In some embryos, the cytoplasm of the inner segment was filled with clear vacuoles. In damaged receptor cells, the mitochondria were observed in various stages of lysis. Commonly, the outer mitochondrial membrane was intact but the cristae were disrupted and vacuous areas were evident in the matrix.

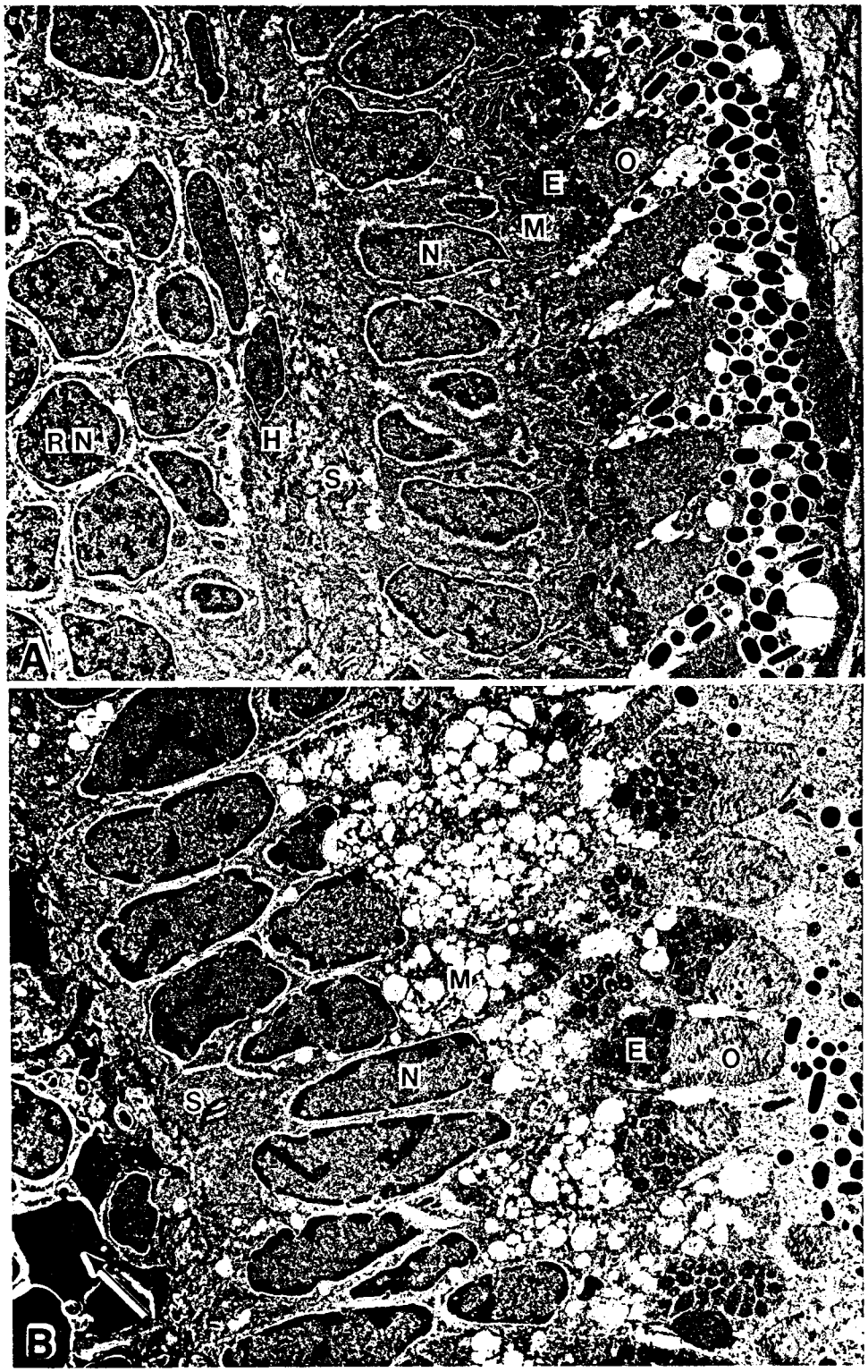
TABLE 43. Percent surf smelt embryos with cellular abnormalities of the eye and brain sampled 21 and 27 days after fertilization (45 and 63 hr oil exposure)

Average hydrocarbon concentration (ppb)	Percent with Cellular abnormalities					
	21 days post-fertilization			27 days post-fertilization		
	Vacuolated retinal receptor cells	Necrotic retinal neurons	Necrotic forebrain neurons	Vacuolated retinal receptor cells	Necrotic retinal neurons	Necrotic forebrain neurons
control	0 (4)[a]	0 (4)	0 (5)	0 (4)	0 (4)	0 (5)
54	0 (6)	83 (6)	72 (7)	80 (5)	80 (5)	80 (5)
113	0 (4)	75 (4)	80 (5)	75 (4)	80 (5)	66 (6)

[a] Number of embryos examined.
(From Hawkes and Stehr 1982)

FIGURE 43A. TEM of a portion of the retina from a normal 28-day-old smelt embryo. Regions of the receptor cells include the outer segment (O), ellipsoid region (E), myoid region (M), nucleus (N), and synaptic junctional complex (S). Neural cells of the retina shown in the micrograph include horizontal cells (H) and retinal neurons (RN). X 5,000. (From Hawkes and Stehr 1982)

FIGURE 43B. TEM of a portion of the retina of a 28-day-old exposed (113 ppb CICO) surf smelt embryo. Vesiculation is evident in the myoid regions (M) of the receptor cells and necrotic neurons are present (arrow). X 5,000. (From Hawkes and Stehr 1982)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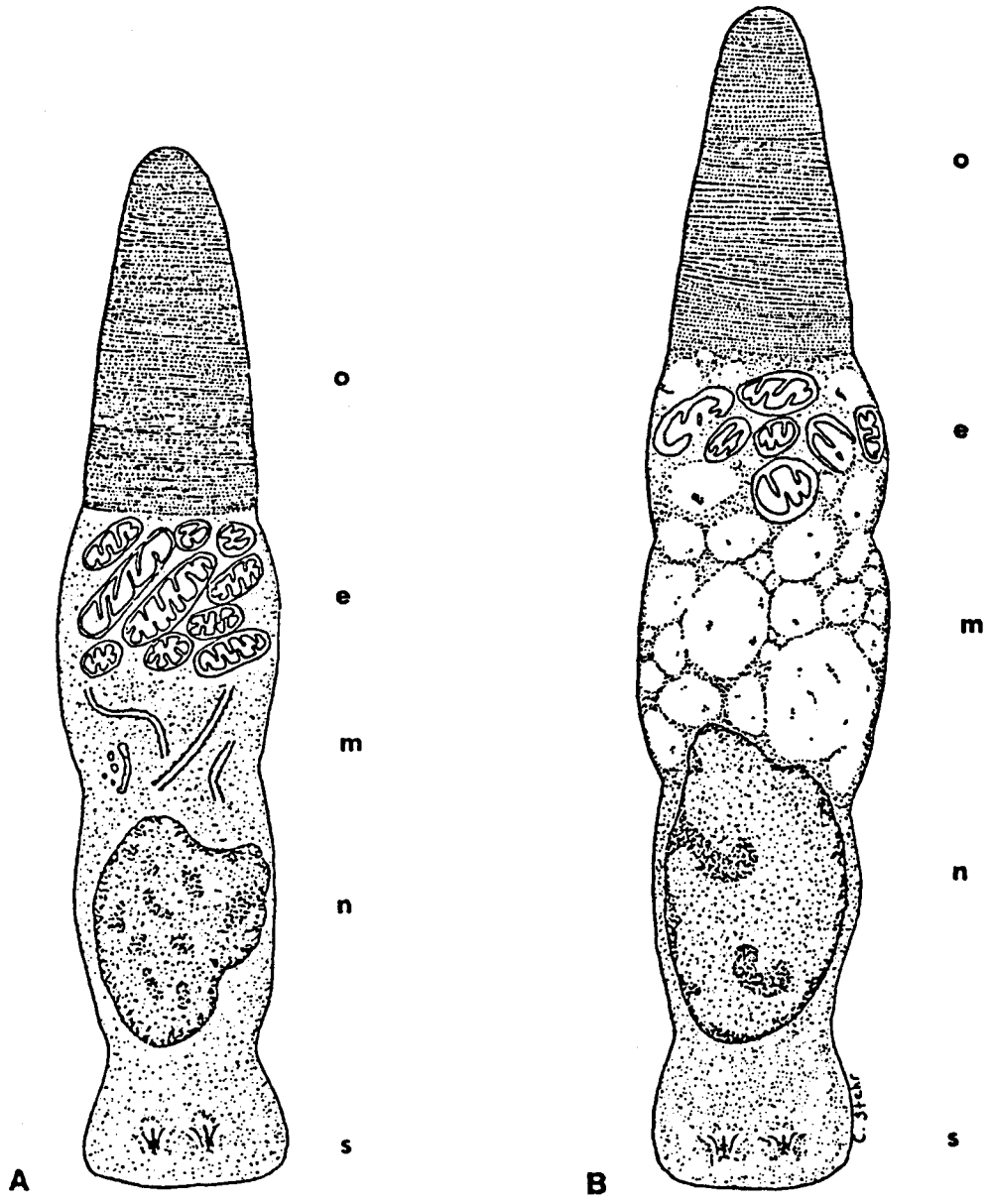

FIGURE 44. Schematic drawing of receptor cells from 28-day-old surf smelt embryos. (A) Control. (B) Exposed to 113 ppb of the SWSF of CICO (hypertrophy, vacuolization and chromatin margination are depicted). Regions of the cells are labelled: outer segment (O), ellipsoid region (e), myoid region (m), nucleus (n), and synaptic complex (s). (From Hawkes and Stehr 1982).

6.3 Behavior and Physiology

6.3.1 Vertebrate Studies

Behavior of Pacific Salmon Exposed to Petroleum Hydrocarbons

(a) Olfactory Disruption in Juvenile Coho Salmon. The first series of electrophysiological tests to evaluate the effect of petroleum hydrocarbons on the olfaction of seawater-adapted juvenile salmon were of an exploratory nature, and the data were not evaluated statistically. Exposure of the olfactory epithelium to both benzene (0.2 to 17.0 ppm) and NPH (0.2 to 2.0 ppm) for 25 seconds induced a rapidly diminishing oscillatory pattern from the olfactory bulb indicating stimulation, but no immediate injury to, or irritation of, the olfactory epithelium. Following perfusion of the nares with either 2 ppm NPH or benzene for 10 minutes, there was no reduction or inhibition of the immediate subsequent response to 10^3 L-serine, L-methionine, or L-alanine. When one part of 1.5 ppm NPH was mixed with an equal part of 10^3 L-serine the EEG response approximated that of the L-serine solution alone.

There was no evidence of the salmon detecting 2,6-dimethylnaphthalene, or 2,3,6-trimethylnaphthalene at concentrations of 0.2 to 2.0 ppm. The EEG response to these aromatics was similar to that of rinsing the nares with filtered saltwater; that is, there was a slight temporary decrease in baseline activity. In contrast, the SWAF of PBCO (1.8 to 20.0 ppm) was detected by the fish; however, there was no decrease in subsequent responses to amino acids. Threshold concentrations for discrimination were not determined.

In the second series of electrophysiological tests, activity of the olfactory bulb (EEG responses) of coho salmon following exposure of the nares to a standard stimulant (10^{-3} M L-serine) were compared with EEG responses induced by different concentrations of an aromatic hydrocarbon mixture representative of the SWAF of PBCO (Table 44). A significant increase in the amplitude of the EEG response to the aromatic hydrocarbon mixture occurred at concentrations between 1.9 and 2.8 ppm; this is less than the 2.8 to 3.7 ppm concentrations which resulted in statistically significant avoidance of the hydrocarbons by juvenile coho salmon tested at the same time of year and at the same temperatures (Maynard and Weber 1981).

Coho salmon exposed to a 4.0 ppm aromatic hydrocarbon mixture for up to 20 min showed no significant change in EEG response to 10^{-3} M L-serine given before and after exposure (Table 45).

(b) Migratory and Homing Behavior in Adult Salmon

Avoidance reaction. Control observations (i.e., percentage of salmon migrating up the east or west fish ladders when aromatic hydrocarbons were not added) and tests with hydrocarbons were made during the peak of salmon migration in Chambers Creek. More than 1,400 salmon ascended the dam during control periods; 56% of these

TABLE 44. Effect of a mixture of monocyclic aromatic hydrocarbons (see Table 1) on the olfactory bulb EEG as measured by amplitude of response to test mixture compared response to control water, and as percent of 10^{-3} M L-serine response.

Hydrocarbon Mixture (ppm)	Number of tests[a]	Average amplitude of EEG response (SD)[b] Control	Test	Significance[c]	% of 10^{-3} M L-serine EEG response (average)
1.9	10	37 (27)	23 (24)	NS	7
2.8	20	35 (24)	87 (52)	***	25
3.7	15	31 (19)	117 (49)	***	32

[a] Equal numbers in each of control and test groups.
[b] Relative values calculated from area (in cm^2) of integrated EEG response between 2 and 6 sec after introduction of stimulant to naris.
[c] NS, not significant ($p>0.05$); ***$P<0.001$
(From Maynard and Weber 1981)

TABLE 45. Comparison of olfactory bulb EEG response to 10^{-3} M L-serine before and after rinsing nares with a 4.0 ppm mixture of monocyclic aromatic hydrocarbons.

	Duration of hydrocarbon mixture rinse (min) <1	10	20
Difference ($\bar{x}\pm$SE)	6.4±16.7	42.7±17.5	-36.2±34.1
Number of fish tested	6	6	5
Significance	NS[a]	NS	NS

[a] Relative values calculated from area (in CM^2) of integrated EEG response between 2 and 6 sec after introduction of L-serine.
[b] NS, not significant (= 0.05).
(From Maynard and Weber 1981)

chose the west ladder (Table 46). Another 1,149 salmon migrated over the dam during test periods when hydrocarbons were added to the water in the west fish ladder.

Of the total number of salmon ascending the two fish ladders during the study period, 99% were coho salmon and the remaining 1% were a mixture of pink, chum, and chinook.

Data was analyzed using hydrocarbon concentrations based on GC analysis rather than the approximately 40% greater concentrations calculated from the amount of hydrocarbon mixture used and water flow. The Francis formula used for calculating water flow in the fish ladder is a rough approximation of weir discharge, and tends to underestimate water volume at high velocities representative of the field study site. Because of the consistency of hydrocarbon concentrations in replicate

TABLE 46. Numbers of salmon ascending fish ladders of Chambers Creek dam when aromatic hydrocarbons were, and were not (control), present in water of west ladder.

Date	Observation and Test Period(s) (h)	Salmon Ascending Ladders			Hydrocarbon Concentration in West Ladder (ppm)		
			%			GC Analysis	
		Total	East	West	Calculated[a]	Top of ladder x̄ + range	Middle of ladder x̄ + range
Control							
29 Oct. to 15 Nov.	6 to 24	1431	44 (±7.8 sd,N=9)	56	0		
Test							
2 Nov.	22.0	236	56	45	1,900	1330 ± 280	b
3 Nov.	6.0	107	69	31	4,900	2690 ± 280	2900 ± 360
8 Nov.	18.5	56	75	25	5,300	2130 ± 30	2130 ± 160
11 Nov.	7.0	59	64	36	2,700	1490 ± 140	950[c]
12 Nov.	16.0	139	55	45	1,900	1160 ± 70	970[c]
13 Nov.	4.5	35	71	29	4,400	2500 ± 10	2250 ± 30
13 Nov.	6.0	61	93	7	6,100	3750 ± 110	3720[c]
14 Nov.	5.0	126	56	44	1,000	530 ± 50	b
16 Nov.	5.0	100	69	31	3,800	2070 ± 110	2080 ± 20
17 Nov.	19.5	149	45	55	300	180 ± 1	180[c]
18 Nov.	20.0	60	57	43	2,600	1530 ± 70	1410 ± 60
19 Nov.	6.0	21	95	5	7,000	4590 ± 150	3960 ± 30

[a] Based on ml/min of hydrocarbon mixture used and water flow in fish ladder
[b] No water sample collected
[c] No replicate water sample collected.
(From Weber et al. 1981)

water samples taken at the top and in the middle of the fish ladder we elected to use the results of chemical analysis as being the more appropriate for representing the hydrocarbon concentration. The hydrocarbon concentration at which 50% of the fish expected to ascend the west ladder would avoid it (EC_{50}) was 3.2 ppm; the EC_{50} value was calculated from a regression equation based on results of individual avoidance tests (Fig. 45). Water samples for hydrocarbon analysis by GC were also collected downstream from the dam at the confluence of flows from the fish ladders and spillway. When hydrocarbons were present in the west fish ladder the concentration in midstream was 0.7 ppm or less. Thus, all fish approaching the dam during a test period may have encountered the aromatic hydrocarbons downstream.

Disruption of homing capability. Results of capture-oil exposure-transport-release experiments indicate that exposure of up to 40 ppm of the FWAF of PBCO for 14-18 hr, or up to 2 ppm of an aromatic hydrocarbon mixture for 8-22 hr, does not impair homing capability.

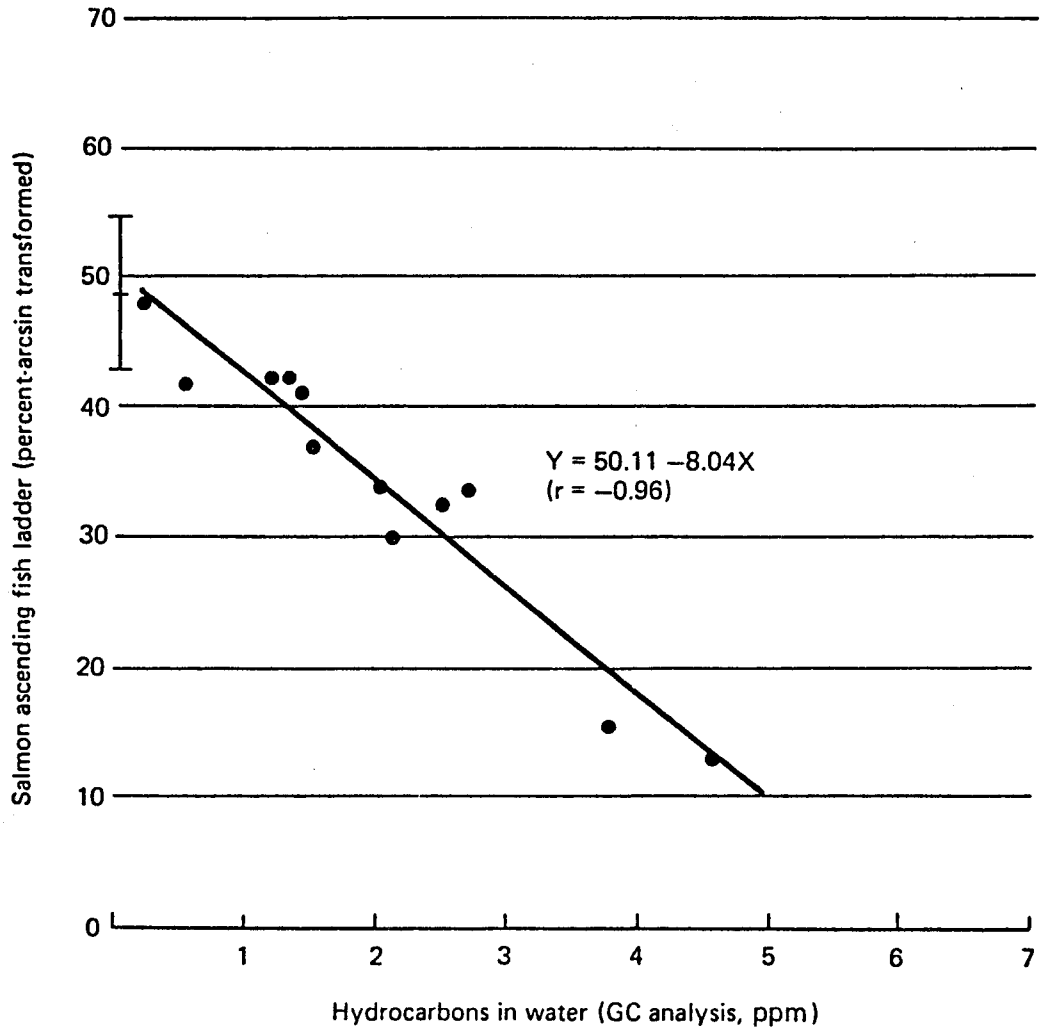

FIGURE 45. Percentage of adult salmon migrating up (west) fish ladder and concentration of aromatic hydrocarbons in the water as determined by GC analysis. Mean and standard deviation for control observations are given at left. (From Weber et al. 1981)

The percentage of chinook salmon returning to the University of Washington homing pond after exposure to 0.5 ppm PBCO was 10% greater than the percentage of returning controls (Table 47). Fish exposed to 5 ppm returned 11% less frequently than their controls, and fish exposed to 40 ppm returned 2% less frequently. These percentages are in marked contrast to the olfactory occluded salmon which returned at a frequency which was 44% less than their controls (6% vs 50%), suggesting that the concentrations of PBCO used for exposure did not adversely affect olfaction. Analysis of data yielded no significant difference ($P>0.05$) between the numbers of returning control fish and fish exposed to either 5 or 40 ppm of PBCO, or in time of return between control and oil exposed fish.

TABLE 47. Release and recovery data for mature chinook salmon at University of Washington following exposure to the FWAF of PBCO.

Release date	Exposure (hr)	Hydrocarbon concentration (ppm) Calc.[a]	Hydrocarbon concentration (ppm) Analyzed[b] x̄±range	Number released Control	Number released Exposed	Number recovered (%) Control	Number recovered (%) Exposed	Average days to return Exposed-Control
Oct. 30	15	0.5	0.005±.004	7	6	4 (57)	4 (67)	+8.5
Nov. 3-8[c]	14-18	5.3	0.045±.02	19	22	9 (47)	8 (36)	+1.1
Nov. 10-16[d]	14-18	40.6	0.100±.05	30	32	10 (33)	10 (31)	+0.4
	Total			56	60	23 (41)	22 (37)	+1.9

[a] Determined from volume of PBCO injected and water flow.
[b] Determined by GC (sum of aromatic hydrocarbons).
[c] Four separate release groups.
[d] Five separate release groups.

TABLE 48. Release and recovery data for jack coho salmon at Tulalip following exposure to a mixture of monocyclic aromatic hydrocarbons (Table 1).

Release date	Number released Control	Number released Test	Exposure (hr)	Transported (km)	Hydrocarbon concentration (ppm)[a]	Number recovered (%) Control	Number recovered (%) Test	Average days to return Exposed-Control
Oct 21	23	23	22	4.7	1.0	14 (61)	16 (70)	-0.4
Oct 28	55	44	22	1.6	1.0	24 (44)	18 (41)	-1.8
Oct 31	62	66	8	1.6	1.0	17 (27)	25 (38)	-5.1
Nov 3	26	25	8	1.6	1.0	15 (58)	9 (36)	-1.9
Nov 4	64	65	8	1.6	2.0	28 (44)	29 (45)	-4.5
Nov 6	52	52	8	1.6	2.0	23 (44)	19 (37)	-2.3
Total	282	275				121 (46)	116 (45)	-2.7

[a] Determined from volume of hydrocarbons injected and water flow.

In the first 4 tests at Tulalip Creek, jack coho salmon were exposed for 8-22 hr at calculated concentrations of aromatic hydrocarbons of 1 ppm, and for the last 2 tests the fish were exposed for 8 hr to 2 ppm. Analysis of individual tests indicated no significant difference ($P>0.05$) between the number of returning control fish versus number of hydrocarbon exposed fish (Table 48). However, the exposed salmon were delayed an average of 3 days in their return to Tulalip Creek ($P\leq 0.05$).

TABLE 49. Comparison of calculated vs. measured concentrations of aromatic hydrocarbons in the Tulalip Creek exposure tank and the relation to number of fish exposed.

Number of fish	Hydrocarbon concentration (ppb)	
	Calculated	GC analysis ($\bar{x}$ ± range) (N=2)
3	1500	1597 ± 76
12	1020	378 ± 25
25	960	154 ± 43
52	2200	214 ± 106
65	2100	24 ± 11

In the Tulalip study, a marked discrepancy (ranging from 0.6 to 2.0 ppm) was found between the calculated concentration of aromatic hydrocarbons present in the flow-through exposure tank and concentrations determined by GC analysis (Table 49). Since the eductor system used to introduce monocyclic aromatic hydrocarbons into the water was shown to be accurate (see Fig. 3, Section 5.3.2[c]), an explanation for this discrepancy was sought. The difference between the calculated concentrations of aromatic hydrocarbons present and the concentrations determined by GC analysis are apparently related to the number of fish present in the exposure tank. Figure 46 shows that the more fish present in the exposure tank the larger the discrepancy between calculated and GC determined concentrations. A test of this relationship with linear regression demonstrates a highly significant ($P<0.01$) correlation between the two variables. It appears that the coho salmon were rapidly taking up the aromatic hydrocarbons from the water, and either the aromatics were retained in the tissues or they were being excreted as metabolic products not detectable by the analytical methods employed.

(c) Predator-Prey Behavior. The SWSF concentrations to which the fry were exposed averaged 350 ppb and ranged from 130 to 620 ppb. Mortality of oil-exposed fish was approximately 5% during exposure compared to <1% for controls. Of 280 oil-exposed fry only 85 survived predation, whereas of 280 control fry, 161 survived predation. The hypothesis that the survival of prey chum fry exposed to the predators is independent of exposure to the SWAF of CICO was tested by chi-square analysis and rejected in every case: at 24 hr, $P<0.01$; 48 hr, $P<0.01$; 72 hr, $P<0.001$; 96 hr, $P<0.05$; entire experiment, $P<0.001$. At 96 hr, however, significantly fewer oil-exposed fish were consumed in comparison to controls.

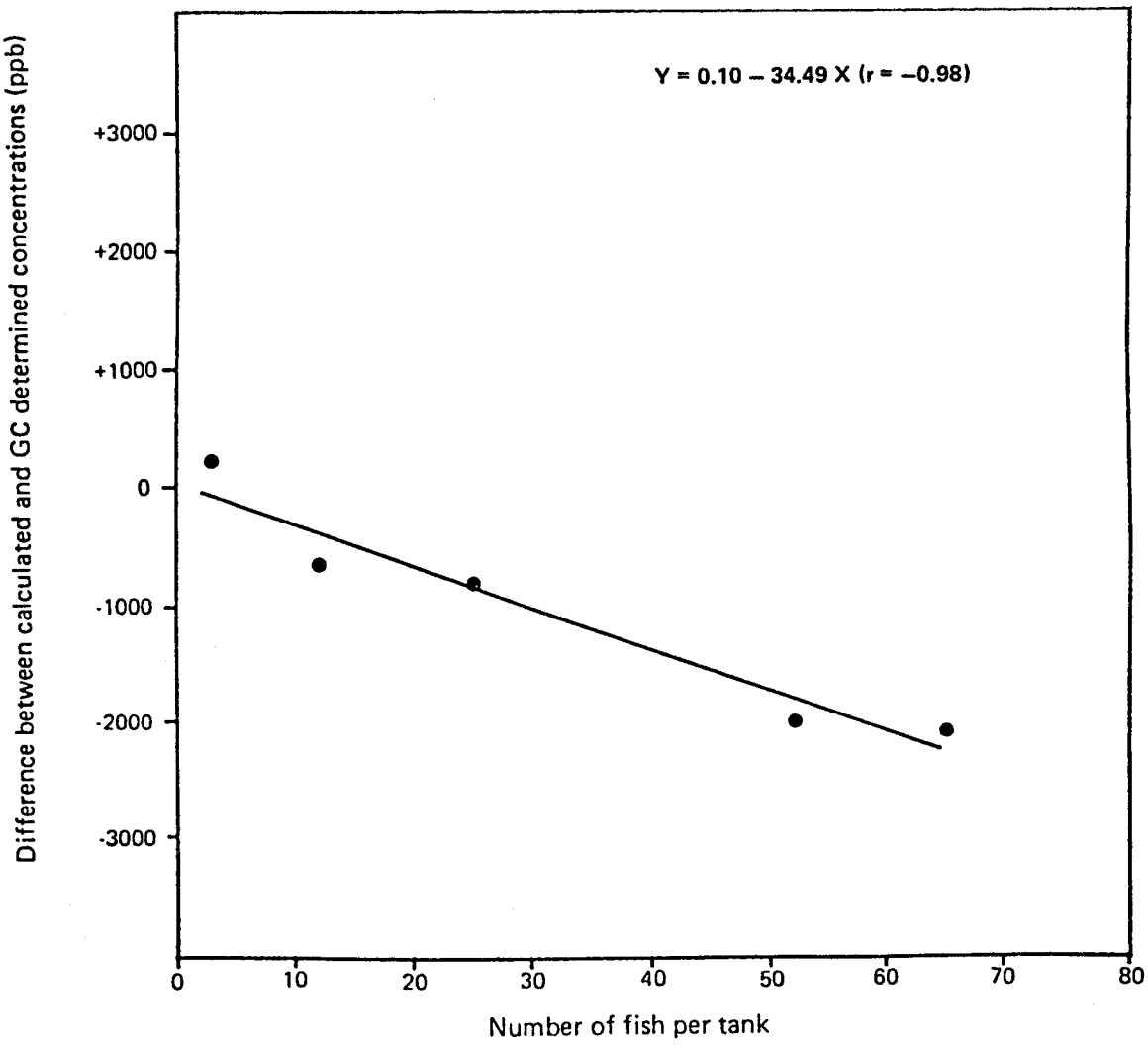

FIGURE 46. Difference between calculated concentrations of aromatic hydrocarbons present in the water and concentrations determined by GC analysis plotted against number of fish exposed.

Many of the coho predators exposed to 343 + 93 ppb ($\bar{x}$ + SD) of the SWSF of CICO began to show behavioral modifications by the tenth day of exposure. In general, the oil-exposed predators appeared lethargic and showed little or no interest in the prey presented to them (these predators were designated as noneaters). However, one of the oil-exposed subgroups (number 6, designated as eaters) demonstrated none of these behavioral modifications and continued to feed at rates comparable with those of the unexposed predators. Similar behavioral responses with eater and noneater groups in coho predators exposed to No. 2 fuel oil were also observed in another experiment (L. Folmar, NWAFC, personal communication).

Figure 47 depicts the numbers of rainbow trout fry consumed during 10 min of exposure to the control or oil-exposed predators at three time intervals. An initial Yates X^2 evaluation showed a significant difference in prey consumption between the control and oil-exposed predator

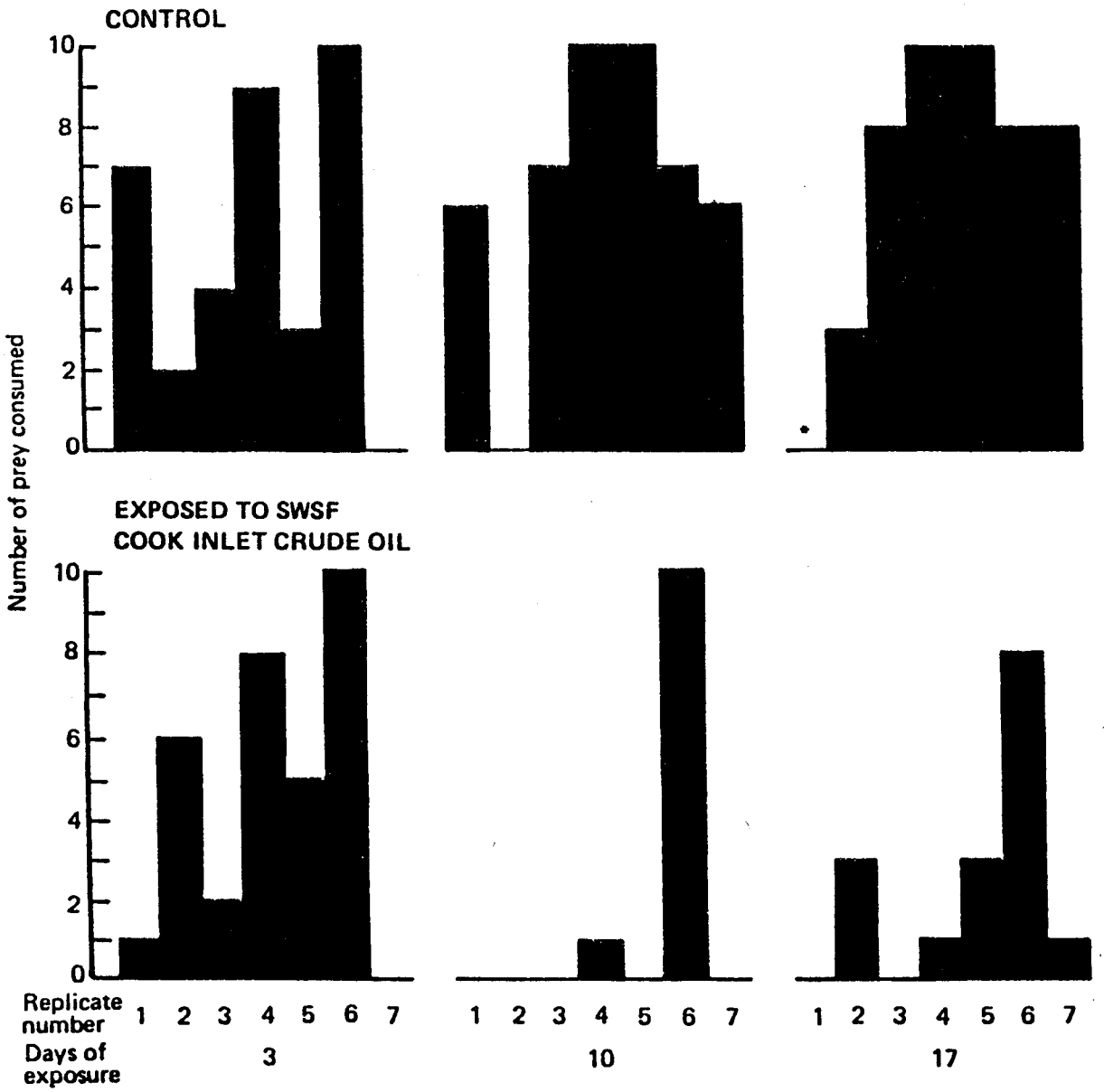

FIGURE 47. Ten rainbow trout fry were offered to each of the seven (3 fish) replicate control and oil-exposed coho salmon predator subgroups after 3, 10, and 17 days of oil exposure. The histograms represent the number of prey consumed by each of the control and oil-exposed subgroups at the three sampling periods. Each numbered subgroup represents the same three predators throughout the experiment. * = no data due to mortalities. Other absences of a bar represent no prey consumed. (From Folmar et al. 1981)

subgroups ($P<0.005$). To determine within- and between-group differences with the small sample sizes, the data were subjected to predictive sample reuse analysis (Geisser and Eddy 1979). With a sample size of seven, this test is designed to select the correct model >95% of the time. The data were used to select one of two models: Model 1 (test populations were equivalent) or Model 2 (test populations were unequal). Model 1 (=) was selected for the control observations at 3, 10, and 17 days, as it was for the comparison tests of control and oil-exposed fish after 3 days of exposure. However, Model 2 ($\neq$) was selected for the control and oil-exposed fish comparison tests after 10 and 17 days of exposure. Model 1 (=) was also selected for the comparison of the 10 and 17 days oil-exposed predators. Even with the bias of Subgroup 6 toward the control values, our results clearly indicate that there was reduced predation by the adult coho salmon exposed to the SWAF of CICO for periods of 10 and 17 days.

(d) Avoidance Behavior of Flatfish Exposed to Oil-Contaminated Sediment

Control tests with uncontaminated sediment on both sides of the choice chamber (Fig. 6, Section 5.3.3[d]) indicated that after 22-24 hr an average of 54% of the fish were found on the side of introduction (Table 50).

Tables 51 and 52 present the results of 3 series of tests with oil-contaminated sediment on one side of the test apparatus and uncontaminated sediment on the other. The first two series were conducted with an initial oil-to-sediment concentration of 2.5%; the third, with 5% oil. At the start of Test 1 (2.5% oil) a sediment sample taken immediately after mixing contained over 23,000 ppm TEPH which decreased by 62% after 2 hr of rinsing. Subsequent sediment samples did not show an appreciable decline in TEPH over an 8 day period. At the

TABLE 50. Percent juvenile English sole found in each side of the testing apparatus when no oil was present in the sediment.

Test dates	No. fish introduced	Hours in test apparatus	% fish	
			left	right
8/9	20	24	45[a]	55
8/23	20	22	55	45[a]
8/24	20	24	70[a]	30
9/6-11	20	24	45	55[a]
		48	50	50
		76	50	50
		117	55	45

[a] Side on which fish were introduced.

TABLE 51. Percent juvenile English sole on each side of the test apparatus when oil-contaminated sediment was present on one side at an initial oil concentration of 2.5% (v/v). Hydrocarbon analysis of sediment was by the gravimetric method; above-sediment water analysis was by GC.

Test dates	No. fish	Hours in test apparatus	% fish		Hours oiled sediment in apparatus	Hydrocarbon concentration (ppm)			
						sediment (dry wt.)		above-sediment water	
			left	right		left	right	left	right
Test 1									
8/14	0				0[a]	23315.9	17.3		
8/14	0				2	8974.8	17.5	.001	<.001
8/16	20	23	25	75[b]	46	8404.0	17.5	.003	.026
8/17	20[c]	21	60	40[b]	71	7140.0	34.1	.078	.040
8/18	20[c]	22	45	55[b]	94	8056.9	43.2	NA[d]	NA
Test 2									
8/29	0				22	12.2	8194.3	.025	.026
8/30	20	22	75[b]	25	46	12.2	8496.0	.032	.037
8/31	20[c]	22	70[b]	30	69	13.3	5258.1	.050	.062
9/1-5	20[c]	23	65[b]	35	93	14.7	6350.2	NA	NA
		98	35	65	192	15.9	6821.8	NA	NA

[a] Sediment sample taken before filling test apparatus with seawater.
[b] Side on which fish were introduced. In each instance fish were initially placed on the non-oil-contaminate side of the apparatus.
[c] Previous 20 fish removed and a new group of 20 fish placed in chamber on same non-oil-contaminated sediment.
[d] NA, not analyzed.

start of Test 3 (5% oil) the TEPH concentration after 4 hr rinsing was 18,600 ppm (Table 52); however, after 26 hr rinsing, with fish in the test apparatus, the oil concentration in sediment was reduced to 8,300 ppm and remained fairly constant thereafter. Thus, regardless of the initial oil-sediment concentration, the carrying capacity of the sediment appeared to be about 8,000 to 10,000 ppm.

At the start of each test series the total extractable organic material from the non-oiled sediment was 12 to 17 ppm. During avoidance testing there was a gradual increase to a maximum of 43 ppm in the uncontaminated tray, indicating a probable transfer of petroleum hydrocarbons from the oil-contaminated side.

In Test 3, after the introduction of fish into the choice apparatus, the average TEPH in the oil-contaminated sediment between 26 and 361 hr was 8,860 $\pm$ 1,240 (SD) ppm. The TEPH found in the sediment-associated water (SAW) of the same oil-contaminated sediment averaged 6,980 $\pm$ 762 (SD) ppm, indicating that only about 20% of the crude oil was bound to the sediment. Of the total extractable organic material found in uncontaminated sediment, less than 20% was found in the SAW. SAW samples from the oil-contaminated sediment were analyzed by GC; no change in the relative composition of aromatic and alkane constituents from the beginning to the end of each test was observed.

TABLE 52. Percent juvenile English sole on each side of the test apparatus when oil-contaminated sediment was present on one side at an initial oil concentration of 5% (v/v). Hydrocarbon analysis of sediment was by the gravimetric method; above-sediment water analysis was by GC.

Test dates	No. fish	Hours in test apparatus	% fish		Hours oiled sediment in apparatus	Hydrocarbon concentration (ppm)			
						sediment (dry wt.)		above-sediment water	
			left	right		left	right	left	right
Test 3									
9/18	0				4	18594.5	17.1	.016	.010
9/19	20	21	30	70[a]	26	8282.8	23.8	.081	.079
9/20	20[c]	21	50	50[a]	46	9323.9	26.0	.266	.062
to		91	40	60	96	8469.5	26.6	.074	.014
10/3	(19)[b]	166	58	42	171	6936.1	28.2	.136	.045
	(18)[b]	214	50	50	219	9668.7	31.8	.109	.014
	(17)[b]	356	41	59	361	10498.3	33.9	.024	.017

[a] Side on which fish were introduced. In each instance fish were initially placed on the non-oil contaminated side of the apparatus.
[b] Reduced number of fish due to mortality in initial group of 20 fish.
[c] Previous 20 fish removed and a new group of 20 fish placed in chamber on same non-oil-contaminated sediment.

Since analysis of the TEPH in the sediment indicated that the sediment was essentially saturated with oil at concentrations of less than 2.5%, all tests were comparable with respect to petroleum hydrocarbon exposure. There was no significant avoidance of oil-contaminated sediment by juvenile flatfish when all 21 to 23 hr tests were combined ($P=0.265$). However, there was a significant difference ($P=0.017$) between numbers of fish on oil-contaminated sediment and numbers of fish on uncontaminated sediment for the first groups of 20 fish introduced into the avoidance apparatus at the beginning of each of the three test series. Prior to initiation of each test series, when the oil-contaminated sediment had not been disturbed, the average percentage of flatfish found on the oil-contaminated side after the first 21 to 23 hr was 27%, compared to 46% for controls. That is, within this experiment a high percentage of fish were found at each observation period on oiled sediment. For those fish repeatedly tested, there was no predictable pattern for preference of the right or left side, regardless of the presence of oil-contaminated sediment. Also, no differences were detected in feeding responses of fish on either oil-contaminated or uncontaminated sediment, and visual assessment of stomach fullness at the end of each test indicated that all fish were feeding except in the test which extended 15 days. During this extended test (Table 52) there was a 15% (3 of 20) mortality, and at termination of the test 24% (4 of 17 of the survivors) were not feeding.

(e) Effect of Petroleum Hydrocarbons on Development of Embryos and Larvae

(1) Hydrocarbon Exposure. In experiments assessing the effects of petroleum hydrocarbons on embryos and larvae, salmon and flatfish were exposed to PBCO and surf smelt were exposed to CICO. A list of the major components in PBCO and CICO as determined gravimetrically is

TABLE 53. Comparison of major petroleum fractions of fresh PBCO and CICO. Analysis determined gravimetrically, and each fraction expressed as percent of total.

Fraction	CICO[b]	PBCO[c]
Naphtha[a]	27.9	18.6
Saturates	39.5	48.9
Aromatics	19.7	19.4
Polars	11.5	13.8
Insolubles	2.3	1.4
	100.9	102.1

[a] Naphtha fraction includes saturates and aromatics boiling at less than 210° C.
[b] Personal communication, Paul Robisch, NWAFC.
[c] Malins et al. (1978)

given in Table 53. A more detailed composition of aliphatic and aromatic fractions of PBCO and CICO, as determined by glass-capillary GC, is presented by MacLeod et al. (1980). The major differences between these two oils are in the naphtha and saturate fractions. In our experiments the low molecular weight saturates through n-C_{11} and most of the naphtha fraction of both PBCO and CICO disappeared from the SWAF in the first 30-48 hr of weathering. The result of weathering on the hydrocarbon content of water-accommodated CICO is shown in Figure 48. Generally, as weathering of CICO progresses, there is a rapid initial loss of both akyl-substituted benzenes and low molecular weight alkanes. With increasing time the naphthalenes disappeared from the SWAF, and after over 500 hr of weathering the dominant hydrocarbons were compounds less volatile than n-C_{14}. PBCO has the same general pattern of weathering as CICO with the more volatile benzenes and naphthalene compounds disappearing over time (Fig. 49).

Chum salmon. Chum salmon embryos and alevins were exposed to the SWAF of weathered PBCO for an average of 3 hr/day, 4 days a week, for 16 consecutive weeks. The concentration of petroleum hydrocarbons remained relatively constant for the first 3 days of exposure and then dropped on day 4 (Table 54). The average hydrocarbon concentration in the water during exposure was 470 ppb.

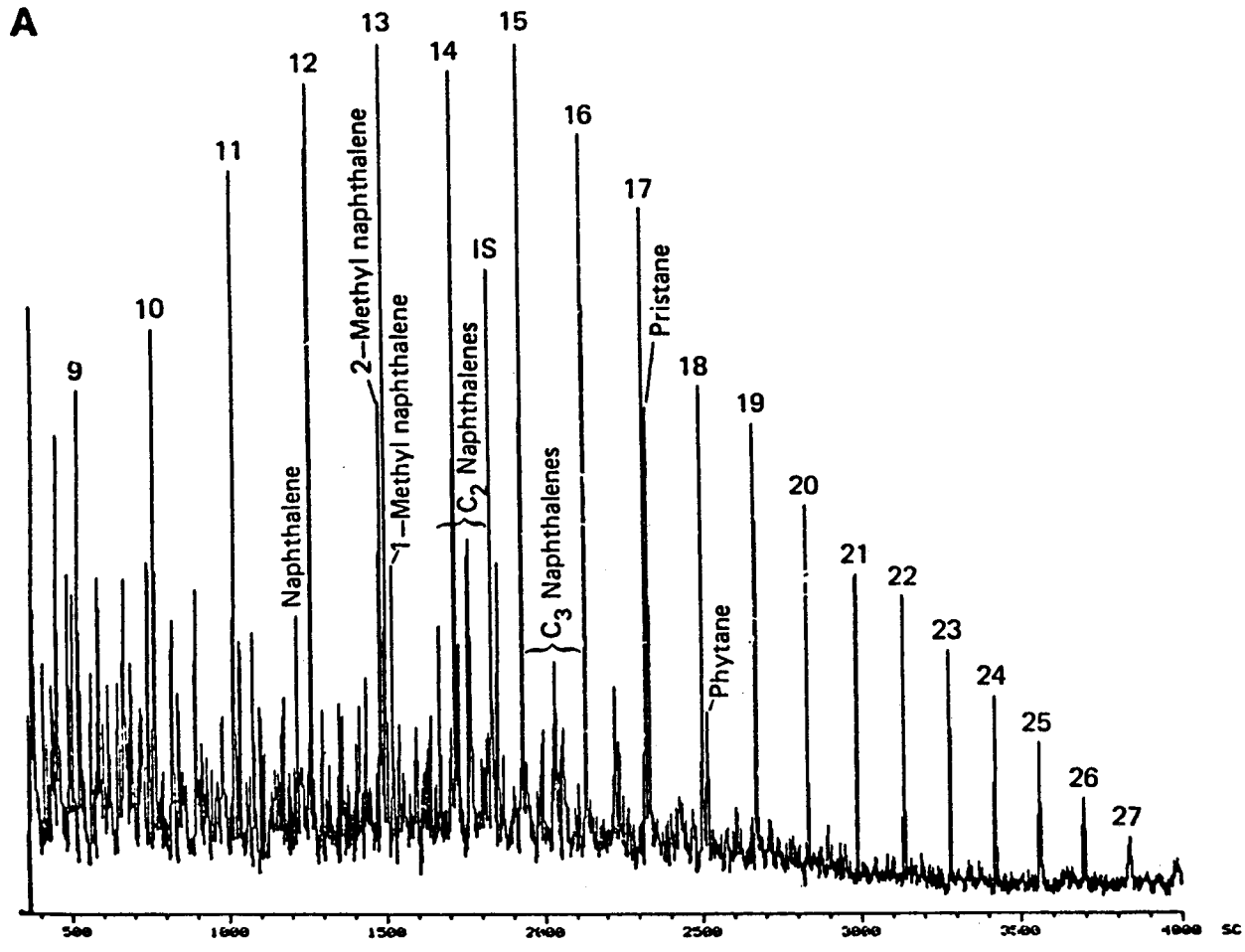

FIGURE 48. Gas chromatograms of fresh CICO and seawater-accommodated CICO, all normalized to n-pentadecane (n-C_{15}). A. Fresh CICO. B. CICO weathered 48 hr. C. Weathered 192 hr. D. Weathered 528 hr.

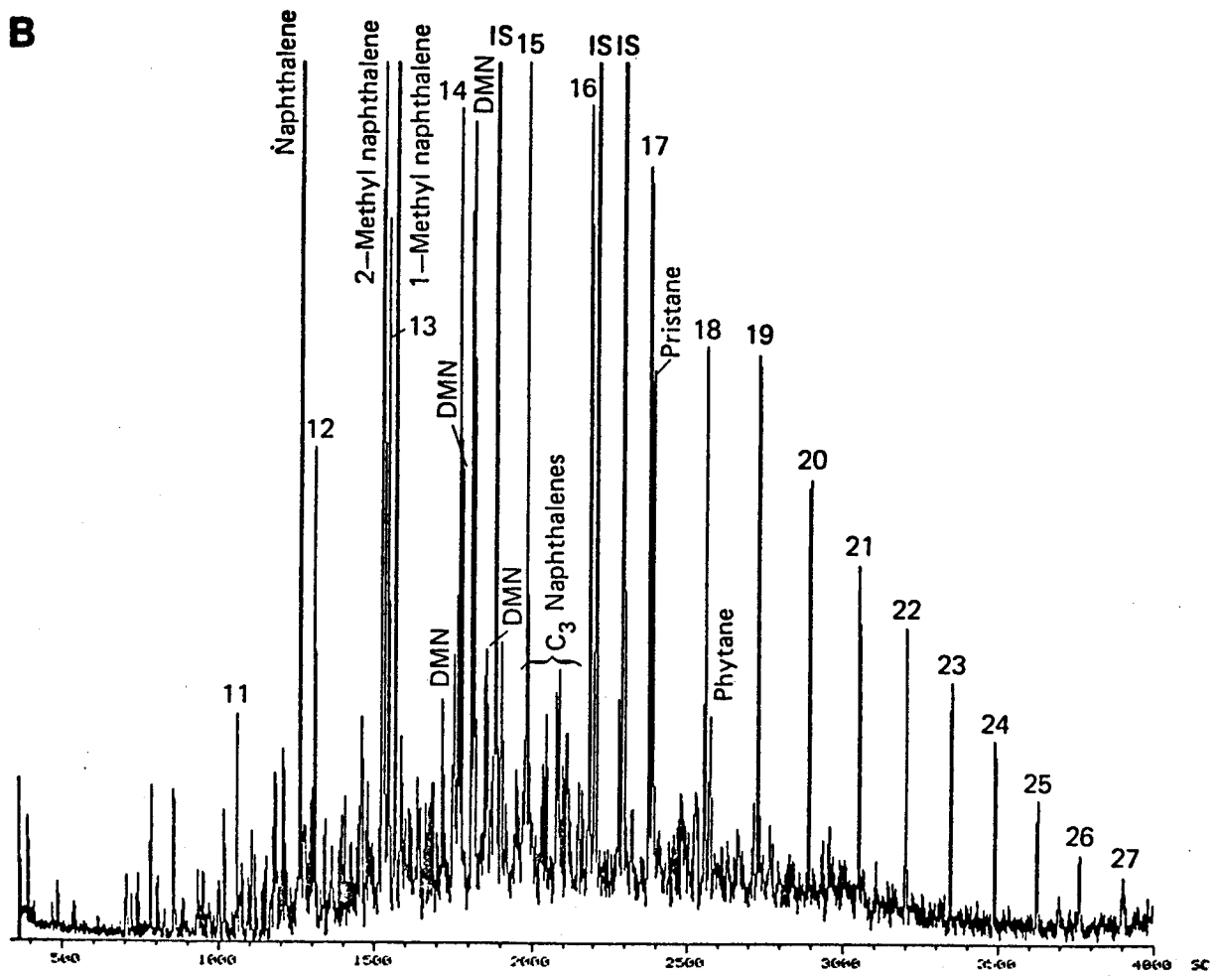

FIGURE 48. B. CICO weathered 48 hr.

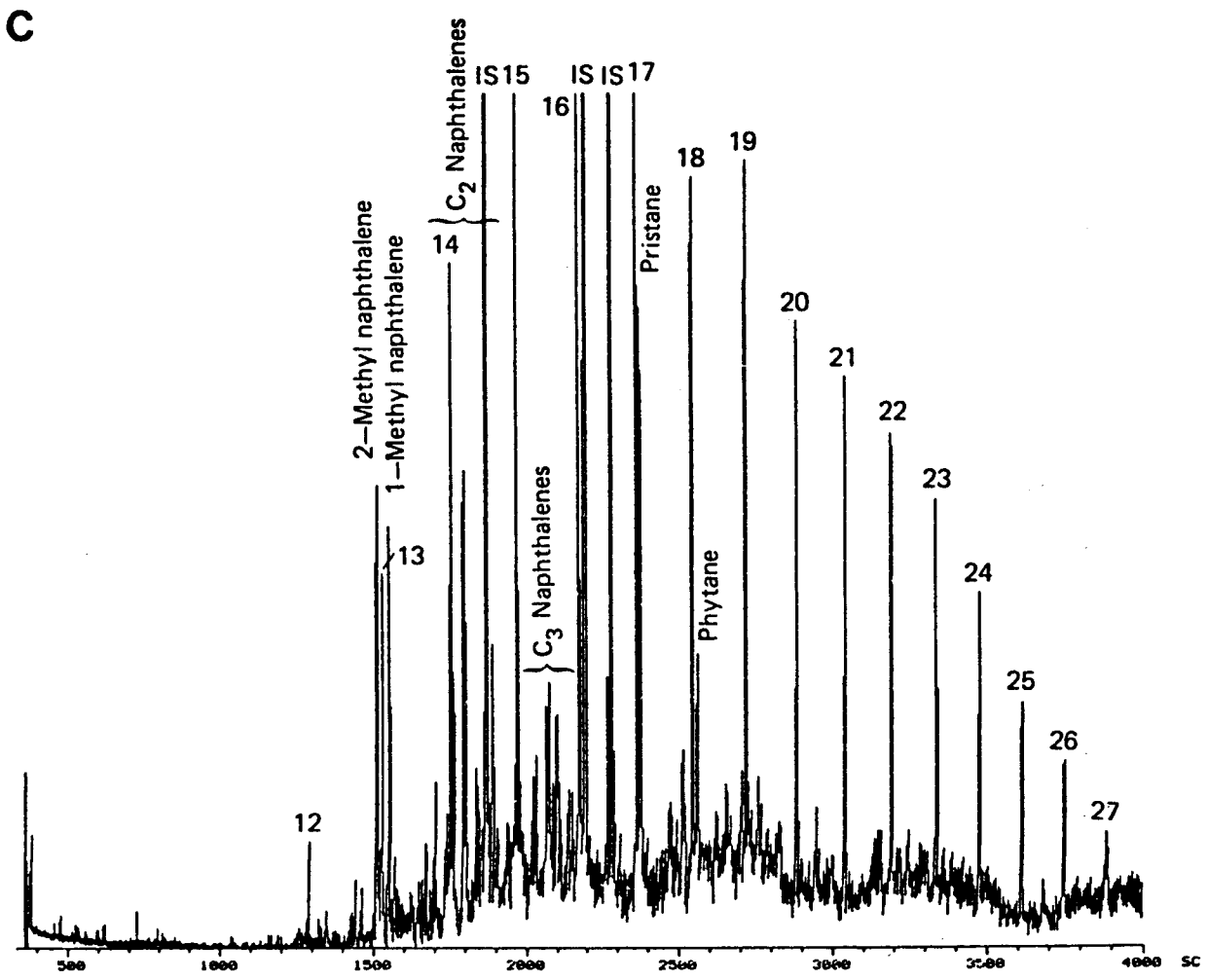

FIGURE 48. C. CICO weathered 192 hr.

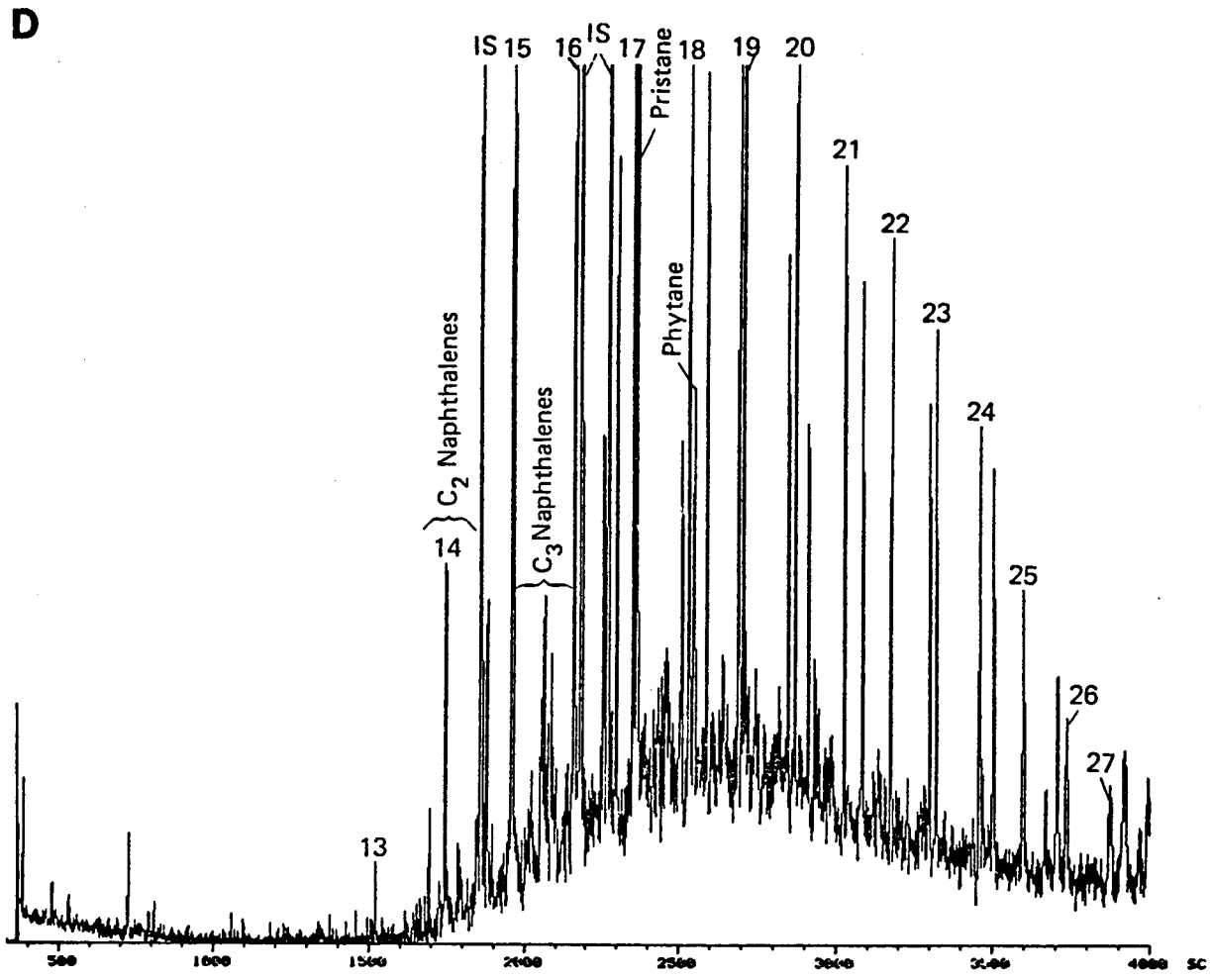

FIGURE 48. D. CICO weathered 528 hr.

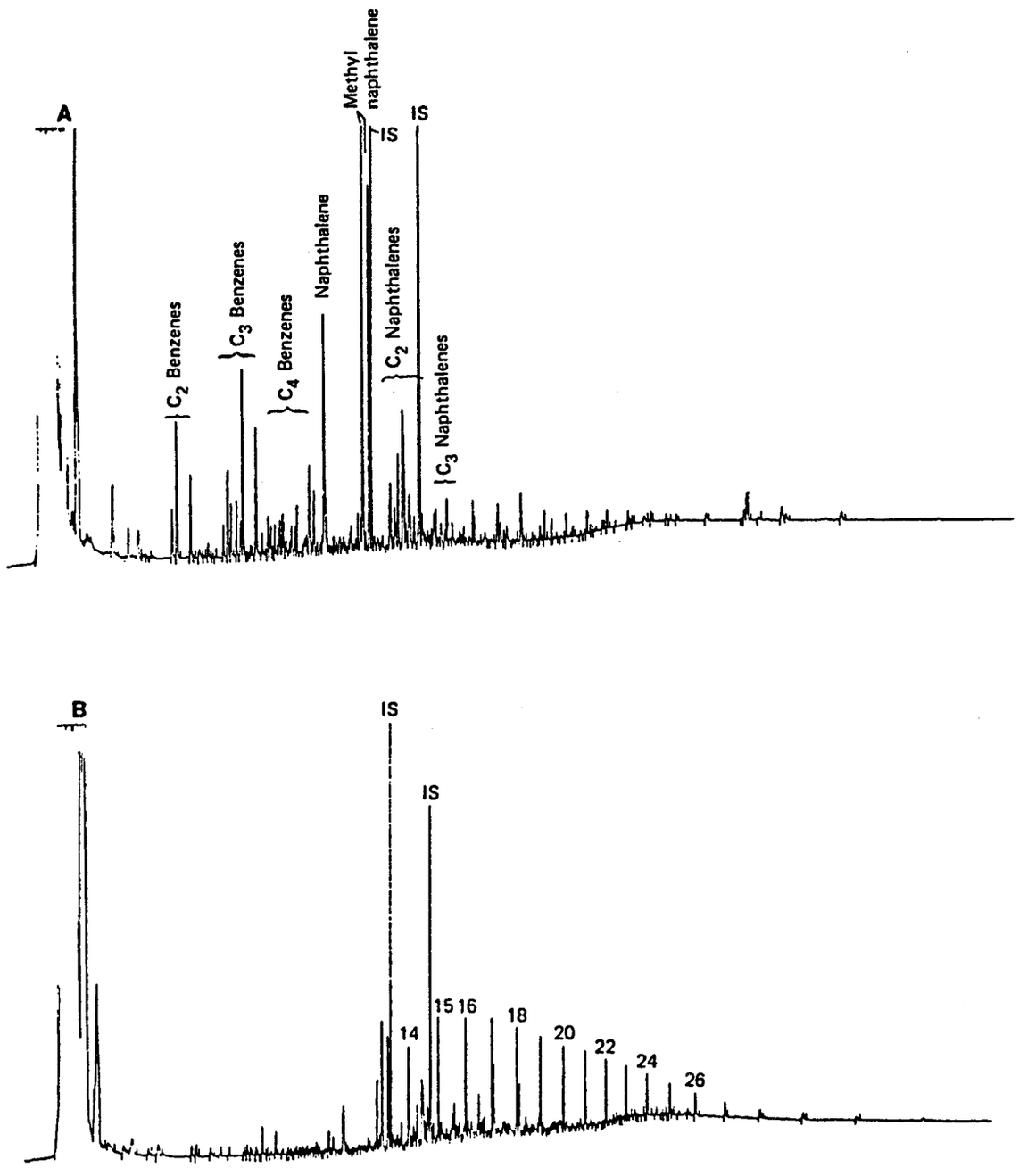

FIGURE 49. Gas chromatograms of the SWAF of PBCO after weathering for: A. 30 hr and B. 54 hr.

TABLE 54. Concentration of petroleum hydrocarbons in the SWAF of PBCO during exposure of chum salmon embryos and alevins.

Day of exposure	Hours oil weathered	Number of samples	Total hydrocarbon concentration in ppb ($\bar{x}\pm SD$)
1	30	10	730±540
2	54	10	420±480
3	78	9	450±510
4	102	5	70±30

Flatfish. Exposure of flatfish embryos to the SWAF of PBCO was initiated one day after fertilization at which time the oil had weathered 48 hr. A water sample taken from one rearing vessel at 72 hr (mid-incubation) indicated almost total loss of hydrocarbons (Fig. 50). At mid-incubation the SWAF was replaced with an aliquot of the original SWAF. The hydrocarbon concentrations found in the water at the start of oil exposure, half-way through incubation, and at time of hatching, are given in Table 55.

TABLE 55. Concentration of petroleum hydrocarbons in the SWAF of weathered PBCO to which English and sand sole embryos were exposed.

Species and treatment	Total hydrocarbon Concentration (ppb) Days post-fertilization 1	4	8	$\bar{X}$	Number of samples
Sand sole					
Control	2	4	13	6	3
Test 1	430	25(193)[a]	11	164	4
Test 2	200	9(91)[a]	15	79	4
English sole					
Control	4	10(2)[a]	4	5	4
Test	278	60(184)[a]	8	133	4

[a] Hydrocarbon concentrations of replacement aliquots of original SWAF, or control water, are in parentheses.

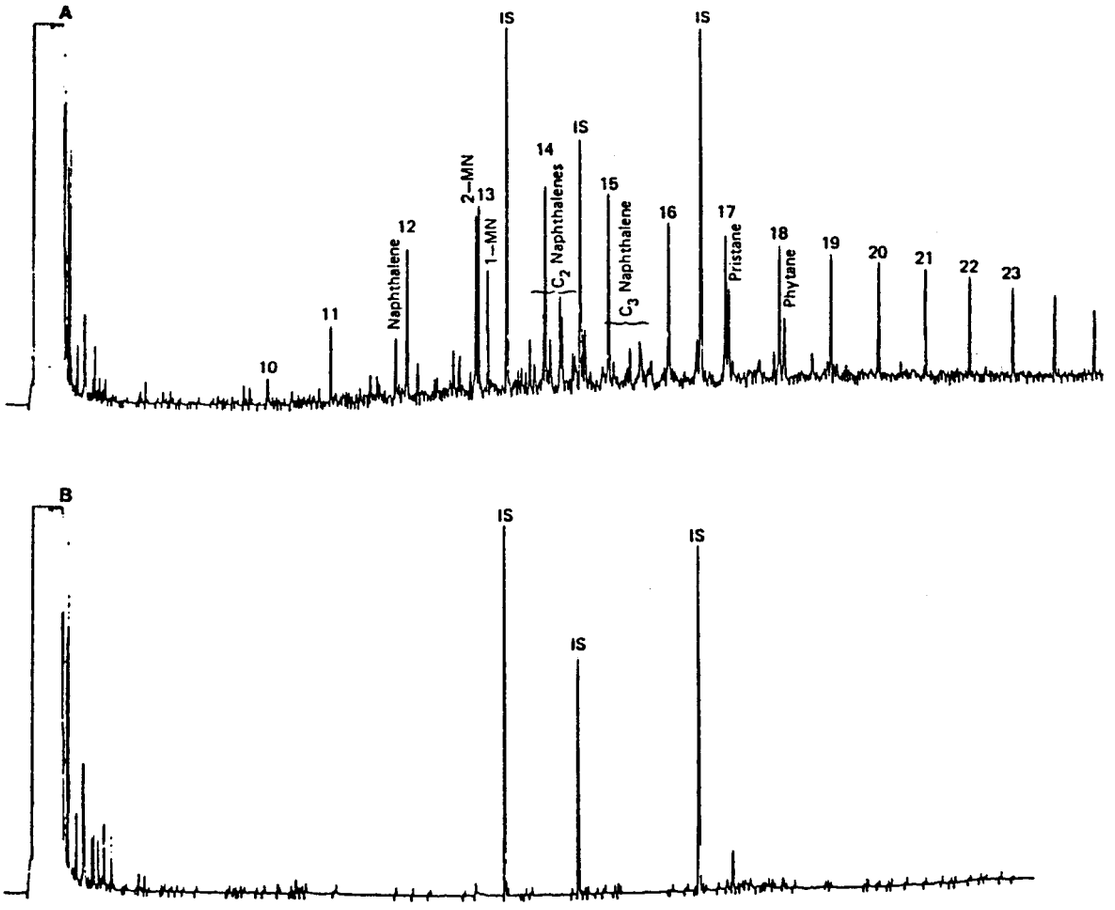

FIGURE 50. Gas chromatograms of the SWAF of PBCO used in exposure of flatfish embryos. A. 48 hr weathered PBCO sampled at initiation of experiment. B. Same SWAF as above sampled half-way through incubation 72 hr later.

Surf smelt. Experiments in which surf smelt embryos were exposed to the SWAF of weathered CICO were replicated, and the hydrocarbon concentrations found in water samples taken throughout each of the two tests are shown in Figure 51. Eggs collected in November were first exposed to the SWAF 4 days after fertilization; the oil having been weathered previously for 48 hr. The undiluted hydrocarbon concentration, as measured by GC analysis, averaged 324 $\pm$ 125 ($\bar{x}$ $\pm$ SD) ppb for the first 7 days of exposure and then dropped to an average concentration of 77 $\pm$ 64 ppb for the remaining two weeks of the test. The overall undiluted hydrocarbon concentration throughout the 23 days of embryo exposure averaged 173 $\pm$ 152 ppb. The SWAF was also mixed directly with uncontaminated seawater to give diluted hydrocarbon concentrations, calculated from water flow as 53% and 25% of the original SWAF. This results in calculated average hydrocarbon concentrations for these dilutions of 92 and 43 ppb, respectively.

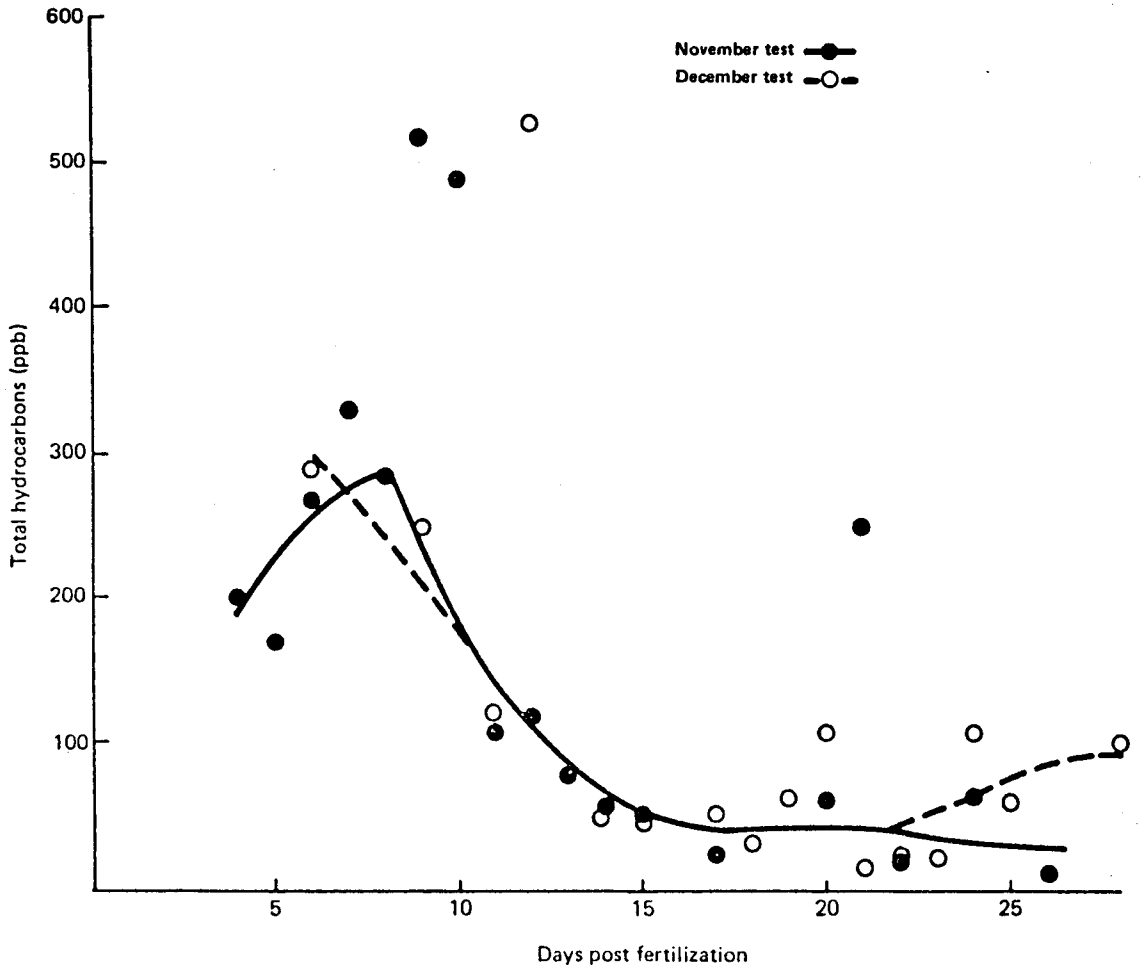

FIGURE 51. Total petroleum hydrocarbons in the SWAF of weathered CICO as related to time of surf smelt embryo exposure following fertilization. Circles represnt hydrocarbon concentration in water samples collected from the undiluted SWAF during each test (Nov. and Dec.). Corresponding solid and dashed lines represent smoothed data calculated by method of Cleveland (1979).

For surf smelt exposed to weathered CICO in December, the general trend of hydrocarbon concentration with time is similar to the November test (Fig. 51). After the oil had weathered for 8 days (12 days post-fertilization) the SWAF concentration in December dropped from an average of 298 ± 148 ($\bar{x}$ ± SD) ppb to an average concentration of 56 ± 32 ppb. The overall hydrocarbon concentration for the December test was 113 ± 128. The calculated dilutions of the original SWAF for the December test were 48% and 23% (54 and 26 ppb, respectively).

(2) Chum salmon. Mortality of embryos from oil-treated and control groups was evaluated at time of hatching (Table 56). Embryos treated for the full 75 day development period (122 hr exposure) showed a significant increase in mortality over controls (P=0.01).

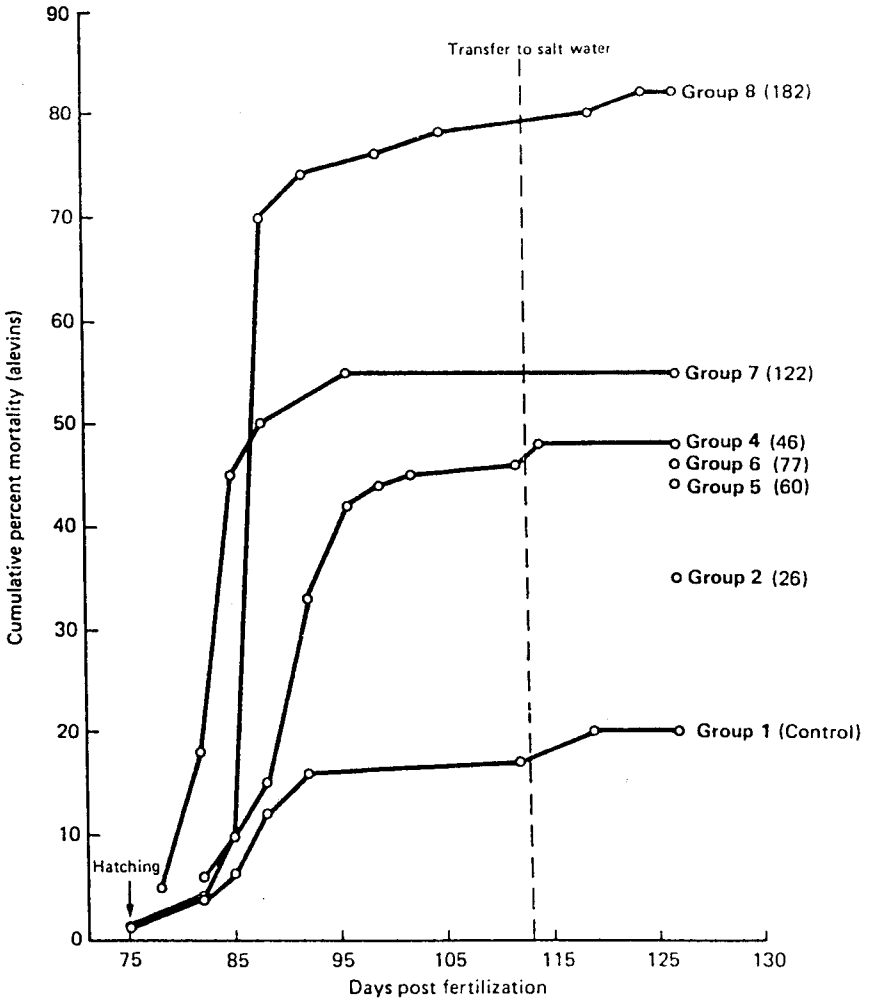

FIGURE 52. Cumulative percent mortality for alevin chum salmon exposed to the SWAF of weathered PBCO as embryos and/or alevins. Refer to Figure 8 for exposure at stage of development (groups). Total hr of exposure for each group are given in parentheses.

All groups of chum salmon embryos began hatching 75 days after fertilization, and on day 76 in the oil-exposed groups, 50% to 95% of the viable embryos had hatched as compared to only 15% of the controls. Six days later the percent of viable embryos hatching in the controls still lagged behind the oil-exposed groups.

After hatching, 5 groups of chum salmon were held for 50 days to observe delayed mortalities. Exposure to the SWAF of weathered PBCO was continued in group 8, which had been exposed continuously since fertilization, and group 5, alevins which had not been exposed to the SWAF as embryos. In group 3, late alevin exposure (day 92 after hatching to day 113), there were no mortalities; the cumulative mortality for all other groups of alevins is shown in Figure 52. Generally, the

TABLE 56. Percent mortality of chum salmon embryos at time of hatching (75 days post-fertilization), and percent of viable eggs hatched on days 76 and 82.

Group(s)[a]	Exposure period (days post-fert.)	Hours exposed	Average percent mortality[b]	Cumulative % hatch of viable eggs on days 76	82
1,3,5	(control)	0	24	15	70
2	(14-26)	26	28	60	95
4	(1-26)	46	25	95	99
6	(26-75)	77	19	80	98
7,8	(1-75)	122	42	50	96

[a] See Figure 8, Section 5.3.3.(e)
[b] Data adjusted for 2.6% of eggs which were unfertilized.

longer the duration of exposure to the SWAF the lower the survival. Highest mortality occurred in group 8, which was exposed continuously from fertilization; in this group 70% of the alevins were dead 12 days after hatching as compared to a 12% mortality in controls. The second highest mortalities occurred in group 7. These were exposed continuously as embryos, but with no oil exposure as alevins; 50% of this group died within 12 days after hatching.

Alevins were removed from each trough 12 days after hatching and examined for gross abnormalities. Three types of abnormalities were evident: malformed yolk sac, "dome" head, and kyphosis (angular curvature of the notochord).

The normal yolk sac is elliptical compared with the foreshortened and bulbous sac observed in some oil-exposed individuals. This abnormality occurred in the majority of oil-exposed groups, but was most frequent (61%) in those exposed continuously from fertilization (Table 57, group 8). Normally developing chum salmon alevins have heads with slight "dome" shape above the optic lobes of the brain (Distler 1954). Oil-exposed individuals had single- or double-"dome" heads which were much more pronounced than in Distler's illustrations or in our controls, and resembled lake charr (Salvelinus namaycush) alevins reared in water with low dissolved oxygen content (Balon 1980). Dissection of alevins with cephalic abnormalities revealed no evidence that the "dome" shape was a result of hydrocephaly. Kyphotic notochords occurred only in alevins exposed to oil continuously (143 hr); other groups did not exhibit any obvious notochord deformities.

Sixteen days after hatching, samples of 10 to 15 alevins from each group were fixed and stained to examine development of fin rays. Fin ray development in alevins exposed to the SWAF of weathered PBCO only during embryonic development was similar to controls; however, for those exposed to oil after hatching (groups 5 and 8) there appears to be an inhibition of ray development, particularly of the dorsal fin (Table 58).

TABLE 57. Gross morphological abnormalities observed in chum salmon alevins 12 days after hatching (87 days post-fertilization).

Group	Exposure period (days post-fert.)	Hours exposed	Sample size	% abnormalities[a]		
				Yolk sac	head	kyphosis
1	(Control)	0	204	0	0	0
2	(14-26)	26	15	0	9	0
3	(1-26)	46	19	16	16	0
4	(75-87)	21	10	10	33	0
5	(26-75)	77	15	0	33	0
6	(1-75)	122	6	33	33	0
8	(1-87)	143	16	61	44	11

[a] Pictorial description of yolk sac and cephalic abnormalities are shown in Malins et al. (1980).

TABLE 58. Effect of weathered PBCO on fin ray counts of chum salmon alevins 16 days after hatching (91 days post-fertilization).

Group	Exposure period (days post-fert.)	Hours exposed	$\bar{X}$ Number of fin rays with calcification[a]		
			Caudal	Anal	Dorsal
1	(Control)	0	17	7.8	8.3
2	(14-26)	26	18.7	11.2	9.2
4	(1-26)	46	12.5	8.7	6.3
5	(75-91)	29	10.0	1.2	0
6	(26-75)	77	20.1	12.7	10.9
7	(1-75)	122	15.0	11.7	6.0
8	(1-91)	151	5.0	3.5	0

[a] Following staining with alizarin red S.

A minimum of 10 alevins from each group were measured at termination of the experiment (127 days after fertilization). There were no differences in growth, as measured by total length, between control and oil-exposed alevins.

(3) Flatfish. Effects of the SWAF of weathered PBCO on English and sand sole embryos and larvae are given in Table 59 and summarized in Figure 53. Flatfish eggs and larvae were categorized using the following nomenclature:

Nondeveloped eggs: Consisted of two types of eggs which were indistinguishable as to the cause of nondevelopment--(1) nonviable eggs which were not successfully fertilized; (2) embryos which died in early cell division as a result of oil exposure or natural failure (embryo not formed).

TABLE 59. Effects of the SWAF of weathered PBCO on egg, embryo, and larval development of English sole and sand sole. Data were collected at time of hatching (end of 8 day exposure) and are reported in percent of total eggs introduced.

Species and hydrocarbon concentration (ppb)	Eggs		Embryo		Larvae			
	Number of eggs introduced	Non-developed (%)	Normal (%)	Abnormal or dead (%)	Normal (%)	Abnormal (%)	Grossly abnormal (%)	Dead (%)
English sole								
Control	348	13.5	0.9	0	74.7	5.2	0	5.7
133	372	22.0	0	3.0	0	0	64.5	10.5
Sand sole								
Control[a]	1469	7.4	0.1	0	89.7	1.8	0	1.0
164	365	15.6	0	4.9	0	43.6	22.2	13.7
79[a]	968	5.5	0.4	0.2	78.9	8.9	1.6	4.5

[a] Tests repeated and data pooled.

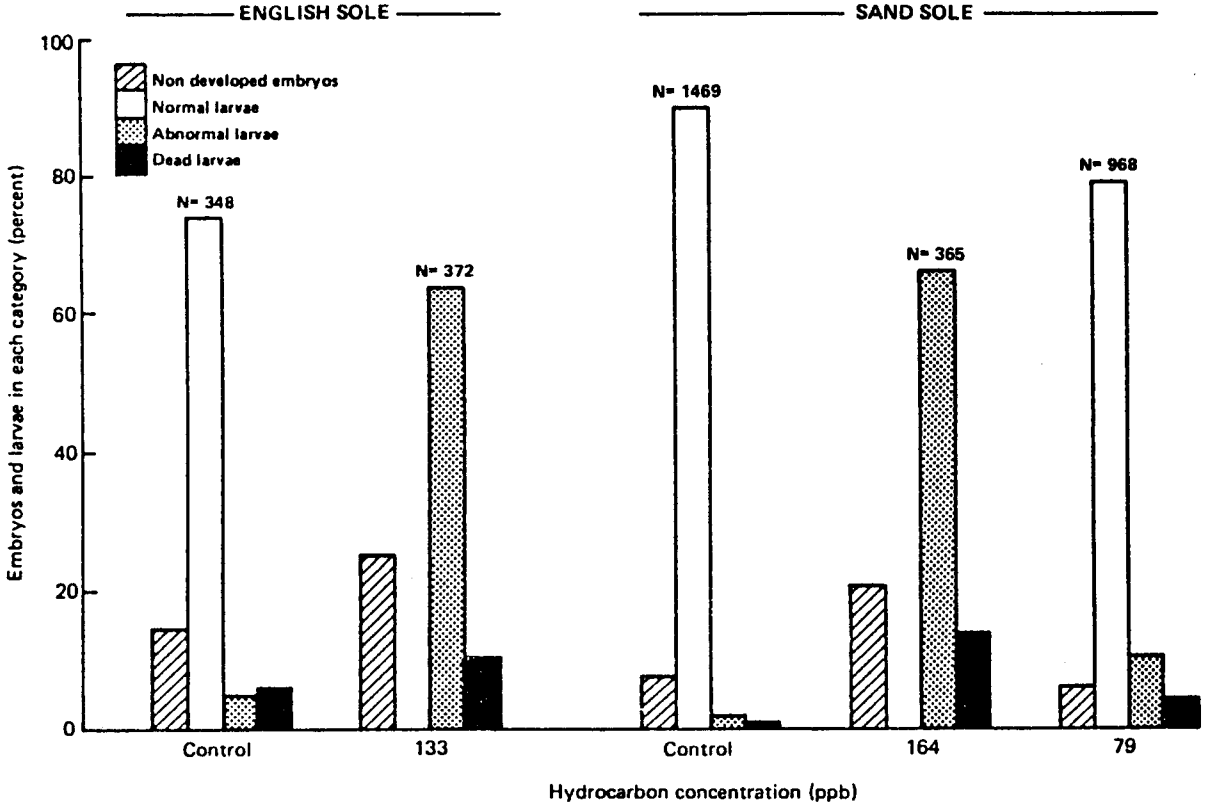

FIGURE 53. Hydrocarbon concentration of SWAF of PBCO and percent English and sand sole in each category at termination of tests. Data from Table 59 (% eggs and embryos, and abnormal larvae categories combined).

Normal embryo: Embryo transparent, with regular heart contractions.

Abnormal or dead embryo: Opaque, often scoliosis evident, and intermittent or no heart contractions.

Normal larvae: Regular heart contractions, notochord straight, finfold continuous, digestive tract complete, and pigmentation complete.

Abnormal larvae: Regular heart contractions, slight curvature of the notochord and entire body, generally a lateral curvature of up to 45°.

Grossly abnormal larvae: Regular heart contractions, but with body curvature exceeding 45° (some with notochord curvature of 180° and double 180° curvatures). Finfold deformed, digestive tract incomplete pigmentation not in patches but scattered, lying motionless on bottom, opaque.

Dead larvae: No heart contraction, generally opaque, and usually contorted.

A single test with English sole eggs indicated that exposure to an average hydrocarbon concentration of 133 ppb resulted in a high percentage of hatching, but all larvae were either abnormal or died shortly after hatching.

In control tests with sand sole an average of 90 + 2% ($\bar{x} \pm$ range) of the eggs hatched into normal larvae. Exposure to the SWAF at a concentration of 79 ppb resulted in an average of 79 + 2% normal larvae and only 10 + 1% deformed larvae. At a SWAF concentration of 164 ppb embryos developed and hatched, but two-thirds of the larvae were deformed; the most common abnormality was scoliosis.

(4) Surf smelt. An accounting of all surf smelt embryos and larvae in the two replicate experiments was conducted when hatching of live control embryos appeared complete. These data are presented in Tables 60 and 61 along with the average hydrocarbon exposure concentrations. A majority of the eggs were unaccounted for at the end of both experiments, and as no intact embryos could be lost from the incubation baskets, this loss is attributed to early embryo death, and subsequent embryo disintegration; for controls this averaged 49 $\pm$ 3% ($\bar{x} \pm$ range).

In both experiments the control embryos and those exposed to the lower hydrocarbon concentrations produced an equal number of live larvae (controls, 44 $\pm$ 4%; the 26 and 43 ppb exposure groups, 43 $\pm$ 1%). However, eggs exposed to the two higher hydrocarbon concentrations produced few live larvae with most dying in the embryonic stage (89 $\pm$ 9% as dead embryos or disintegrated eggs).

Cumulative hatching rates for both experiments are shown in Figures 54 and 55. Control larvae in the December experiment hatched approximately 6 days later than those in the November experiment, but the total percent hatching was similar. At an average hydrocarbon

TABLE 60. Effects of a 57 hr exposure to the SWAF of weathered CICO on hatching success of surf smelt embryos (November 1979 experiment). Data compiled at completion of hatching in controls, and reported in percent of total embryos introduced.

Hydrocarbon concentration (ppb)	Embryos				Larvae	
	Initial no. (N)	Alive (%)	Dead (%)	Disintegrated (%)	Alive[a] (%)	Dead (%)
Control	440	3.2	3.6	51.9	40.6	0.7
43	425	0	3.0	52.1	42.2	2.7
92	420	9.6	13.8	66.1	9.1	1.4
173	425	1.7	47.1	50.5	0.7	0

[a] Live larvae were collected daily immediately after hatching, and the percent presented is cumulative.

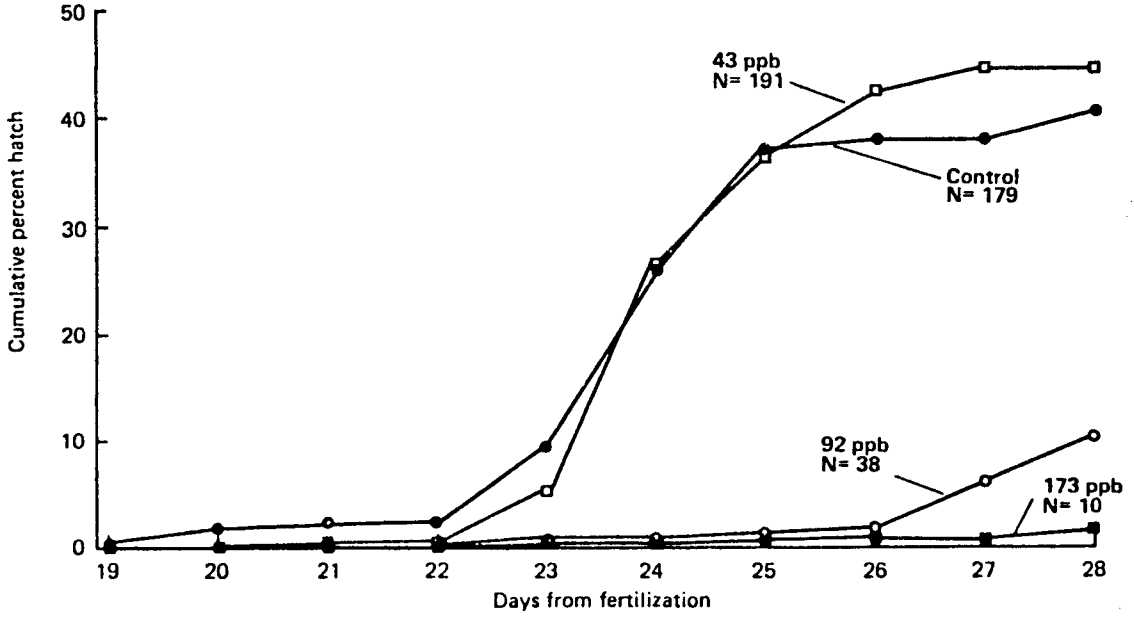

FIGURE 54. Cumulative percent hatching of surf smelt embryos exposed to 3 different concentrations of the SWAF of weathered CICO for a total of 57 hr (November 1979 experiment). Average hydrocarbon concentration for undiluted SWAF (173 ppb) was determined by GC analysis. The two lower SWAF concentrations (92 and 43 ppb) were calculated from proportional dilutions of the undiluted SWAF.

TABLE 61. Effects of a 63 hr exposure to the SWAF of weathered CICO on hatching success of surf smelt embryos (December 1979 experiment). Data compiled at completion of hatching in controls, and reported in percent of total embryos introduced.

Hydrocarbon concentration (ppb)	Embryos				Larvae	
	Initial no. (N)	Alive (%)	Dead (%)	Disintegrated (%)	Alive[a] (%)	Dead (%)
Control	461	0	2.2	46.2	47.7	3.9
26	458	0.7	12.9	28.6	43.2	14.6
54	455	4.0	12.8	71.1	5.9	6.2
113	445	0.7	4.9	86.8	4.0	3.6

[a] Live larvae were collected daily and held in uncontaminated seawater to evaluate post-exposure survival; the percent represented is cumulative.

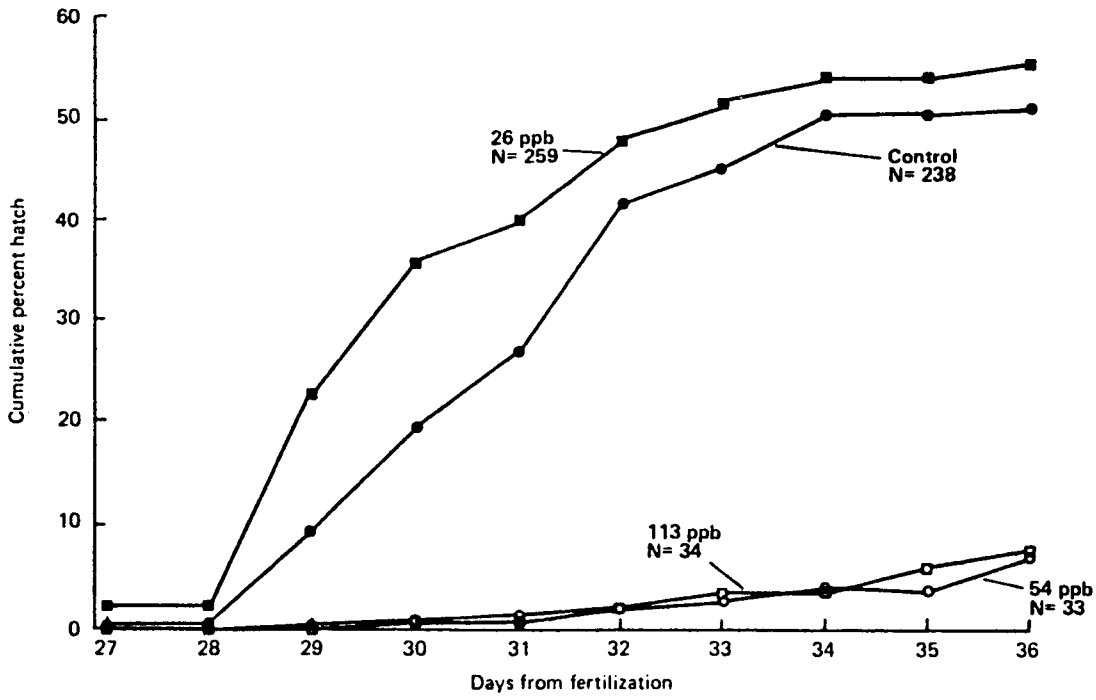

FIGURE 55. Cumulative percent hatching of surf smelt embryos exposed to 3 different concentrations of the SWAF of weathered CICO for a total of 65 hr (December 1979 experiment). Average hydrocarbon concentration for undiluted SWAF (113 ppb) was determined by GC analysis. The two lower SWAF concentrations (54 and 26 ppb) were calculated from proportional dilutions of the undiluted SWAF.

concentration of 26 ppb (December experiment) there was an apparent acceleration in hatching similar to that observed for chum salmon embryos exposed to the SWAF of weathered PBCO.

Samples of developing embryos were collected at two points during the December experiment (Table 62). On day 14 post-fertilization, mortality in control embryos was greater than in oil-exposed embryos; however, in embryos sampled one week later (approximately two-thirds of the way through incubation), this was reversed. The most appropriate statistical model (Geisser and Eddy 1979) for the 22 day post-fertilization data indicated that the percent abnormal and dead embryos in the control group and those exposed to 26 ppb petroleum hydrocarbons were similar, but differed from embryos exposed to 54 and 113 ppb. These latter two in turn differed from each other.

In the December 1979 experiment, three consistent types of gross abnormalities were observed in the oil-exposed embryos: reduced coiling (an index of growth by length); absence of eye rotation and closure of the choroid fissure; and diffused pigment. The most frequent abnormality was reduced coiling. As embryos develop, the body coils inside the chorion attaining a maximum of 2.5 coils just prior to hatching. All abnormal embryos, exposed to a hydrocarbon concentration of 113 ppb exhibited reduced growth, with only 1.5 coils after 22 days of development; the same number of coils was observed one week earlier in controls. Approximately 50% and 20% of abnormal embryos exposed to hydrocarbon concentrations of 54 and 26 ppb, respectively, also exhibited reduced growth after 22 days incubation. The eyes of affected embryos did not develop beyond the 15-day post-fertilization stage. Arrested eye development was the predominate abnormality in embryos incubated in 26 ppb total hydrocarbons.

Surf smelt embryos have a single row of contracted, dark, melanophores along the ventral midline, and smaller, stellate-shaped melanophores scattered over the ventral surface of the yolk. Fifty percent of the abnormal embryos exposed to 54 and 113 ppb had diffused pigment in these melanophores.

In order to observe any latent effects of oil exposure, newly hatched larvae from the December 1979 experiment were held in uncontaminated water, and fed rotifers (Brachionus plicatilus). After holding an average of 10 days (depending on time of hatching) survivors were counted (Table 63). Although the percent of normal appearing larvae hatching in the 26 ppb concentration of the SWAF was nearly identical to the control group, their survival as larvae was significantly less ($P<0.001$); only 8.7% survived compared with 42.5% in controls.

Surf smelt embryos from the December 1979 experiment were sampled 14 days after fertilization and whole eggs (embryo and egg envelope) were analyzed for petroleum hydrocarbons by GS/MS. Aromatic hydrocarbons found in control eggs and eggs exposed to the SWAF of weathered CICO are given in Table 64. Alkanes are commonly found in marine organisms, and prominent differences were not observed in concentrations from either oil-exposed or control eggs.

TABLE 62. Percent normal, abnormal, and dead surf smelt embryos sampled from the December 1979 experiment 14 and 22 days after fertilization (28 and 53 hr exposure).

Hydrocarbon concentration (ppb)	Sampling date (post-fertilization) 14 days (N)	Normal (%)	Dead (%)	22 days (N)	Normal (%)	Abnormal (%)	Dead (%)
Control	99	83	17	27	85	0	15
26	103	97	3	26	69	19	12
54	100	99	1	26	23	19	58
113	100	100	0	28	4	32	64

TABLE 63. Percent survival of surf smelt larvae hatched from embryos exposed to three concentrations of the SWAF of weathered CICO. Percent survival was determined 10 days after hatching.

	Control	Average hydrocarbon concentration (ppb) during embryo incubation 26	54	113
Original number of larvae introduced	219	196	23	18
Percent survival	42.5	8.7	8.7	5.6

TABLE 64. Concentrations of aromatic hydrocarbons in control surf smelt eggs and eggs exposed for 26 hr to the SWAF of weathered CICO.

Hydrocarbon	Hydrocarbon concentration in Nanograms/g(Wet Wt.) Control	Oil-exposed[a]
Naphthalene	33	22
2-Methylnaphthalene	<30	72
1-Methylnaphthalene	<15	46
Biphenyl	<35	37
2,6-Dimethylnaphthalene	<15	110
2,3,5-Trimethylnaphthalene	<15	125
Fluorene	<15	66
Phenanthrene	<15	63

[a] Sample size of 99 eggs from control group and 100 eggs from 113 ppb oil-exposed group.

(f) Effect of Crude Oil Ingestion on Salmonid Reproductive Success

Fifteen of the 48 test fish died 1 to 3 months after spawning; all of these animals were heavily infected with fungus. None of the 12 controls were similarly affected.

Maturation and Reproductive Success. The first males were in spawning condition by mid-December 1975; the first females were ripe 2-3 weeks later (Fig. 56). Although the first ripe fish were from the test group, there appeared to be no pronounced acceleration or retardation of maturity related to petroleum exposure. Eggs were collected from ripe females starting in early January 1976, and collections continued weekly through mid-February.

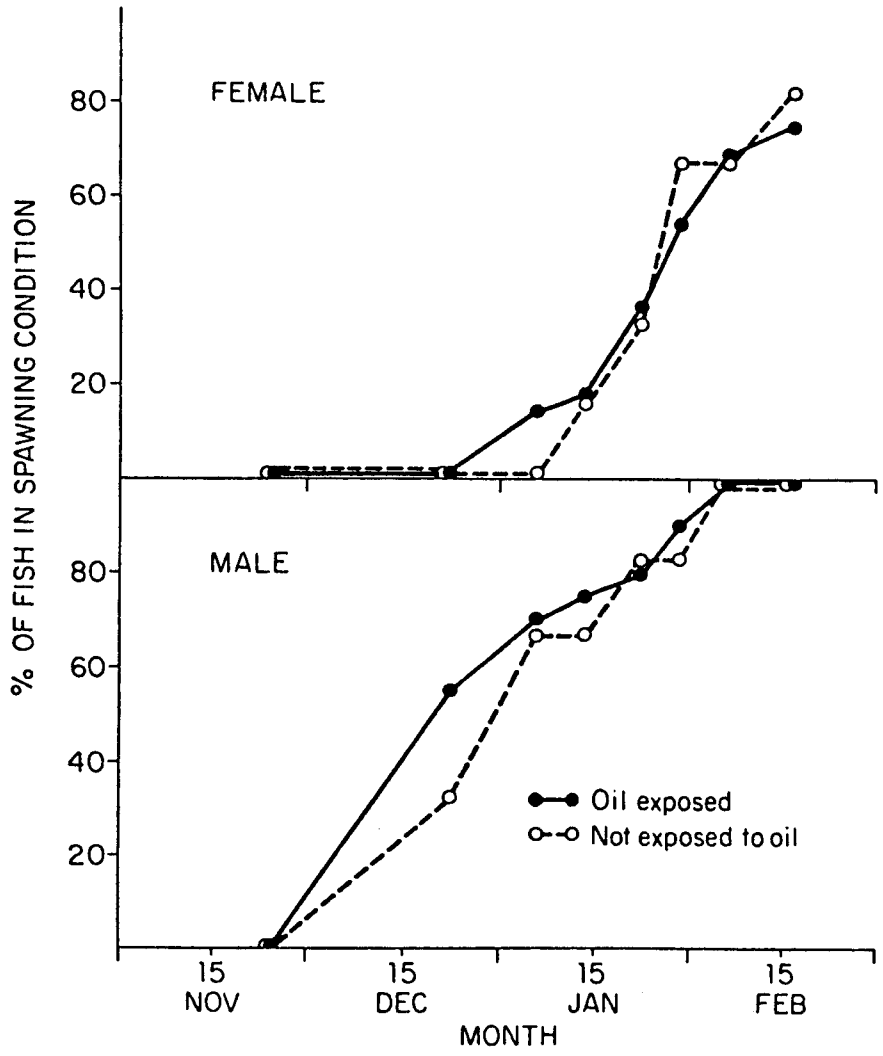

FIGURE 56. Timing of maturation for petroleum-exposed and non-petroleum exposed rainbow trout. (From Hodgins et al. 1977)

No significant difference (P=0.10) in hatching success (percent survival) among crosses in which sperm was used from petroleum-fed and control males was observed (Table 65). One particular cross did, however, result in very low survival (5.1%) and slightly lowered the average percent hatching success of eggs fertilized with sperm from control males.

Hatching success ranged from 32.4% to 99.5% for eggs from petroleum-exposed females and from 79.2% to 96.8% for control eggs (Table 66), but the respective means of 86.4% and 90.3% were not significantly different (P=0.10). Lowered survival of eggs from 2 test females reduced the average survival of the test group.

Average survival of alevins was higher, although not significantly (P=0.10), for control than for test fish (Table 67). Again, low survival occurred in one petroleum-exposed group.

Chemical Analyses. In using the spectrofluorometric method of analysis, interference from non-hydrocarbon fluorescing compounds prevented precise quantitation of PBCO in adult trout muscle and eggs; only qualitative and semi-quantitative results were possible. An emission maximum (364 nm) superimposed on the background of fluorescing compounds was observed for all samples from fish fed PBCO; this maximum was not observed for any of the samples from fish fed the control diet (Fig. 57). The ratios of the average relative intensities at an excitation wavelength of 262 nm and an emission wavelength of 364 nm of petroleum-fed fish to

TABLE 65. Survival of eggs fertilized with sperm from petroleum-exposed and non-petroleum exposed male rainbow trout.

Female	% Survival through hatching	
	Crossed with petroleum-exposed males	Crossed with non-petroleum-exposed males
Non-petroleum exposed	96.8	96.7
	81.3	77.3
	95.7	96.3
	89.9	87.7
	88.5	94.4
Petroleum exposed	99.4	99.6
	98.2	98.1
	98.6	95.2
	95.9	95.2
	36.9	37.3
	58.9	5.1
	95.8	98.3
	94.7	94.5
	78.5[a]	72.5[b]
	$\bar{x}$ = 86.4	$\bar{x}$ = 82.0
	SD = 18.0	SD = 27.7

[a] Pool of eggs from two females
[b] Pool of eggs from three females.
(From Hodgins et al. 1977)

control fish for muscle tissue and eggs were 2.8:1 and 3.8:1, respectively. A total of 14 analyses of muscle and 7 analyses of eggs from petroleum-fed fish and 4 analyses of muscle and 2 of eggs from control fish were performed. The background of fluorescing compounds was sufficiently high for the control and petroleum-impregnated food so that no definitive results could be obtained via spectrofluorometry.

TABLE 66. Survival of eggs through hatching from petroleum-exposed and non-petroleum exposed female rainbow trout.

% Survival for eggs from non-petroleum-exposed trout	% Survival of eggs from petroleum-exposed trout
96.8	96.8
79.2	98.4
96.0	98.9
88.4	98.5
91.3	99.5
	98.1
$\bar{x}$ = 90.3	97.0
SD = 7.1	95.6
	37.2
	32.4
	97.0
	94.6
	75.6[a]
	89.8[b]
	86.5[c]
	$\bar{x}$ = 86.4
	SD = 21.9

[a] Pool of eggs from two females.
[b] Pool of eggs from three females.
[c] Pool of eggs from four females.
(From Hodgins et al. 1977)

TABLE 67. Survival of alevins from petroleum-exposed and non-petroleum-exposed female rainbow trout.

% of offspring surviving from hatching to swim-up	
Non-petroleum exposed	Petroleum exposed
99.0	97.4[a]
99.4[a]	96.2[a]
89.1	90.3[a]
76.3	87.3[c]
	81.1[a]
$\bar{x}$ = 91.0	79.8[b]
SD 10.9	74.1
	68.0
	61.7[a]
	25.0[a]
	$\bar{x}$ = 76.1
	SD = 21.4

[a] Pool of offspring from two females.
[b] Pool of offspring from three females.
[c] Pool of offspring from four females.
(From Hodgins et al. 1977)

6.3.2 Invertebrate Studies

Defense Behavior of Sea Urchins Exposed to Petroleum Hydrocarbons.

Aromatic hydrocarbons were found to markedly inhibit the chemosensory-mediated defense response of green sea urchins at concentrations in the low ppb range. The effect of the SWSF of PBCO and its major identified monocyclic aromatic hydrocarbon components are shown in Figures 58-60.

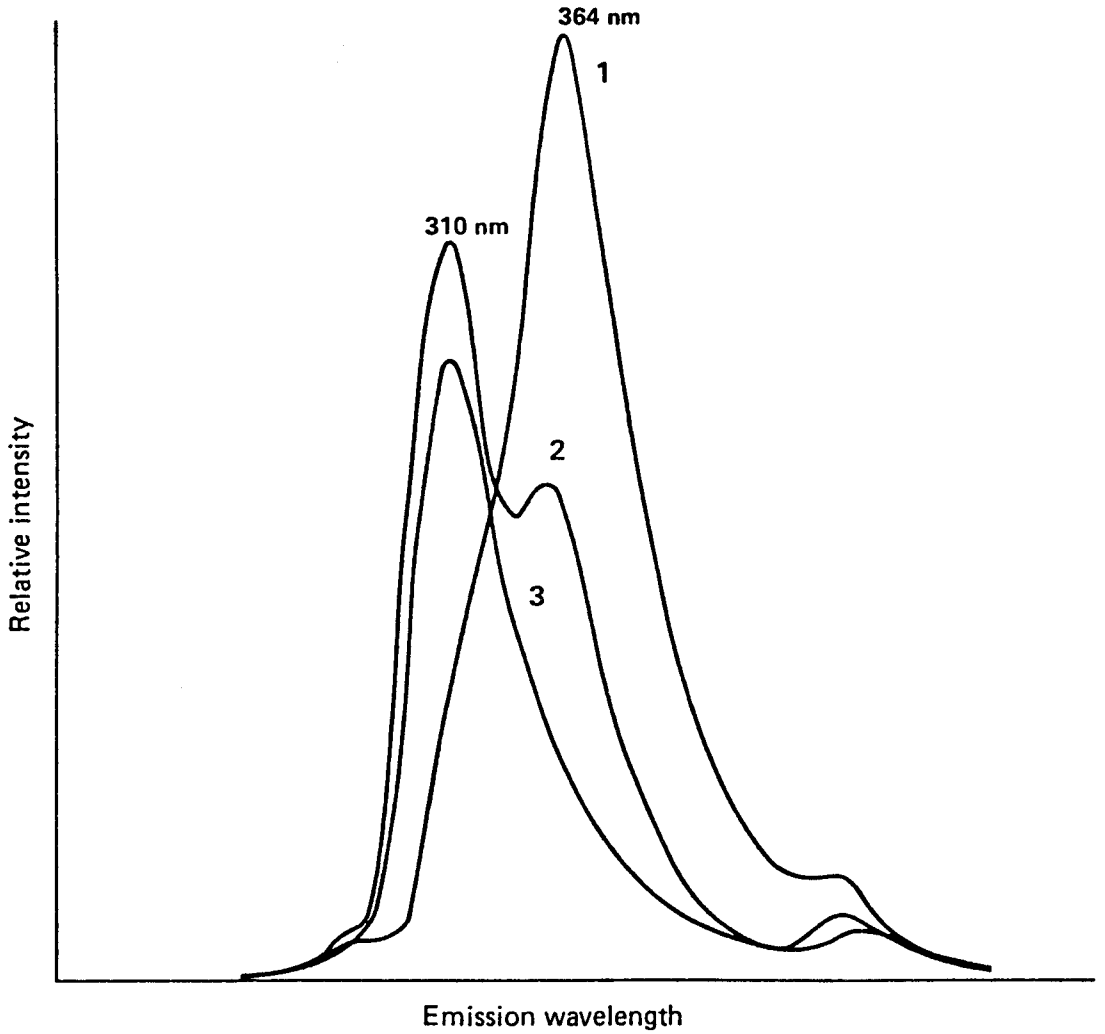

FIGURE 57. Spectrophotofluorometric curves of PBCO and extracts of trout eggs.
No. 1 - Prudhoe Bay crude oil
No. 2 - Oil-fed fish egg extract
No. 3 - Control fish egg extract
(From Hodgins et al. 1977)

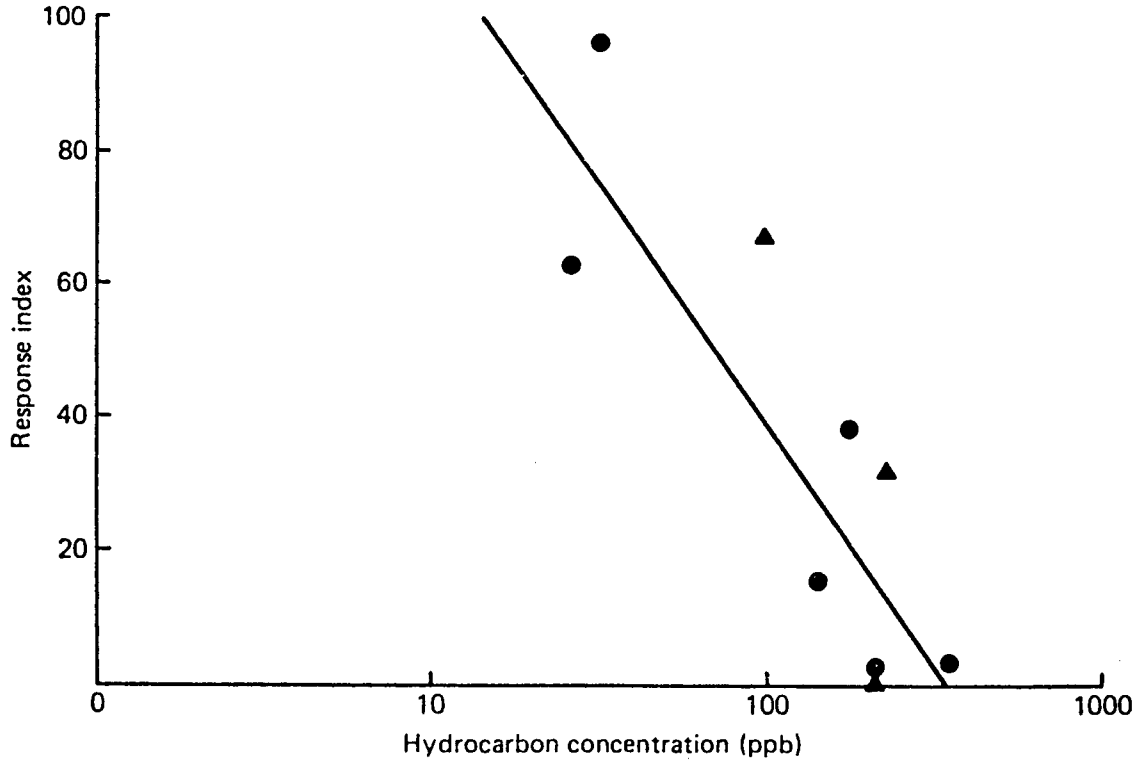

FIGURE 58. Effect of the SWSF of PBCO on the pedicellarial defense response of green sea urchins. ● = 24 hr exposure; ▲ = 10 min exposure. The 10 min exposures are not included in calculation of regression line: $Y = 182 - 72 \log_{10}$ (ppb SWSF); $r = -0.88$.

The relationship between the logarithm of the hydrocarbon concentration and the resulting response index was apparently unaffected by the duration of exposure to the hydrocarbons. In general, 10 min exposure periods produced inhibitions of the defense response equal to those following 24 hr exposures. Thus, it can be assumed that the urchins, in terms of their assayed defense behavior, equilibrate with the hydrocarbons very quickly.

Based on the calculated regression given in Figure 58, the PBCO SWSF concentration at which the pedicellarial response is inhibited by 50% (EC_{50}) is 60 ppb. The EC_{50} for toluene, the major constituent of the SWSF, is 200 ppb. For o-xylene, ethylbenzene, and trimethylbenzene, the EC_{50}'s are 350, 400, and 350 ppb, respectively.[1]

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[1] Analysis of dissolved benzene concentrations were insufficient to permit a regression analysis. Benzene analyses that were completed averaged 194 ± 62 ppb ($\bar{x}$ ± range) and the corresponding average pedicellarial response indices were 32% for 24 hr exposures and 32% for 10 minute exposures.

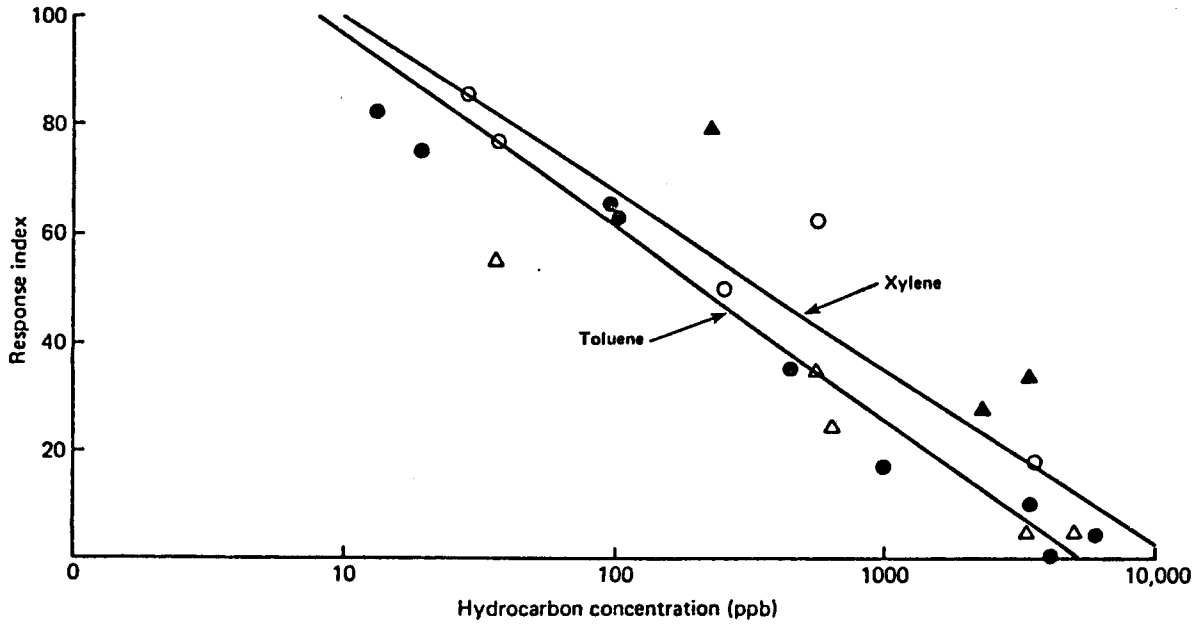

FIGURE 59. Effect of toluene and o-xylene on the pedicellarial defense response of green sea urchins. Closed circles and triangles are for toluene exposure, and 10 min exposures are not included in calculation of regression lines. ● = 24 hr toluene exposure; ▲ = 10 min exposure; Y = 133 - 36 $\log_{10}$ (ppb toluene); r = -0.98; o = 24 hr xylene exposure; △ = 10 min exposure; Y = 131 - 32 $\log_{10}$ (ppb xylene); r = -0.89.

Four groups of 5 sea urchins each were also exposed to 1-methylnaphthalene for either 1 or 24 hr. Although naphthalenes were usually detected in the SWSF of PBCO, their concentrations normally amounted to only 3% or less of the total SWSF. The effect of 1-methylnaphthalene on the pedicellarial response is shown in Figure 61. The EC_{50} (60 ppb) is similar to that found for the SWSF of PBCO. It is doubtful that naphthalenes are responsible for the effects noted in the SWSF tests since their concentrations in the SWSF were generally less than 2 ppb; such a concentration would probably not inhibit pedicellarial response.

In order to determine the competence of the pedicellariae to respond, 0.5 ml isotonic KCl was injected into the body cavity of 3 groups of 5 sea urchins each. Injections of KCl into the control group, which previously showed an average 64% response to starfish extract, caused 100% opening of the pedicellariae. In the two parallel groups of urchins which had been exposed for either 10 min or 24 hr to 210 ppb of the SWSF an average of 93% responded. These tests indicate that pedicellariae which demonstrated reduced behavioral sensitivity as a result of hydrocarbon exposure are fully competent to respond if artificially provoked.

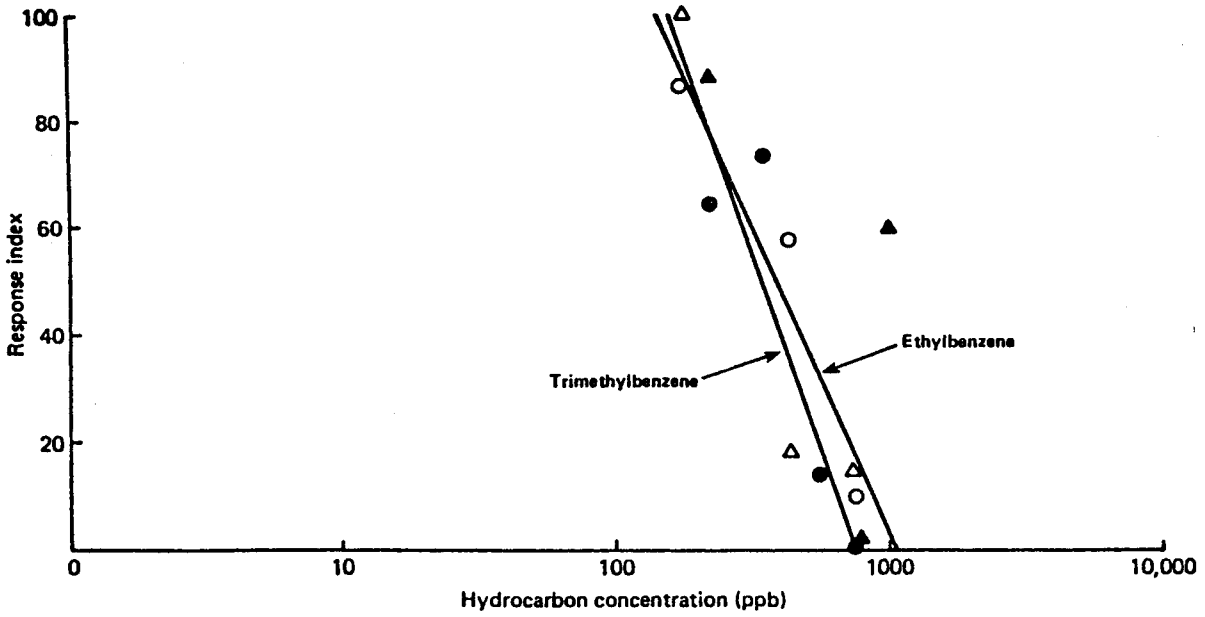

FIGURE 60. Effect of 1,2,4-trimethylbenzene and ethylbenzene on the pedicellarial defense response of green sea urchins. Closed circles and triangles are for trimethylbenzene exposure, and 10 min exposures are not included in calculation of regression lines. ● = 24 hr toluene exposure; ▲ = 10 min exposure; $Y = 429 - 148 \log_{10}$ (ppb trimethylbenzene); $r = -0.88$; o = 24 hr ethylbenzene exposure; △ = 10 min exposure; $Y = 363 - 120 \log_{10}$ (ppb ethylbenzene); $r = -0.96$.

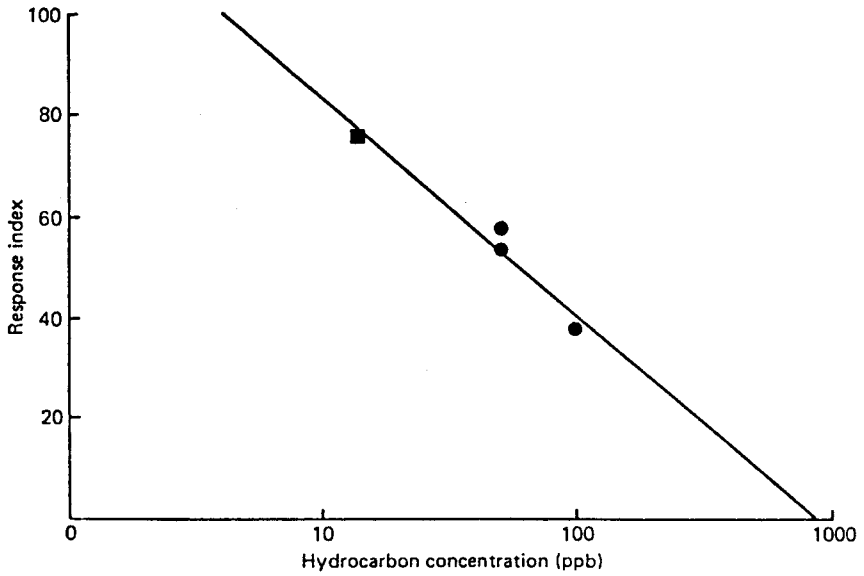

FIGURE 61. Effect of 1-methylnaphthalene on the pedicellarial defense response of green sea urchins. ● = 24 hr exposure; ■ = 1 hr exposure: $Y = 128 - 44 \log_{10}$ (ppb methylnaphthalene); $r = -0.98$.

Hydrocarbon-induced inhibition of the pedicellarial response was found to be reversible if the urchins were allowed to depurate in clean, flowing seawater. The results of 3 assays involving various depuration periods and hydrocarbon treatments are shown in Figure 62. These data show that the hydrocarbon-induced pedicellarial inhibitions diminished much more slowly than they ensued.

In addition to the assays of defense behavior considered above, numerous observations of hydrocarbon-treated urchins were made concerning other types of activity. In general, urchins exposed to less than 1 ppm hydrocarbon were able to feed, respond to touch, adhere to the glass substrate, right themselves, and move about in an apparently normal fashion. No urchins were killed by the hydrocarbon treatments employed in this research, and narcosis was generally evident only at hydrocarbon concentrations approaching or exceeding 1 ppm.

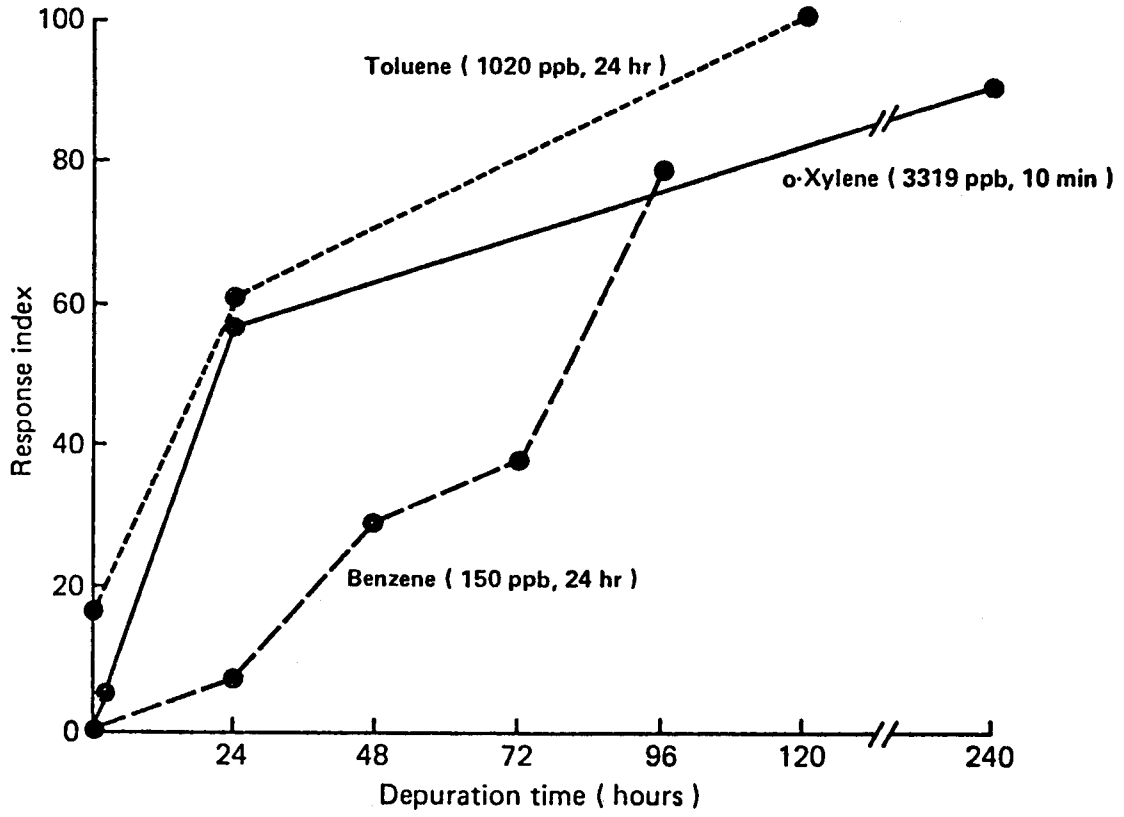

FIGURE 62. Recovery of the pedicellarial defense response as a function of depuration time in clean flowing seawater. Points represent average response index for each group of five green sea urchins. Lines are labeled with hydrocarbon treatments administered to each group.

In predation experiments equal numbers of control urchins and urchins exposed to the SWSF of PBCO were placed into a tank with three Pycnopodia. The results showed that more hydrocarbon-treated urchins were eaten than untreated controls in each of the four trials (Table 68). The combined data for each of these trials indicates that predation of PBCO-treated urchins was significantly greater than on control urchins (P=0.01).

Reproductive Behavior of Dorid Nudibranchs Exposed to Petroleum Hydrocarbons

(a) Chemosensory disruption. Prior to testing of individual dorid nudibranchs for their behavioral response to an aggregate of conspecifics, the individuals were exposed for 24 hr to 3 different concentrations of the SWSF of PBCO; 15 ± 3 ppb ($\bar{x}$ ± range), 60 ± 20 ppb, and 420 ± 250 ppb. There was no direct relation between hydrocarbon concentration and chemotatic response; thus, the data are combined (Table 69).

TABLE 68. Effect of 24 hr exposure to a SWAF of PBCO on predation of sea urchins by Pycnopodia.

Exposure concentration (ppb)	Trial duration (hr)	Treated		Control	
		N	% eaten	N	% eaten
470	2	7	100	7	43
359	2	5	100	5	40
233	24	5	60	5	40
145	2	5	100	5	60
$\bar{x}$ = 302			88		49

TABLE 69. Chemotatic response of control and SWSF exposed (15 to 420 ppb) dorid nudibranchs to an aggregate of reproductive conspecifics in "stimulus" chamber.

Treatment	Number tested	Percent movement		
		Toward "stimulus" chamber	Toward "blank" chamber	No choice
Control	19	84	5	11
24 hr exposure	24	62	33	4

The percentage of nudibranchs moving into the "stimulus" chamber (toward the conspecific aggregate) after exposure to the SWSF of PBCO was significantly different from that of controls (P=0.05). Their mobility (denoted by the number that did not make a choice) was not affected by exposure to petroleum hydrocarbons. For these tests a 50-50 distribution is interpreted as random movement.

(b) Embryological development. Exposure of mature dorid nudibranchs to increasing concentrations of the SWSF of PBCO resulted in two macroscopically measureable effects: a delay in egg laying in the highest exposure group, and an immediate and long-term decrease in the total weight of eggs laid (Table 70). The egg laying delay was not a factor in total weight of eggs deposited after 12 days since all groups had sharply declined oviposition by the end of the 18 day experiment.

Microscopically, exposure of mature dorids and their subsequent spawn to the SWSF of PBCO resulted in a direct relationship between the number of abnormal embryos and SWSF concentration (Fig. 63). There was no evidence of an increase in embryo abnormalities with duration of exposure to the SWSF at any concentration tested. Nearly half (44%) of the abnormalities observed in eggs spawned by adults exposed to 225 ppb did not have capsules. This type of abnormality was never observed in control samples, and occurred at a frequency of only 0.7 and 1.1% in the 7 and 22 ppb exposure groups, respectively.

Hatching commenced 9 to 10 days after egg deposition and was completed by the 11th day in all groups except the one exposed to the highest SWSF concentration. Embryos exposed to 225 ppb of the SWSF exhibited a marked delay in development, with only 25% of those which appeared normal reaching the shelled stage; none were observed to hatch after 11 days of development.

TABLE 70. Effect of the SWSF of PBCO on egg deposition of dorid nudibranches. Each exposure group contained 20 mature nudibranches with a total wet weight of 2.1 g per group.

Hydrocarbon concentration (ppb)	First egg masses laid			Total egg mass weight over 12 days (mg)
	Time after start of adult exposure (days)	Number of egg masses	Wet weight of masses (mg)	
Control	1	16	370	938
7[a]	1	14	320	832
22[a]	1	10	345	821
225	6	5	185	516

[a] Concentrations based on dilutions of highest concentration which was 225±80 ($\bar{x}$±SD, N=4).

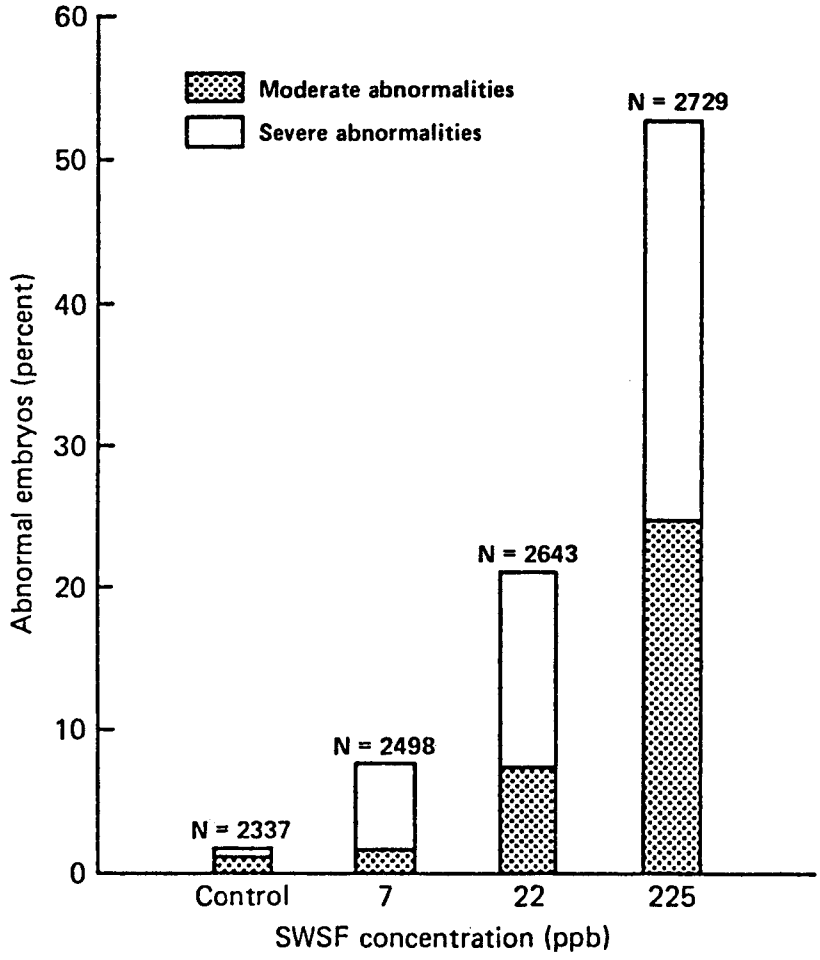

FIGURE 63. Effect of the SWSF of PBCO on embryonic development of the dorid nudibranch. Percent abnormal embryos averaged from all samples taken in first 10 days of exposure.

Feeding Behavior of Shrimp Exposed to Petroleum Hydrocarbons

Results of experiments on the feeding response of spot shrimp indicate that exposure to the SWSF of PBCO causes a decrease in feeding activity, particularly those activities involving searching and contact with the stimulus source (Fig. 64). Both of these higher order behaviors show an EC_{50} of about 25 ppb.

These results represent a composite of observations taken over a 6-day exposure period at each SWSF concentration. Generally there was not a progressive increase or decrease in searching or feeding behavior with time. At the highest concentration several of the shrimp exhibited a loss of equilibrium following 3 days' exposure; observations on these shrimp are not included.

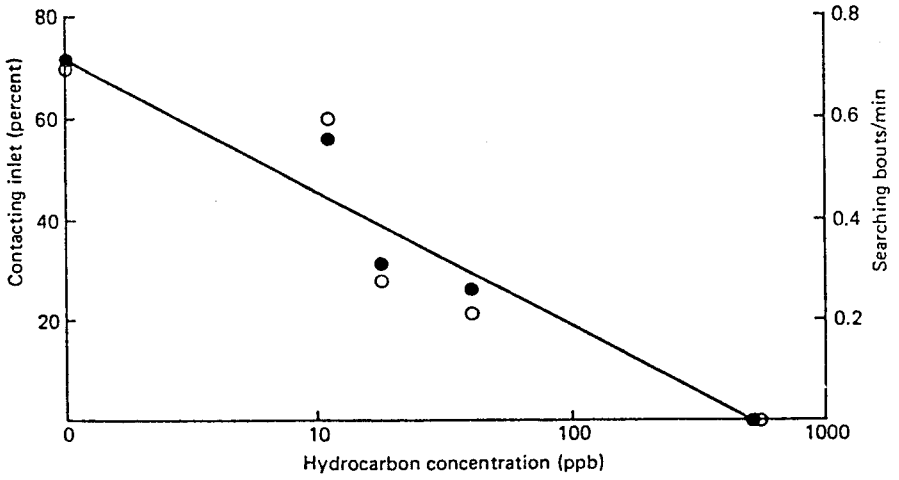

FIGURE 64. Effect of the SWSF of PBCO on pereopod searching behavior (bouts/min) and percent of spot shrimp contacting stimulus inlet in response to squid extract. Each point reflects response to stimulus minus background activity. Regression line based on data of percent shrimp contacting stimulus inlet (closed circles): $Y = 73 - 27 \log_{10}$ (ppb SWSF); $r = -0.97$. For searching behavior (open circles): $Y = 0.72 - 27 \log_{10}$ (ppb SWSF); $r = -0.93$.

7. DISCUSSION

7.1 Chemistry

7.1.1 Accumulation and Biotransformation of Specific Aromatic Hydrocarbons in Salmonids

Results (Roubal et al. 1977a) indicated that benzene, NPH, and anthracene are readily deposited in coho salmon tissues. Concentrations of these compounds increased in tissues, such as liver and brain, in the order of benzene < NPH < anthracene. This finding suggests that within certain molecular weight ranges, the accumulation of aromatic hydrocarbons in salmonid tissues may be directly related to the number of benzenoid rings in the molecule; however, as discussed below, the degree of alkyl-group substitution on the parent hydrocarbon also appears to influence hydrocarbon concentrations in tissues of fish (Roubal et al. 1978).

The hydrocarbon metabolites appeared in considerable amounts in all tissues examined, including the brain, of coho salmon. A substantial decline in hydrocarbon concentrations over time was accompanied by a steady increase in the proportions of metabolic products in all tissues examined.

This study showed that metabolites of NPH formed by salmonids are similar to those formed by mammals (Boyland and Solomon 1955, 1956, Terriere et al. 1961, Sims 1964). Moreover, the study showed for the first time, that conjugated metabolites of NPH were formed in fish.

7.1.2 Accumulation of Petroleum Hydrocarbons by Fish Exposed to SWSF of PBCO

Roubal et al. 1978 reported that substituted benzenes, NPH, and substituted NPH accumulated both in coho salmon and starry flounder when the fish were exposed to the SWSF of PBCO. Moreover, as the number of alkyl substituents increased on benzene and naphthalene, so did the accumulation of these compounds.

The C_4- and C_5-substituted benzene fraction of PBCO was the most prominent fraction accumulated in muscle of both coho salmon and starry flounder. However, substantially greater concentrations of this fraction accumulated in the muscle of starry flounder than in muscle of coho salmon. Hydrocarbons of the SWSF were not detected in the liver and gills of the test coho salmon. However, substantial amounts of the substituted benzenes, NPH, and substituted NPHs were found in the liver and gills of starry flounder. The ability of starry flounder to bioconcentrate aromatic hydrocarbons suggests that starry flounder residing on sediments contaminated with petroleum hydrocarbons may accumulate substantial concentrations of petroleum hydrocarbons in various tissues.

7.1.3 Metabolism of NPH by Coho Salmon

This study (Collier et al. 1978) represents the first use of HPLC for characterizing NPH metabolites in fish. The presence of both non-conjugated and conjugated derivatives in the liver and gall bladder indicates that coho salmon have a significant ability to metabolize NPH and excrete the metabolites. However, the question still remains to what extent low levels of these metabolites are retained for extended periods in tissues of salmonid fish.

7.1.4 NPH and Its Metabolites in Fish Skin and Mucus

Skin. The results (Varanasi et al. 1978) show that within a few hours of NPH exposure, appreciable concentrations of both the hydrocarbon and its metabolites were present in the skin of coho salmon, starry flounder, and rainbow trout, regardless of the mode of exposure or the salinity of the medium. Moreover, comparison of these results with those obtained for liver of rainbow trout (Collier et al. 1980) and starry flounder (Varanasi et al. 1979) reveals that the pattern of uptake and release of NPH and its metabolites in skin was similar to that observed for the liver. NPH concentrations in skin declined more rapidly than the concentrations of NPH metabolites, demonstrating a tendency of skin to preferentially retain metabolites. This finding is in agreement with results of Lee and coworkers (1976), Sanborn and Malins (1977), and Roubal et al. (1977a), which showed that the parent hydrocarbon was more rapidly discharged either directly or via biotransformation and that small but detectable concentrations of metabolites persisted in tissues of crustaceans and fish over a long period. Whether metabolic products detected in the skin of our test fish arise from the direct transport of these compounds by blood to the skin and/or from biotransformation of NPH by AHM that may be present in the skin

remains to be seen. It should be noted that maxima in concentrations of both NPH and its metabolites in the skin were preceded by the corresponding maxima in the livers (Varanasi et al. 1978, Collier et al. 1980). It appears therefore that injected or ingested NPH was first transported to the liver and subsequently transported to the skin via the blood stream.

Mucus. Epidermal mucus of fish serves as a physical and perhaps an immunological barrier to pathogenic organisms (Harris and Hunt 1973). It is also believed to be associated with osmoregulation (Jakowska 1963) and is an important factor in controlling the swimming speed of the fish (Rosen and Cornford 1971). The results (Varanasi et al. 1978) from the force-feeding and injection studies, which showed that NPH and its metabolic products were present in epidermal mucus of rainbow trout for several days after the initial exposure to NPH, strongly suggest that epidermal mucus of salmonids is involved in excretion of hydrocarbons and their metabolites. Epidermal mucus is in constant flux; its relative importance in the excretory mechanisms of fish would depend on the rate of discharge of these compounds from the mucus into the surrounding water as well as on the turnover or sloughing rate of the mucus itself. Varanasi and Markey (1978) reported that the presence of certain metals in water induces increased mucus production in fish, thereby accelerating the turnover of the mucus layer. Whether hydrocarbons exert such an influence on mucus production in fish is unknown; however, copious amounts of mucus were produced by soft-shell clams exposed to No. 2 fuel oil in water (Stainken 1975).

7.1.5 Uptake and Biotransformation of NPH by Flatfish

The results (Varanasi et al. 1979) show that pleuronectids, like salmonids (Roubal et al. 1977a, Varanasi et al. 1978, Collier et al. 1978), are able to absorb and metabolize dietary NPH, and that NPH and its metabolic products are broadly distributed in tissues and body fluids of the exposed fish. Rock sole contained statistically significant larger concentrations of NPH and its metabolites than did starry flounder and almost 4 times as much radioactivity remained in the digestive tract of rock sole compared to that in the starry flounder at 24 hr after feeding of ^{3}H-NPH. The digestive tract of rock sole has pyloric caeca, whereas that of starry flounder does not; in addition to other species-specific differences, perhaps structural and functional variations in the digestive tracts (Barrington 1957) of these fish are responsible for the observed differences in the levels of accumulated radioactivity. In fact, a major fraction of the administered radioactivity may have been directly discharged from the gastrointestinal tract of starry flounder.

Support for this observation comes from results showing that when the same dose of NPH was administered to starry flounder via i.p. injection, subsequent concentrations of NPH in tissues (e.g., liver) were comparable to those obtained for NPH exposed rock sole and were much higher than those for starry flounder force-fed NPH. It may be that absorption of NPH over a longer period, from the gastrointestinal (GI) tract in the case of rock sole in the feeding study and from the abdominal cavity

in the case of starry flounder in the injection study, was responsible for the results showing that the decline in the concentrations of NPH from the tissues (e.g., liver, skin, and blood) of these fish was much slower than that for starry flounder force-fed NPH.

In terrestrial mammals, the route of administration can alter the pharmacokinetics of a given compound (Gibaldi and Perrier 1974). The mode of hydrocarbon-exposure did influence concentrations and relative proportions of NPH and its metabolic products as well as the types of metabolites accumulated in pleuronectid fish. Metabolism of NPH in the GI tract may be responsible for the observed differences in the pattern of metabolites accumulated in livers of starry flounder in the injection and feeding experiments at 12°C. Thus, it can be speculated that differences in patterns of metabolites may occur when the major route of uptake of PAH is other than the GI tract (e.g., gills and skin when PAH is present in water or sediment).

Dietary aromatic hydrocarbons are cleared from terrestrial animals primarily via biliary and to a lesser extent, via renal excretion (Daniel et al. 1967, Guarino et al. 1972). As with salmonids (Roubal et al. 1977a, Melancon and Lech 1978, Statham et al. 1978, Collier et al. 1978), bile of flatfish in both dietary and i.p. exposures accumulated large amounts of NPH metabolic products. This suggests that biliary excretion was one of the major routes by which the hydrocarbon was cleared from these pleuronectids. Metabolites in the bile of the NPH-exposed flatfish were characterized by the preponderance of glucuronides (>80%). Radioactivity excreted in urine was not measured, but considerable radioactivity was present in the kidney of the test fish over a long period (up to 6 wk). Excretion of both NPH and metabolic products via epidermal mucus (Varanasi et al. 1978) and gills (Thomas and Rice 1981) seems to occur in salmonids. Epidermal mucus and gills of pleuronectids exposed to NPH contained detectable concentrations of NPH and, more importantly, its metabolic products, which suggests that in addition to biliary and renal excretion, clearance via epidermal mucus and gills may also take place in these fish. The relative importance of each of these pathways in clearance of xenobiotics in fish remains to be assessed.

The concentrations of NPH and its metabolic products in muscle were one-tenth of those for the liver of the individual species, indicating that no marked tendency was shown by muscle to accumulate the hydrocarbon or its metabolites. Liver and various extra-hepatic tissues of marine organisms are shown to possess AHM (Varanasi and Malins 1977, Bend and James 1978), but the presence of this enzyme system in the muscle of fish has not yet been demonstrated. Our results show that the pattern of metabolites accumulated in muscle were qualitatively similar to that in the liver of the same fish. However, in both species, the proportion of non-conjugates, specifically 1,2-dihydro-1,2-dihydroxy-NPH (dihydrodiol), was higher in the muscle than in the liver. If the presence of metabolic products in muscle was primarily due to their transport from the liver, then selection seems to take place in the types of metabolites that are deposited in muscle.

In agreement with the results with salmonids (Roubal et al. 1977a), proportions of metabolites relative to NPH increased with time after exposure in tissues (e.g., liver) of flatfish. Moreover, the findings show that the types of metabolites accumulated in flatfish liver were dependent on the time elapsed after the administration of NPH. With time, the pattern of metabolites changed in the liver of test fish, regardless of species or mode of administration of NPH; there was an increase in the proportion of conjugates--specifically sulfate/glucoside fraction--and a decrease in the proportion of the dihydrodiol. Because certain dihydrodiols of PAH are known to be mutagenic and carcinogenic (Swaisland et al. 1974, Levin et al. 1976), a decreased proportion of the dihydrodiol may imply increased detoxification of the hydrocarbon.

7.1.6 Effect of Temperature on Disposition of NPH and Its Metabolites in Fish

Water temperature had a pronounced effect on NPH accumulation and retention in the major organs of coho salmon exposed to NPH via force feeding (Collier et al. 1978). An inverse relationship between environmental temperature and NPH retention was observed for brain, liver, kidney and blood. The work of Fucik and Neff (1977) with clams, Rangia cuneata, and Harris et al. (1977) with copepods, Calanus helgolandicus, indicated a similar relationship between temperature and NPH retention in marine invertebrates. Thus, it seems that the nature and severity of any toxic effects of NPH in marine organisms may be different in colder environments; cold-adapted organisms may retain NPH longer than those in warmer waters.

In another study, Varanasi et al (1981a) reported that a decrease in water temperature from 12° to 4°C resulted in the retention of substantially higher concentrations of NPH in tissues of NPH-exposed starry flounder. The increase in NPH concentrations could be due to several factors: Differences in rates of absorption of the ingested dose, differences in rates of excretion of NPH from tissues, and differential rates of biotransformation of NPH at these two temperatures.

Environmental temperature is known to have a marked effect on the activity of the alimentary canal of fish; both the rate of passage of food and the rate of its absorption are much slower in fish at lower temperatures (Barrington 1957). The results of Varanasi et al (1981a) indicate that both absorption and elimination of the ingested dose from the alimentary canal were slower for fish at 4°C than at 12°C. This may explain the slower decline in NPH concentrations from 24 to 168 hr in tissues of starry flounder at the lower temperature. Lowering the water temperature may also influence the rate of elimination of NPH from tissues of fish. For example, Collier et al. (1978) did not observe any effect of lowered water temperature on the retention of ingested NPH in the gut of coho salmon, but did report statistically significant higher concentrations of NPH in tissues of fish at 4°C compared to those at 10°C.

The effect of decreased temperature on metabolite concentrations in starry flounder tissues was not as marked as that observed for the NPH concentrations, which suggests that a much smaller proportion of NPH was biotransformed by liver of starry flounder held at the lower temperature. However, such a result may also be due to altered rates of excretion of NPH and its metabolites at the lower temperature. Nevertheless, when considering consequences (e.g., toxicity) of increased concentrations and increased residence times of NPH in cold-acclimated fish, the decreased bioconversion of NPH should be taken into account.

A change in environmental temperature is also known to alter the activities of certain enzymes in fish (Hochachka and Somero 1971). Stegeman (1979) reported that in vitro activities of hepatic BaP hydroxylase measured at 25°C were significantly greater for Fundulus heteroclitus maintained at 6.5°C than those at 16.5°C. Egaas and Varanasi (1982) reported that lowering the environmental temperature increased both the initial hydrocarbon metabolism and the time needed for the effect of the chemical inducer to be evident. A change in the water temperature of a fish brings about changes in proportions of polyunsaturated fatty acids (PUFA) in phospholipids associated with membranes (Hazel 1979a, 1979b), which may result in altered rates of absorption and excretion of lipophilic PAHs and their metabolites. Wills (1980) reported a linear relation between the rate of BaP oxidation and PUFA content in endoplasmic reticulum in rat. Thus, environmental temperature can alter concentrations of individual metabolites formed and retained in tissues of fish exposed to PAHs. A decrease in water temperature brought about marked changes in individual metabolite classes in flatfish, which would have gone undetected if only total metabolite concentrations were determined.

Virtually no information is available on the toxicity of different NPH metabolites on fish. However, in mammals phenols and dihydrodiols of certain PAHs [e.g., benzo(a)pyrene] are more toxic than the parent compound, and some of these metabolites also interact with cellular macromolecules (Burke et al. 1977, Kapitulnik et al. 1977, Varanasi et al. 1981b). Certain hydroxylated metabolites of NPH also irreversibly bind to cellular protein (Hesse and Mezger 1979). Accordingly, the results showing a statistically significant increase in the concentrations of the dihydrodiol derivative of NPH in liver and muscle of cold-maintained starry flounder is noteworthy. Moreover, it should be noted that 1 wk after NPH-exposure, fish held at 12°C contained very low concentrations of NPH, whereas tissues (e.g., liver and muscle) of fish at 4°C still retained substantial concentrations of NPH, a potential source of metabolic products.

7.1.7 Uptake and Metabolism of Sediment-Associated NPH and BaP by Flatfish

Several studies report on the uptake and metabolism of NPH by fish (Lee et al. 1972, Roubal et al. 1977a, 1978, Melancon and Lech 1978, Collier et al. 1978, 1980, Varanasi et al. 1979, 1981a). Studies on uptake of BaP are fewer (Lee et al. 1972, Lu et al. 1977, Gerhart and Carlson 1978, Martin 1980) and to our knowledge, until the work of Varanasi and Gmur (1981a), no information was available on the extent

of in vivo metabolism of BaP by fish liver. In this study, when English sole were exposed simultaneously to ^{14}C-NPH and ^{3}H-BaP in sediment, a substantial percentage of NPH-derived radioactivity in the liver of fish was present in the form of the parent hydrocarbon, whereas most of the BaP in the liver of the same fish was biotransformed. Moreover, the results show that the ratio of radioactivity in bile vs liver was substantially higher for BaP than that for NPH, demonstrating that BaP was more extensively metabolized in liver and transferred to bile. A large proportion of NPH may have been excreted, prior to metabolism, via gill (Thomas and Rice 1981) and skin/mucus (Varanasi et al. 1978).

These results (Varanasi and Gmur 1981a), together with our previous results (Varanasi et al. 1979), show that whether the fish were exposed to NPH or BaP, singly or in the presence of other hydrocarbons, the liver metabolized BaP more extensively than NPH. This probably explains why BaP is detected in very low concentrations or not detected at all in liver of fish (Veldre et al. 1979, Malins et al. 1980) sampled from areas containing considerable concentrations of BaP, since the currently available techniques can detect only the parent hydrocarbon. It is obvious from the results that if fish tissues are examined for hydrocarbons alone to assess whether the organisms have been exposed to these compounds, grossly misleading results may be obtained; the magnitude of error will be dependent on a number of factors (e.g., tissue type, duration of exposure, type of hydrocarbon). Therefore, metabolite analyses together with hydrocarbon analyses should be included in order to assess properly the levels of xenobiotics in marine organisms.

One of the major difficulties in tissue analyses of PAHs, such as BaP, has been the presence of lipids. A new method using two-dimensional TLC was developed to separate BaP and its metabolites from each other and from lipids present in liver (Varanasi and Gmur 1981a). This method may be adapted to separate PAHs and their metabolites from liver and muscle of fish sampled in field studies.

Metabolites of NPH in the bile of English sole in the present study were similar to those reported for starry flounder and rock sole exposed to dietary NPH (Varanasi et al. 1979, 1981a). The glucuronide of 1,2-dihydro-1,2-dihydroxynaphthalene was the major metabolite. The tendency of pleuronectid fish to produce a greater proportion of glucuronide conjugates than sulfate conjugates was also evident when bile samples were analyzed for both NPH and BaP metabolites. Glucuronidation is the major detoxification pathway in hamster embryo cells (Baird et al. 1977) and rat hepatocytes (Burke et al. 1977); however, marine invertebrates tend to produce large proportions of sulfate conjugates (Corner et al. 1960, Malins and Roubal 1982). Moreover, a preliminary report (Von Hofe et al. 1979) has shown that a higher proportion of sulfate conjugates than glucuronide conjugates was present in bile of Fundulus heteroclitus injected (i.p.) with BaP. Species specific differences as well as differences in mode of exposure to hydrocarbons may be responsible for the observed differences in types of conjugates formed by pleuronectids and Fundulus.

A number of metabolites known to be toxic to mammals were detected in liver and bile of English sole exposed to sediment-asociated BaP. Studies with mammals have demonstrated that BaP-7,8-dihydrodiol is further metabolized to BaP-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, which is known to bind covalently with DNA (Sims et al. 1974). The diolepoxide is hydrolyzed to 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene. HPLC analyses of polar metabolites in the present study revealed the presence of tetrols in bile of English sole, indicating that the diolepoxide, which is implicated as the ultimate carcinogen of BaP, was produced in liver of these fish. Recently Varanasi et al. (1981b) have demonstrated that English sole metabolizes BaP into reactive metabolites that bind to liver DNA. Such interaction of BaP with DNA is believed to be an important step in chemical-induced neoplasia.

In contrast to the extensive conversion of BaP by English sole liver, BaP in sediment and sediment-associated water remained primarily as the parent compound over a period of several days. The results also show that while BaP concentrations in sediment did not change markedly, NPH concentrations declined steadily. Greater susceptibility to microbial degradation (Herbes and Schwall 1979, Lee 1977) and higher water solubility (Brown and Weiss 1978) of NPH compared to BaP are two likely causes for the observed decline in NPH concentrations in sediment during the experiment.

The statistically significant decrease in concentrations of NPH-derived radioactivity in most tissues of English sole from 24 to 168 hr was attributed to the net release of NPH and/or its metabolites from tissues and not to decreased availability of NPH because NPH concentrations in SAW did not decline markedly. In contrast to NPH, BaP-derived radioactivity was continually accumulated and retained by English sole. This tendency to retain and bioconcentrate BaP-derived radioactivity is not unexpected. Roesijadi et al. (1978) reported that clams bioconcentrated radioactivity due to PHN, chrysene and BaP in the order of increasing molecular weight of PAH. Roubal et al. (1977a) with coho salmon fed benzene, NPH, or anthracene found that the degree of retention of hydrocarbon-derived radioactivity in fish tissues was in direct correlation with increasing molecular weight of the parent hydrocarbon.

7.1.8 BaP Metabolism by English Sole

English sole force-fed BaP converted it into a number of metabolites that have been characterized as mutagenic and carcinogenic in a variety of biological assays. Both liver and muscle contained toxic metabolites such as BaP 7,8-dihydrodiol, BaP 9,10-dihydrodiol and phenols. The two findings showing that concentrations of BaP metabolites decreased significantly with time in muscle and that a number of toxic metabolites were present in edible tissue of flatfish are important from a human health point of view. Further studies on the characterization of, mutagenic potency of, and rate of release of PAH metabolites from muscle of fish are needed to gain a better understanding of the fate and effects of these hydrocarbons in edible tissue of fish.

7.1.9 Binding of BaP Intermediates to DNA Catalyzed by Liver Enzymes

The results show that liver enzymes of coho salmon, starry flounder and English sole converted BaP into reactive intermediates that bind to DNA (Varanasi and Gmur 1980, Varanasi et al. 1980). The metabolites formed by fish liver extracts were characterized by a preponderance of non K-region dihydrodiols (e.g., BaP 7,8-dihydrodiol and BaP 9,10-dihydrodiol). It should be noted that mainly non K-region metabolites of PAH covalently bind to DNA of PAH-exposed mammals.

Many environmental and species-specific differences may influence the xenobiotic metabolizing capabilities of aquatic animals; it would be imprudent to attribute the marked difference observed in the binding of values for the three fish species to any particular factor. Moreover, wide variations in AHM activity have been reported to occur among different strains of a single species of fish or mice. However, it is possible that starry flounder and English sole, being demersal fish, are habitually exposed to a multitude of xenobiotics (some of which are inducers of AHM) present in bottom sediments, which could result in an initially high value for the in vitro covalent binding of BaP to DNA. This would also explain why pre-exposure of flatfish to 3-methylcholanthrene (MC) or BaP did not result in an increase in binding as great as that for coho salmon. The results (Varanasi et al. 1980) showing that the increase in the formation of reactive DNA binding intermediates was much greater for English sole after treatment with PBCO than that for starry flounder suggest that English sole was more sensitive to petroleum exposure than starry flounder. This can be compared with results described in the Pathology Section 6.2.2 showing that English sole were sensitive to PBCO when exposed to PBCO contaminated sediment.

Our results (Varanasi and Gmur 1980, Varanasi et al. 1980) and those of others (Ahoka et al. 1979) show that the magnitude of increase in in vitro binding of BaP to DNA on pre-exposure of fish or rat to PAH (MC or BaP) was relatively greater than the increase in AHM activity. Covalent binding of BaP to DNA may prove to be more sensitive than AHM activity as an index of pre-exposure of fish to certain toxic chemicals. Moreover, the binding value serves as a useful index for correlating metabolism with the carcinogenicity of a PAH. The carcinogenic potential of a compound to mammals is roughly correlated with the extent of covalent binding to DNA (Buty et al. 1976, Pelkonen et al. 1978). Accordingly, studies to assess the extent of covalent binding of a variety of xenobiotics to cellular DNA in various target tissues of marine organisms may give useful information. In a recent study (Varanasi et al. 1981a) binding of BaP intermediates to DNA in English sole liver was reported to be as high as that for mammalian tissues susceptible to BaP carcinogenesis.

7.1.10 Activities of Aryl Hydrocarbon Monooxygenases (AHM) in Different Species

Investigations of the biotransformations of aromatic hydrocarbons and the fate of PAH metabolites in marine organisms should also include

analysis for the presence or absence of aryl hydrocarbon monooxygenase enzyme systems, e.g., benzo(a)pyrene monooxygenase (BPMO). In the last decade (Philpot et al. 1976, Payne 1977, Gruger et al. 1977, Kurelec et al. 1977, Bend et al. 1977, Bend and James 1978) it has been demonstrated that many freshwater and marine fish species possess hepatic and extrahepatic AHM activity. The results of Sanborn and Malins (1977, 1980) and Malins and Roubal (1982) discussed elsewhere in this report show the ability of several marine invertebrates to metabolize NPH and 2,6-DMN *in vivo*. Bend and James (1978) pointed out that those marine invertebrates that do metabolize xenobiotics *in vivo* by cytochrome P-450-dependent mixed-function oxidases do so at a slower rate than do fish. Microsomal mixed-function oxidation and aryl monooxygenation *in vitro* are also very slow in crustacean hepatopancreas, relative to fish hepatic microsomes. The present research adds eight more species to the growing list of aquatic organisms which possess aryl hydrocarbon monooxygenase activity.

7.1.11 Uptake, Metabolism and Toxicity of Hydrocarbons in Invertebrates

Exposure of Mollusc Larvae and Gametes to NPH. Mussel and oyster gametes and the resultant larvae were sensitive to NPH at concentrations as low as 1 ppb. The major effect at low concentrations was a reduction in survival of the larvae. At higher concentrations, a decrease in fertilization of eggs resulted when NPH exposed sperm was used. Embryological abnormalities also were more prevalent at concentrations greater than 1 ppb. All of these factors indicate a reduction in the ecological fitness of petroleum-contaminated mollusc gametes and their resulting larvae.

Exposure of Crustaceans to Hydrocarbons. Larval forms of spot shrimp and Dungeness crabs were found to be extremely sensitive to water-borne NPH and NPH bound to protein (Sanborn and Malins 1978). The compounds were lethal in the low ppb range to Stage I and Stage V spot shrimp and newly hatched Dungeness crab zoea. These results are consonant with the generally accepted belief that early developmental stages are more sensitive to petroleum than adult marine organisms (Malins and Hodgins 1981). The findings also suggest that Stage I and Stage V spot shrimp are capable of accumulating from 25 to 100 times the environmental concentrations of NPH, depending upon whether the NPH is free or protein-bound (Sanborn and Malins 1978). The findings showing that NPH is readily released from Stage I and Stage V spot shrimp and the metabolites retained for several days (Sanborn and Malins 1978) are similar to those observed with fish (Roubal et al. 1977a, Varanasi et al. 1979, Collier et al. 1980).

The findings with *P. platyceros* suggest that the mature animals readily accumulate hydrocarbon components of the SWSF of crude oil (Sanborn and Malins 1980). The thoracic segments contained both low molecular weight benzenes and NPHs. It is likely that the hepatopancreas located in the thorax is a major site of hydrocarbon accumulation and metabolism. Abdominal segments also contained low molecular weight benzenes and NPHs indicating that shrimp sequester water-soluble hydrocarbons in edible tissue. Bioconcentration values are low in comparison to

values observed with fish (Roubal et al. 1978). The bioconcentration of substituted benzenes was in direct relationship to the degree of alkylation, a finding that corresponds to results obtained with fish (Roubal et al. 1978).

Both adult and larval spot shrimp are capable of forming a wide variety of metabolic compounds (Sanborn and Malins 1980). The profile of metabolites in adult shrimp was not markedly dissimilar from that observed in NPH-exposed fish (Varanasi et al. 1979, Collier et al. 1980). The fact that larvae formed metabolites indicates that early developmental stages have well developed enzyme systems capable of converting NPH to both conjugated and nonconjugated metabolites.

7.1.12 Food Chain Transfer of 2,6-DMN to Sea Urchins via Algae

The present work (Malins and Roubal 1982) shows that ^{3}H-2,6-DMN readily accumulates in Fucus exposed to this hydrocarbon through the water column. Moreover, the 2,6-DMN is not metabolized in the algae and thus was transferred to the echinoderms as the parent compound (Malins and Roubal 1982).

It was not expected that the sea urchins would accumulate a large proportion of the ingested tritium in the exoskeleton, particularly because the 2,6-DMN was administered through the diet and no evidence was found for radioactive hydrocarbons and metabolites in surrounding sea water. However, this finding raised the question of whether tritium exchange occurred with the result that tritiated polar compounds (e.g., water) accumulated in the exoskeleton. This possibility was dismissed because the $^{3}H/^{14}C$ ratios in exoskeleton and soft tissues in the dual-label experiment indicated that such an exchange did not occur to a significant degree (Malins and Roubal 1982). The question of whether the tightly bound aromatic fraction in the exoskeleton is available to interfere with normal cellular functions of soft tissues remains to be answered.

The preferential formation in echinoderms of 2,6-DMN metabolites arising from oxidation of the aromatic ring is an interesting finding. In rat liver, 7,12-dimethylbenzanthracene is converted primarily to methanol derivatives, although a shift to ring oxidation occurs after pretreatment with other aromatic hydrocarbons (Jellinck and Goudy 1967). Moreover, Kaubisch et al. (1972) demonstrated that in rat liver microsomes oxidation of 1- and 2-methylnaphthalene and 1,2-DMN leads principally to naphthoic acids and methylnaphthoic acids, respectively. Little comparable information is available on marine organisms; however, Gruger et al. (1981) showed that starry flounder accumulate primarily methanol derivatives in bile after oral administration of 2,6-DMN. Thus, the metabolism of methyl-substituted NPHs in this species of echinoderm appears to be similar to that by aromatic hydrocarbon-pretreated rat.

Sulfotransferases are responsible for the conjugation of a variety of compounds (Pasternak et al. 1963, Wortman 1961). Little information exists on these metabolic transformations in marine invertebrates;

however, the lobster (Homarus americanus) is reported to have a poor ability to convert aromatic hydrocarbons to oxygen-containing derivatives (Elmamlouk and Gessner 1978). Yet, if hydroxy compounds (e.g., p-nitrophenol) are administered, they are readily conjugated through the sulfotransferases (Elmamlouk and Gesser 1978). The present work indicates that echinoderms are different from lobsters in having active enzyme systems for biosynthesizing the hydroxy compounds, as well as for converting these oxidation products to aryl sulfates.

7.1.13 Biological Fate of Metals in Fish

The results (Varanasi and Markey 1978, Varanasi 1978, Reichert et al. 1979) demonstrated that fish held at lower temperatures accumulated relatively smaller concentrations of metals. These findings suggest that the effect of temperature on the turnover and retention of metals should be taken into account when considering the overall effect of trace metals on arctic biota. These studies also show that fish exposed to low levels (ppb) of water-borne metals readily accumulate substantial concentrations (ppm) in tissues. High levels of lead, a neurotoxin, were found in the brain of starry flounder, which suggests possible neurological damage that could have behavioral consequences (Varanasi 1978). Moreover, the results indicate that coho salmon exposed to either water-borne lead or cadmium accumulate about twice as much metal in the posterior kidney as in the anterior kidney (Reichert et al. 1979). These results cannot be adequately explained at present; however, it is suggested that this tendency may be associated with the excretory function of the posterior kidney in salmonids (Smith and Bell 1976).

Termination of exposures to metals does not automatically mean that metal concentrations will necessarily remain constant or decline in the kidney and possibly other tissues. Even when a decline in cadmium or lead concentrations was observed, e.g., in gills, considerable levels still remained in gills after 5 wk depuration in control seawater (Reichert et al. 1979). The strong tendency to sequester cadmium and lead in gills (Reichert et al. 1979) during uptake and depuration periods raises questions about possible interference with osmoregulation and oxygen consumption (Thurberg et al. 1973).

More than 50% of lead and cadmium accumulated in the scales of coho salmon was still present after 5 wk of depuration (Varanasi and Markey 1978). It is known that in salmonids, skin and scales play an important role in calcium transport and regulation (Podoliak and Holden 1965). The persistence of high concentrations of lead and smaller concentrations of cadmium may interfere with transport and perhaps regulation of calcium in fish. Moreover, scales of salmonids are known to be resorbed during maturation and the mineral components of the scales are utilized for general metabolism and production of gametes (Wallin 1957). In the metal-exposed fish, resorption of scales may result in the release of toxic metals in the bloodstream at the time of stress. The consequences of release of such toxic metals on eggs and sperm remains to be assessed. It should be noted that increased levels of environmental or dietary calcium (Varanasi and Gmur 1978)

significantly reduces uptake and retention, and presumably toxicity, of lead in coho salmon.

The finding (Varanasi and Markey 1978) that coho salmon had lower epidermal mucus concentrations of metals than did starry flounder, along with the results on rainbow trout (Varanasi et al. 1975) indicated that the level of metals accumulated in epidermal mucus are dependent largely on a particular species of fish rather than the salinity of the medium. In addition, the results (Varanasi and Markey 1978) show that short-term exposure to pollutants triggers increased synthesis of mucus; however, the consequence of long-term exposure on mucus production remains to be assessed. Sherwood and Bendele (1975) reported that in Dover sole (Microstomus pacificus) collected from Palos Verdes, an area of known metal contamination, fin erosion was accompanied by reduction in epidermal mucus (visual observation). Whether or not there exists a relationship between altered rate of mucus production and pathological surface conditions is not known.

Epidermal mucus of salmonids exists in a state of continuous flux (Pickering 1976)--that is, small amounts of mucus are continuously sloughed off and renewed. Thus, our results showing that metals were present in the mucus of the test fish for several days after termination of exposure suggested that metals were discharged in epithelial mucin via the mucous cells and excreted in the mucus (Varanasi and Markey 1978, Varanasi 1978). The intriguing possibility exists that a certain critical concentration of a pollutant in blood at a given temperature may trigger increased synthesis of epithelial mucin, resulting in rapid turnover of mucus which would expedite discharge of the pollutant. With respect to this hypothesis, it should be noted that NPH and its metabolic products are also shown to be released in epidermal mucus of NPH-exposed salmonids (Varanasi et al. 1978) and flatfish.

Heavy metal-binding proteins in gills of marine species have been reported previously (Marafante 1976, Bouquegneau et al. 1975, Noel-Lambot et al. 1978). Noel-Lambot et al. (1978) found extremely low concentrations of cadmium bound to a cadmium binding protein (CdBP) in the gills of eels that had been exposed to 200 ppm of cadmium for 5 hr. Our studies with unexposed coho salmon indicate that CdBP concentrations in gills are low. Yet within 24 hr after metal challenge there is an appreciable increase in the accumulation of CdBP-bound cadmium, indicating induction of CdBP. Accordingly, it is likely that CdBP may play an important role in binding cadmium in the salmonid gill; this reaction may serve to protect vital physiological processes (e.g., osmoregulation) from the potentially destructive influences of this metal.

In rat liver, the proportion of cytosolic cadmium bound to CdBP (identified as metallothionein) increases steadily for at least 48 hr after injection (Frazier and Puglese 1978, Cempel and Webb 1976). Similar to studies with rat, induction of CdBP in the liver and kidney of salmonids was observed after the fish were exposed to cadmium (Reichert et al. 1979). Also, unexposed coho salmon apparently have appreciable concentrations of CdBP present in liver. However, there

appears to be no specific low molecular weight protein like metallothionein in the cytosolic fraction of the kidney, liver, and gills of coho salmon that has a high affinity for lead. Similar observations have been made in mammals. Consequently, this metal binds with a variety of proteins in the cytosol and may alter protein structures.

In conclusion, the biochemical fate and biological effects of metals are probably influenced by temperature, calcium and other metal ions in the marine environment. Synergistic and antagonistic effects associated with multiple systems of metals on marine organisms are important subjects for future study.

7.2 Pathology

7.2.1 Effects of Petroleum on Disease Resistance

The results of a series of tests designed to assess the effects of petroleum hydrocarbons on disease resistance of salmonids, juvenile and adult flatfish, and adult spot shrimp failed to identify a marked impairment. Whether or not a lowered host resistance could occur under other stress and exposure conditions and to different diseases cannot be predicted from the present data. These data do, however, strengthen an argument that exposure to environmentally realistic concentrations of petroleum does not markedly impair disease resistance in the tested and related species.

In contrast, a preliminary assay with a representative petroleum dispersant (Corexit 9527) suggested the potential for an adverse effect of oil-dispersant mixtures on disease resistance of salmon.

7.2.2 Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment

Flatfish were shown in these series of experiments to take up petroleum hydrocarbons in muscle and liver tissue following contact with PBCO-contaminated sediments. Bioavailability and bioaccumulation appears to be dependent on a number of factors including aromatic hydrocarbon structure, sediment characteristics, and biotransformation mechanisms in the flatfish tissues.

The differential uptake and retention of 1- and 2-methylnaphthalenes and tetramethylbenzene suggests that aromatic structure influences bioavailability. Such selectivity has been reported for starry flounder by Roubal et al. (1978), and has also been inferred from other studies (see Section 6.1.2). The reduction of tissue levels of petroleum hydrocarbons over time, despite a persistently high sediment concentration of hydrocarbons, further suggests that increased clearance rates and/or increased metabolic transformation of petroleum hydrocarbons to oxidation products are occurring (see Section 6.1.7).

The silt content of the sediments clearly influence the dynamics of release of sediment-associated petroleum. There appears to be a slower and more continuous release of hydrocarbons from high-silt sediment than from high-sand sediment.

The significance of severe hepatocellular lipid vaculoization (HLV) is unknown. Although this condition has been observed to occur at a higher frequency in crude oil-exposed English sole than in controls, it was observed at approximately the same frequency in both oil-exposed and control rock sole and starry flounder. HLV can be induced by a variety of causes, including nutritional deficiencies (Snieszko 1972), exposure to pesticides (Couch 1975), and by contact with polluted environments (Pierce et al. 1978). Also, under the experimental conditions employed severe HLV appears to be reversible.

7.2.3 Cytopathology

(a) Adult Fish Exposures

Gill. Both salmon and flatfish exposed to 100 ppb concentrations of the SWSF of PBCO exhibited gill lesions resulting from loss of surface cells. Similar conditions have been observed in a number of fish species exposed to waterborne contaminants. Trace metals such as cadmium, nickel, and lead damage gills by causing sloughing of epithelial cells (Voyer et al. 1975, Schwiger 1957, Haider 1964). Excessive mucus production is also characteristic of exposure of fish to trace metals (Varanasi et al. 1975, and Section 6.1.13 of this report) and is thought to cause death by suffocation (Gardner 1975, Haider 1964). Similarly, epithelial sloughing and discharge of mucous glands was observed in marine fish captured near an oil slick off the Texas and Louisiana coast (Blanton and Robinson 1973). The secretory cells of the pseudobranch of the Atlantic silverside (Menidia menidia) degenerated after exposure to either 0.14 ppm Texas-Louisiana crude oil or 100 ppm waste motor oil (Gardner 1975, Gardner et al. 1975); light micrographs revealed severe vacuolization of the entire pseudobranch but no data on adjacent gill tissue was presented.

The effects of phenol on gill tissue have been documented in several freshwater species. Rainbow trout sloughed epithelial cells and developed a general inflammation of the gills after exposure to 6.5 to 9.6 ppm phenol (Mitrovic et al. 1968). Another freshwater fish, the bream (Abramis brama), was exposed to lower concentrations of phenol for a longer duration and suffered similar destruction of the gill epithelial cells; in addition test fish suffered damage to the blood vessels and extravasation of blood in the gill lamellae (Waluga 1966). Fourteen species of fish sampled from phenol-polluted portions of the Rhine and Elbe Rivers revealed discharged mucous glands and generalized gill inflammation (Reichenbach-Klinke 1965).

Liver. Increases in rough endoplasmic reticulum in the hepatocytes of rainbow trout were observed after exposing the fish to high peroral doses of PBCO for 8 months. In one of the few field studies

that included ultrastructural data, a similar increase in rough endoplasmic reticulum was reported in the hepatocytes of Fundulus heteroclitus sampled from an area which had received an oil spill 8 years earlier (Sabo and Stegeman, 1977). Also, the hepatocytes of channel catfish (Ictalurus punctatus) showed proliferation of endoplasmic reticulum and "bizarre" whorls of both the smooth and rough endoplasmic reticulum after chlorobiphenyl exposure via gastric intubation for 21 days (Hinton et al. 1978, Klaunig et al. 1979). Circular arrays of smooth endoplasmic reticulum in liver cells have been reported following dietary exposure of rainbow trout to aflatoxin B (Scarpelli 1976), and to phenylbutazone (Scarpelli 1977). Hawkes et al. (1980) showed that after 28 days exposure to a mixture of 5 ppm chlorobiphenyls in diet, the liver cells of chinook salmon had vesiculated rough endoplasmic reticulum and circular arrays of smooth surfaced membranes that closely resembled the arrays of smooth endoplasmic reticulum reported in catfish (Hinton et al. 1978, Klaunig et al. 1979). Such changes in the endoplasmic reticulum, and particularly the changes resulting in circular arrays of smooth endoplasmic reticulum, have been suggested to be a response to substances that induce the mixed-function oxidase system (Klaunig et al. 1979).

The process that results in changes in endoplasmic reticulum has been hypothesized to include the disruption of lipoprotein synthesis. Triglycerides normally utilized for lipoprotein formation are thought to accumulate in the hepatocytes (Tanikawa 1979). An increase in hepatocyte lipid content has been reported in English sole exposed to oiled-sediment (McCain et al. 1978), in channel catfish (Hinton et al. 1978) and rats (Kasza et al. 1978) exposed to dietary chlorobiphenyls, and in humans and other mammals exposed to a variety of toxic substances (Lombardi 1966; Tanikawa 1979). In contrast, our studies showed exposure of rainbow trout to dietary crude oil resulted in depletion of lipid in the hepatocytes, and although there was an increase in rough endoplasmic reticulum, no vesiculation of the cisternae was found (Hawkes 1977).

Lens. Opacities in lenses of mammals are preceded by hydration of the lens fiber cells (Hollwich et al. 1975); lens changes observed in rainbow trout perorally exposed to petroleum also appear to include hydration. Generally, long-term peroral exposure of trout to PBCO caused progressive degeneration of lens tissue characteristic of early cataract formation. Payne et al. (1978) also reported tissue changes in the lens of cunner (Tautogolabrus adspersus) exposed to an oil slick for 6 mo. In mammals, lens abnormalities associated with exposure to naphthalene have been well documented (Hollwich et al. 1975). The sequence of metabolic events that precedes cataract formation in rats and rabbits after exposure to naphthalene appears to include enzymatic oxidation reactions that lead to the formation of 1,2-dihydroxynaphthalene, a compound which may be autooxidized to 1,2-naphthoquinone; this compound may react with lens components. The enzymes that perform some of the oxidation reactions have been found in the eye of rabbits (Van Heyningen 1979, Van Heyningen and Pirie 1967) and, recently, in the eyes of fish (Stegeman, personal communication).

(b) Embryonic Fish Exposures

Olfactory epithelium. Scanning electron microscopy of sand sole larvae exposed to 164 ppb of the SWAF of weathered PBCO during embryogenesis revealed degenerative changes in the chemosensory cilia and a loss of the microridges that circumscribe the perimeter of the epithelial cells surrounding the olfactory organs. Whether olfaction was impaired in these larvae is not known; however, the degree of structural alteration observed indicated severe damage to the receptor organelles.

These findings are in accordance with those of Gardner (1978), who identified the olfactory epithelium of fish as a site of damage from petroleum hydrocarbons. Gardner exposed the Atlantic silverside, Menidia menidia, to whole crude oil, the water-soluble fraction of crude oil, and the water-insoluble fraction of crude oil for 7 days. Pathological changes in the olfactory organ differed with exposure regime; whole crude oil induced a marked hyperplasia; epithelial metaplasia developed after exposure to the water-soluble faction; and submucosal blood vessels were dilated and congested after exposure to the water-insoluble fraction.

Brain and Eye. The late appearance of cytopathological changes in the retinal receptor cells of surf smelt exposed to the SWAF of CICO may be related to the differentiation times of these cells. Typically, teleost retinas are relatively undifferentiated throughout embryogensis and, in some species (Ali 1959, Blaxter 1974), do not begin to mature until late in larval development. In surf smelt, the receptor cells develop outer segment membranes (a necessary structure before the cells can function as photon receptors) between day-15 and day-21 of embryogenesis. The first eye damage was observed in 21-day old embryos; this suggests that receptor cell damage from exposure to petroleum hydrocarbons is not evident cytologically until these cells are fully differentiated.

In addition to morphological alterations of the receptor cells, the effects of exposure to the SWAF of CICO on surf smelt embryos were reflected by necrosis in the neurons of both the brain and eye. In some fish and mammals, neural tissue appears to be particularly vulnerable to certain petroleum hydrocarbons; brain tissue has been reported to bioaccumulate relatively large amounts of these compounds. For example, naphthalene was sequestered in the brain of salmonids in amounts comparable to that in the liver (Roubal et al. 1977a, Collier et al. 1980, and Section 6.1.6 of this report). Neurotoxic effects of low-molecular-weight hydrocarbons in mammals have been attributed to accumulation of either the parent hydrocarbons or their metabolites in brain tissue (Savolainen 1977). Although the effect of changes in the forebrain of the surf smelt is difficult to assess, the observed neuronal damage may have been sufficient to account for the five-fold increase in mortality of larvae exposed to the SWAF of CICO as embryos as compared to unexposed controls (See Section 6.3.1 [e]).

7.3 Behavior and Physiology

7.3.1 Vertebrate Studies

Behavior of Pacific Salmon Exposed to Petroleum Hydrocarbons

(a) Olfactory disruption. The waterborne aromatic hydrocarbon components of petroleum are thought to be a likely cause of chemosensory disruption in aquatic organisms (Takahashi and Kittredge 1973). Although the mechanism of disruption is unknown, it has been suggested that such contaminants may mask the chemoreceptive sites, thus blocking incoming chemical signals at the receptor level (Sutterlin 1974). It is also possible that olfactory lesions, such as those observed by Gardner (1975) in Atlantic silversides exposed for 7 days to the SWSF of Texas-Louisiana crude oil, might account for disruption. Our data indicate that the olfactory receptor sites of salmon responsive to certain amino acids are not masked by aromatic hydrocarbons; further, comparison of our EEG data with results of similar studies using other pollutants indicates that short-term exposure (20 min) to a 4 ppm aromatic hydrocarbon mixture does not result in disruption of the olfactory epithelium. In contrast, when Hara (1972) infused the nares of juvenile coho and sockeye (*O. nerka*) salmon for 10 sec with either 2.7 ppm $HgCl_2$ or 1.6 ppm $CuSO_4$, the olfactory response to all stimulants was eliminated. Also, Hara et al. (1976) found a significant decrease in rainbow trout EEG response to stimulants after 30 min exposure to 0.3 ppm $HgCl_2$.

A statistically significant increase in olfactory bulb EEG response was observed at hydrocarbon concentrations of 2.8 ppm and greater. However, an olfactory EEG response does not preclude the fishes' use of other sensory modalities (such as taste) for identification and concomitant behavioral responses to hydrocarbons. Also, the olfactory bulb EEG response is the result of integrated neural activity and does not necessarily reflect the minimal olfactory detection of aromatic hydrocarbons.

(b) Migratory and Homing Behavior in Adult Salmon

Avoidance reaction. Fifty percent of the mature salmon migrating up Chambers Creek during the peak of the run avoided a mixture of hydrocarbons in the water at concentrations greater than 3.2 ppm. These tests represent avoidance of hydrocarbons under estuarine rather than strictly freshwater conditions since movement of adult salmon into Chambers Creek occurred predominantly at high tide when seawater intrusion covered 1/2 to 3/4 of the fish ladders. Whether or not similar relationships between hydrocarbon concentration and avoidance hold for higher salinity waters is not known.

Disruption of homing capability. The concentrations of hydrocarbons used in our homing tests are well below the acutely toxic levels reported for Pacific salmon (Morrow 1973, Bean et al. 1974, Moles et al. 1979). Sublethal concentrations of petroleum, however, have been shown to cause a number of behavioral, physiological, and

histological changes in salmonids (Bean et al. 1974, Rice et al. 1976, Cardwell 1973, Hawkes 1977). Thus, the 3 day delay of coho returning to Tulalip Creek following exposure to monocyclic aromatics may be attributed to a variety of causes. Several lines of evidence suggest that the most likely cause is central nervous system disruption in the form of narcosis. The three major components of the aromatic hydrocarbon mixture (toluene, xylene's, and benzene) have all been established as vertebrate narcotics. Other investigators have noted a similar "narcosis" in fish exposed to monocyclic aromatics (Strushaker 1977, Strushaker et al. 1974, Pickering and Henderson 1966), and there is strong evidence that central nervous system tissue concentrates monocyclic aromatics (Korn et al. 1976, Roubal et al. 1977b, and Section 6.1.2 of this report). Also, observations made during hydrocarbon exposure and at time of release in our studies indicated a consistently reduced level of activity of salmon exposed to petroleum hydrocarbons.

In the Tulalip study the difference between the calculated concentration of aromatic hydrocarbons in the exposure tank and the concentrations determined by GC analysis (Table 9, Section 6.3.1[b]) would indicate that the coho salmon were not exposed to 1-2 ppm hydrocarbons, but to a series of lower concentrations ranging from 25 to 375 ppb. Analysis of the data using the latter hydrocarbon exposure concentrations suggests that the percent returning to Tulalip Creek was inversely related to prior exposure concentration. (For details of this method of analysis see Malins et al. 1978.) However, the overall results of our studies indicate that, outside of a possible slight delay, short-term exposure of Pacific salmon to petroleum hydrocarbons does not impair homing ability.

(c) Predator-Prey Behavior

Exposure of chum salmon fry to low levels of SWAF of CICO for periods of 24 to 72 hr resulted in a statistically significant increase in their consumption by non-oil-exposed coho predators. However, the longest exposure period (96 hr) did not cause the greatest loss to predation. Predation by coho predators was impaired only after the predators were exposed to the SWAF of CICO for longer than 72 hr.

An interesting observation associated with predation by oil-exposed predators was that levels of the parent hydrocarbons were markedly higher in the tissues of predators actively eating compared to those that were not eating. Brain and liver hydrocarbon concentration differences between the eater and noneater subgroups suggest differential uptake, excretion and/or metabolism of these chemicals. These results are in apparent contrast with reported findings that acute neurotoxic effects (Savolainen 1977) and behavioral changes (Dixit and Anderson 1977) were related to accumulation of the parent hydrocarbon compounds.

Avoidance Behavior of Flatfish Exposed to Oil-Contaminated Sediment

Twenty-five to 60% of the juvenile English sole tested did not avoid sediment contaminated with 8,000-10,000 ppm PBCO. Although this oil concentration is high , it is environmentally attainable. In a review

of the literature on observed concentrations of petroleum in the marine environment, Clark and MacLeod (1977) presented data which show that hydrocarbon levels in sediment of polluted coastal areas are usually less than 1,000 ppm; however, they have been measured at concentrations exceeding 12,000 ppm.

The significance of avoidance or non-avoidance cannot be definitively resolved in a laboratory situation because of numerous factors in the natural environment that cannot be duplicated. For example, Rice (1973) has shown that pink salmon fingerlings will avoid the seawater-soluble fractions of PBCO and suggests that this may cause them to move into offshore waters where food supplies are less abundant and predation may occur -- a situation which may promote return to an oil-contaminated area. It is evident, in any event, that without consideration of behavioral parameters, predictions of effects of petroleum hydrocarbons on many marine organisms may be grossly exaggerated or, conversely, overlooked.

Effect of Petroleum Hydrocarbons on Development of Embryos and Larvae

(a) Chum salmon. Embryos and alevins were exposed 3 hr/day in a flow-through system to an average of 470 ppb of the SWAF of weathered PBCO. Survival of embryos exposed during either the first third or last two-thirds of development was similar to controls, but continuous exposure throughout development reduced embryo survival. The primary effect of oil exposure during embryonic development was observed in alevins in the first 10 to 15 days after hatching. Of embryos and alevins exposed continuously, only 20% of those actually hatching survived through yolk sac absorption; 85% of the control alevins survived during a comparable period. Oil exposure of newly hatched alevins, not previously subjected to oil contamination, resulted in mortality double that of controls.

A somewhat comparable study was conducted by Rice et al. (1975) assessing the effect of PBCO on pink salmon embryos and alevins. They found that, generally, embryos and alevins were equally resistant to the SWAF of PBCO. These findings are similar to ours in that chum salmon alevin mortality was 44-48% regardless of whether they were exposed to the SWAF of PBCO as embryos (46 to 77 hr exposure) or as alevins (60 hr exposure). In contrast to our studies, Rice et al. (1975) noted a difference in growth between control pink salmon alevins and those exposed for 10 days to sublethal hydrocarbon concentrations (average of 725 ppb measured by infrared spectrophotometry). Although we did not observe any differences in total length of oil-exposed and control chum alevins, the lack of calcification of fin rays in chum alevins exposed to the SWAF of PBCO may be a reflection of developmental retardation.

(b) Flatfish. Eggs were exposed to the SWAF of weathered PBCO using a static-relacement regime with the SWAF being renewed at mid-incubation. Incubation of embryos at a concentration of 80 ppb resulted in a percentage of normal larvae comparable to that of controls. At hydrocarbon concentrations of 130 to 165 ppb embryo survival and percent hatching

was high, but all hatched larvae were either abnormal or died shortly after hatching. Similar events were observed by Kuhnhold (1972) during exposure of cod embryos to slightly weathered Iranian crude oil. Even though the percent hatching of oil-exposed cod embryos was high, most of the larvae were deformed, unable to swim normally, and died within one day.

(c) Smelt. In duplicate experiments, surf smelt embryos were exposed in a flow-through system to the SWAF of weathered CICO for 3 hr/day during embryonic development. At the highest hydrocarbon concentrations (55 to 175 ppb) less than 10% of the embryos hatched into normal larvae. At the lowest hydrocarbon concentrations (25 to 45 ppb), 43% of the exposed embryos hatched into apparently normal larvae -- a percentage similar to that of controls. The hatched larvae were held in uncontaminated water and the survivors counted 10 days later. Over 40% of the control larvae survived, whereas survival was less than 10% for any group of larve exposed to the SWAF of weathered oil as embryos.

One day prior to hatching, normal appearing surf smelt embryos from the above experiment were sampled and examined microscopically. Ultrastructural analysis indicated that up to 80% of the oil-exposed embryos had cellular abnormaltities of the eye and brain; no abnormalities were observed in the controls (see Section 6.2.3). In a similar study Cameron and Smith (1980) exposed Pacific herring embryos to the SWAF of slightly weathered PBCO at a concentration of 680 ppb and examined the newly hatched larvae for cytopathological changes. Although the oil-exposed larvae showed no gross abnormalities, there were ultrastructural disruptions which the investigators believed would have severely decreased larval survival.

(d) Mortality of Controls. In all our experiments there were varying levels of mortality in controls, ranging from a low of 10% for sand sole to a high of 50% for embryos of surf smelt. These mortalities are attributed, in part, to stresses induced by simulating natural environmental conditions. For example, chum salmon embryos and alevins were subjected daily to wide fluctuations in salinity, and surf smelt embryos were subject to desiccation and thermal stress. Thus, the effects we observed are most likely the result of stress from a combination of chemical, physical, and biological factors, and not from petroleum acting alone.

Effect of Crude Oil Ingestion on Salmonid Reproductive Success

The quantities of petroleum components consumed by experimental fish in the reproduction study clearly exceeded that which would be encountered in natural food supplies; however, it was our intention to examine an extreme case of exposure. The fish readily consumed the petroleum-impregnated food and continued to grow and develop. Although there were no mortalities of petroleum-fed fish prior to spawning, the post-spawning mortality of petroleum-exposed trout with fungus infections suggests some possible interaction betweeen petroleum exposure and recovery

from spawning. It is also possible, however, that the differential post-spawning mortality between the test and control groups may have been related to a greater density of fish in the test tank compared to that in the control tank.

There was no statistically significant impairment of hatching success related to the petroleum exposure. Survival percentages of 86 to 90% compare well with survivals of 90 to 95% for the hatchery program from which the fish were obtained (M. Albert, Hatchery Manager, personal communication, 1976), as well as with published values for other studies using rainbow trout (Anon., 1973). However, eggs from two of the test females had low survivals, and it may be that certain individual fish were adversely affected by the petroleum exposure. There is no indication that the dietary petroleum exposure had any effect on male fertility; in fact, the lowest survival was associated with a control male.

Many other behavioral and physiological aspects of natural reproduction were not examined in these studies. Clearly, activities such as homing, mate selection, redd-building behavior, and territoriality could be disrupted by petroleum consumption and contribute to poor reproductive success in the natural environment.

The fluorescence spectra associated with the trout muscle indicated that certain fluorescing compounds were mobilized from the food through the circulatory system in the fish, and localized in the tissues. Similarly, the evidence suggests that trout are capable of transporting certain hydrocarbons into eggs when the fish are exposed to petroleum in food.

There is no evidence from these studies to suggest that chronic dietary exposure to concentrations of the less volatile components of PBCO that are likely to occur in the environment would result in reproductive failure of rainbow or steelhead trout; however, the histological abnormalities of eye lenses and livers observed in some of these fish (see Section 6.2.3) exposed to petroleum are potentially deleterious.

7.3.2 Invertebrate Studies

In marine organisms the chemosensory system plays a prominent role in behavioral activities related to feeding, avoidance and escape responses, and to reproduction. These responses can be induced in laboratory-maintained animals by specific compounds at levels of parts-per-trillion (Kittredge et al. 1971) and have been abolished by water-soluble oil fractions at levels of 1 ppb (Jacobson and Boylan 1973). In our studies the SWSF of PBCO was found to disrupt, at low ppb concentrations, chemosensory mediated behavior in three diverse invertebrate organisms: the green sea urchin (an echinoderm); the dorid nudibranch (a mollusc); and the spot shrimp (an arthropod).

Defense Behavior of Sea Urchins Exposed to Petroleum Hydrocarbons

The pedicellarial defense behavior of the green sea urchin is mediated by chemical substances released by the starfish; following exposure of urchins to hydrocarbons, this pedicellarial response is moderated. At hydrocarbon concentrations of less than 1 ppm detected behavioral effects were limited to pedicellarial responses; urchin mobility was not markedly impaired, nor was the competence of the pedicellariae to respond eliminated (as shown by elicitation of pedicellarial response following the injection of isotonic KCl). The mode of action of hydrocarbons on the pedicellarial system is not known but the results are provocative in several respects. Inhibition of the defense response ensues within 10 min and thereafter the response index shows no marked change following continuous exposure of up to 48 hr, indicating that equilibrium is quickly established between the pedicellarial response system and the hydrocarbon concentration. In contrast to the rapid onset of inhibition, recovery of the defense reponse during depuration is relatively slow.

Of the five aromatic hydrocarbons tested individually for defense reponses in the sea urchin, only 1-methylnaphthalene approximates the responses elicited by the total SWAF. The concentration of 1-methylnaphthalene found in the SWSF, however, is not sufficient by itself to account for the inhibition of the pedicellarial response. The same lack of sufficient concentration in the SWSF applies to ethyl benzene and trimethylbenzene which, when tested individually, are ineffective at concentrations less than 100 ppb. The greatest concentration reached by either of these latter two aromatics in the SWSF was 21 ppb.

In experiments on interaction between sea urchins and Pycnopodia sp. there was an 80% difference in predation of oil-exposed urchins over controls. This is probably attributable to the inhibition of the pedicellarial defense response, but there is the possibility that Pycnopodia are attracted to, and prefer, oil-tainted urchins. (The attraction to low concentrations of hydrocarbons has been observed in some marine organisms, particularly crustacea.) It is also possible that oil-treated urchins are less successful at avoiding the starfish. Regardless of the causes, the results show selective predation for oil-exposed sea urchins.

Reproductive Behavior of Dorid Nudibranchs Exposed to Petroleum Hydrocarbons

The movement of a nudibranch toward an aggregate of other mating conspecifics is thought to be a chemotactic response mediated by a sex pheromone. As little as 1 day of exposure to the SWSF of PBCO at a concentration of less than 15 ppb significantly decreased the percentage of nudibranchs responding to the aggregate. This effect of SWSF exposure appears to involve the chemoreceptive system and is not a general narcosis, as evidenced by equal movement for both SWSF-exposed and control animals.

As a follow-up to the dorid mating behavior experiment, the effect of the SWSF on egg laying and embryonic development was also studied. At the highest exposure level (225 ppb) egg deposition and development was retarded, and approximately half of the eggs laid were either not encapsulated or showed other abnormalities. At lower exposure concentrations the effect of the SWSF on egg development was reduced accordingly.

Feeding Behavior of Shrimp Exposed to Petroleum Hydrocarbons

Observations concerning the effect of the SWSF of PBCO on the feeding behavior of spot shrimp generally showed a decrease in responsiveness by the shrimp as hydrocarbon concentrations increased from 0 to 500 ppb. The actual physiological mechanisms involved in alteration of the observed behaviors is unknown and may involve either narcosis or simple masking effects at the chemoreceptor level.

In an attempt to differentiate between general narcosis and chemosensory disruption, we exposed 3 crayfish (Astacus pacifastacus) to 2-4 ppm of a model mixture of aromatic hydrocarbons (Table 1, Section 5.3.2) and monitored reflex reactions of the heart and scaphognathite to physical and chemical stimuli using the methods of Larimer (1964) and Wilkins et al. (1974). The results indicated that both under control conditions, and during exposure to hydrocarbons, changes in heart and ventilation frequencies to sound and light stimuli were similar, occurring 68 $\pm$ 12% (range) of the time. The 75% control reflex response to chemical stimuli was reduced to 29% when hydrocarbons were present. The decrease in reflex response to chemical stimuli, and not light or sound stimuli, may indicate specific action of petroleum hydrocarbons on the chemosensory system.

8. CONCLUSIONS

8.1 Chemistry

8.1.1 Accumulation and Biotransformation of Aromatic Hydrocarbons by Marine Species

Coho salmon, starry flounder and adult shrimp exposed to <1.0 ppm of a SWSF of PBCO accumulated substantial concentrations of aromatic hydrocarbons representing a broad spectrum of individual compounds in all tissues examined (Roubal et al. 1978, Sanborn and Malins 1980). Starry flounder accumulated substantially greater concentrations of aromatic hydrocarbons than did either coho salmon or shrimp. For example, bioconcentration values for naphthalenes in the muscle of starry flounder and coho salmon where about 500 and 30 respectively, and the value for the abdomen of shrimp was about 80. In addition, the results indicate that fish (Gruger et al. 1977) and shrimp (Sanborn and Malins, 1978, 1980) have a capability for metabolizing aromatic hydrocarbons to potentially toxic products, as indicated by enzyme (AHM) studies and determination of total and individual metabolites in tissues. Characterization of the individual NPH metabolites in coho

salmon showed that hydroxylated and conjugated compounds were formed (Roubal et al. 1977a, Collier et al. 1978); these compounds are similar to metabolites found in studies with mammals. These findings showing that fish and shellfish accumulate hydrocarbons and metabolic products in a variety of body tissues suggest that potentially deleterious effects on the organisms could arise, and raises questions about the suitability for human consumption of fish and shellfish exposed to petroleum.

8.1.2 Factors Influencing Uptake and Metabolism of Naphthalene by Fish

Starry flounder and rock sole readily accumulated and extensively metabolized dietary NPH. The extent of biotransformation of naphthalene and the types of metabolites remaining in tissues of these flatfish were shown to be greatly influenced by both the mode of exposure and elapsed time after exposure (Varanasi et al. 1979). However, regardless of mode of exposure, species, or structure of hydrocarbon, it was demonstrated that hydrocarbon metabolites remain in tissues of fish and crustaceans over a longer period than do the parent compounds (Roubal et al. 1977a, Collier et al. 1978, 1980, Sanborn and Malins 1978, 1980, Varanasi et al. 1979, 1981, Varanasi and Gmur 1981a,b,). Thus, when evaluating the overall consequences of petroleum exposure to marine fish, it is extremely important to include determinations of tissue metabolite concentrations together with the concentration of parent hydrocarbon.

Lowering the water temperature resulted in higher levels of NPH in several organs of NPH-fed coho salmon (Collier et al. 1978). In addition, starry flounder maintained at a lower temperature showed an increase in both concentrations and resident times of NPH and its metabolites in tissues of fish exposed to dietary NPH; however, the increase in concentration was much greater for NPH than for its metabolites, indicating that the bioconversion of NPH was considerably less at the lower temperature. Lowering of the temperature also influenced the relative concentrations of metabolite classes accumulated in tissues of starry flounder (Varanasi et al. 1981b). It is evident from these studies that environmental temperature may sharply influence both the nature and severity of toxic effects of NPH in fish.

8.1.3 Naphthalene and Its Metabolites in Fish Skin and Mucus

The results demonstrate that the skin of salmonids and flatfish was actively involved in the retention and discharge of NPH and its metabolites. A notable finding was that in starry flounder four times more metabolites as parent hydrocarbon were present in the skin one week after exposure, indicating either preferential retention of metabolites or extensive metabolism of naphthalene. In addition, data on the role of epidermal mucus in the excretion of naphthalene and its metabolites in fish suggest that mucus must also be considered as one of the routes of excretion (Varanasi et al. 1978, Varanasi and Gmur 1978).

8.1.4 Uptake and Metabolism of Sediment-Associated Naphthalene and Benzo(a)pyrene by Flatfish

English sole exposed to NPH and BaP in oil-contaminated sediment took up and readily metabolized these hydrocarbons (Varanasi and Gmur 1981a,b). A number of mutagenic and carcinogenic metabolites of BaP were identified in the liver. The metabolites of BaP were retained in flatfish liver over a much longer period than NPH and its metabolites. Furthermore, BaP tends to remain largely unconverted in sediment and thus can be available for continued uptake by benthic organisms. Continued uptake, greater retention, and more extensive metabolism of BaP than NPH by benthic fish indicate that although BaP is a minor component of petroleum, its derivatives can be bioconcentrated in tissues of demersal organisms. The substantial bioconversion of BaP in fish liver very probably explains why BaP is usually not detected in fish tissues even when a considerable concentration of BaP is detected in the environment. The ultimate consequences of the presence of BaP metabolites in fish tissues are not known, but implications on toxicity can be drawn from biological activities of these metabolites from various assays (e.g., Ames Test, cytotoxicity, carcinogenicity).

8.1.5 BaP Metabolism by English Sole

When English sole were force-fed BaP, a number of toxic metabolites were found in both the liver and edible muscle of the fish. The concentration of BaP-derived radioactivity in muscle was much lower than in liver and the decrease in concentration of BaP and its metabolites in muscle with time was greater than in liver. These findings indicated that muscle of English sole may not be a major storage site of BaP and its metabolites, which is important, since muscle of English sole is a consumer product.

8.1.6 Examination of Aryl Hydrocarbon Monooxygenase Activity in Different Species and Binding of BaP Intermediates to DNA Catalyzed by Liver Enzymes

Examination of tissue aryl hydrocarbon hydroxylase activities of marine species from Alaskan waters revealed that highly variable enzyme activities are common to hepatic tissues of vertebrate species tested.

In other studies (Section 6.1.9), liver enzymes from flatfish were able to convert benzo(a)pyrene (BaP) into reactive intermediates that bind to DNA *in vitro*, and the level of binding increased with pre-exposure of flatfish to PAH or PBCO. Binding of metabolites of PAH to DNA is a presumed prerequisite step in the chain of events leading to chemical-induced genetic damage and neoplasia.

8.1.7 Uptake, Metabolism and Toxicity of Hydrocarbons in Invertebrates

The acute toxicity (mortality preceded by narcosis) of about 10 ppb of naphthalene to early life stages of crustacea suggests that these life forms are highly sensitive to this component of the SWSF of crude

oil. The tendency of early developmental stages of shrimp to metabolize and retain NPH metabolites at unchanged concentrations while concentrations of the parent hydrocarbon decline is of concern because evidence links aromatic hydrocarbon metabolites to toxicity in mammals. The high susceptibility of larval and other developmental stages to aromatic hydrocarbons must be considered an important factor in the environmental effect of arctic and subarctic petroleum operations.

8.1.8 Food Chain Transfer of 2,6-DMN from Algae to Sea Urchins

Sea urchins feeding on 2,6-dimethylnaphthalene-exposed algae accumulated and metabolized this hydrocarbon to hydroxy compounds and their conjugates, thus demonstrating a food chain transfer of an aromatic hydrocarbon (Malins and Roubal 1982).

8.1.9 Fate of Metals in Fish

Salmon and starry flounder readily accumulate lead and cadmium from seawater (Reichert et al. 1979). The metals in liver and kidney were stored to a substantial degree in cellular fluids where biochemical and physiological processes occur (e.g., cytosol). Cadmium was preferentially bound by low molecular weight proteins (9,000 daltons), but was also associated with high molecular weight proteins of the cytosol. Lead showed a strong preference for neural tissue (e.g., brain). The findings imply that low concentrations of metals entering marine waters are likely to increase the metal burden of key tissues of fish and thereby possibly alter normal physiological processes.

Epidermal mucus of coho salmon is involved to some extent in excretion of lead and cadmium (Varanasi and Markey 1978). The importance of this route in relation to other excretory tissues (e.g., kidney), however, remains to be assessed. Exposure to sublethal levels of lead and cadmium for periods of up to two weeks resulted in increased production of epidermal mucus in coho salmon. This increased rate of production may induce alterations in physicochemical and rheological properties of mucus.

Our results also show that skin and scales act as storage and perhaps detoxification sites for metals in both salmonids and flatfish. Substantial amounts of metals persisted in the skin and scales several weeks after fish were returned to clean water. Whether persistence of high concentrations of toxic metals would have adverse effects on skin structure remains to be seen.

8.2 Pathology

8.2.1 Effects of Petroleum on Disease Resistance

Laboratory studies evaluating the effects of petroleum hydrocarbons on immunocompetence and disease resistance of selected fish and shellfish

failed to demonstrate a marked impairment. Preliminary assays did, however, suggest petroleum dispersants may enchance the incidence of infectious disease in salmon.

8.2.2 Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment

Experimental exposures of 3 species of flatfish (English sole, rock sole, and starry flounder) to PBCO-contaminated sediments indicates that English sole are the most likely to be adversely affected. Although the pathological changes observed appeared to be reversible and not directly life-threatening, they do reflect alterations that may reduce fitness, and thereby survival, of flatfish in heavily oiled environments.

8.2.3 Cytopathology

Cytopathological changes occurred in flatfish and smelt embryos exposed throughout embryogenesis to low ppb concentrations of the SWAF of crude oil. The olfactory cilia of flatfish developed abnormally and extensive necrosis was evident in the eye and brain of smelt embryos. Structural changes were also observed in liver and lens tissues of adult rainbow trout perorally exposed to high levels of petroleum hydrocarbons for 2 to 12 mo.

8.3 Behavior and Physiology

8.3.1 Vertebrate Studies

Behavior of Pacific Salmon Exposed to Petroleum Hydrocarbons

Mature Pacific salmon migrating upstream during the peak of the run substantially avoided a mixture of monocyclic aromatic hydrocarbons in the water at concentrations of 3.2 ppm (EC_{50}) and higher. Also, electrophysiological recordings from the olfactory bulb of juvenile salmon indicated that short-term exposure to these high concentrations of aromatic hydrocarbons would not be likely to disrupt the olfactory system.

Mark-recapture experiments were conducted to assess the ability of adult chinook and coho salmon to repeat their spawning migration following exposure to petroleum hydrocarbons. Chinook salmon exposed for 14 to 18 hr to FWAF of PBCO showed no alteration of homing capability. Similarly, the spawning migration of coho salmon was not disrupted by 8 to 22 hr exposure to a mixture of monocyclic aromatic hydrocarbons. However, the return of the coho to their captive site was delayed by 3 days.

Effects of Petroleum Hydrocarbons on Predator-Prey Behavior of Salmon

Exposure of chum salmon fry to the SWSF of CICO at a hydrocarbon concentration of 350 ppb for periods of 24 to 72 hr resulted in a

statistically significant greater consumption of the oil-exposed prey by coho predators compared to the consumption of controls.

Similarly, exposure of coho salmon predators to 340 ppb of the SWSF of CICO for over 3 days resulted in a reduction in numbers of salmonid fry eaten. Concentrations of parent hydrocarbons were higher in both liver and brain of actively eating oil-exposed predators compared to those not eating. This suggests that the parent hydrocarbons were not the compounds primarily influencing a decline in feeding behavior.

Avoidance Behavior of Flatfish Exposed to Oil-Contaminated Sediment

Although there is evidence of initial avoidance of oil-contaminant sediment by juvenile English sole, generally the fish did not avoid oil-sediment mixtures at concentrations up to 10,000 ppm - the sediment's apparent oil-carrying capacity. At these concentrations, some mortality did occur over a 15-day period, but the majority of flatfish were active and fed readily.

Effect of Petroleum Hydrocarbons on Development of Embryos and Larvae

Embryos of chum salmon, English sole, and sand sole exposed to water-borne hydrocarbon concentrations ranging from 100 to 500 ppb showed either high embryonic mortality, abnormal development, or delayed larval mortality. In addition, hydrocarbon concentrations of less than 100 ppb severely reduced the larval survival of surf smelt.

Effect of Crude Oil Ingestion on Salmonid Reproductive Success

Long-term peroral exposure of maturing male and female rainbow trout to high levels of PBCO had no marked effects on reproduction, as measured by hatching success and alevin survival.

8.3.2 Invertebrate Studies

Defense Behavior of Sea Urchins Exposed to Petroleum Hydrocarbons

Results of studies on the sea urchins' defense behavior following exposure to the SWSF of PBCO and its major aromatic components indicate that no single principal component of the SWSF is responsible for the total inhibition observed by exposure to the SWSF alone; that at low ppb concentrations the pedicellarial defense inhibition resulting from hydrocarbon exposure is apparently a chemosensory specific reaction and not general narcosis; and that SWSF-exposed sea urchins are preyed upon by Pycnopodia to a greater extent that non-exposed urchins.

Reproductive Behavior of Dorid Nudibranchs Exposed to Petroleum Hydrocarbons

Interference with the reproductive processes of dorid nudibranchs by the SWSF of PBCO occurred at several different levels. First, there was disruption of the chemotatic responses necessary to form mating

aggregations. Second, there was disruption of the egg laying process as noted by the lack of egg encapsulation, and delay in egg deposition. Third, eggs exposed to the SWSF showed retarded development and increases in abnormalities. Disruptive effects on the reproductive biology of dorid nudibranchs occurred at hydrocarbon concentrations as low as 15 ppb.

Feeding Behavior of Shrimp Exposed to Petroleum Hydrocarbons

Exposure of spot shrimp to the SWSF of PBCO resulted in a reduction in feeding activity. At 25 ppb there was a 50% reduction in overt behavioral activity elicited in response to food stimuli, and at higher concentrations of the SWSF there was a still further decline in feeding responses, with some loss of equilibrium and mortality occurring at 500 ppb.

9. PAPERS PUBLISHED, DISSERTATIONS, THESES, AND PRESENTATIONS GIVEN UNDER RU73 OCSEAP SPONSORSHIP

9.1 Papers Published

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9.3 Presentations

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ACKNOWLEDGEMENTS

We wish to acknowledge the following scientific staff (past* and present) who contributed to the studies presented in this report.

Warren E. Ames
John W. Blackwell*
Donald H. Bovee*
Robert C. Clark, Jr.
Donovan R. Craddock*
Donald D. Dungan*
David A. Federighi*
Teresa Federighi*
John S. Finley
Andrew J. Friedman
Donald D. Gennero*
Kirsten M. Gleim*
Dennis J. Gmur*
Lucinda Grant*
Victor D. Henry
Eric Henderson*
Tom Hom
Dr. Frederick G. Johnson*
Dr. Russell F. Kappenman
Neva L. Karrick*
Kate King*
Dr. Vyacheslav Konchin*
Daniel Lazuran*
Dr. William D. MacLeod, Jr.
Dennis Markey*
Desmond J. Maynard*
Mark S. Meyers
James L. Mighell
Stephen E. Miller*
David A. Misitano
Laura M. Mumaw*
Frank J. Ossiander*
Kenneth V. Pierce*
Patty G. Prohaska*
L. Scott Ramos*
Linda D. Rhodes
Paul A. Robisch
Philip Scordelis*
George F. Slusser*
Carla M. Stehr
Susan I. Stranahan*
Dr. Lawrence C. Thomas*
Patrick A. Treseler*
Michael Uhler*
Marianne Y. Uyeda*
Ellen M. Warinsky*
Dr. Marleen M. Wekell*
Keith K. Wong*

We also thank the following personnel who provided administrative, editorial, and clerical support.

Coleen M. Annis
Susan M. DeBow
Elaine Eldridge
Carol E. Hastings
M. Annette Hodgson
James Peacock
Gloria D. Sergneri
Gail E. Siani
Ethel F. Terashita
Marianne Tomita
Marylyn West
Marci Worlund

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THE NATURE AND BIOLOGICAL EFFECTS OF WEATHERED PETROLEUM

by

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Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 619

December 1983

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1. SUMMARY OF OBJECTIVES, CONCLUSIONS, AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

1.1 Summary of Objectives

Determine under laboratory conditions the toxicities of: a) Alaskan crude oil "weathered" by exposure to ultraviolet (UV) light compared to non-UV-exposed Alaskan crude oil, b) Alaskan crude oil weathered under simulated natural conditions compared to nonweathered Alaskan crude oil, and c) No. 2 fuel oil refined from an Alaskan crude oil exposed to UV light, in order to compare crude oil with a material (i.e., fuel oil) known to be capable of extensive photooxidation.

Determine: a) changes in concentrations of total hydrocarbons as a result of exposure to UV light, and b) the nature of oxidized compounds formed from Alaskan crude oils and No. 2 fuel oil and found in the underlying seawater.

Determine the relative uptake by selected marine organisms of hydrocarbons and oxidized components from weathered and nonweathered petroleum materials. Determine the nature of oxidized components in the organisms used for toxicity assays.

1.2 Summary of Conclusions

The concentrations of oxidized components released to seawater beneath Cook Inlet crude oil (CICO) and Prudhoe Bay crude oil (PBCO) as the result of UV irradiation were relatively low. UV irradiation of No. 2 fuel oil, in contrast, resulted in the formation and release to underlying seawater of substantially greater concentrations of oxidized products than were formed and released from UV-irradiated crude oils. A study was conducted to determine why a fraction of crude oil (i.e., fuel oil) produced higher concentrations of oxidized compounds after UV irradiation than did the parent crude oil. When polar compounds were removed from Alaskan crude oil and the resulting aromatic/paraffinic (A/P) fraction was subjected to UV irradiation, the concentration of oxidized components increased to levels intermediate between the crude oils and the refined product. These findings suggested that naturally occurring polar compounds in the crude oils inhibit photooxidation.

Experiments with radiotracers showed that petroleum components and oxidized products were substantially bioaccumulated by fish larvae. However, little or no mortality of English sole (*Parophrys vetulus*) embryos and larvae, or of surf smelt (*Hypomesus pretiosus*) larvae, resulted from short-term laboratory exposures to environmentally realistic levels of components from either UV-irradiated or nonirradiated crude or fuel oils. Changes in swimming behavior of surf smelt larvae were seen, but only after exposures to unweathered crude oil or fuel oil. Overall, there is no evidence from these studies that photooxidation would signficantly enhance the toxicity of petroleum in the marine environment.

1.3 Implications with Respect to OCS Oil and Gas Development

The results indicate that, while UV irradiation (sunlight) can produce photooxidized products from CICO and PBCO in seawater, the amounts produced do not appear to be sufficiently large to cause -- at least in the short-term -- marked environmental damage. Environmentally realistic concentrations of crude oil and oxidation products obtained in the seawater-accommodated fractions (SWAF's) did not cause extensive short-term mortality of English sole embryos and larvae, or of surf smelt larvae, under the laboratory conditions used. Swimming behavior of surf smelt larvae was affected only by SWAF's from freshly prepared reference oils.

The implication is, therefore, that it appears unlikely that under most conditions that photooxidized products formed from Alaskan crude oils spilled into the Northeast Pacific Ocean would constitute a serious threat to fish. Environmental irradiation of a refined product, such as No. 2 fuel oil, however, could induce the release of much higher concentrations of soluble components into the water column than would be released from crude oils. These high concentrations could under worst-case conditions cause both mortality and behavioral modifications in fish, especially in early life stages in low energy environments (i.e., sheltered bays and estuaries).

2. INTRODUCTION

This two-year study was initiated in FY 82 to investigate: (1) changes in the chemical structures and concentrations of petroleum-derived compounds in the SWAF of petroleum during weathering and (2) the effects of these chemical changes on toxicity to marine species.

During the first year, two types of weathering regimes were employed: (1) CICO was exposed to ambient environmental conditions and (2) CICO and No. 2 fuel oil were exposed to UV light under controlled laboratory conditions in both static and flow-through modes. The SWAF's were analyzed for petroleum components using microgravimetric (MGA) and gas chromatographic (GC) techniques. Radiolabeled compounds (phenanthrene [PHN] and p-cresol) were used as markers in weathering experiments to provide information on oxidative changes. The SWAF's were assayed for acute toxicity and sublethal effects on embryos and newly hatched larvae of fish.

It was demonstrated that CICO was only slightly photooxidized under controlled laboratory weathering conditions while No. 2 fuel oil was extensively photooxidized. Technical problems with the ambient environmental weathering system required modification of the protocol to one of laboratory exposures where light intensity, day length, air circulation and temperature could be closely controlled. Thus, during the second year more detailed, controlled studies were conducted and a second Alaska crude oil, Prudhoe Bay crude oil (PBCO), was also examined.

3. BACKGROUND

Petroleum entering the oceans undergoes a series of changes (i.e., evaporation, dissolution, photooxidation, microbial degradation) termed "weathering". These processes are believed to alter its toxicity. The oxidized products of petroleum hydrocarbons that are formed during weathering may be more water soluble than the parent hydrocarbons and may reach high concentrations in seawater. However, little is known about the chemical structure of many of these oxidized petroleum hydrocarbons or their concentrations in seawater because they are generally not detected in routine chemical analyses of petroleum-derived compounds.

Numerous oxidized products have been identified from petroleum which has been subjected to UV radiation under both natural (sunlight) conditions and simulated environmental conditions in the laboratory (Hansen 1975, Hendry et al. 1976, Larsen et al. 1977, Patel et al. 1978,). Ahmadjian et al. (1976) demonstrated that petroleum weathered under simulated natural conditions was similar to that weathered in the environment. While the oxidized products of petroleum hydrocarbons are frequently more water soluble than the parent hydrocarbons and oxidation will thus tend to help disperse the oil, it has been reported that some of the oxidized products are more toxic than the parent compounds (Lacaze and Villedon de Naïde 1976). There are also reports (Larsen et al. 1977, Patel et al. 1978) that the acute toxicity of underlying water increases with the weathering of crude oils and of hydrocarbon fractions refined from crude oils (e.g., No. 2 fuel oil).

Crude petroleum contains naturally occurring nonhydrocarbons; a few crude oils may contain as much as 50% nonhydrocarbon components. Certain metallo-organic compounds promote oxidation while others, such as sulfur-containing organic compounds, tend to inhibit oxidation (Clark and MacLeod 1977). Oxygen-containing compounds found in petroleum include acids, phenols, ketones, esters, lactones, ethers, and anhydrides (Clark and Brown 1977).

The oxygen content of the fractions of crude petroleum increases with boiling point, and the greater part of petroleum oxygen is found in high molecular weight distillation fractions boiling above 400°C. Hence, No. 2 fuel oils (boiling range, 185-345°C) usually have negligible amounts of oxygen-containing organic compounds. Oxygen-containing structures may have high molecular weights and long saturated hydrocarbon-type segments which cause their solubilities to be greater in the oil slick than in underlying seawater. This higher solubility in oil than in seawater is not true for of many of the lower molecular weight photooxidized products. Hence, high molecular weight polar compounds in crude petroleum tend to be retained in an oil slick whereas photooxidized lower molecular weight polar byproducts of the slick tend to dissolved into the underlying seawater as soon as they are formed.

4. STUDY AREA

Experiments were performed at either the Northwest and Alaska Fisheries Center (NWAFC) in Seattle, or at the NWAFC's saltwater field station at Mukilteo, WA.

Organisms and seawater were obtained from Puget Sound.

5. METHODS

5.1 Reference Mixtures of Seawater and Oils

The reference mixtures of seawater and CICO, PBCO, and No. 2 fuel oil were prepared under conditions which minimized the oxidation of petroleum hydrocarbons. The seawater was filtered through a 0.45 µm membrane filter and some batches were autoclaved. Ten grams of oil were added to 2 L of either nonsterilized or sterilized seawater in 3.8 L clean brown glass bottles. After the bottles were shaken on a mechanical shaker for 2 hr at 4°C, the contents of a bottle were transferred to a 2 L separatory funnel and the two phases were allowed to separate for 2 hr at 4°C. The underlying SWAF was then separated from the remaining surface layer of oil and stored in the dark at 4°C.

Radiotracers ([^{14}C]-*p*-cresol, [^{3}H]-phenanthrene, and ^{14}C-hexadecane) were added to the initial preparations so that the amount of time needed for the small oil droplets (unaccommodated oil) suspended throughout the seawater to float to the surface could be readily determined by liquid scintillation counting (LSC). In addition, the radiotracers provided another means for determining the reproducibility of the preparation method. During the preparation, the oil/water mixtures were protected from UV light. The preparations of the SWAF's (Table 1) were then removed and divided into individual aliquots for replicate analyses to determine the precision of the procedures.

All solvents used were "distilled in glass" grade from Burdick and Jackson (Muskegan, Michigan). The [9-^{14}C]-phenanthrene (specific activity, 9.4 mCi/mmol) and [ring-UL-^{14}C]-*p*-cresol (Wizard Laboratories, Davis, CA), as well as [UL-^{3}H]-phenanthrene (Moravek, Brea, CA) were purified, immediately prior to use, by silica gel column chromatography (Varanasi and Gmur 1980). The *n*-[1-^{14}C]-hexadecane (specific activity, 53.6 mCi/mmol; Amersham, Arlington Heights, IL) was used without further purification.

The total amount of oil incorporated and total extractable organic material was determined by both MGA (Cahn Electrobalance, Model 4700) and GC analyses. Concentrations of selected individual compounds were determined by GC (Malins et al. 1980).

5.1.1 Effects of Separation Time and Filtration on Composition of Reference Mixtures

Two hundred µL of a standard solution of [^{14}C]-hexadecane were added to each of three preweighed Erlenmeyer flasks containing (1) 10.6 g of CICO, (2) 10.7 g of No. 2 fuel oil, or (3) 10.6 g of PBCO. The hexadecane was

TABLE 1. Codes for the seawater-accommodated fractions (SWAF's) sampled from beneath oils and phenanthrene in static, agitated, and shaken reference oil/seawater experiments.

Code	Description
[ICICO$_{S120}$]	Irradiated, Cook Inlet crude oil, static, 120-hr exposure.
[ICICO A/P$_{S120}$]	Irradiated, Cook Inlet crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[IPBCO$_{A120}$]	Irradiated, Prudhoe Bay crude oil, agitated, 120-hr exposure.
[IPBCO$_{S120}$]	Irradiated, Prudhoe Bay crude oil, static, 120-hr exposure.
[IPBCO A/P$_{S120}$]	Irradiated, Prudhoe Bay crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[IFO$_{A120}$]	Irradiated, No. 2 fuel oil, agitated, 120-hr exposure.
[IFO$_{S120}$]	Irradiated, No. 2 fuel oil, static, 120-hr exposure.
[IFO$_{S475}$]	Irradiated, No. 2 fuel oil, static, 475-hr exposure.
[IPH$_{S120}$]	Irradiated, Phenanthrene, static, 120-hr exposure.
[NCICO$_{S120}$]	Nonirradiated, Cook Inlet crude oil, static, 120-hr exposure.
[NCICO A/P$_{S120}$]	Nonirradiated, Cook Inlet crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[NPBCO$_{A120}$]	Nonirradiated, Prudhoe Bay crude oil, agitated, 120-hr exposure.
[NPBCO$_{S120}$]	Nonirradiated, Prudhoe Bay crude oil, static, 120-hr exposure.
[NPBCO A/P$_{S120}$]	Nonirradiated, Prudhoe Bay crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[NIFO$_{A120}$]	Nonirradiated, No. 2 fuel oil, agitated, 120-hr exposure.
[NIFO$_{S120}$]	Nonirradiated, No. 2 fuel oil, static, 120-hr exposure.
[NIFO$_{S475}$]	Nonirradiated, No. 2 fuel oil, static, 475-hr exposure.
[NIPH$_{S120}$]	Nonirradiated, Phenanthrene, static, 120-hr exposure.
[RCICO]	Reference seawater solution, Cook Inlet crude oil, (^{14}C-hexadecane).
[RPBCO]	Reference seawater solution, Prudhoe Bay crude oil, (^{14}C-hexadecane).
[RFO]	Reference seawater solution, No. 2 fuel oil, (^{14}C-hexadecane).

thoroughly mixed into the oil by swirling. Each flask was poured into its designated 3.8 L bottle containing filtered (0.45 μm) and autoclaved (sterilized) seawater; the air above the water was then displaced with dry N_2.

The bottles were shaken for 2 hr in an Eberbach shaker which was placed in a dark, 4°C walk-in refrigerator. The contents of each bottle were then poured into a 2 L separatory funnel. Zero time samples of 1-2 mL were immediately withdrawn from the bottom of the separatory funnel into 20 mL scintillation vials; additional samples were taken at 15, 30, 45, 60, 120, 180, and 240 min.

After 240 minutes, the seawater was filtered to determine if the residual radioactivity was removable as entrained oil. The water was slowly drained from the separatory funnel through a 2000 mL Buchner funnel containing a coarse (40-60 μm) frit into a clean, N_2-filled 3.8 L bottle.

5.1.2 Effects of Storage on Composition of [RCICO]

A preparation of [RCICO] [Table 1] was divided into three 500 mL aliquots and stored at 4°C for up to 65 days under four separate conditions. These conditions were: (1) [RCICO] made with sterilized seawater, (2) [RCICO] made from sterilized seawater to which 20 mL of methylene chloride (CH_2Cl_2) and 1 mL of 6N hydrochloric acid (HCl) had been added, (3) [RCICO] made with nonsterilized seawater, and (4) [RCICO] made with nonsterilized seawater to which 20 mL of CH_2Cl_2 and 1 mL of 6N HCl had been added. All bottles were rinsed with 1N HCl, methanol (CH_3OH), and CH_2Cl_2 consecutively, before use.

After storage for up to 65 days the [RCICO] was extracted and analyzed as described in Section 5.2.4.

5.2 Weathering of Oils and Hydrocarbon Fractions of Oils

Several different modes of weathering were used to expose CICO, PBCO, No. 2 fuel oil, and the A/P (aromatic/paraffinic; see Section 5.2.3) fractions of crude oil. In some experiments, ^{14}C-phenanthrene, ^{3}H-phenanthrene, or ^{14}C-*p*-cresol were added to the test oil prior to the weathering to facilitate characterization of oxidation products formed. The radiotracers were added on the assumption that they would be affected in a manner representative of compounds of their respective classes (i.e., phenanthrene representative of mid-range aromatic hydrocarbons; *p*-cresol representative of phenols).

5.2.1 Flow-through Conditions

A 25 L and a 5 L wave machine were constructed from stainless steel and used to produce waves by the movement of a motor-driven paddle within the containers. These machines were placed outdoors where the oils were weathered under simulated natural environmental conditions in a water bath maintained at ambient Puget Sound water temperature (10-12°C). In addition, a second 25 L container and 5 L container, identical to the above wave machines with the exception of not having a wave-producing paddle,

were placed in a laboratory water bath maintained at ambient Puget Sound water temperature and irradiated with filtered UV light (six, 1.2 m long sunlamps, Sylvania FS 40 placed 25 cm above the oil-water surface). To simulate natural sunlight, cellulose triacetate film was used to filter out wavelengths below 270 nm (Hansen 1975). The filter was changed after each experimental run.

In addition, a third 25 L container and 5 L container identical to the second pair of containers were kept in the dark. All containers were provided with a constant flow of unfiltered, unsterilized Puget Sound seawater (salinity, 28-29 °/oo; temperature, 10-12°C). In each system, the 25 L container was used for weathering oil without added radiotracers, while the 5 L container was used for weathering oil which contained radiotracers.

The weathering experiments during the first year were initiated by layering CICO (5 g CICO in pentane/L seawater) over the unfiltered, nonsterilized seawater in each container. The flow of seawater was started after 10 min by which time most of the pentane had evaporated. The oil in the 5 L containers contained ^{3}H-phenanthrene and ^{14}C-*p*-cresol. Water samples from the outlet pipe, which drew water from the bottom of the water column of the 5 L tanks, were collected at various intervals during the weathering processes to assess incorporation of radioactivity from oil into the underlying seawater. The radioactivity was determined by LSC.

5.2.2 No-flow Conditions

5.2.2.1 Static: The weathering of oils under laboratory conditions was carried out so that the primary weathering factor was photooxidation by UV light. Two Pyrex glass trays (19 X 30 cm), each containing 1 L of filtered, sterilized seawater, were placed in a water bath maintained at 12°C. The water bath was enclosed in a wooden box containing six 0.6 m long sunlamps (Sylvania FS 20 placed 25 cm above the oil-water surface), filtered as previously mentioned in 5.2.1.

During the first year, a solution consisting of 5 g of oil (CICO or No. 2 fuel oil, in pentane or hexane) was poured on the surface of the filtered, sterilized seawater in each tray. After 10 min, by which time most of the added solvent had evaporated, one tray was covered with aluminum foil, the other tray was left uncovered and the sunlamps were then energized for the duration of the experiment.

During subsequent studies in the second year, the oil was poured directly onto the seawater surface and a hexane rinse of the oil container was added. The wooden box was redesigned so that only one tray was irradiated with the light; the other tray was kept dark without having to be covered with aluminum foil. The light was filtered through cellulose triacetate as described in Section 5.2.1. One experiment was conducted in which only a mixture of radioisotopically labelled and nonlabelled phenanthrene dissolved in pentane was layered onto the seawater. The duration of all exposures was 120 hr.

5.2.2.2 Agitated: During the second year, two 5 L wave machines were used and they were located outdoors in a water bath maintained at ambient Puget Sound water temperature (10-12°C). Both wave machines were covered with a lightproof and waterproof wooden box; one box, however, contained four 0.6 m long Sylvania FS 20 sunlamps located 21 cm above the oil/water surface. The light was filtered as previously described. The same oil/water ratios and times were used as those for the laboratory static exposures (5 g/L and 120 hr). It had been determined during experiments using radiotracers that the time required for settling or separation of the aqueous and oil phases was 2-4 hr, depending on the oil used. At the conclusion of the exposures, therefore, the two phases were allowed to separate for a minimum of 4 hr.

5.2.3 Removal of Polar Compounds from Crude Oils Prior to Irradiation (A/P Fractions)

Twenty grams of PBCO or 20 grams of CICO were mixed with 20 mL of pentane and passed through 500 mL attapulgite clay (Harrison and Crossfield, Los Angeles, CA) columns to separate the aromatic/paraffinic (A/P) fraction from the asphaltene and naturally occurring crude oil polar compounds which were retained on the column (Pancirov 1974). After removal of the eluting solvent, pentane, 13.7 g of the PBCO and 14.7 g of the CICO remained in the form of a moderately viscous, clear yellow A/P fraction of the original crude oil.

5.2.4 Extraction of Seawater-Accommodated Fractions

Seawater-accommodated fractions (SWAF's) were placed in separatory funnels, the pH was adjusted to 2 with 1N HCl and the seawater was extracted 3 times with CH_2Cl_2 (seawater: CH_2Cl_2 = 9:1). The extract was concentrated to 15 mL in a water bath at 65°C, then to 1 mL in a concentrator tube with a tube heater (Kontes, Vineland, NJ). A 100 µL portion of the concentrated extract was set aside for chemical analysis. The remaining 900 µL were chromatographed on silica gel (Malins et al. 1980) to yield fractions containing mainly (1) alkanes, (2) aromatic hydrocarbons and olefins, and (3) compounds containing oxygen. Samples were analyzed by GC and MGA.

5.3 Characterization of Oxidation Products of Radiotracers

In experiments in which radiotracers were added prior to the weathering, thin layer chromatography (TLC) analyses were conducted on all SWAF extracts and on the remaining oil (surface slicks). All samples were spotted on channeled, 5 x 20 cm silica gel plates (KL6DF, Whatman, Clifton, NJ).

During analysis of the ^{14}C-phenanthrene and its oxidized products, the TLC plate was first developed in 100% toluene. The plate was removed; the solvent was allowed to evaporate, and a 4 cm band (R_f 1.00 to 0.75) containing the parent hydrocarbon was scraped from the plate. The plate was redeveloped in toluene:ethyl alcohol (92:8 v/v) which separated the different classes of oxidized products. The remaining silica gel was then scraped off the plate in 2 cm bands. Phenanthrene, 9-fluorenone, phenanthraquinone, and diphenic acid were used as TLC standards.

Infrared analyses of the extracts were conducted using a Perkin-Elmer Infrared Spectrophotometer (Model 681). Microprocessor-controlled ordinate scale expansion was used to detect low levels of oxygenated components.

5.4 Uptake Experiments

The bioaccumulation studies with newly hatched surf smelt larvae were conducted using SWAF's from either fresh CICO ([RCICO]) or weathered CICO ([$ICICO_{S120}$]) plus the added radiotracers. Duplicate static exposures were used, each having an initial ^{3}H-phenanthrene concentration of 140 pg phenanthrene equivalents/g of [RCICO] or [$ICICO_{S120}$]. Samples were taken at various intervals during the uptake experiments to determine the amount of radiolabeled compounds in the SWAF's and in the surf smelt tissue.

The larvae were removed from the SWAF and placed in uncontaminated flowing seawater to flush away radioactivity not directly associated with the larvae. An aliquot of the final rinse water was also collected and analyzed by LSC; any residual radioactivity present in this final rinse water was subtracted from the total radioactivity for the larvae to correct for contaminated water associated with the larvae as a result of sampling. The larvae in 1-2 mL seawater were then placed in a vial containing approximately 200 µL tricaine methanesulfonate and counted. The tissue, after being either homogenized with a Brinkman Polytron Homogenizer or solubilized chemically by Soluene 350, was then analyzed by LSC. At various intervals during the experiments approximately 100 larvae were sampled, washed and frozen for future extraction of tissue and analysis by TLC.

Newly hatched surf smelt were collected on a 64 µm screen. Three groups of ten larvae were removed from the screen and weighed. The mean weight of the larvae was determined to be 320 $\pm$ 30 µg ($\bar{X} \pm$ S.D.).

5.5 Toxicity Assays

5.5.1 Surf Smelt Larvae

Surf smelt eggs were collected from Puget Sound 1-2 days after deposition and 14-20 days prior to each bioassay. Eggs and sand-and-gravel substrate to which the eggs were attached were transferred to the Mukilteo Field Facility for incubation, where holding containers with the eggs were flooded with unfiltered ambient seawater (10-12°C and 28-29°/oo) for 3-5 hr per day (Misitano 1977).

Nine hundred mL of undiluted SWAF of each of the three different oils (CICO, PBCO, No. 2 fuel oil) and duplicate dilutions of each SWAF (50, 25 12, and 6% of original SWAF in filtered [5 µm] seawater) were placed in 1 L glass beakers (11 cm water depth). A known number of newly hatched larvae (100-200) were added to each beaker, and the beakers were placed in a 10°C water bath for the duration of the 48 hr exposure period. The bioassay included 6 control groups (larvae held in uncontaminated filtered [5 µm] seawater) interspersed among 30 oil-exposed groups.

At 2 and 24 hr after the start of exposure, visual estimates were made of the percent larvae swimming in the upper 1-2 cm of the water column. In addition, at the termination of the 48-hr exposure, all larvae were counted and assigned to one of four categories: (1) positive phototaxis with active swimming in the upper 1-2 cm of the water column; (2) active, but uncoordinated swimming on the bottom of the holding container; (3) inactive, with heart beat, and usually partly opaque; (4) dead, i.e., no heart beat. All larvae except those inactive or dead were then transferred to 900 ml of filtered (5 μm) uncontaminated seawater and held for an additional 72-96 hr. At termination of the bioassay (120-144 hr after start of the exposure), the larvae were again counted and categorized on the basis of swimming behavior and survival.

5.5.2 English Sole Embryos

Mature English sole were caught by trawl in Port Orchard, Puget Sound, Washington. Eggs were immediately stripped from ripe females and mixed with sperm from several males. The fertilized eggs were held at 8-10°C during transportation to the Mukilteo Field Facility where the eggs were incubated in 8°C seawater in 3 L containers, each containing 2,000-4,000 eggs. Approximately 100 three-day old viable embryos were transferred to 200 mL beakers containing 50 mL of filtered (0.45 μm) seawater in an 8°C water bath. Crude oil SWAF's or No. 2 fuel oil SWAF's were added so as to achieve a desired dilution in a total of 150 mL of liquid.

All exposures were conducted in triplicate. The embryos in test and control media were incubated at 8°C. After 48 hr, each beaker was examined under a dissecting microscope for (1) dead eggs, larvae, or embryos, (2) moribund individuals, and (3) viable embryos of larvae. Moribund individuals included embryos and larvae having a whitish color, larvae on the bottom of the container, and larvae that were unresponsive to touch.

5.5.3 English Sole Larvae

Larvae hatched from five-day old eggs held at 8°C were exposed and evaluated in a fashion similar to that described above (Sec. 5.5.2).

Data were statistically analyzed using either a chi-square analysis or analysis of variance with a Student-Newman-Keuls multiple comparison test.

5.6 Cytopathology

Samples of surf smelt larvae for histopathological and ultrastructural analyses were collected after 48 hr of exposure to the CICO SWAF and again at the termination of each bioassay experiment (120 hr). Larvae were fixed in a solution containing 0.75% glutaraldehyde, 3% formalin, 0.5% acrolein and 0.1 M sodium cacodylate buffer (pH 7.4) with 0.02% calcium chloride, and 5.5% sucrose. Larvae were postfixed in 1% osmium tetroxide in buffer, dehydrated with ethanol, embedded in Spurr medium (Spurr 1969) and sectioned with glass or diamond knives. Semi-thin (1 μm) sections were stained with Richardson's mixture. Tissues were first examined by light microscopy. Selected larvae were then thin-sectioned (80 nm), triple-stained with lead citrate, uranyl acetate, and again with lead citrate, and examined by transmission electron microscopy (TEM).

6. RESULTS

6.1 Reference Mixtures of Seawater and Oils

The data from GC and MGA for total organic extractable material and from GC for selected individual compounds indicated that the protocol was suitable for preparing reproducible concentrations of essentially non-weathered and nonoxidized reference mixtures. In subsequent discussions, the use of MGA for the total extractable organic material is assumed unless otherwise stated.

The total hydrocarbon (GC) concentrations in undiluted SWAF's for three reference oils used for the surf smelt larvae assays were: 2.2 µg/mL for PBCO; 3.4 µg/ml for CICO; and 3.9 µg/mL for No. 2 fuel oil (see Fig. 1).

6.1.1 Effects of Separation Time and Filtration on Composition of Reference Mixtures

Only 2-4% of the total ^{14}C-hexadecane-derived radioactivity added to the nonweathered crude oils was transferred to the aqueous phase SWAF's after 2 hr of mechanical shaking and 2 hr of separation of the two phases (Fig. 2). However, the more soluble No. 2 fuel oil had 13% of the total radioactivity remaining in the seawater under the same conditions. After 4 hr of settling, filtration did not change the amount of residual radioactivity in the SWAF's.

6.1.2 Effects of Storage on Composition of [RCICO]

The data in Table 2 and Appendix Tables 1A and 2A, show that [RCICO] can be stored for up to 15 days at 4°C with little change in the concentration of total extractable organic material as determined by GC and MGA or in the concentration (GC) of selected individual compounds -- except for naphthalene in the nonsterilized, nonpreserved sample. Major differences were not seen in these parameters between preparations made with sterilized and nonsterilized seawater and between preserved and nonpreserved samples. However, there were differences in the absolute values of total extractable organic material determined by GC compared to those determined by MGA. This was not unexpected because MGA is a measure of total extractable organic material extracted from the SWAF's, whereas the GC resolves only those components which can be injected into a gas chromatograph and resolved by flame-ionization detection.

After 65 days of storage, there were no changes in nonsterilized, non-preserved [RCICO] based on the results from MGA; however, there were sharp reductions in the extractable material based on the results from GC and GC for selected individual compounds. Specifically, total aromatic hydrocarbon concentrations were sharply reduced after 65 days of storage, whereas concentrations of selected phenolic compounds did not change, except for o-cresol which increased.

TABLE 2. Effects of storage at 4°C on [RCICO] (reference preparation of seawater and CICO).[a]

[RCICO]	Total Extractables (Pre-chromatography)		Total Aliphatic (Compounds)		Total Aromatic (Compounds)		Total Polar (Compounds)	
	GC	MGA	GC	MGA	GC	MGA	GC	MGA
	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL
Sterilized seawater								
0 days, preserved	2.5 ± 0.1[b]	2.6 ± 0.1	0.14 ± 0.01	0.38 ± 0.01	1.5 ± 0.1	0.46 ± 0.03	0.32 ± 0.03	1.7 ± 0.1
0 days, non-preserved	2.4 ± 0.1	2.7 ± 0.1	0.15 ± 0.01	0.39 ± 0.10	1.4 ± 0.1	0.37 ± 0.17	0.24 ± 0.03	1.5 ± 0.1
Blank	0.07	0.44	0.001	0	0.002	0.01	0.11	0.60
15 days, preserved	2.8 ± 0.1	3.7 ± 0.4	0.10 ± 0.01	0.29 ± 0.08	1.6 ± 0.0	0.41 ± 0.03	0.37 ± 0.04	1.2 ± 0.2
15 days, non-preserved	2.8 ± 0.2	3.7 ± 0.3	0.10 ± 0.02	0.30 ± 0.04	1.4 ± 0.1	0.42 ± 0.03	0.30 ± 0.02	1.1 ± 0.1
Blank	0.06	0.89	0.001	0	0.01	0	0.09	0.24
Non-sterilized seawater								
0 days, preserved	2.1 ± 0.2	1.5 ± 0.0	0.13 ± 0.01	0.40 ± 0.07	1.4 ± 0.1	0.56 ± 0.05	0.30 ± 0.01	0.84 ± 0.11
0 days, non-preserved	2.0 ± 0.1	1.7 ± 0.2	0.14 ± 0.01	0.33 ± 0.06	1.4 ± 0.1	0.39 ± 0.04	0.25 ± 0.01	0.93 ± 0.18
Blank	0.01	0.27	0.001	0	0.004	0	0.02	0
15 days, preserved	3.0 ± 0.4	2.0 ± 0.0	0.09 ± 0.01	0.30 ± 0.05	1.6 ± 0.2	0.33 ± 0.12	0.25 ± 0.01	0.90 ± 0.09
15 days, non-preserved	2.6 ± 0.1	2.0 ± 0.1	0.04 ± 0.01	0.21 ± 0.02	1.5 ± 0.0	0.32 ± 0.07	0.33 ± 0.03	0.94 ± 0.16
Blank	0.07	0.47	0.0003	0.03	0.003	0.01	0.06	0.05
65 days, non-preserved	0.92	2.4	0.03	0.29	0.37	0.36	0.45	1.5

[a] For concentration data for selected individual compounds, see Appendix, Tables 1A and 2A.

[b] $\bar{X} \pm$ S.D., n = 3.

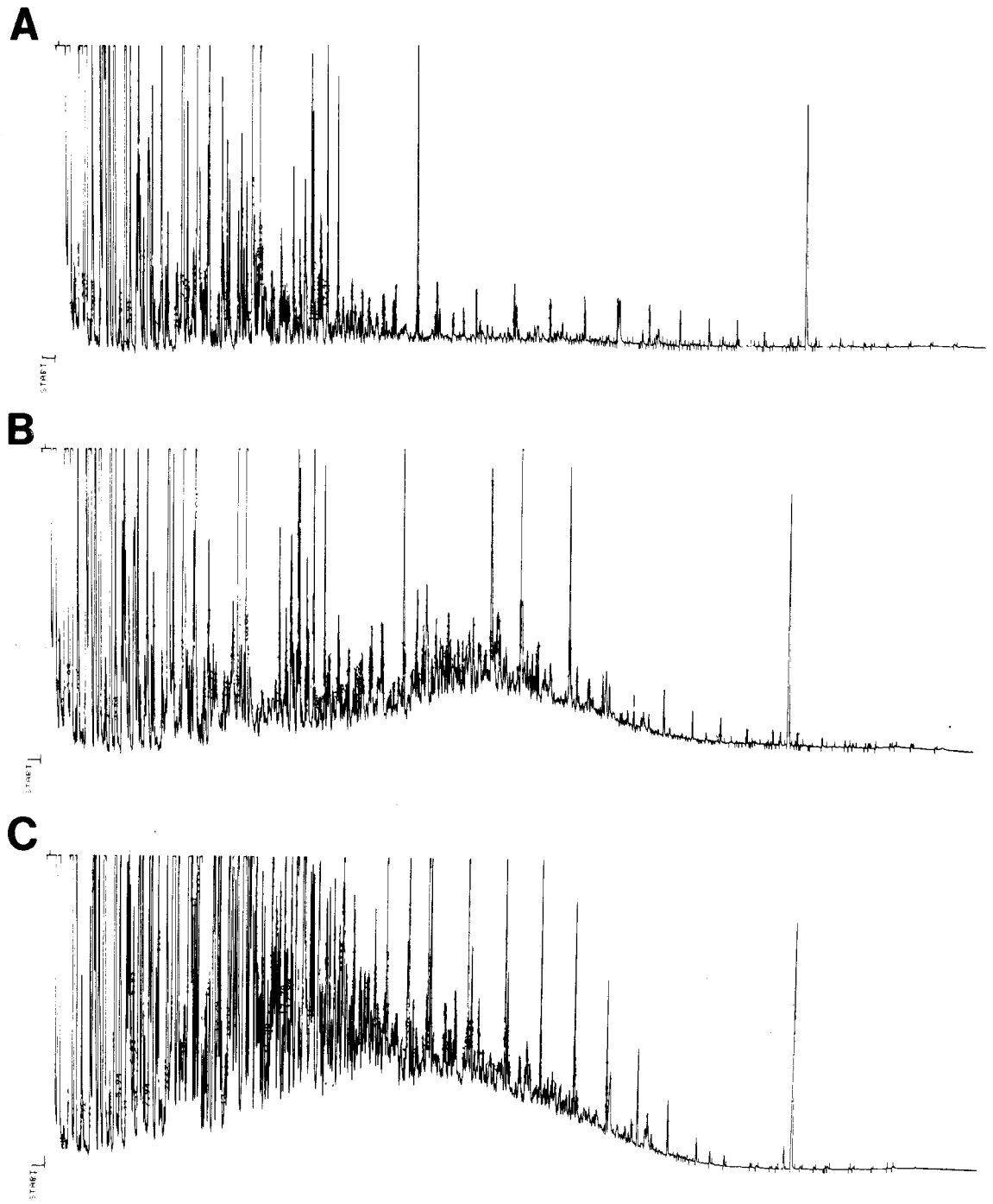

FIGURE 1. Gas chromatograms of the reference SWAF's of Prudhoe Bay crude oil [RPBCO] (A), Cook Inlet crude oil [RCICO] (B), and No. 2 fuel oil [RFO] (C).

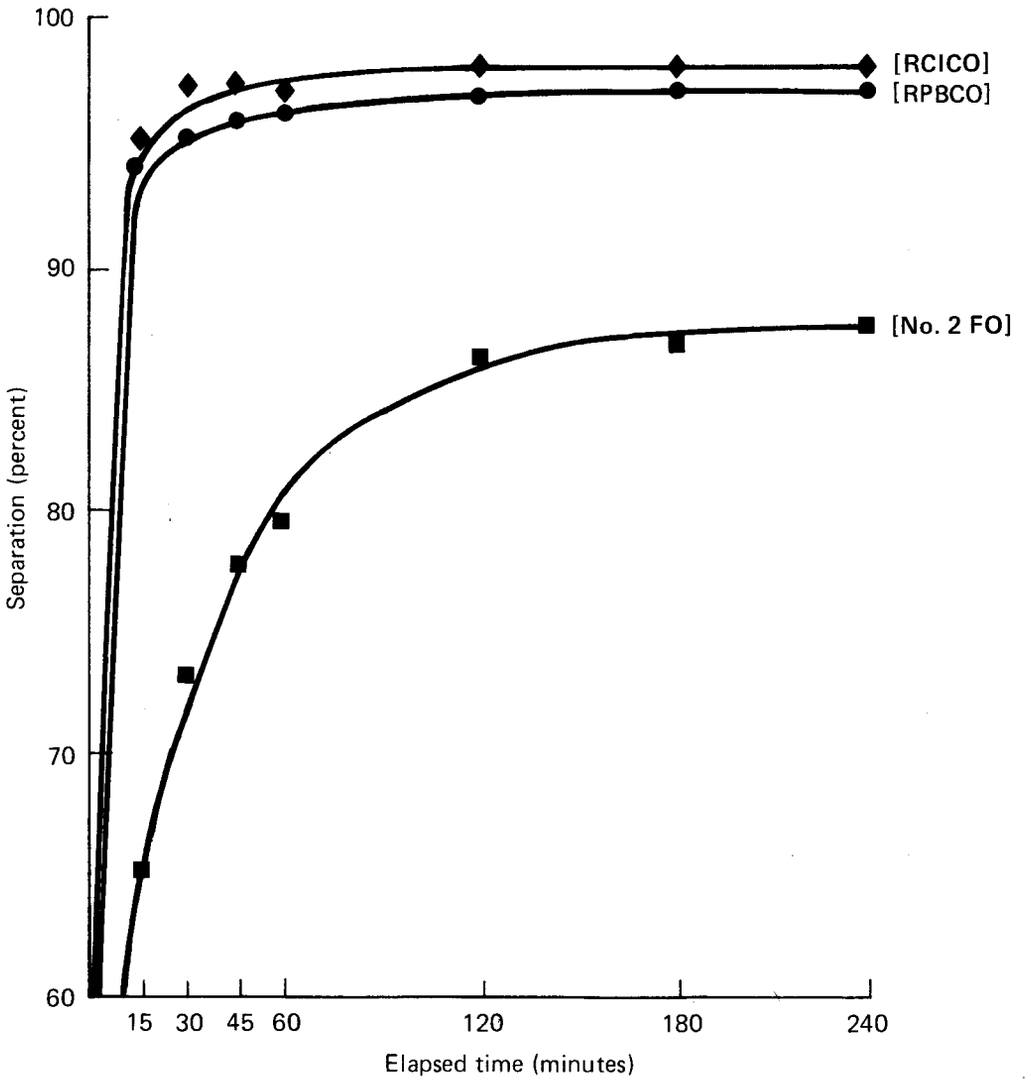

FIGURE 2. Separation times for reference SWAF's of Prudhoe Bay Bay crude oil [RPBCO], Cook Inlet crude oil [RCICO], and No. 2 fuel oil [RFO].

6.2 Weathering of Oils and Hydrocarbon Fractions of Oils

6.2.1 Flow-through Conditions

The MGA-determined total extractable organic material in the CICO SWAF produced by environmental weathering was markedly more variable than the content of this material present in any other preparation. High levels of total extractable organic material present in the seawater after environmental weathering appear to be due to unaccommodated oil. Chemical analyses revealed high concentrations of total aliphatic compounds in seawater environmental weathering compared to low concentrations in the other preparations.

6.2.2 No-flow Conditions

6.2.2.1 Static: The amount of total extractable organic material entering the seawater varied greatly depending on the type of oil layered on the surface and the presence of UV light (Table 3). There were also differences in the physical appearance between irradiated and nonirradiated oil. For instance, nonirradiated CICO spread out evenly over the entire surface of the seawater, whereas the irradiated CICO formed a layer on only a portion of the water's surface. In the case of No. 2 fuel oil, both irradiated and nonirradiated oil spread out as a thin film over the entire surface of the seawater, whereas the nonirradiated fuel oil remained clear while the irradiated fuel oil and A/P fractions of crude oils turned a dark yellowish brown with a concomitant increase in the surface film tensile strength.

After 120 hr of exposure with no irradiation, the amount of total extractable organic material recovered from the seawater underlying the No. 2 fuel oil ($[NIFO_{S120}]$) was only slightly more than that extracted from the CICO SWAF ($[NCICO_{S120}]$) (Table 3); however, after 120 hr of weathering with irradiation of the oils, the amount of total extractable organic material recovered from the No. 2 fuel oil system ($[IFO_{S120}]$) was nearly 14 times more than the amount extracted from the CICO system ($[ICICO_{S120}]$).

After 120 hr of weathering, the seawater beneath irradiated No. 2 fuel oil contained about 24 times as much total extractable organic material as did the seawater beneath the nonirradiated fuel oil. In contrast, the CICO SWAF contained about 3 times more material in the irradiated SWAF and PBCO was similar with 5 times more material in the irradiated SWAF than in nonirradiated SWAF.

When ^{14}C-phenanthrene was added to the oils, the ratios of radioactivity in irradiated versus nonirradiated SWAF's were similar to ratios of total extractable organic material determined by MGA (Table 3). When ^{14}C-phenanthrene was added to CICO, 0.4% of the phenanthrene-derived radioactivity was incorporated into $[ICICO_{S120}]$, and 0.2% was incorporated into $[NCICO_{S120}]$ (Table 3 and Fig. 3). Similarly for PBCO, 0.7% of the radioactivity was incorporated into $[IPBCO_{S120}]$ and 0.2% into $[NPBCO_{S120}]$. However, when ^{14}C-phenanthrene was added to No. 2 fuel oil, 11.6% of the phenanthrene-derived radioactivity was incorporated into $[IFO_{S120}]$ compared to 1.2% for $[NIFO_{S120}]$ (Table 3 and Fig. 3).

TABLE 3. Concentrations of total extractable organic material and percent radioactivity incorporated in seawater underlying oil. Tests conducted with and without exposure to UV light and under static conditions.

Surface Layer	Exposure Time (hr)	Source of ^{14}C	Incorportion of ^{14}C (%)	Total Extractable organic material (µg/mL)	Radioactivity extraction efficiency (%)
Cook Inlet crude oil[a]					
Irradiated [$ICICO_{S120}$]	120	Phenanthrene	0.4	25.7	76.3
Nonirradiated [$NCICO_{S120}$]	120	Phenanthrene	0.2	9.2	98.0
Cook Inlet crude oil					
Irradiated [$ICICO_{S120}$]	120	p-cresol	53.3	14.9	77.6
Nonirradiated [$NCICO_{S120}$]	120	p-cresol	67.1	4.2	78.0
Cook Inlet A/P fraction					
Irradiated [ICICO A/P_{S120}]	120	Phenanthrene	5.7	147	97.4
Nonirradiated [NCICO A/P_{S120}]	120	Phenanthrene	0.9	29.0	100.0
Prudhoe Bay crude oil					
Irradiated [$IPBCO_{S120}$]	120	Phenanthrene	0.7	12.0	73.2
Nonirradiated [$NPBCO_{S120}$]	120	Phenanthrene	0.2	2.3	99.9
Prudhoe Bay crude oil					
Irradiated [IPBCO A/P_{S120}]	120	Phenanthrene	5.3	161	97.6
Nonirradiated [NPBCO A/P_{S120}]	120	Phenanthrene	0.5	6.9	100.0
No. 2 fuel oil (2)[a]					
Irradiated [IFO_{S120}]	120	Phenanthrene	11.6	356	67.7
Nonirradiated [$NIFO_{S120}$]	120	Phenanthrene	1.2	12.5	83.9
Phenanthrene without oil[a]					
Irradiated [IPH_{S120}]	120	Phenanthrene	3.9	3.6	68.5
Nonirradiated [$NIPH_{S120}$)	120	Phenanthrene	0.6	1.2	98.7

[a] These data used in Figure 3.

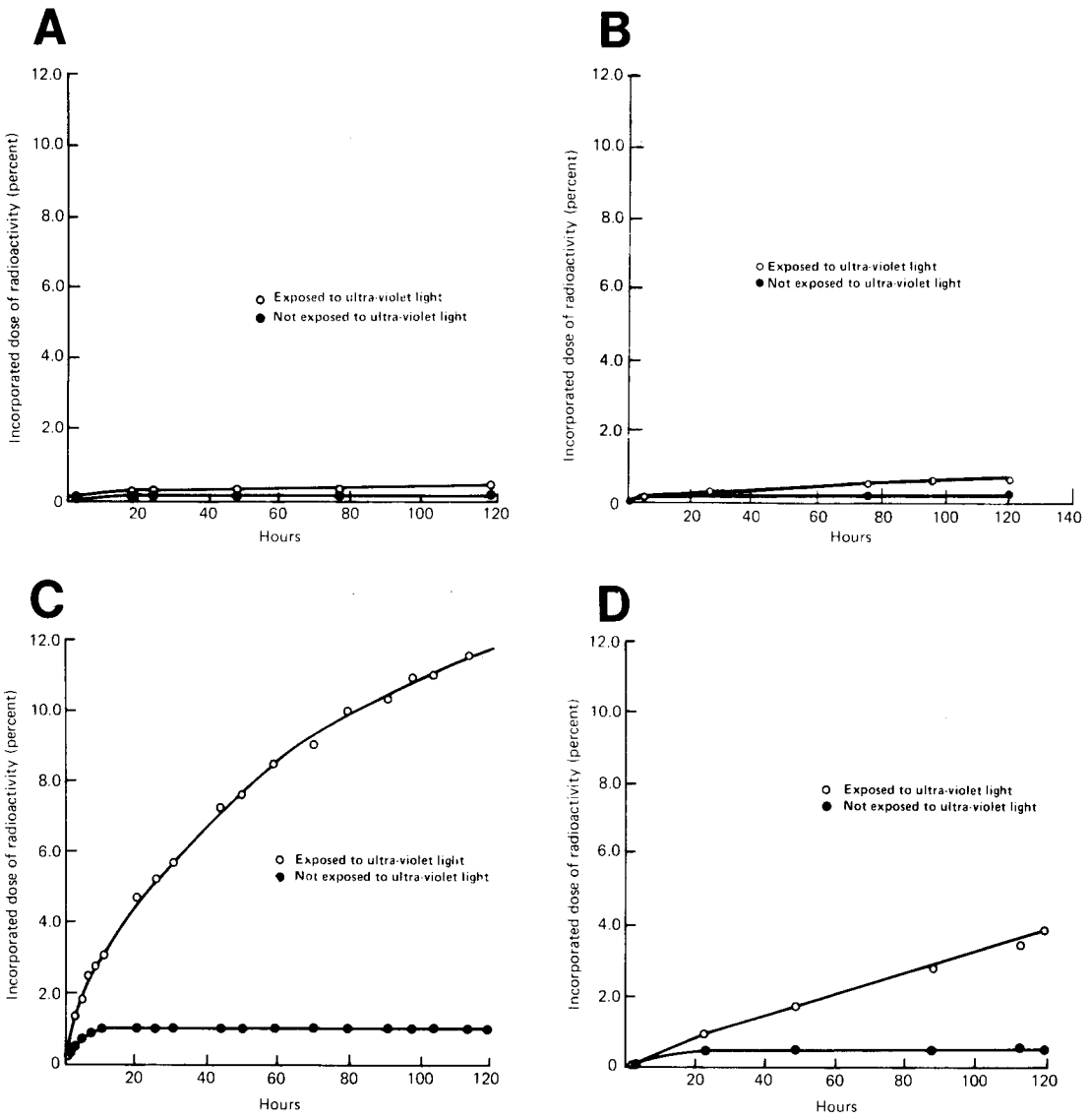

FIGURE 3. Incorporation of ^{14}C-phenanthrene-derived radioactivity into sterilized seawater with time under static conditions. The phenanthrene was layered on the seawater either mixed with CICO (A), PBCO (B), No. 2 fuel oil (C) or without oil (D) with and without exposure to UV irradiation.

When ^{14}C-phenanthrene (in pentane) was layered onto seawater in the absence of petroleum, the amount of radioactivity recovered in the total extractable organic material from the seawater following irradiation ([IPH_{S120}]) was nearly seven times higher than from the material from the nonirradiated system ([$NIPH_{S120}$]).

When ^{14}C-*p*-cresol, a polar component of crude oil, was added to CICO and then layered onto seawater, the incorporation of radioactivity into the underlying water was rapid, being slightly faster and greater in the nonirradiated system (Fig. 4). Within 4-5 hr the underlying seawater of both irradiated and nonirradiated CICO contained over 50% of the added radioactivity and a maximum was attained within 20 hr.

The extraction efficiency was determined by monitoring the amount of radioactivity remaining in the seawater after solvent extraction (Table 3). The extraction efficiency for the irradiated samples was considerably lower than for the samples which were not irradiated. The determination of the chemical composition of the nonextractable (using CH_2Cl_2, at pH = 2) radioactivity was beyond the scope of this study.

6.2.2.2 Agitated: Preliminary experiments to establish suitable periods of mixing suggested that UV-irradiated SWAF contained a relatively constant level of total extractable organic material with only 10 hr of wave machine agitation. However, a 120-hr mixing period and a 4-hr separation time (Fig. 5) for the disappearance of dispersed petroleum droplets from the water column were experimentally determined to be suitable.

In a radiotracer study of No. 2 fuel oil, the nonirradiated SWAF ([$NIFO_{A120}$]) contained more ^{14}C-phenanthrene-derived radioactivity than did the irradiated SWAF ([IFO_{A120}]); however, the total organic extractable material from the irradiated SWAF was twice that from the nonirradiated SWAF. The amounts of total extractable organic material found in the agitated systems were markedly different (21.4 µg/mL for nonirradiated and 41.5 µg/mL for irradiated SWAF) from those found in the static system (12.5 and 356 µg/mL, respectively). When PBCO was weathered for 120 hr in the wave machine, concentrations of total extractable organic material from nonirradiated ([$NPBCO_{A120}$]) and irradiated ([$IPBCO_{A120}$]) SWAF were 3.2 and 11.2 µg/mL, respectively. For comparison, a reference No. 2 fuel oil SWAF contained 3.9 µg/mL total extractable organic material.

6.2.3 Removal of Polar Compounds from Crude Oil Prior to Irradiation (A/P Fraction)

The two crude oils were subjected to a further chromatographic separation producing an A/P fraction which was essentially free of naturally occurring polar compounds. This fraction was then investigated in the same way as were the whole oils.

When the A/P fractions of CICO and PBCO were layered onto seawater, the amounts of extractable material entering the underlying seawater in both irradiated and nonirradiated systems were considerably higher than those for the unfractionated oils (Table 3). The total extractable organic material, 147 and 161 µg/mL, respectively, in both [ICICO A/P_{S120}] and [IPBCO A/P_{S120}] approached the values obtained with the irradiated No. 2

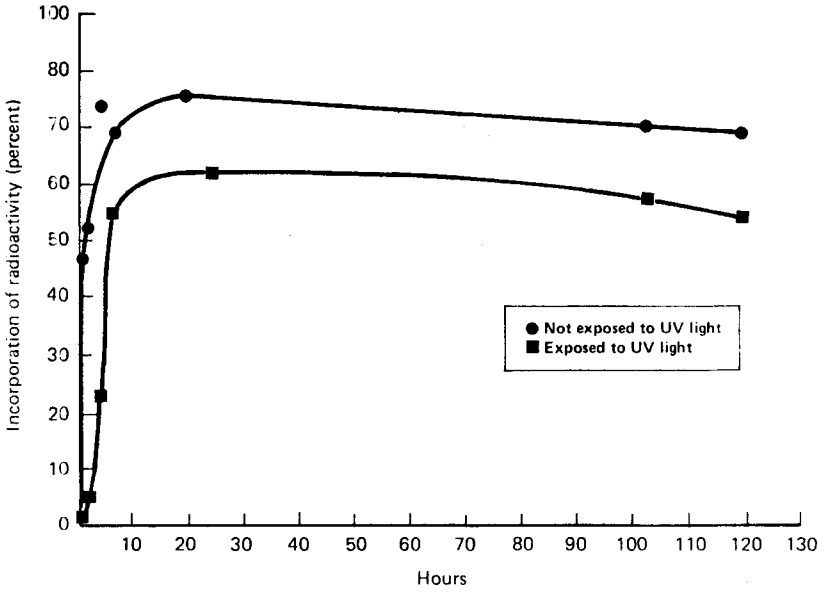

FIGURE 4. Incorporation of ^{14}C-p-cresol-derived radioactivity under static conditions into sterilized seawater underlying CICO with and without exposure to UV irradiation.

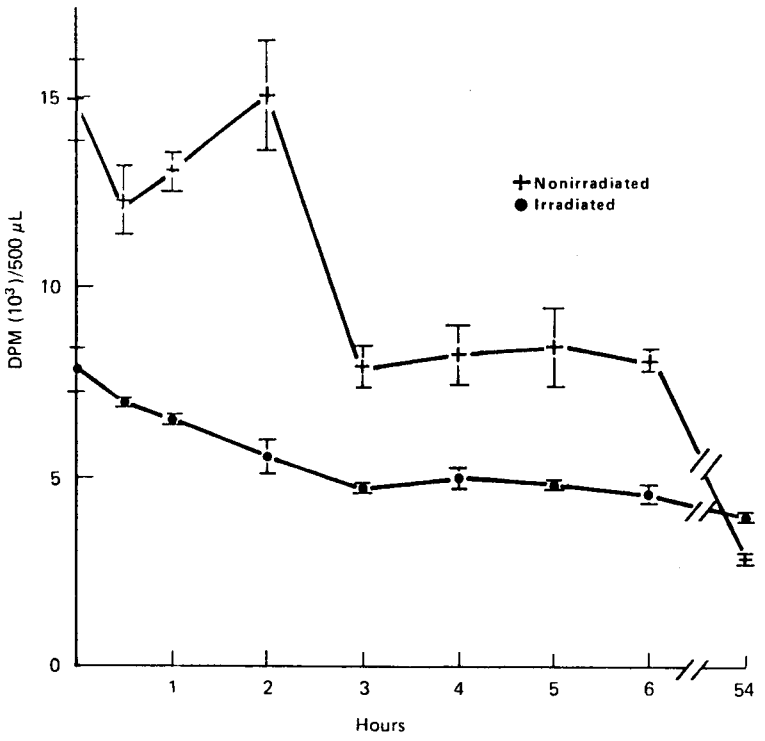

FIGURE 5. Radioactivity (^{14}C-phenanthrene-derived) in sterilized seawater after various periods of separation following 23 hr of no-flow agitated mixing. The phenanthrene was initially mixed with No. 2 fuel oil and layered onto the seawater, with and without exposure to UV irradiation ($\bar{X} \pm$ S.D.).

fuel oil [IFO_{S120}] (356 µg/mL). The incorporation of ^{14}C-phenanthrene into the irradiated SWAF's increased to 5.7% for [ICICO A/P_{S120}] and 5.3% for [IPBCO A/P_{S120}], respectively. This represents an increase of approximately 14 and 8 times over the corresponding values for the respective irradiated whole crude oils. However, the incorporation of ^{14}C-phenanthrene from A/P fractions into the nonirradiated SWAF's remained under 1%.

The results of the incorporation of ^{14}C-phenanthrene in the seawater under irradiated and nonirradiated A/P crude oil fractions are given in Table 4. A greater amount (3.4x and 11x) of the radioactivity was incorporated from the A/P fractions into the underlying seawater than from the irradiated whole crude oils; the nonirradiated enrichments from the A/P SWAF's were less (ca. 2x). In the case of the irradiated A/P fractions, nearly half (45%) of the radioactivity remained in the seawater after the methylene chloride extraction; however, in the nonirradiated SWAF's no radioactivity remained.

The TLC analyses of the extracts of the A/P SWAF's of the two crude oils showed that the bulk (92-97%, $R_f>0.62$) of the radioactivity in the nonirradiated SWAF's was present as the parent ^{14}C-phenanthrene (Fig. 6). The disposition of the radioactivity in the irradiated SWAF's of the two crude oil A/P fractions was nearly identical. The broader distributions of radioactivity in the irradiated SWAF's suggested the possible formation of several oxidized products ($R_f<0.5$) from these fractions, such as diphenic acid and quinone components.

6.3 Characterization of Oxidation Products of Radiotracers

Studies were made of the chemical composition of the radioactive compounds in the surface oil slick and in the total extractable organic material from the underlying seawater. The TLC analyses of the surface oil slicks ([$NCICO_{S120}$] and [$NIFO_{S120}$]) and phenanthrene without oil ([$NIPH_{S120}$]) showed that the radioactivity was largely ($\geq$ 99%) associated with the parent compound, phenanthrene, under static, nonirradiated conditions (Table 5). After irradiation with UV light, the CICO ([$ICICO_{S120}$]) and phenanthrene without oil ([IPH_{S120}]) underwent a 5- to 9-fold increase in the amount of oxidized materials, although the concentrations were low in the surface slick after 120 hr of exposure. The No. 2 fuel oil ([IFO_{S120}]) showed a 15-fold increase in oxidized products in the irradiated surface slick.

The distribution of radioactivity between parent compound and oxidized products extracted from the underlying seawater under static conditions (Table 5) was quite different from the distribution in the surface oils. After irradiation with UV light the proportion of radioactivity due to oxidized products of phenanthrene in [$ICICO_{S120}$], [ICICO A/P_{S120}], [IPBCO A/P_{S120}], [IPH_{S120}], and [IFO_{S120}] was noticeably increased.

Infrared analysis confirmed the presence of oxygenated compounds, and gas chromatographic/mass spectrometric (GC/MS) analyses identified several specific phenanthrene-derived oxidized products in [IPH_{S120}], such as carbonyl, quinone, and carboxylic acid structures (Fig. 7).

TABLE 4. Incorporation of ^{14}C-phenanthrene-derived radioactivity in seawater beneath irradiated and nonirradiated slicks of aromatic/paraffinic (A/P) fractions of two crude oils.

	Incorporated into SWAF's			Whole oil	Ratio of SWAF / Whole oil
	Total extractables (using CH_2Cl_2) (%)	Remaining in seawater (%)	Total (%)	(%)	
IRRADIATED					
Cook Inlet crude oil [ICICO A/P_{S120}]	3.1	2.6	5.7	1.7	3.4
Prudhoe Bay crude oil [IPBCO A/P_{S120}]	2.9	2.4	5.3	0.5	11
NONIRRADIATED					
Cook Inlet crude oil [NCICO A/P_{S120}]	0.9	0	0.9	0.5	2
Prudhoe Bay crude oil [NPBCO A/P_{S120}]	0.5	0	0.5	0.2	2

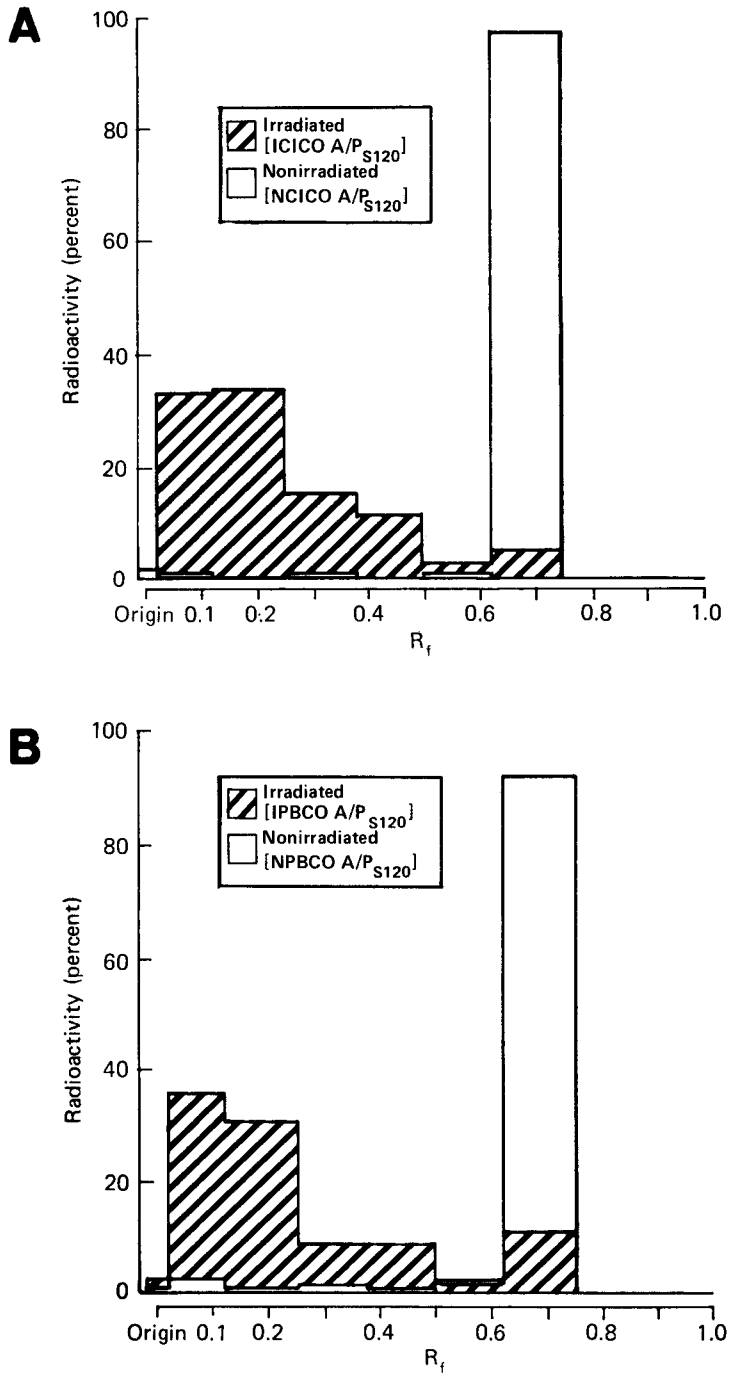

FIGURE 6. Radioactivity in TLC extracts of SWAF's from irradiated and nonirradiated A/P fractions of Cook Inlet crude oil (A) and Prudhoe Bay crude oil (B).

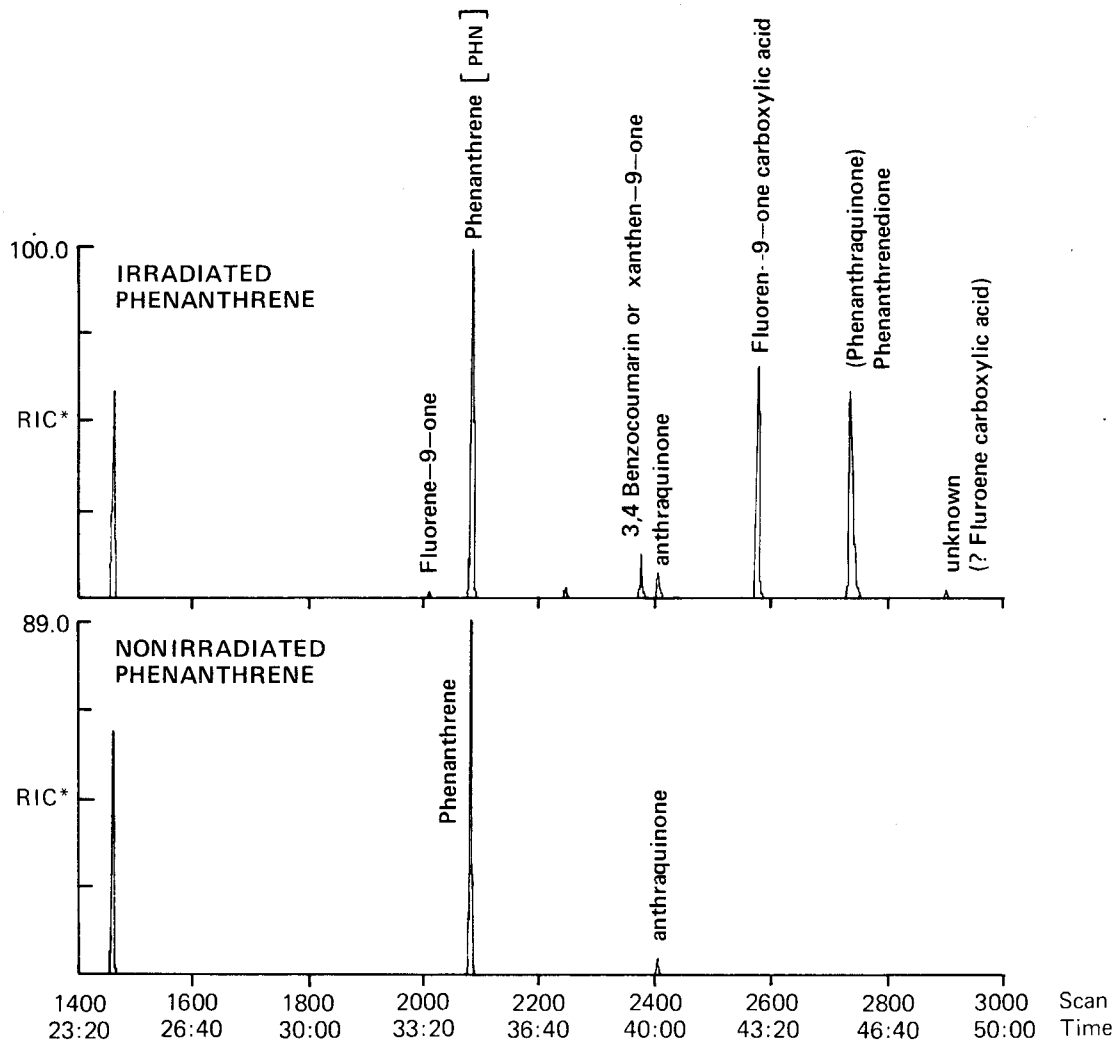

FIGURE 7. Reconstructed ion chromatograms (RIC) of phenanthrene-derived products extracted from seawater underlying phenanthrene with and without exposure to UV irradiation.

In the wave machine-produced SWAF's, the ratios of parent hydrocarbon: oxidized products were slightly different from the static system ratios; however, the overall profiles of the phenanthrene-derived oxidized products were similar. For instance, in the No. 2 fuel oil SWAF, 95% of the non-irradiated ^{14}C-phenanthrene remained whereas 75% remained unoxidized in the irradiated system.

In summary, the nonirradiated surface oil slicks and SWAF's generally had lower levels of oxidized products than did the irradiated slicks and SWAF's (Table 5). In the case of the higher levels of radioactivity associated with "oxidized" material detected in the nonirradiated No. 2 fuel oil SWAF, infrared analyses failed to detect the presence of any oxygenated compounds in the solution ([NIFO$_{S120}$]). Moreover, the GC/MS of [NIPH$_{S120}$] (Fig. 7) showed no oxygenated compounds except for trace amounts of anthraquinone.

6.4 Uptake Experiments

Larval surf smelt were exposed to [RCICO] which contained added ^{3}H-phenanthrene (Fig. 8). Duplicate static exposures were used, each having an initial ^{3}H-phenanthrene concentration of 140 pg phenanthrene equivalents/g [RCICO]. The concentration of ^{3}H-phenanthrene in the SWAF of [RCICO] did not change markedly over the 24 hr exposure period (Fig. 8). The concentration of ^{3}H-phenanthrene associated with the surf smelt larvae (pg phenanthrene equivalents/g wet weight larvae) increased rapidly for the first 2 hr, and then remained constant at approximately 3,000 times the concentration of phenanthrene (pg phenanthrene equivalents/g water) in the water for the remainder of the 24-hr exposure.

Larval surf smelt were exposed to SWAF's of CICO from low-flow weathering experiments conducted during the first year. Prior to 24 hr of weathering, ^{3}H-phenanthrene and ^{14}C-*p*-cresol were added to the CICO (see Section 5.2.1). During the 24-hr uptake experiment, concentrations of ^{3}H-phenanthrene and ^{14}C-*p*-cresol-derived material in the irradiated and nonirradiated SWAF's remained relatively constant at 1.5 pg phenanthrene equivalents/g of seawater and 24 ng *p*-cresol equivalents/g of seawater, respectively (Fig. 9). The concentration of ^{3}H-phenanthrene-derived material in the fish exposed to the CICO increased to a maximum value of 560 pg phenanthrene equivalents/g wet weight larvae after 6 hr of UV irradiation, compared to a maximum concentration (19 hr) of 240 pg phenanthrene equivalents/g larvae exposed to nonirradiated CICO SWAF. Similarly, the concentration of ^{14}C-*p*-cresol-derived material reached a maximum value of 400 ng *p*-cresol equivalents/g wet weight of larvae after 19 hr of exposure in the irradiated CICO, compared to the value 150 ng *p*-cresol equivalents/g wet weight larvae in the nonirradiated CICO SWAF.

6.5 Toxicity Assays

6.5.1 Surf Smelt Larvae

6.5.1.1 Mortality: The survival of surf smelt larvae was routinely high until concentrations of total hydrocarbons (GC) exceeded about 1 µg/mL (Fig. 10). Approximately 80% of the mortality occurred within the

TABLE 5. Characterization of phenanthrene-derived radioactivity in the surface oil and SWAF's with and without exposure to UV light.[a]

Surface Layer	Exposure Time (hr)	Surface oil slick		Underlying seawater (SWAF's)	
		^{14}C as phenanthrene (%)	^{14}C as oxidized products (%)	^{14}C as phenanthrene (%)	^{14}C as oxidized products (%)
Cook Inlet crude oil					
Irradiated [$ICICO_{S120}$]	120	99.1	0.9	57.5	42.5
Nonirradiated [$NCICO_{S120}$]	120	99.9	0.1	96.6	3.4
Cook Inlet A/P fraction					
Irradiated [ICICO A/P_{S120}]	120	-	-	7.3	92.7
Nonirradiated [NCICO A/P_{S120}]	120	-	-	97.7	2.3
Prudhoe Bay A/P fraction					
Irradiated [IPBCO A/P_{S120}]	120	-	-	12.3	87.7
Nonirradiated [NPBCO A/P_{S120}]	120	-	-	94.4	5.6
No. 2 Fuel Oil					
Irradiated [IFO_{S120}]	120	90.9	9.1	8.2	91.8
Nonirradiated [$NIFO_{S120}$]	120	99.4	0.6	51.9	48.1
Phenanthrene without oil					
Irradiated [IPH_{S120}]	120	99.5	0.5	17.9	82.1
Nonirradiated [$NIPH_{S120}$]	120	99.9	0.1	89.8	10.2

[a] Analyses performed by TLC (see Methods).

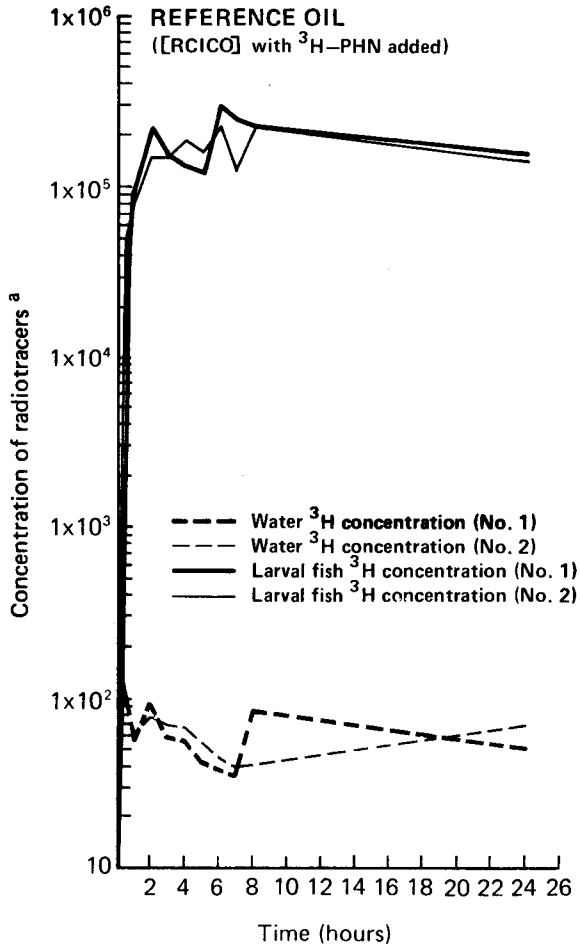

FIGURE 8. Uptake of ^{3}H-phenanthrene-derived material by surf smelt larvae from [RCICO]. ^{3}H-phenanthrene was added after [RCICO] was prepared.

[a] The concentrations of phenanthrene-derived radioactivity in seawater are expressed as pg (10^{-12}g) phenanthrene equivalent/mL of seawater and in larval fish tissues as pg phenanthrene equivalents/g of larvae (wet weight).

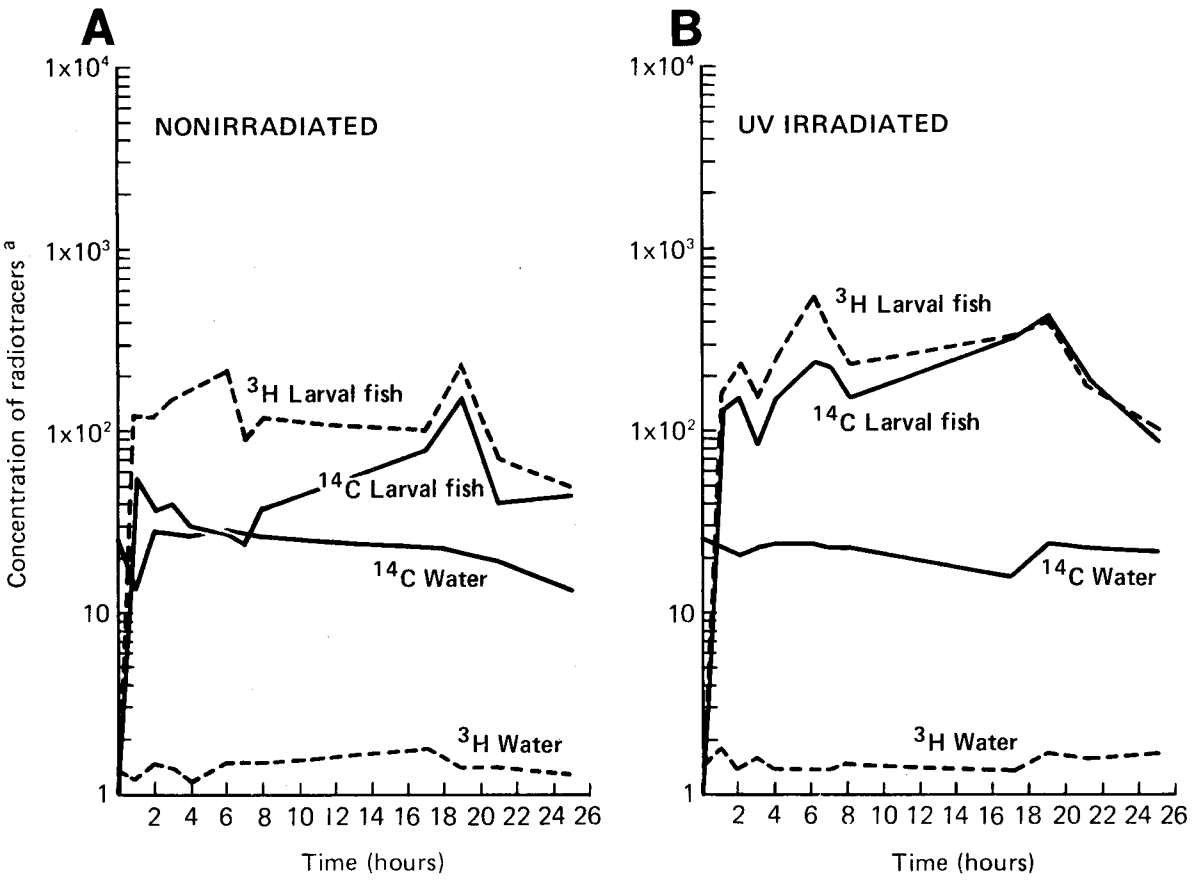

FIGURE 9. Uptake of ^{3}H-phenanthrene-derived and ^{14}C-p-cresol-derived radioactivity by surf smelt larvae from low-flow, weathered nonirradiated (A) and irradiated (B) Cook Inlet crude oil.

[a]Concentrations of phenanthrene-derived radioactivity in seawater are expressed as pg (10^{-12}g) phenanthrene equivalents/mL of seawater and in larval fish tissues as pg phenanthrene equivalent/g of larvae (wet weight). Concentrations of cresol-derived radioactivity in seawater are expressed as ng (10^{-9}g) cresol equivalent/mL of seawater and in larval fish tissues as ng cresol equivalent/g of larvae (wet weight).

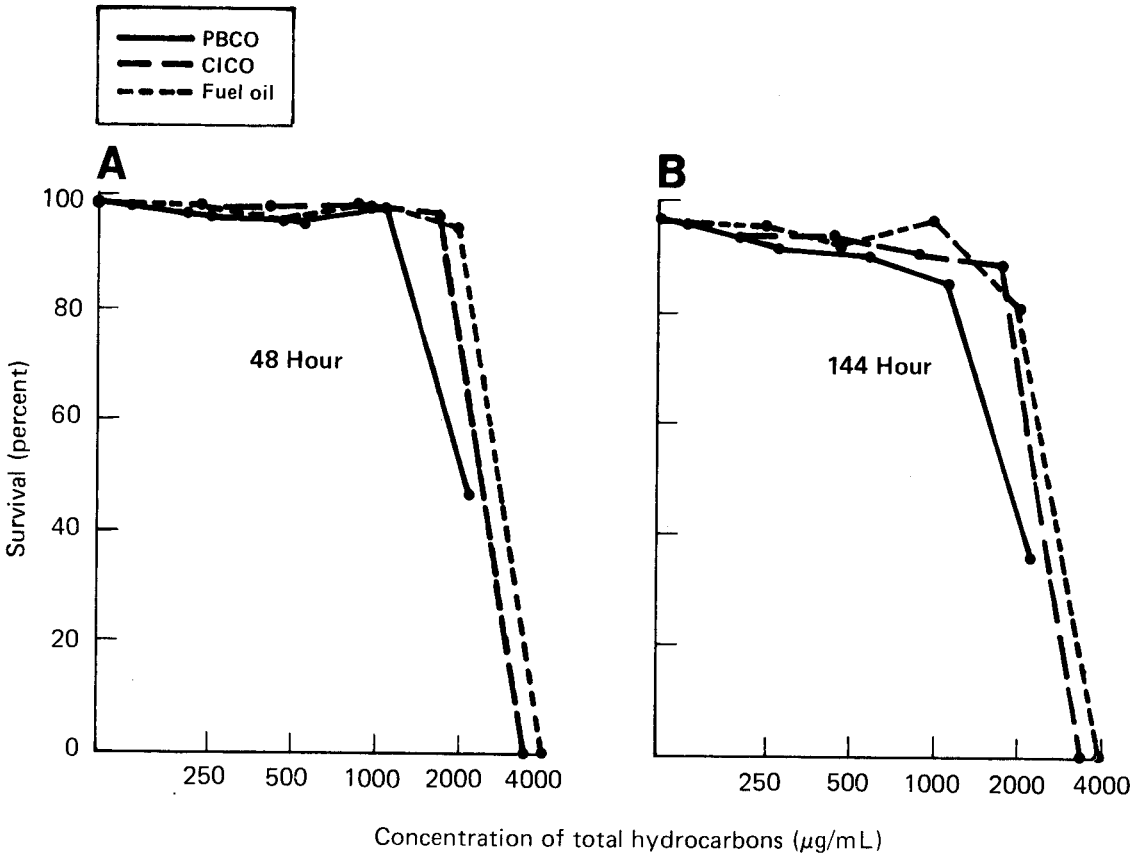

FIGURE 10. Percent survival of surf smelt larvae after (A) 48-hr exposure to the reference SWAF's of PBCO, CICO, and No. 2 fuel oil and (B) 96 hr of depuration following exposure (144 hr total). Concentrations of total hydrocarbons determined by GC analysis. Each data point represents an average of the percent survival in duplicate tests. The average percent differences between duplicate oil-exposed groups was 4.4% (± 3.7% S.D.), and 1.1% (± 0.7% S.D.) for controls.

initial 48 hr of exposure. The estimated 48-hr EC_{50} (value where 50% of the larvae died) varied from 2.1 µg/mL total hydrocarbons for fresh, nonweathered PBCO SWAF ([RPBCO]) to 2.7 µg/mL for fresh, nonweathered No. 2 fuel oil ([RFO]).

6.5.1.2 Swimming Behavior: The position of surf smelt larvae in the water column was evaluated after 2, 24, and 48 hr of exposure and again after 96 hr of depuration. The results shown in Figure 11A indicated that after 2 hr of exposure to nonweathered and undiluted SWAF's of PBCO, CICO or No. 2 fuel oil, 97-100% of the larvae had developed a pronounced change in swimming behavior, called ataxia, whereby they were unable to maintain their usual position in the water column. The estimated hydrocarbon concentration at which 50% of the larvae exhibited ataxia (EC_{50}) ranged from 0.5 to 1.0 µg/mL. By 24 hr (Fig. 11B), the estimated EC_{50} had dropped to 0.25 to 0.35 µg/mL (roughly 250-350 ppb). Thereafter, the EC_{50}'s increased, possibly as the more volatile and toxic hydrocarbons were lost from the water column by evaporation (Fig. 11C). After 96 hr of depuration (Fig. 11D), over 75% of the larvae previously exhibiting ataxia had regained normal swimming behavior.

6.5.2 English Sole Embryos

6.5.2.1 Hatching Success: When fertilized eggs of English sole were exposed to high concentrations of fuel oil SWAF's ($[IFO_{A120}]$) and $[NIFO_{A120}]$ for 48 hr, the hatching success was significantly ($p \leq 0.05$) different from the 98% hatching success in control seawater (Fig. 12). In this experiment, the nonirradiated fuel oil SWAF ($[NIFO_{A120}]$) reduced hatching success more than the irradiated SWAF ($[IFO_{A120}]$). The differences were statistically different over the concentration ranges 1-5, 6-10, 11-15, and >15 µg/mL. The hatching success of eggs exposed to the nonirradiated fuel oil was not markedly different from the hatching success of the controls at SWAF concentrations of the total extractable organic material below 4 µg/mL. Under static conditions with UVirradiated PBCO, hatching success of English sole eggs was less than 50% in the 1-2 µg/mL of total extractable organic material from both the irradiated SWAF ($[IPBCO_{S120}]$) and the nonirradiated SWAF ($[NPBCO_{S120}]$).

6.5.2.2 Mortality: The toxicities of SWAF's from irradiated and nonirradiated wave machine-weathered No. 2 fuel oil were not statistically significantly different, based on mortalities from 48-hr exposures of English sole embryos (Table 6). Mortalities were not different from controls (2%) when the embryos were exposed for 48 hr to concentrations of 21 µg/mL or less of total extractable organic material from the UV-irradiated SWAF ($[IFO_{S120}]$) or nonirradiated SWAF ($[NIFO_{120}]$). An environmental unrealistic level of 35 µg/mL from the irradiated SWAF ($[IFO_{S120}]$) caused 26% mortality. This concentration of No. 2 fuel oil SWAF materials probably reflects a hydrocarbon content of 10-15 µg/mL which is at the high end of laboratory-prepared solutions (Anderson et al. 1974b) and several orders of magnitude higher than levels routinely measured after oil spills (Clark and MacLeod 1977). Until these high levels were reached, English sole embryos were not detectably affected during 48-hr assays.

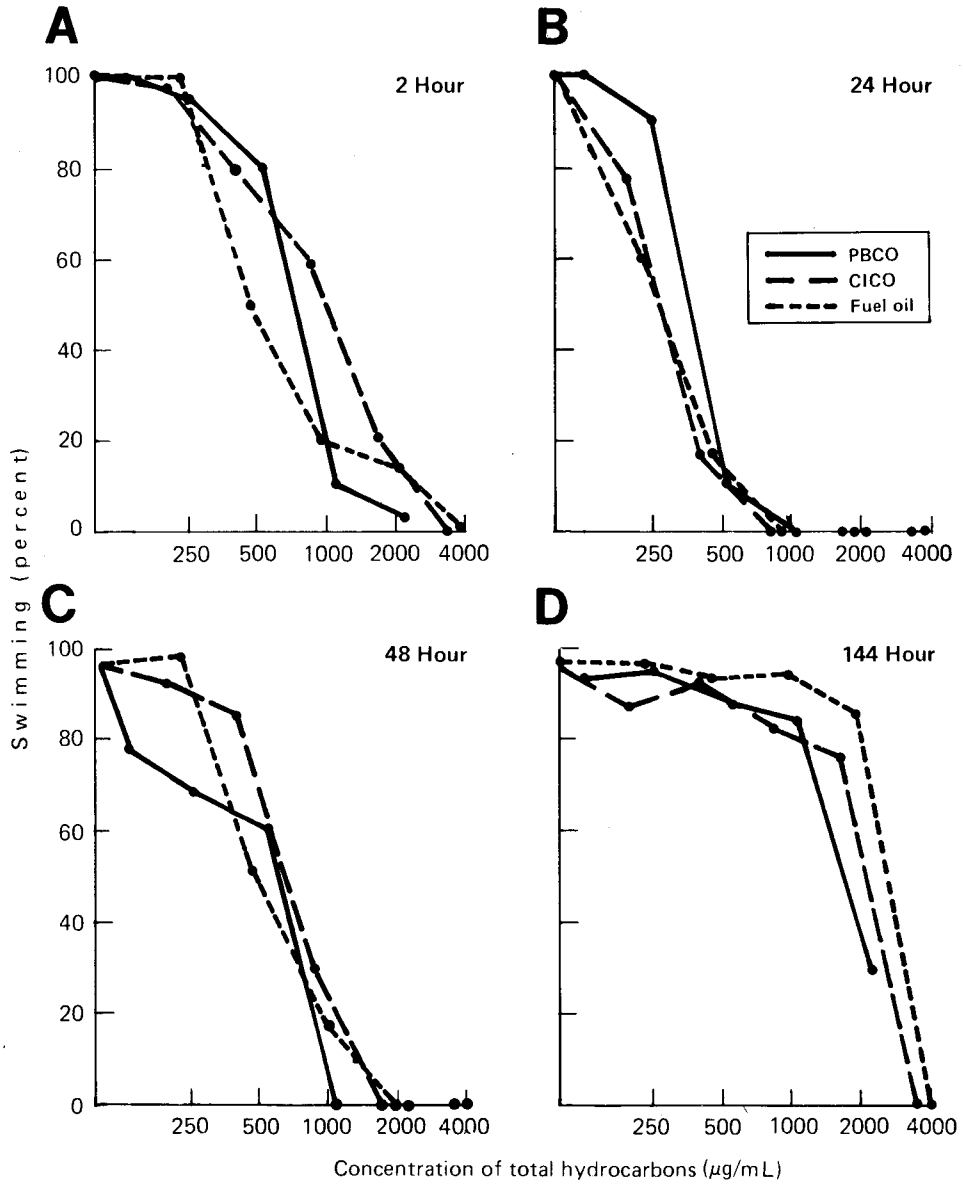

FIGURE 11. Percent of surf smelt larvae swimming in the upper 2-4 cm of the water column and respective total hydrocarbon concentrations in the reference SWAF's of PBCO, CICO, and No. 2 fuel oil: (A) 2-hr exposure, (B) 24-hr exposure, (C) 48-hr exposure, and (D) 96-hr depuration following 28-hr exposure (144 hr total). Concentrations of total hydrocarbons determined by GC analysis. Data points include mortalities, and each point is an average of the percent swimming in duplicate tests. The average percent difference between duplicate oil-exposed groups was 10.5% (± 14.7% S.D.), and 1.2% (± 1.2% S.D.) for controls.

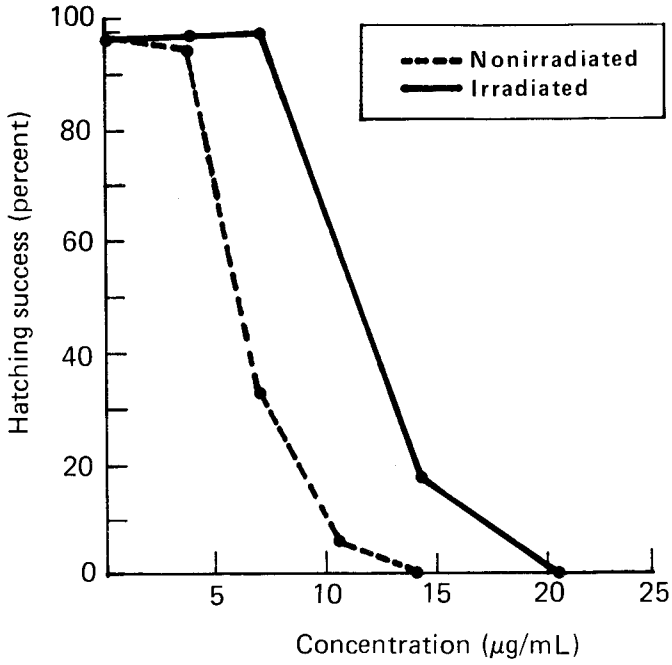

FIGURE 12. Hatching success of English sole eggs after exposure to irradiated [IFO_{S120}] and non-irradiated [$NIFO_{S120}$] SWAF's of No. 2 fuel oil under static conditions.

Very high levels of total extractable organic material were obtained from the static UV-irradiated No. 2 fuel oil weathering system (Table 6). In order to accommodate levels as high as 160 μg/mL of SWAF without having dispersed oil droplets, most of the total extractable organic material must have consisted of oxidized products.

English sole embryos exposed for 48 hr to SWAF's from wave machine-agitated UV-irradiated PBCO ([$IPBCO_{A120}$]), which contained considerably lower levels of extractable organic material than fuel oil SWAF's, had a mortality not markedly different from the mortality seen in the controls (Table 7). Similar results were seen for PBCO SWAF obtained under static conditions. The highest levels of total extractable organic material obtained from PBCO SWAF under these two different weathering regimes were similar.

When the nonweathered, nonirradiated reference preparations of No. 2 fuel oil ([RFO]) and PBCO ([RPBCO]) obtained by mechanical shaking (Section 5.1) were used in the English sole embryo assay, the mortalities were not markedly different from those for the controls (Table 8). The highest concentrations of total extractable organic material obtained in the reference preparations were one to two orders of magnitude lower than those obtained either in the static or agitated controlled irradiation systems.

TABLE 6. Mortality of English sole embryos exposed for 48 hr to irradiated or nonirradiated SWAF's of No. 2 fuel oil produced under agitated or static conditions.

	Concentration (µg/mL)	Animals		Mortality (%)
		Total	Dead	
AGITATED CONDITIONS				
Irradiated	3	333	4	1
[IFO_{A120}]	7	319	7	2
	14	302	14	5
	21	329	9	3
	28	304	7	2
	35[a]	312	81	26
Nonirradiated	4	286	8	3
[$NIFO_{A120}$]	7	349	13	4
	11	292	13	4
	14	298	11	4
	18	341	13	4
	21[a]	165	5	3
Control	0	680	13	2
STATIC CONDITIONS				
Irradiated	13	293	52	18
[IFO_{S120}]	25	272	147	54
	54	289	218	75
	107	310	310	100
	161[a]	303	303	100
Nonirradiated	2	181	4	2
[$NIFO_{S120}$]	3	204	20	10
	6	312	34	11
	7[a]	327	67	20
Control	0	507	55	11

[a] Maximum concentration of total extractable organic material removed from the SWAF's.

TABLE 7. Mortality of English sole embryos exposed for 48 hr to irradiated or nonirradiated SWAF's of Prudhoe Bay crude oil produced under agitated or static conditions.

	Concentration (µg/mL)	Animals		Mortality (%)
		Total	Dead	
AGITATED CONDITIONS				
Irradiated	2	280	14	5
[$IPBCO_{A120}$]	4	310	24	8
	6	310	14	5
	8[a]	290	27	9
Nonirradiated	0.5	250	7	3
[$NPBCO_{A120}$]	1	310	17	5
	2	310	20	6
	2.1[a]	260	10	4
Control	0	507	55	11
STATIC CONDITIONS				
Irradiated	1	280	13	5
[$IPBCO_{S120}$]	2	320	8	3
	4	290	17	6
	6	310	12	4
	8[a]	210	13	6
Nonirradiated	0.2	270	9	3
[$NPBCO_{S120}$]	0.4	330	12	4
	0.8	320	18	6
	1	310	11	4
	2[a]	230	14	6
Control	0	680	13	3

[a] Maximum concentration of total extractable organic material removed from the SWAF's.

TABLE 8. Mortality of English sole embryos exposed for 48 hr to reference SWAF's from No. 2 fuel oil or Prudhoe Bay crude oil.

	Concentration (µg/mL)	Animals Total	Animals Dead	Mortality (%)
No. 2 fuel oil [RFO]	0.2	297	7	2
	0.4	307	3	1
	0.6	323	6	2
	0.7	291	11	4
	0.9[a]	267	6	2
Prudhoe Bay crude oil [RPBCO]	0.07	322	7	2
	0.1	327	7	2
	0.2	290	9	3
	0.3	250	6	2
	0.33[a]	250	9	4

[a] Maximum concentration of total extractable organic material removed from the reference SWAF's.

6.5.3 English Sole Larvae

In the experiment where newly hatched English sole larvae were exposed to irradiated ([IFO_{A120}]) or nonirradiated ([$NIFO_{A120}$]) fuel oil SWAF's, the mortalities differed significantly (ANOVA, Student-Newman-Keuls $p \leq 0.05$) from those for the seawater control (Table 9). The mortalities of the larvae occurred at higher levels of SWAF than the levels necessary to produce mortality in the apparently more resistant English sole embryos. For instance, larvae exposed for 48 hr to starting total extractable organic material concentrations of 4 µg/mL and 2 µg/mL from the irradiated SWAF ([IFO_{A120}]) experienced 84% and 34% mortalities respectively, compared to 8% for controls. Exposure of the larvae to the nonirradiated SWAF ([$NIFO_{A120}$]) from No. 2 fuel oil produced 100% mortality at concentrations of 2 µg/mL and 18% mortality at 0.6 µg/mL (roughly 600 ppb).

TABLE 9. Mortality of English sole larvae exposed for 48 hr to irradiated or nonirradiated SWAF's of No. 2 fuel oil produced by a wave machine.

	Concentration (µg/mL)	Animals		Mortality (%)
		Total	Dead	
Irradiated [IFO_{A120}]	2	210	72	34
	4	120	101	84
	6	227	227	100
	8[a]	153	153	100
Nonirradiated [$NIFO_{A120}$]	0.6	209	37	18
	1	187	111	59
	2	216	216	100
	2.3[a]	198	198	100
Control	0	203	16	8

[a] Maximum concentration of total extractable organic material removed from the SWAF's.

6.6 Cytopathology

Eight control surf smelt larvae and 25 larvae exposed to 2.7 µg/mL of CICO ([RCICO]) were examined by light microscopy. Tissues from larvae which were actively swimming on the bottom or in the water column following a 48-hr exposure to reference oil SWAF ([RCICO]) appeared normal and similar to controls (Fig. 13A & B). Ninety-one percent (10 of 11) of the inactive larvae exhibited necrotic neurons in either the eye, brain, and/or olfactory placode (Fig. 13C). Eighty-two percent (9 of 11) of the inactive larvae also had compressed muscle bundles that were more intensely stained with Richardson's solution (Fig. 13D) than were those of controls. Limited examination with TEM showed that neurons of the eye and brain of inactive larvae exhibited condensed chromatin and numerous lysosomes, which is indicative of necrosis, and that there were severe disruptions in regions of myofibrils and sarcomeres of the muscle.

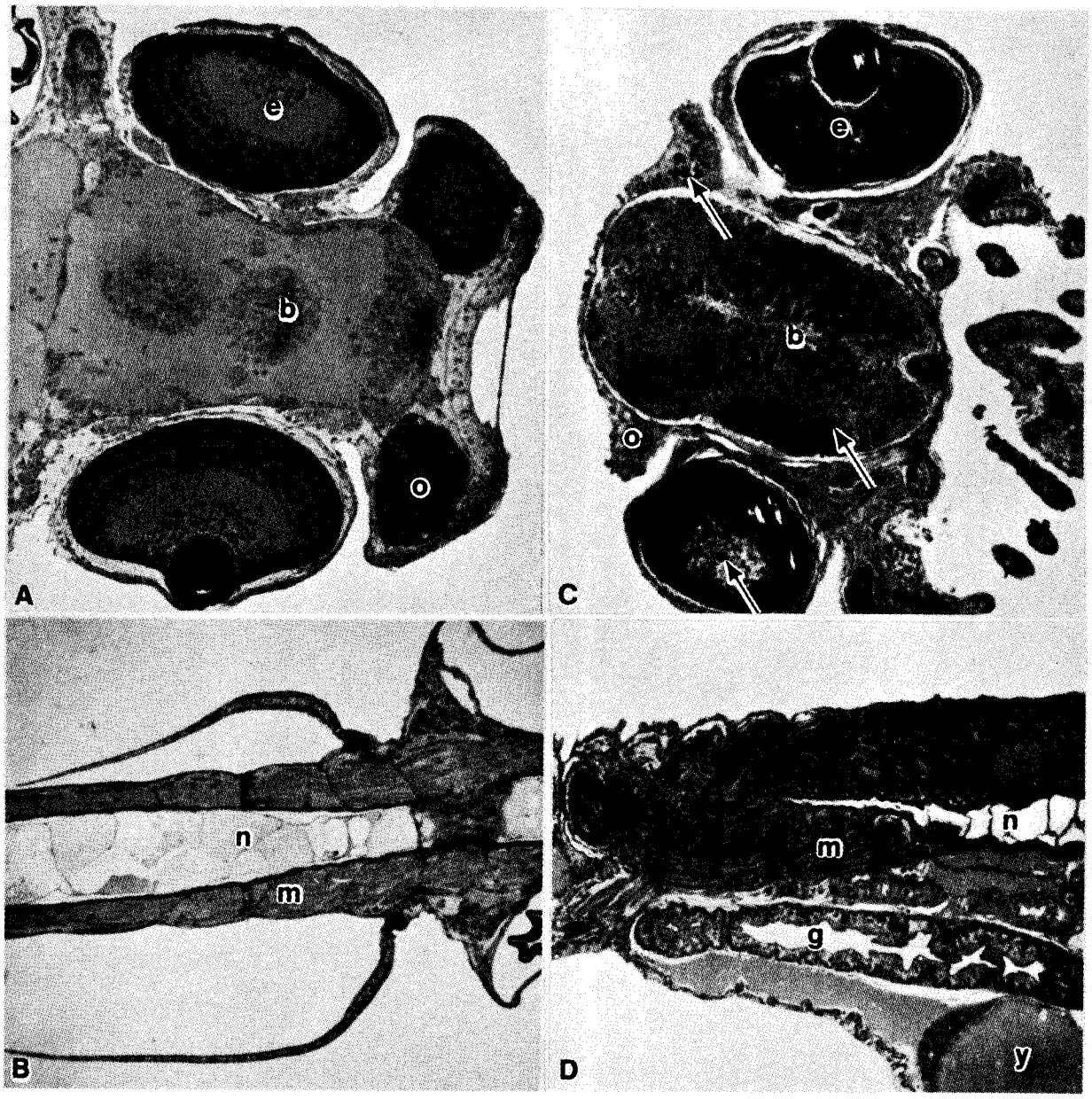

FIGURE 13. Microphotographs of a surf smelt larva. Frontal sections, X160. (A) Head of control larva with normal eye (e), brain (b), and olfactory placode (o). (B) Normal body musculature (m) and notochord (n) of a control larva. (C) Head of inactive surf smelt larva exposed to the reference SWAF of Cook Inlet crude oil [RCICO] for 48 hr showing dark staining necrotic cells (arrows) in the eye (e), brain (b), and olfactory placodes (o). (D) Compressed muscle bundles (m) of an inactive larvae with notochord (n), yolk (y), and gut (g) visible.

DISCUSSION

7.1 Reference Mixtures of Seawater and Oils

Detailed studies with CICO SWAF's ([RCICO]) showed that the [RCICO] was essentially stable when stored at 4°C for up to 15 days, when sterilized or when a preservative was added. This suggested that samples of seawater collected from under an oil spill can be stored at 4°C for up to 15 days before chemical analyses without any marked changes occurring. In the absence of sterilization or a preservative, considerable loss of naphthalene occurred from the SWAF even when samples were stored at 4°C. Microbial degradation of hydrocarbons occurs even at low temperature (Karrick 1977) and, therefore, the addition of preservatives is necessary during storage.

The results from partitioning ^{14}C-hexadecane into seawater showed that 2 hr of separation was sufficient for the preparation of crude oil SWAF's; however, for No. 2 fuel oil a longer separation time was necessary. It was determined that a 4 hr separation time was suitable for preparing stable SWAF's from all three oils. Moreover, it was noted that filtration of the SWAF's did not alter the amount of ^{14}C-hexadecane-derived radioactivity in the seawater thereby indicating that SWAF's did not contain appreciable amounts of oil droplets.

7.2 Weathering of Oils and Hydrocarbon Fractions of Oils

7.2.1 Flow-through Conditions

Studies conducted with CICO during the first year of this investigation revealed that high concentrations of total extractable organic material were present in the seawater from the agitated, environmentally weathered system. However, the high values may be due to the presence of oil droplets as indicated by high concentrations of aliphatic hydrocarbons in the SWAF. Moreover, the variability of natural exposure conditions (i.e., sunlight, wind, rain, temperature), low production of photooxidized products from CICO, and the rapid flushing out of these oxidized products in the flow-through system prompted us to replace this system during the second year with a no-flow laboratory weathering system.

7.2.2 No-flow Conditions

Two modes of no-flow, UV irradiated exposures were selected: (1) static and (2) wave machine agitated. These modes were designed to simulate in the laboratory two important natural environments: (1) highly sheltered bays and estuaries where wave and wind action is restricted and spilled oil could spread out in a thin film as was simulated in the static system, and (2) open water areas, where the action of wind and waves could break up the oil into droplets which would be suspended in the water column as occurs in the water column of the wave machine.

The radiometric and gravimetric analyses with No. 2 fuel oil suggest that the effects of UV light on oil are markedly dependent on the conditions of exposure. The intensity of UV light in seawater rapidly decreases with depth. For extensive photooxidation of oils to take place, it was important

to have an undisturbed surface oil film. For example, after 120 hr of irradiation under static conditions, the seawater underlying the fuel oil film contained 7 times more total extractable organic material than did seawater beneath wave machine-agitated fuel oil. This was consistent with proposed mechanisms of photooxidation reactions of air/oil and oil/water interfaces (Aksnes and Iversen 1983).

It was shown that compared to either CICO or PBCO, UV irradiation of No. 2 fuel oil produced SWAF's with higher amounts of oxidized products. Because aromatic hydrocarbons are in general more soluble in seawater and are more easily photooxidized than paraffins (Atlas 1981), UV irradiation would be expected to be more pronounced on oils having the highest concentration of aromatic hydrocarbons. The No. 2 fuel oil contained a higher percentage of aromatic hydrocarbons than did CICO and PBCO (Pancirov 1974).

7.2.3 Removal of Polar Compounds from Crude Oils Prior to Irradiation (A/P Fraction)

The low concentrations of photooxidized products from irradiated CICO observed during the first year of this investigation suggested that certain physico-chemical characteristics of CICO had an inhibitory effect on the oxidation of crude oil. To test this hypothesis, A/P fractions separated from CICO and PBCO were subjected to UV irradiation; the amounts of total extractable organic material in the underlying seawater was considerably greater than that found in seawater under irradiated whole crude oils (Table 10).

The precise reasons for the increased oxidation of A/P fractions compared with oxidation of the whole crude oils are unknown; however, it appears that some of the naturally occurring polar components in these crude oils inhibit the photooxidation process in some way, such as acting as scavengers for free radicals in UV light-induced oxidations (Parker et al. 1971).

7.3 Characterization of Oxidation Products of Radiotracers

The extremely complex chemical nature of crude oils and refined products, such as No. 2 fuel oils (Clark and Brown 1977), makes it essentially impossible to (1) monitor photooxidative changes on an individual compound basis and (2) assess uptake of oxidized products by organisms used in toxicity toxicity studies. Therefore, we devised a radiotracer study using ^{14}C-phenanthrene as a model petroleum compound which was added to the oils being weathered. There is precedence for this approach. In previous studies, single compounds were exposed to UV light in the presence of seawater and oxidative products were determined: Aksnes and Iversen (1983) used diphenylmethane and 1,2,3,4-tetrahydronaphthalene and Patel et al. (1978, 1982) used phenanthrene. Chromatographic and mass spectral analyses revealed the presence of several carboxyl derivatives, quinones, and acids of phenanthrene in seawater underlying irradiated phenanthrene. These results are in agreement with those of Patel et al. (1978, 1982) who identified similar photooxidation products of phenanthrene. The presence of oxidized products of phenanthrene in seawater suggested that oxidized products of other aromatic hydrocarbons may also be present in SWAF's from weathered oils.

TABLE 10. Amounts of total extractable organic material in SWAF's from various oils under different weathering conditions (120 hr, unless noted).

	Total extractable organic material		Ratio: Irradiated / Nonirradiated
	Irradiated (µg/mL)	Nonirradiated (µg/mL)	
STATIC CONDITIONS			
Cook Inlet crude oil - whole	25.7	9.2	2.8
- A/P fraction	147	29.0	5.1
Prudhoe Bay crude oil - whole	12.0 & 8.0	2.3 & 1.9	5.2 & 4.2
- A/P fraction	161	6.9	23
No. 2 fuel oil - whole	356	12.5	28
- whole[a]	474	17.9	26
AGITATED CONDITIONS			
Cook Inlet crude oil - whole	1.3	0.6	2.2
Prudhoe Bay crude oil - whole	7.5	2.1	3.6
No. 2 fuel oil - whole	35	21	1.7

[a] After 425 hr of weathering.

7.4 Uptake Experiments

Uptake of two radiotracers, ^{3}H-phenanthrene and ^{14}C-*p*-cresol, from SWAF's of weathered CICO was studied during the first year of this investigation. Both radiotracers, when added directly to [RCICO] SWAF or when present as oxidized products in SWAF's of weathered oils, were taken up by surf smelt larvae. However, considerable differences in bioconcentration values were noted. For example, the uptake of ^{3}H-phenanthrene from [RCICO] SWAF by surf smelt larvae was dramatically different from the uptake of phenanthrene-derived radioactivity from low-flow environmentally weathered CICO preparations. Larval fish accumulated maximum concentrations of ^{3}H-phenanthrene equivalents of approximately 3,000 times the seawater concentrations when exposed to [RCICO] containing ^{3}H-phenanthrene. However, in fish exposed to phenanthrene-derived radioactivity in the environmentally weathered preparation, the concentrations reached were never greater than 250 times the water concentration. This difference in uptake by the larval fish could be due to a number of factors; however, differences in lipophilicity between phenanthrene and its weathered products may be of primary importance.

The extent of uptake by larval surf smelt of phenanthrene-derived radioactivity was much greater than the uptake of ^{14}C-*p*-cresol-derived radioactivity. The maximum concentration of *p*-cresol-derived radioactivity in the larval fish was 18 times the concentration of *p*-cresol-derived radioactivity in seawater. The maximum concentration of ^{3}H-phenanthrene-derived radioactivity in the same larvae was 400 times the concentration of phenanthrene-derived radioactivity in seawater. Whether or not *p*-cresol and its weathered products were taken up, rapidly metabolized and excreted, or were less bioavailable than phenanthrene and its weathered products requires further investigation.

7.5 Toxicity Assays

7.5.1 Surf Smelt Larvae

Based on the results of radiotracers added to nonweathered, nonirradiated reference oils, it was demonstrated that larval surf smelt could bioconcentrate selected petroleum hydrocarbons during exposure in a static system. At initial concentrations of 2-3 µg/mL (*ca.* ppm) of total extractable organic material in the SWAF's, short-term (48 hr) survival of the larvae was high (>95%). However, an order of magnitude lower concentration (0.25-0.35 µg/mL) caused a pronounced sublethal effect involving altered swimming (ataxia). These observations point out the need to differentiate between acute toxicity test results and long-term sublethal impacts at environmentally realistic levels. Reduced swimming activity and/or ataxia in marine organisms following exposure to petroleum hydrocarbons is well documented (Percy 1976, Patten 1977). In our experiments, the observed ataxia was reversible; however, if organisms are so affected and sink out of their usual position in the water column, they may lose their accustomed food supply as well as be subject to increased predation (Frank and Leggett 1982). Though the exact mechanism(s) involved in this swimming behavioral change is not clear, it is generally thought to be induced by aromatic compounds (Anderson et al. 1974b, Johnson 1977). The SWAF's of each of the

oils tested contained large quantities of aromatic compounds, and the reference (nonweathered) preparations of all the oils affected the larval swimming behavior to a similar degree.

7.5.2 English Sole Embryos

Hatching success of English sole eggs was affected only at levels of total extractable organic material exceeding 1 µg/mL. Alterations in the hatching parameters can be caused by a number of stresses other than toxicant stresses, including changes in temperature, changes in salinity, and lowered oxygen concentrations (Rosenthal and Alderdice 1976). The protocol used in the assays in the present studies was designed, therefore, to minimize stresses related to temperature, salinity, oxygen and light.

Mortality of English sole embryos from SWAF's of different oils was low at environmentally high levels (>20 µg/mL) of total extractable organic material. Clark and MacLeod (1977) reported ambient levels of petroleum hydrocarbons in the world's oceans to be a few µg/L (ca. ppb) but in close proximity to large oil spills, the levels reach several µg/mL (ca. ppm). One difficulty with comparing published data to our experimental results is that different investigators have reported their values based on different parameters (e.g., total extractable material using different solvents, total hydrocarbons, saturates, n-alkanes, aromatics, total and unresolved envelope by GC, selected individual compounds). While 5-20 µg/mL appears to be an upper range for laboratory-produced SWAF's (as petroleum hydrocarbons) depending on the petroleum used, the total extractable organic material content can be several times this value. The exact amount found in a SWAF is a function of the specific petroleum, duration and intensity of photooxidation, type and degree of mixing, and other weathering processes unique to each experiment or spill. Therefore, while the absolute concentrations of total extractable organic material produced in our experiments may appear to be high when compared to petroleum hydrocarbon content, they are undoubtedly attainable in actual oil spill situations, especially in high energy locations in close proximity to a spill site. Under open ocean conditions characteristic of the Northeast Pacific, dispersed petroleum would form only a portion of the total extractable organic material and levels of petroleum would probably only reach ppm levels in close proximity to a major spill.

7.5.3 English Sole Larvae

Newly hatched English sole larvae were more sensitive in these assays than the embryos to both irradiated ([IFO_{S120}]) and nonirradiated ([$NIFO_{S120}$]) No. 2 fuel oil. This is not surprising because larval organisms are frequently more sensitive to toxicants than are other life stages; however, there is considerable variability in this regard among species (Rosenthal and Alderdice 1976, Rice et al. 1979).

8. CONCLUSIONS

The concentrations of oxidized components released to the underlying seawater beneath Cook Inlet crude oil (CICO) and Prudhoe Bay crude oil (PBCO) during UV irradiation were relatively low. In contrast, UV irradiation of No. 2 fuel oil resulted in the formation and release to underlying seawater of substantially greater amounts of oxidized components. An attempt was made, therefore, to determine why a fraction of crude oil (i.e., fuel oil) produced much higher concentrations of oxidized compounds after UV-irradiation than did the parent crude oil. When polar compounds were removed from Alaskan crude oils and the resulting aromatic/paraffinic (A/P) hydrocarbon fractions subjected to UV irradiation, the concentration of oxidized components increased to levels intermediate between the crude oils and the comparable, refined product. These results suggest that naturally occurring polar materials in crude oils inhibit crude oil photooxidation.

The simulated natural weathering protocol tested during the first year of this two-year investigation was modified because of unresolved technical problems which resulted in unacceptable variations. The successful weathering protocol used during the second year involved placing crude oil and No. 2 fuel oil slicks under (1) static or (2) agitated conditions with or without exposure to controlled UV irradiation under no-flow conditions. UV irradiation under these conditions induced measurable increases (2-5x nonirradiated levels) in total extractable organic material in the seawater-accommodated fractions (SWAF's) from CICO or PBCO. However, UV irradiation of No. 2 fuel oil under static, but not under agitated, conditions resulted in much higher concentrations (>100 µg/mL) of total extractable organic material in SWAF's compared to the concentrations in the nonirradiated No. 2 fuel oil SWAF's. As indicated above, after removal of polar materials from crude oils, UV-irradiation (static conditions) resulted in a clear increase in total extractable organic material in SWAF's compared to the concentration in the nonirradiated SWAF's.

Newly hatched surf smelt larvae and the embryos and larvae of English sole exposed to concentrations below 1 µg/mL (ca. 1 ppm) of total extractable organic material in the seawater from beneath an irradiated oil slick did not undergo mortalities statistically different from mortalities of non-oil-exposed controls. However, at sublethal total hydrocarbon levels (0.25-0.35 µg/mL), pronounced effects on the swimming behavior of surf smelt larvae were observed during exposure to reference SWAF's (nonirradiated and nonweathered) from both crude oil and No. 2 fuel oil. Cytopathological examination of the affected larvae showed both necrosis of sensory tissues and compressed muscle bundles.

English sole embryos exposed to environmentally high levels (>20 µg/mL of total extractable organic material) in the SWAF of No. 2 fuel oil still exhibited short-term toxic effects, but only in the static exposures. Newly hatched larvae showed a 50% mortality in the range of 1-3 µg/mL with no significant difference in mortality of larvae between irradiated or nonirradiated SWAF's. Exposure to No. 2 fuel oil SWAF reduced the hatching success of English sole embryos, but again, only when exposed to high concentrations of oil-derived material.

Radiotracer studies were used to determine the nature of the oxidized products formed as a result of several of the weathering regimes and to measure the relative uptake of hydrocarbons and oxidized components by selected marine organisms. The highest concentration of weathered ^{3}H-phenanthrene-derived material bioaccumulated in larval surf smelt was 370 times the concentration in the seawater. The maximum uptake of ^{14}C-*p*-cresol-derived material in the larval fish was only 18 times that in the seawater.

Finally, although UV irradiation of the two crude oils and the fuel oil clearly induced formation of a variety of oil-derived oxidized products, there is no evidence from the present studies to lead to a conclusion that photooxidation under most natural conditions would significantly enhance the toxicity of petroleum in the marine environment of the Northeast Pacific Ocean.

ACKNOWLEDGEMENTS

We wish to acknowledge the following who provided technical, editorial, and clerical support for the studies presented in this report: Carla Stehr, Carol Hastings, James Peacock, Sandra Bohenstiel, and Marylyn West.

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APPENDIX

TABLE 1A. Effects of storage at 4°C on the concentration of selected individual compounds in reference SWAF of CICO [RCICO] prepared from sterilized seawater and determined by GC analysis.

Compound	Time of Storage			
	0 Days		15 Days	
	Preserved (ng/mL)	Non-Preserved (ng/mL)	Preserved (ng/mL)	Non-Preserved (ng/mL)
Isopropylbenzene	15 ± 1 [a]	14 ± 1	15 ± 0	14 ± 1
n-propylbenzene	24 ± 2	22 ± 2	24 ± 1	22 ± 1
Indan	11 ± 1	11 ± 1	11 ± 1	11 ± 0
1,2,3,4-tetramethylbenzene	10 ± 1	9.5 ± 0.3	9.9 ± 0.2	9.5 ± 0.2
Naphthalene	83 ± 6	81 ± 8	84 ± 1	82 ± 1
Benzothiophene	2.5 ± 0.2	2.4 ± 0.2	2.6 ± 0.1	2.5 ± 0.1
2-methylnaphthalene	45 ± 3	46 ± 1	45 ± 1	45 ± 1
1-methylnaphthalene	29 ± 2	29 ± 1	30 ± 1	30 ± 1
Biphenyl	4.8 ± 0.4	5.0 ± 0.3	5.0 ± 0.1	5.0 ± 0.2
2,6-dimethylnaphthalene	6.4 ± 0.5	6.8 ± 0.4	8.7 ± 0.2	8.5 ± 0.2
Acenaphthene	1.7 ± 0.2	1.8 ± 0.1	0.15 ± 0.01	0.15 ± 0.01
2,3,5-trimethylnaphthalene	1.1 ± 0.1	1.2 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Fluorene	1.3 ± 0.1	1.6 ± 0.2	1.5 ± 0.1	1.5 ± 0.1
Phenanthrene	0.9 ± 0.1	1.3 ± 0.3	1.3 ± 0.1	1.3 ± 0.1
o-cresol	21 ± 1	21 ± 1	23 ± 0	25 ± 1
m- and p-cresol	9.7 ± 0.3	9.7 ± 0.5	11 ± 1	11 ± 1
2-ethylphenol	3.6 ± 0.2	3.9 ± 0.1	5.0 ± 0.3	4.7 ± 0.2
2,4- and 2,5-dimethylphenols	26 ± 4	29 ± 4	39 ± 1	42 ± 2
3- and 4-ethylphenols and 3,5-dimethylphenol	7.0 ± 0.3	7.0 ± 0.3	5.4 ± 0.4	6.7 ± 0.5
2,3-dimethylphenol	4.1 ± 1.3	3.3 ± 1.6	4.6 ± 1.2	4.6 ± 1.2
3,4-dimethylphenol	2.9 ± 0.2	3.3 ± 0.3	3.4 ± 0.1	3.9 ± 0.2
2,4,6-trimethylphenol	6.7 ± 1.9	9.3 ± 3.5	21 ± 4	21 ± 0
2-n-propylphenol	1.6 ± 0.3	1.8 ± 0.0	1.8 ± 0.2	1.9 ± 0.1
4-n-propylphenol	3.7 ± 0.3	3.6 ± 0.2	3.9 ± 0.1	4.2 ± 0.2
2,4,5-trimethylphenol	5.5 ± 1.5	6.7 ± 1.3	9.7 ± 0.4	10 ± 1
2,3,5-trimethylphenol	1.9 ± 0.6	1.5 ± 0.6	2.6 ± 0.2	3.0 ± 0.4
2,6-dimethylphenol	11 ± 2	13 ± 2	19 ± 1	19 ± 2
2,3,6-trimethylphenol	2.2 ± 0.8	2.9 ± 0.6	13 ± 1	14 ± 1

a $\bar{X} \pm$ S.D., n = 3.

TABLE 2A. Effects of storage at 4°C on the concentration of selected individual compounds in reference SWAF of CICO [RCICO] prepared from nonsterilized seawater and determined by GC analyses.

Compound	Time of Storage: 0 Days, Preserved (ng/mL)	0 Days, Non-Preserved (ng/mL)	15 Days, Preserved (ng/mL)	15 Days, Non-Preserved (ng/mL)	65 Days, Non-Preserved (ng/mL)
Isopropylbenzene	13 ± 1 [a]	14 ± 2	16 ± 2	16 ± 0	8.2
n-propylbenzene	22 ± 2	23 ± 2	26 ± 3	25 ± 0	1.4
Indan	11 ± 1	11 ± 1	12 ± 2	12 ± 0	5.8
1,2,3,4-tetramethylbenzene	9.4 ± 0.6	9.6 ± 0.4	10 ± 1	11 ± 0	10
Naphthalene	80 ± 4	82 ± 4	90 ± 14	1.3 ± 0.5	0.43
Benzothiophene	2.4 ± 0.1	2.5 ± 0.2	2.8 ± 0.3	2.6 ± 0.1	3.7
2-methylnaphthalene	45 ± 2	45 ± 2	49 ± 7	45 ± 2	1.6
1-methylnaphthalene	29 ± 1	29 ± 1	32 ± 4	30 ± 1	0.87
Biphenyl	5.1 ± 0.1	4.9 ± 0.2	5.2 ± 0.6	4.8 ± 0.1	<0.07
2,6-dimethylnaphthalene	8.5 ± 0.4	8.3 ± 0.2	8.8 ± 0.9	8.4 ± 0.2	<0.09
Acenaphthene	1.5 ± 0.2	1.6 ± 0.2	0.13 ± 0.01	0.11 ± 0.02	0.06
2,3,5-trimethylnaphthalene	1.1 ± 0.1	1.0 ± 0.0	1.7 ± 0.2	1.7 ± 0.1	0.45
Fluorene	1.4 ± 0.2	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	<0.07
Phenanthrene	1.3 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	<0.07
o-cresol	19 ± 1	20 ± 2	19 ± 2	26 ± 1	110
m- and p-cresol	6.9 ± 3.4	7.3 ± 3.4	9.4 ± 0.6	10 ± 0	9.9
2-ethylphenol	3.6 ± 0.0	3.9 ± 0.2	4.2 ± 0.3	4.2 ± 0.2	6.6
2,4 and 2,5-dimethylphenols	39 ± 1	39 ± 2	39 ± 3	24 ± 1	9.1
3- and 4-ethylphenols and 3,5-dimethylphenol	7.9 ± 0.1	7.8 ± 0.1	6.7 ± 0.4	7.8 ± 0.2	4.1
2,3-dimethylphenol	4.9 ± 0.2	4.8 ± 0.0	3.8 ± 0.3	3.7 ± 0.2	4.0
3,4-dimethylphenol	3.4 ± 0.1	3.5 ± 0.1	3.0 ± 0.2	3.4 ± 0.1	2.3
2,4,6-trimethylphenol	27 ± 1	25 ± 1	20 ± 2	22 ± 1	19
2-n-propylphenol	1.5 ± 0.0	1.5 ± 0.0	1.9 ± 0.2	2.0 ± 0.1	2.4
4-n-propylphenol	4.4 ± 0.2	4.2 ± 0.1	3.5 ± 0.3	3.8 ± 0.1	10
2,4,5-trimethylphenol	12 ± 1	11 ± 1	10 ± 0.9	11 ± 0	15
2,3,5-trimethylphenol	3.9 ± 0.2	3.5 ± 0.7	3.0 ± 0.6	4.4 ± 0.2	4.2
2,6-dimethylphenol	17 ± 0	17 ± 1	17 ± 1	17 ± 1	11
2,3,6-trimethylphenol	7.9 ± 0.2	7.8 ± 0.3	NA [b]	NA	5.5

a $\bar{X}$ + S.D., n = 3.
b NA = not analyzed

EFFECTS OF OILED SEDIMENT ON JUVENILE KING CRAB

by

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Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 620

18 October 1983

ABSTRACT

This 1-year project to determine effects of oiled sediment on juvenile king crab began in April 1982. Most of the experiments and observations were completed by October 1982. Chemical and statistical analyses were completed in FY 83. Juvenile king crab (Paralithodes camtschatica) were exposed to the water-soluble fraction of Cook Inlet crude oil (flow-through, stable concentrations for 30 days) or to oiled sediments for 3 months. The higher exposure concentrations of the water-soluble fraction were toxic and affected survival, growth, feeding rate, and scope for growth. Adverse effects were visible in just a few days. In contrast, the oiled sediments did not cause any measurable adverse effects on survival, feeding rate, growth, molting success, or scope for growth during the 3-month exposure, including those crabs exposed to the highest concentration--2%. Aromatic hydrocarbons were detected in some tissues of the crabs, including the crabs exposed to oiled sediment. Most experimental evidence suggests that exposures to oiled sediment will have minimal impact directly on survival and growth of juvenile king crab. However, the fact that hydrocarbons were detected in the tissues means that there is some contact with the hydrocarbons, and adverse effects are possible, although exposures longer than 3 months would be required.

INTRODUCTION

Oil may enter the environment in a variety of ways and subsequently cause short-term or long-term effects on both the habitat and the resident animals. Short-term effects on animal survival or physiology are caused by water-soluble fractions (WSF's) of oil, which contain toxic aromatic hydrocarbons. However, toxic effects of WSF's in the environment are usually transient because of rapid dilution, evaporation, and biodegradation. In contrast, oiled sediment may persist for years. Because the hydrocarbons in sediment are not as readily available to animals as in the WSF, biological effects may not be evident until animals have been exposed for long periods.

There are few accounts in the literature on the long-term effects of oiled sediment on survival and physiology of marine animals, but there is substantial documentation that oil in sediment can persist for years. Krebs and Burns (1977) traced the effects of a spill of fuel oil at West Falmouth, Massachusetts, on the fiddler crab (Uca pugnax) that burrowed in the resultant contaminated sediment. After 7 yr, the oiled sediment still held enough hydrocarbons to have a serious impact on the crabs. Anderson (1982) set pans of artificially oiled sediment in the intertidal zone of Sequim Bay, Washington, and found that if the particle sizes of the sediment were very fine, the concentration of hydrocarbons dropped only about 20% in nearly 10 months. McCain et al. (1978) set up laboratory tanks of running seawater with oiled sediment and found that 75% of the initial concentration persisted after 4 months.

The outer continental shelves of Alaska are rich in commercial fishery resources, including king crab, and the same areas are expected to become important producers of petroleum. Although we can predict that spilled oil will persist in sediments for a long time, the long-term effects of this exposure on the survival and growth of king crabs are unknown. This is the final report of the study on the effects of oiled sediment on juvenile king crab that was contracted by OCSEAP to the Auke Bay Laboratory.

The specific objectives of the study were to (1) determine the effects of long-term exposures of oiled sediment on the survival, molting success,

growth, feeding rates, and energetics (scope for growth) of juvenile king crab; (2) determine the effects of long-term exposures of the WSF of the same oil to the same parameters of juvenile king crab; and (3) determine the uptake of hydrocarbons into the tissues of king crab exposed to oiled sediments and WSF's.

The probability of WSF exposures in the environment is much less than the oiled sediment exposures, but there is more literature on WSF tests. We included the same measurement of juvenile king crab response to both types of exposures so that they could be compared and put into perspective.

METHODS

Experimental Design

Juvenile king crab were exposed either to oiled sediment for 3 months or to the WSF of crude oil for 1 month and compared with unexposed control crabs. Several exposure concentrations were used in each test: five in the oiled sediment exposures (0-2% oil added) and eight in the WSF exposures (0-3 ppm). Sample size was 15-16 crabs in each dose. The crabs were monitored daily for survival, molting success, and feeding rate. Growth, energetics (scope for growth), general behavior, and condition were measured weekly in the WSF tests and biweekly in the oiled-sediment tests. Each crab was tagged so that data could be collected on individuals. In addition, uptake of hydrocarbons in crab tissues was measured from crabs sampled periodically from the highest sublethal concentrations of both WSF and oiled sediment.

Oil concentrations in sediment were analyzed by infrared spectrophotometry and in the WSF by gas chromatography. Tissue concentrations were measured by gas chromatography.

Oiled Sediment

The sediment for experiments was collected by dredge from an area in Auke Bay known to be used by crabs. It was kept frozen in 5-gal. buckets until

use. Five concentration of oiled sediment were prepared by mixing measured volumes of oil (2%, 0.8%, 0.2%, 0.05%, and 0%) with sediment in a fiberglass-lined cement mixer. Experiments were conducted in fiberglass tanks (three replicate tanks per dose) with the bottoms covered by 5-cm layers of sand, pea gravel, and oiled sediment to a total depth of 15 cm, with the oiled sediment on top. Clean running seawater from Auke Bay was supplied to each tank for 18 d before the crabs were added.

Samples of oiled sediment were taken periodically from the tanks and were analyzed for hydrocarbon content. The surface 1 cm and subsurface sediments (three replicated each) were analyzed from each of the three replicate exposure tanks (18 analyses per dose). An aqueous slurry of each sample was acidified, extracted into Freon, and its absorbance at 2,930 nm measured by infrared spectrophotometer for comparison with similar extractions of whole crude oil. The changes in concentration over time for each initial concentration were developed by combining analyses of samples from all experiments.

Water-Soluble Fractions

The WSF of Cook Inlet crude oil was generated using our standard method, which involves dripping seawater through a floating layer of the oil (Moles et al., in press). The WSF and diluent seawater were delivered to continuous-flow exposure tanks through glass tubing. All WSF concentrations were monitored in terms of ppm of aromatic hydrocarbons using fluorescence spectrophotometry and gas chromatography. For specifics of analysis methods see Gordon et al. (1973).

Crabs

The crabs were all juvenile red king crab (Paralithodes camtschatica), between 25 and 50 mm in shell length and weighing 10-80 g. They were collected from Auke Bay by scuba divers. Each crab was marked by gluing a numbered 3x5-mm plastic tag to the carapace with Eastman 910 "super glue." Within a few days after any crab molted, its retrieved tag was replaced on its new carapace so each individual could be identified throughout the 3-month experiment.

Test crabs were fed on mussels (Mytilus edulis) throughout the tests. Excess mussels were offered so crab feeding was never limited by availability of food. We have held juvenile king crab for as long as 11 months, feeding them nothing but mussels. During this time, they molted 3-4 times, grew to 6-10 times their original weight, and always appeared normally active; therefore, we are confident that a diet of mussels provided adequate nutrition for the 3 months of oiled-sediment exposure.

Test crabs were exposed at ambient seawater temperatures of Auke Bay, which ranged from 6° to 9°C.

Observations and Measurements

Survival: Deaths of crabs were recorded and all dead crabs removed from test tanks daily. Mean lethal concentrations (LC50's) were determined from mortality counts, using logit analysis (Silverstone 1957) when possible (when two or more concentrations contained both live and dead crabs). The Spearman-Karber method (Finney 1971) was used when less than two concentrations contained both live and dead crabs.

Growth and Molting: Molts were noted and removed from tanks daily. Growth of WSF-exposed crabs was measured weekly and biweekly in oiled-sediment-exposed crabs. Carapace lengths were measured from the posterior edge of the eye socket to the center posterior and live weights measured after gently shaking off excess water. Missing legs, general condition, and incidence of disease were also recorded at the same time as growth data.

Feeding Rate: Crabs were fed daily on mussels that were split open but still attached to the shell. The shells, with any uneaten mussel tissue attached, were retrieved 23 h later. All mussels fed to crabs were weighed before and after feeding to determine feeding rate. Mussels that have been split open will, consequently, lose some weight just by soaking in water 24 h. We determine that weights of the remains of mussel tissue retrieved after feeding, should be corrected by a factor of 0.929 before subtracting from prefeeding weight. The resulting measure of wet mussel tissue eaten was converted to dry weight by multiplying by a factor of 0.238. The calculated

dry weights of tissue eaten by each group of crabs were then divided by the total live weight of those crabs to calculate a feeding rate comparable to rates for other groups of crabs; this rate was then multiplied by the live weights of individual crabs to determine approximate individual feeding rates for calculating scope for growth.

Energy Balance: Scope for Growth: Scope for growth was calculated for individual crabs by subtracting maintenance energy (respiration and excretion) from the calories consumed. Crabs in the oiled sediment tests were measured at the end of each month of exposure. Crabs in WSF tests were measured before exposure and after 1, 2, 3, and 4 weeks of exposure. Each crab was isolated for 6 h in a 350-ml plastic chamber through which waterflow could be precisely controlled and its oxygen consumption and nitrogen excretion rates measured. Absorption efficiencies were determined from samples of feces collected from the chambers after crabs were returned to their test tanks. Scope for growth is defined as C-(F+R+U) where C = food energy consumed, F = energy lost as feces, R = energy respired, and U = energy excreted, all in calories. Detailed methods are given in Shirley and Stickle (1982a, b).

Tissue Levels of Hydrocarbons: Tissue samples from each of seven crabs were taken periodically from groups of 30 crabs held on 2% oiled sediment or in 0.5-ppm WSF. Crabs were sampled from the sediment group after 4, 10, 30, and 90 d of exposure and from the WSF group after 1, 4, 10, and 30 d of exposure. Unexposed crabs were also sampled. The crabs were killed and preserved by placing them in clean glass jars and freezing them. Hydrocarbon levels in leg muscle tissue and hepatopancreas (digestive gland) were determined later. Crabs were thawed and dissected and one pooled sample of muscle tissue and one of hepatopancreas tissue collected from each sample of seven crabs. Samples were weighed, heated, and digested in 10N NaOH, extracted in hexane, and had their aromatic and aliphatic contents separated using columns of silica gell. Individual hydrocarbons were separated and quantified by packed column gas chromatography.

RESULTS

Chemical

Exposure concentrations remained relatively constant in both the WSF and the oiled-sediment tests. As hydrocarbons in the WSF tests were lost from the test containers, they were continuously replenished by new solution during the month-long tests.

Hydrocarbons in the sediment exposures were added only once, at the beginning of the test, and persisted at relatively high levels during the 3-month test (Fig. 1). Surface sediments were the least stable, losing about 35% of the initial concentration. Subsurface sediments lost about 15% of the initial hydrocarbons. The highest concentrations lost more hydrocarbons than lower concentrations, and losses occurred primarily within the first month. The highest concentration approached the upper limit of hydrocarbon-carrying capacity of the sediment; 2% oil was the highest oil concentration that we could mix into the sediment without producing a surface oil slick when water was added. There was a large amount of variation in the hydrocarbon content of the sediments between replicate tanks and between replicate samples from one tank (as shown by large standard deviations in Figure 1). The variability in the initial samples indicates that it is difficult to mix oil into sediments in a uniform manner.

Response of Crabs

All tested concentrations of oil in sediment were sublethal. No crabs exposed to the highest concentration of 2% crude oil in sediment, died during the tests. None of our measurements of growth, feeding, or scope for growth showed any significant differences between the crabs exposed to 2% and the control crabs (Table 1). These negative results are clear and consistent even though the crabs remained in constant contact with the contaminated sediment. They continually stirred up the surface of the mud by walking on it and dug themselves into slight depressions in it. They also ate some sediment (perhaps inadvertently with mussels), as indicated by the presence of sediment in their feces.

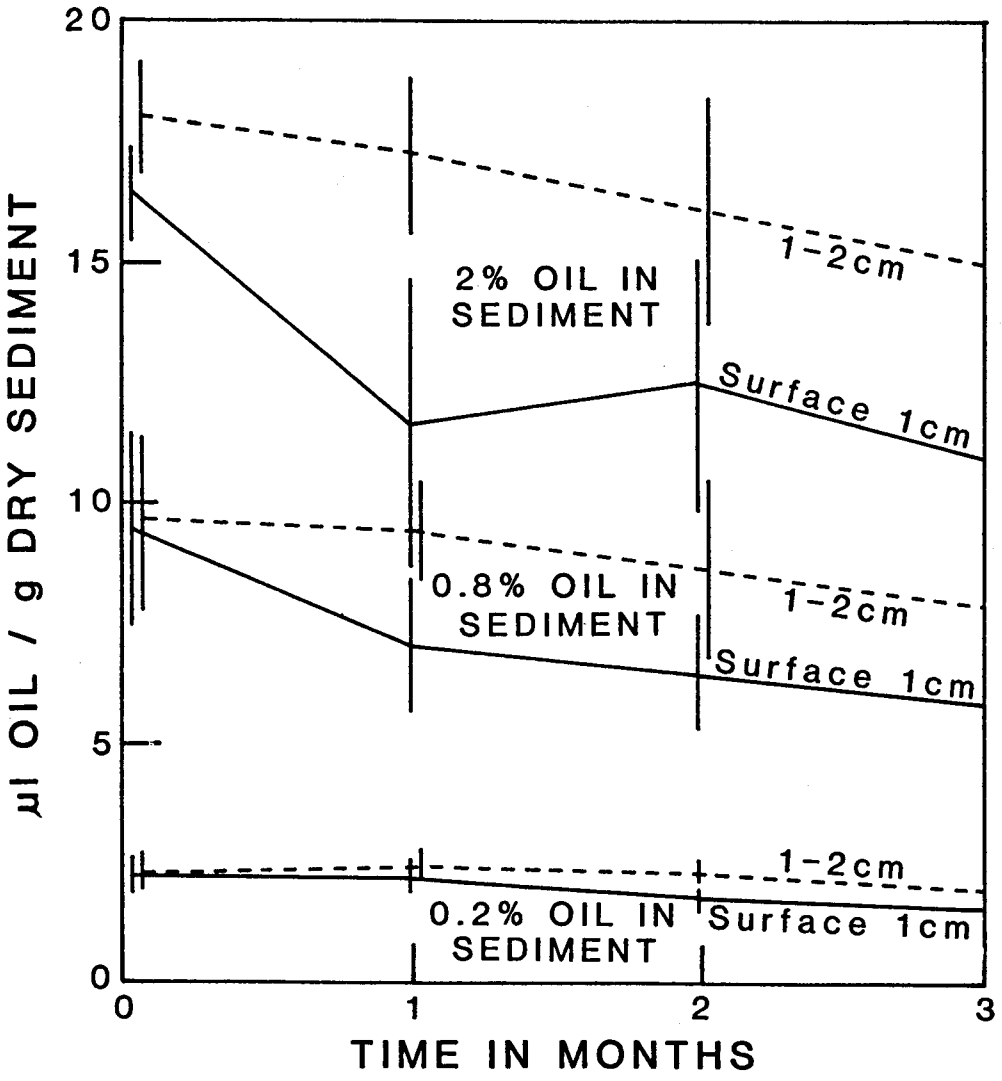

Figure 1.--Loss of oil hydrocarbons over time from three concentrations of Cook Inlet crude oil mixed into sediment. Samples were taken from the top centimeter of sediment and from the centimeter immediately below. All points are means of replicate samples within tanks and between tanks of several similar experiments. Bars are standard deviations of the means.

Table 1.--Comparison of juvenile king crab living for 11 weeks on sediment mixed with 2% Cook Inlet crude oil with crab living on uncontaminated sediments. The two groups do not differ significantly on any parameter.

	CONTROL	2% OIL IN SEDIMENT
DEATH RATE		
% dying of all causes, 24 May - 10 Aug.	13% (3/24)	0% (0/16)
MOLT RATE		
% molting at least once	100%	100%
% molting twice	13% (3/24)	6% (1/16)
GROWTH IN LENGTH		
Carapace length on 10 Aug.	$\bar{X}$ = 1.27	$\bar{X}$ = 1.27
Carapace length on 24 May (excluding crabs molting twice)	s = .036	s = .068
GROWTH IN WEIGHT		
Live weight on 10 Aug.	$\bar{X}$ = 2.14	$\bar{X}$ = 2.12
Live weight on 24 May (excluding crabs molting twice)	s = .118	s = .169
FEEDING RATE		
mg dry mussel tissue eaten / g live crab / day	$\bar{X}$ = 8.45	$\bar{X}$ = 7.87
	s = 1.41	s = 1.48
SCOPE FOR GROWTH		
Calories / crab / day	$\bar{X}$ = 663	$\bar{X}$ = 782
	s = 155	s = 191

The higher tested WSF concentrations were lethal. The crabs had an LC50 of 1.5 ppm of aromatic hydrocarbons for 4 d of exposure and an LC50 of 1.4 ppm for 1 week of exposure. Although the highest concentration used for measurement of sublethal effects --0.5 ppm--did not kill any crabs in the first 3 weeks of exposure, it did stop the crabs from feeding.

Feeding rate of crabs was inversely related to WSF concentration (Fig. 2). This relationship may be seen most clearly when inconsistencies in the WSF concentrations within a single dose are considered. Figure 2 compares the mean WSF concentration for each week of exposure for each group of crabs with the mean feeding rate of that group for that week. Feeding rates for the "0.1-ppm" group were similar to those for the control group. Feeding rates for the "0.3-ppm" group were low at first and then returned to control level at least partially because WSF concentrations fell slightly and perhaps also due to some acclimation or compensation by the crabs. Crabs in the "0.5-ppm" group did not eat at all except in the third week when their exposure levels dropped for a few days.

Failure to feed had a direct effect on other measured parameters. Scope for growth paralleled feeding rate, but there were no concentration-related effects on oxygen consumption, nitrogen excretion, or absorption efficiency. The WSF concentrations >0.3 ppm caused reduced scope for growth, and concentrations >0.5 ppm caused negative scope for growth or a net loss of calories to the crabs (Fig. 3). Failure to feed would obviously have a direct effect on any measure of growth rate; however, the length of the period of exposure was too short to clearly demonstrate differences in growth between feeding (controls) and nonfeeding crabs. Failure to feed would also eventually be lethal and, indeed, there were deaths in the 0.5-ppm group during the fourth week of exposure, bringing the 28-d LC50 down to 0.64 ppm of aromatic hydrocarbons.

Uptake of Hydrocarbons by Crabs

Aromatic and aliphatic hydrocarbons were found in the tissues of crabs exposed to either 0.5-ppm WSF or 2% oil in sediment. However, the patterns of

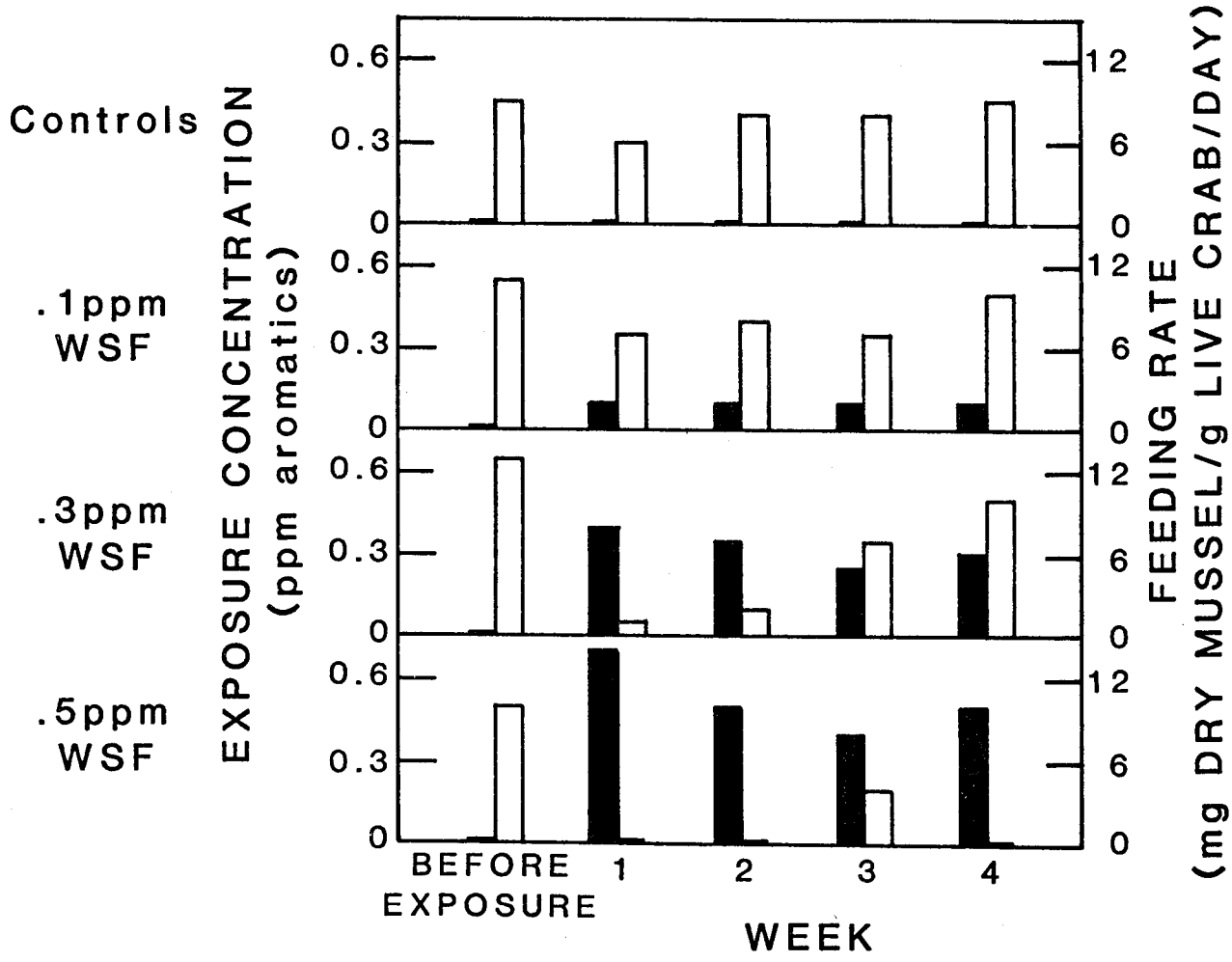

Figure 2.--Effect of weekly mean exposure level (solid bars) on weekly mean feeding rate (white bars) for juvenile king crab fed on blue mussels during exposure to the water-soluble fraction of Cook Inlet crude oil.

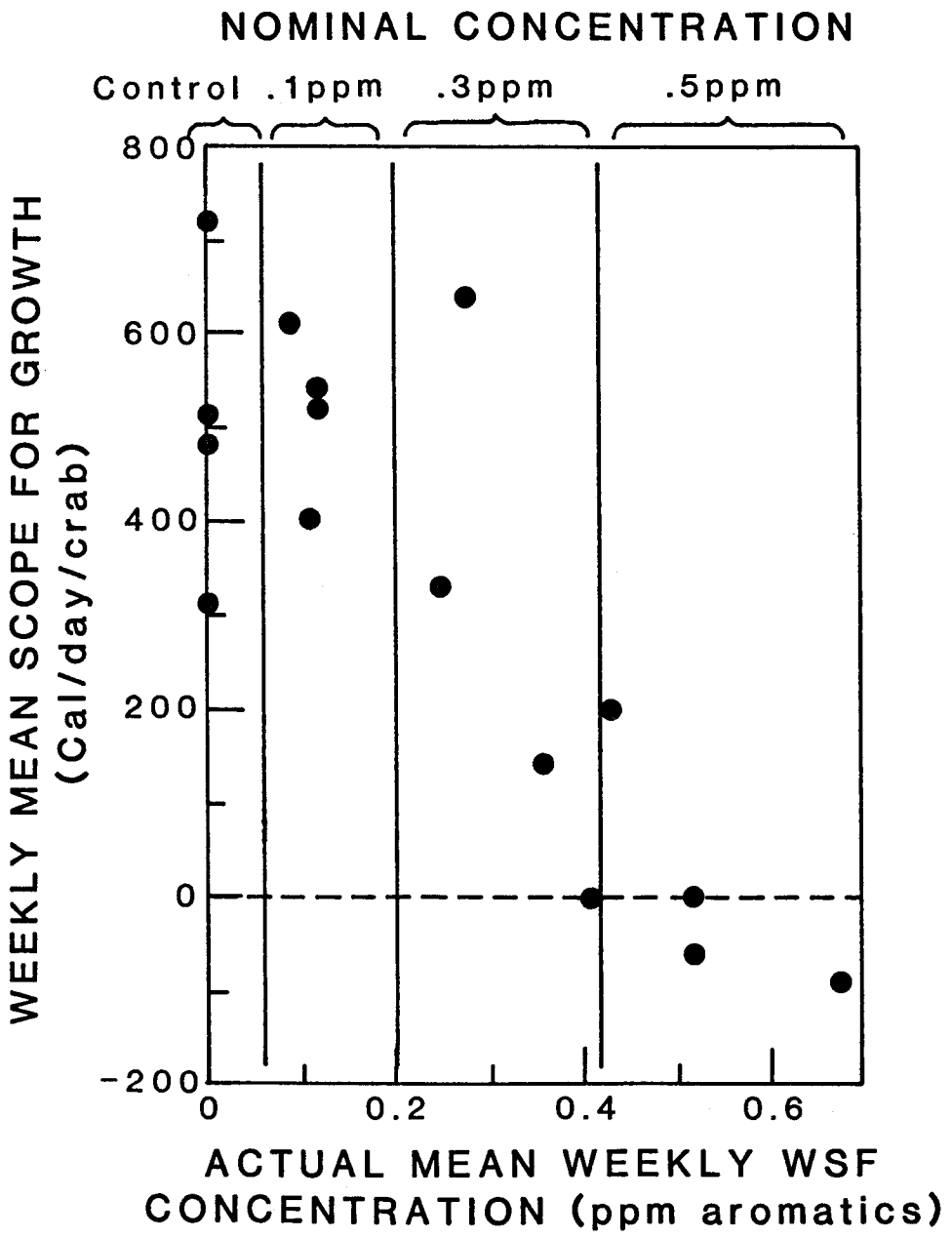

Figure 3.--Weekly scope for growth plotted against the mean exposure concentration for the same week.

uptake were quite different for crabs in the two different exposure methods (Table 2).

The hydrocarbon accumulation in muscle tissues of crabs exposed to either WSF or oiled sediment was low (Fig. 4). Muscles of WSF-exposed crabs had slightly elevated levels of lighter aromatic hydrocarbons but control level concentrations of aliphatics. In contrast, muscles of sediment-exposed crabs had elevated aliphatic levels compared with control crabs. The aromatic hydrocarbon levels in control and oiled-sediment-exposed crabs were low.

The accumulation of aromatic and aliphatic hydrocarbons was much greater in the hepatopancreas than in muscle. Hepatopancreas tissues of WSF-exposed crabs were consistently high in hydrocarbons (Fig. 5). They contained lower boiling aromatic hydrocarbons, the lowest boiling aliphatics, and a low envelope of unresolved higher boiling aliphatics.

In sediment-exposed crabs, the hydrocarbon concentrations in hepatopancreas tissue increased over time (Fig. 6). These samples contained unresolved envelopes (groups of peaks that overlap each other so much that they cannot be differentiated) of aromatic hydrocarbons in a higher boiling range than the aromatic peaks of WSF crabs that increased from 210 ppm at 4 d to 270 ppm at 10 d and to 370 ppm at 90 d. Hepatopancreas tissue also contained aliphatic peaks throughout the sampled range with unresolved envelopes in the midrange. Aliphatics increased from 160 ppm at 4 d to 380 ppm at 10 d and to 670 ppm at 90 d. Accumulation on hydrocarbons in the sediment-exposed crabs was expected because some sediment was consumed and passed through the digestive system. However, the surface sediments were decreasing in concentration over time while the concentrations in hepatopancreas tissue were increasing.

DISCUSSION

Juvenile red king crab can be affected by oil exposure, particularly if the exposure is to a WSF of oil. The WSF of Cook Inlet crude oil was toxic at the higher concentrations tested, and sublethal exposures stopped the feeding of juvenile crabs within the first few days of exposure. If feeding is inter-

Table 2.--Hydrocarbon content of tissues from oil-exposed juvenile king crab.

		HYDROCARBONS IN PPM (g HC/10^6g TISSUE)						
	DAYS OF EXPOSURE	TOLUENE	XYLENE (O,M&P)	NAPHTHALENE	2-METHYL-NAPHTHALENE	1-METHYL-NAPHTHALENE	TOTAL AROMATICS	TOTAL ALIPHATICS
MUSCLE TISSUES								
CONTROL	0	0	0	0	0	0	3	0
EXPOSED TO WSF (.5ppm AROMATICS)	1	1	1	0	0	0	7	0
	4	2	1	0	0	0	6	0
	10	1	1	0	0	0	4	1
	28	1	1	0	0	0	3	-
EXPOSED TO 2% OILED SEDIMENT	4	0	1	0	0	0	3	5
	10	1	1	0	0	0	3	5
	90	4	1	0	0	0	2	2
HEPATOPANCREAS								
CONTROL	0	1	1	0	0	0	6	2
EXPOSED TO WSF (.5ppm AROMATICS)	1	56	58	21	11	10	260	110
	4	39	46	17	19	20	300	82
	10	1	17	12	16	16	180	110
	28	3	8	5	7	8	79	110
EXPOSED TO 2% OILED SEDIMENT	4	0	1	0	1	0	210	160
	10	1	2	0	1	0	270	380
	90	1	1	0	2	1	370	670

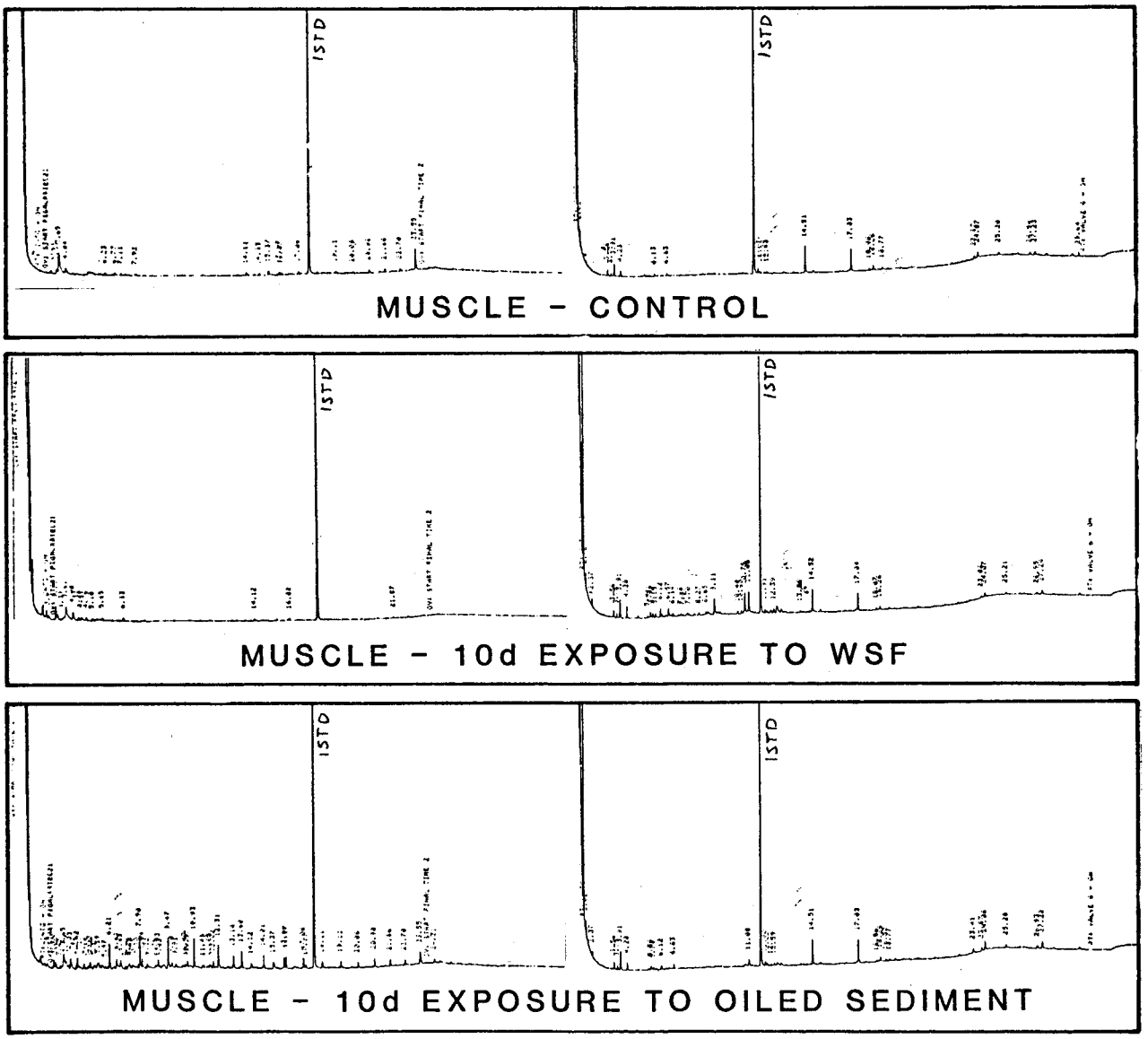

Figure 4.--GC scans of hydrocarbon content in muscle tissue of juvenile red king crab exposed to Cook Inlet crude oil either as WSF of 0.5 ppm aromatics or mixed with sediment at 2% concentration. Samples taken at all other exposure times (1-28 days for WSF exposure and 4-90 days for oil sediment exposure) showed scans extremely similar to those shown for the same exposure method.

ISTD = internal standard. Other peaks visible in control scans are contaminants and artifacts common to all scans shown.

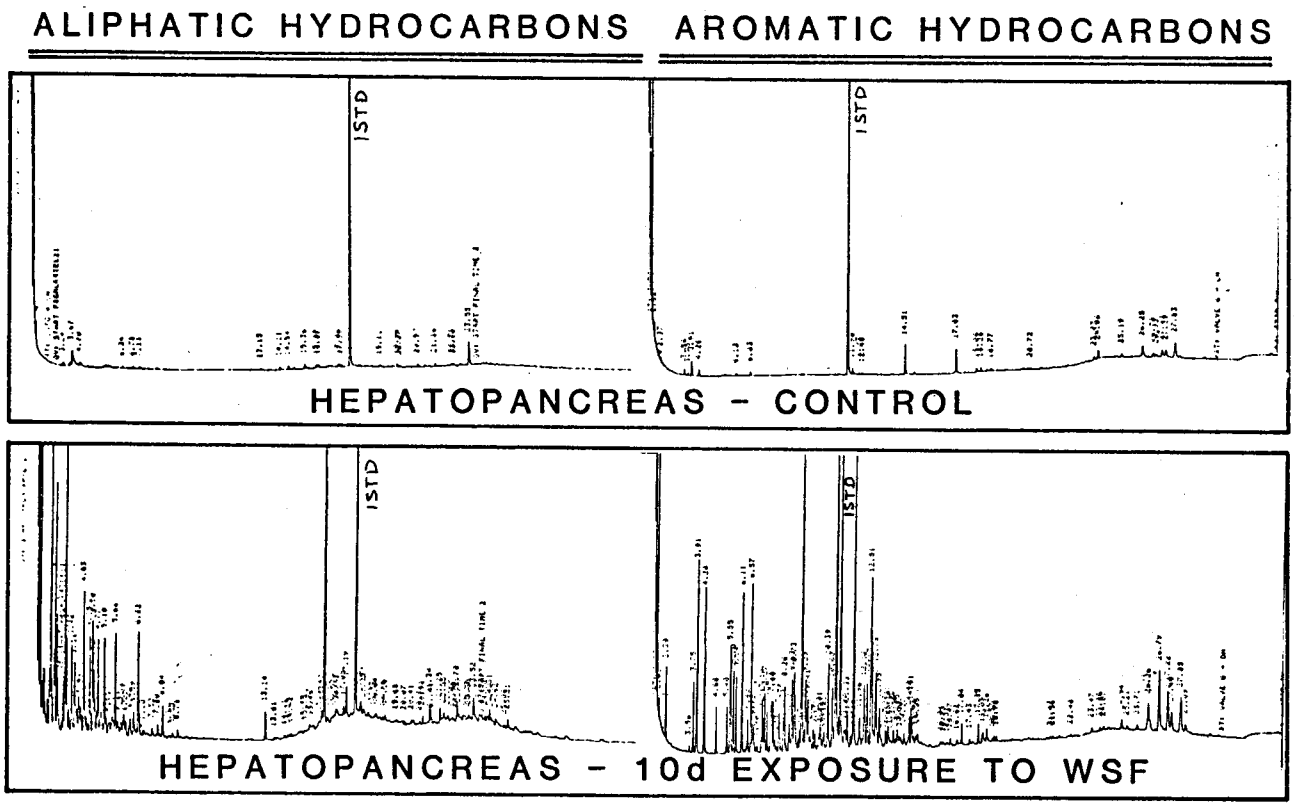

Figure 5.--GC scans of hydrocarbon content in hepatopancreas (digestive gland) of juvenile red king crab exposed to Cook Inlet crude oil WSF of 0.5 ppm aromatics. Samples taken 1, 4, 10, and 28 days after the start of exposure showed extremely similar scans.
ISTD = internal standard. Other peaks visible in control scans are contaminants and artifacts common to all scans shown.

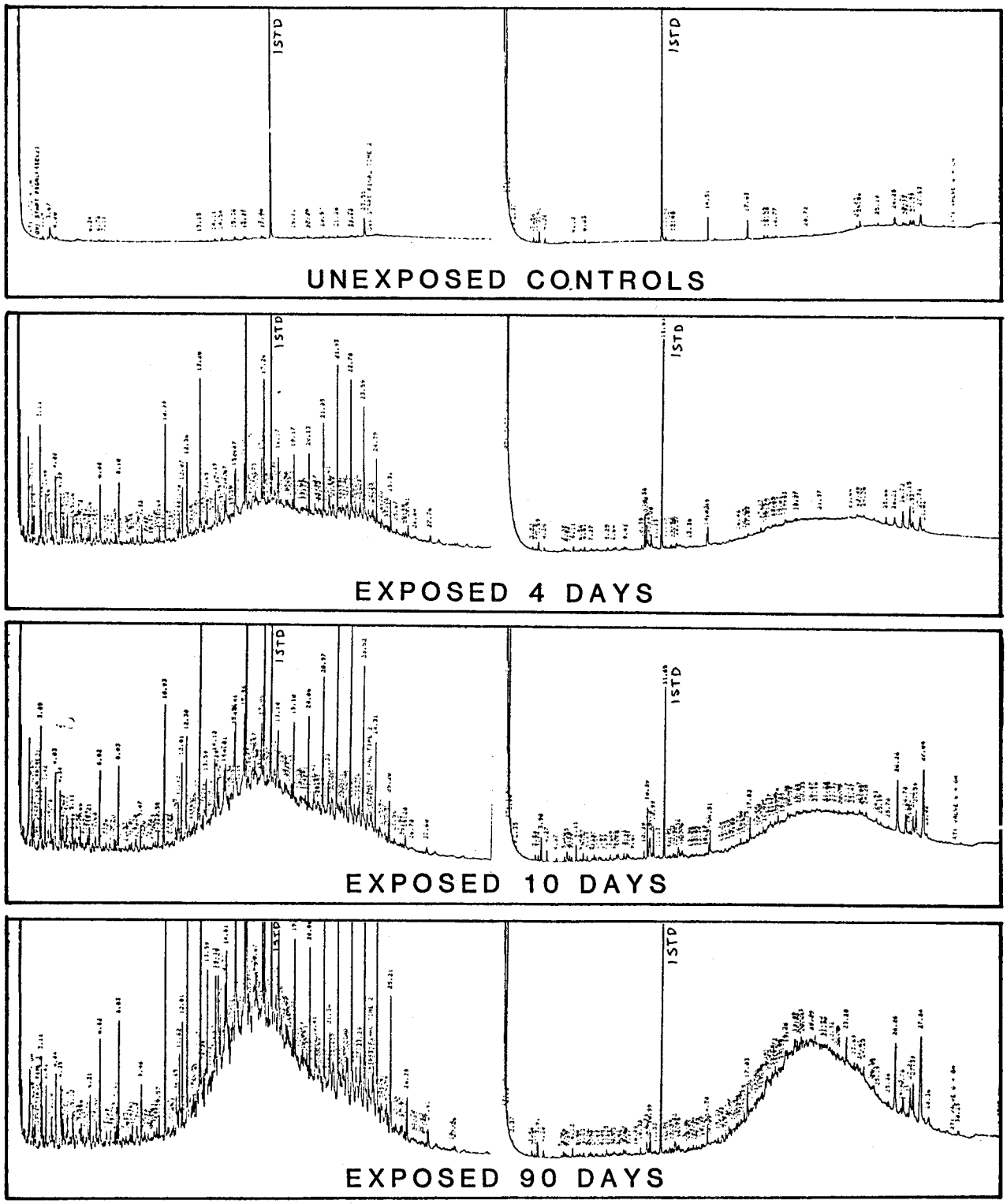

Figure 6.--GC scans of hydrocarbon content of hepatopancreas (digestive gland) of juvenile red king crab exposed to Cook Inlet crude oil mixed with sediment at 2% concentration for up to 3 months.

ISTD = internal standard. Other peaks visible in control scans are contaminants and artifacts common to all scans shown.

rupted, it is not surprising that long-term exposures to the WSF of oil would have an effect on energetics (scope for growth) and eventually on growth and survival. The WSF of oil contains the lighter toxic aromatic hydrocarbons which, when present in the water column, are rapidly taken up by marine organisms.

In contrast, oiled sediments have a hydrocarbon composition that is similar to unweathered parent oil which is less available to marine organisms than WSF's. Our tests with juvenile king crab exposed to oiled sediment suggest that the oil did not adversely affect the crabs during the 3-month exposures. Survival, molting success, growth, and scope for growth were the same for control crabs as for those exposed to the highest concentration of oil (2%). Although this is a high concentration of oil, concentrations measured from actual spills have been reported to be of similar magnitude (Gilfillan et al. 1976; Krebs and Burns 1977).

King crabs live on the surface of the sediment, so direct contact with hydrocarbons in the sediment matrix is minimal. We measured hydrocarbon accumulation in muscle and in the hepatopancreas. We found some sediment in the guts of juvenile crab and, therefore, were not surprised that some hydrocarbons accumulated in tissues. But these accumulations had no apparent adverse effects on survival and growth. In other studies, oiled sediments have caused adverse effects on several species, but unlike king crab, the animals lived and burrowed in the sediment (Krebs and Burns 1977; Gilfillan and Vandermeulen 1978; McCain et al. 1978; Augenfeld et al. 1980; Fletcher et al. 1981).

Although WSF exposures can be detrimental to crab survival and sublethal exposures can be damaging physiologically, the potential for harmful effects is minimal because exposures would be transient. A large spill could be violently mixed by wind and waves to produce an oil-water dispersion, but the toxic water would usually be rapidly diluted and the compounds rapidly degraded. In contrast, oiled sediment could persist for years. Our results indicate that exposures to oiled sediment for 3 months would not be harmful to survival and molting success of juvenile king crab. However, the tissue accumulation of hydrocarbons from the oiled sediments suggests that long-term

effects from prolonged exposure are possible, even though lengthy exposures may be required before damage is evident. For example, McCain et al. (1978) reported increased incidence of disease (tumors) in English sole (Parophrys vetulus) exposed to oiled sediments. Some of the hydrocarbons in oiled sediments are known carcinogens. Fletcher et al. (1981) have suggested that animals exposed to oiled sediments may be much more vulnerable to additional stress than unexposed animals. The flounders they studied survived oiled-sediment exposure at cold temperatures but succumbed at temperatures near their maximum tolerance level.

The hydrocarbon accumulation in the tissues of sediment-exposed crabs could cause tainting, and there could be some risk to consumers of the crabs because some hydrocarbons are carcinogens.

Additional studies of the potential for damage to king crab stocks exposed to oil should focus on very long-term (1 yr or more) exposures to oiled sediments. The uptake of hydrocarbons into several crab tissues should be measured, and the incidence of disease and tissue abnormality should be determined at the end of exposure. The ability of exposed crabs to withstand further stress, such as extremes of temperature, overcrowding, or limited food, should also be tested.

The risk to king crab populations from oiled sediment may be negligible in exposures of less than 3 months, but there could be adverse effects from very long exposures. Such effects, if any, are likely to be subtle and very difficult to assess.

CONCLUSIONS

(1) WATER-SOLUBLE FRACTION EXPOSURES: Survival, feeding, and energetics of juvenile king crab were effected by WSF exposures, 4-d LC50 = 1.5 ppm; 28-d LC50 = 0.64 ppm. Feeding stopped at 0.5 ppm.

(2) OILED-SEDIMENT EXPOSURES: The highest oiled-sediment concentration (2%, which was the maximum amount of oil that we could mix into the sediment) did not affect survival, molting success, feeding rates, growth, or energetics of juvenile king crab exposed to oiled sediment for 3 months. This concentration remained relatively constant during the 3-month study.

(3) TISSUE ACCUMULATION OF HYDROCARBONS: Hydrocarbons were accumulated into muscle and into the hepatopancreas of crabs exposed to WSF and oiled sediment. The patterns of uptake were different for the two exposure methods. The accumulation of hydrocarbons in tissues of oiled-sediment-exposed crabs proves that these crabs were in contact with oil possibly through ingestion of some sediment. However, no adverse effects were observed during the 3-month exposure.

(4) Although no adverse effects were observed in the 3-month oiled-sediment exposure of juvenile king crab, there is potential for long-term effects from the accumulated hydrocarbons in the tissues. Greater incidence of disease and other effects which require long time periods before they are visible, could be caused by the accumulated hydrocarbons.

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EFFECTS OF PETROLEUM HYDROCARBONS ON ALASKAN AQUATIC ORGANISMS: A COMPREHENSIVE REVIEW OF ALL OIL-EFFECTS RESEARCH ON ALASKAN FISH AND INVERTEBRATES CONDUCTED BY THE AUKE BAY LABORATORY, 1970-81

by

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Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 72

1 April 1983
(Revised November 1984)

SUMMARY

This report reviews and summarizes all oil-effects research by the Auke Bay Laboratory from the beginning of these studies in 1970 through 1981. Both published and unpublished results from 62 studies are included, regardless of funding source. Research is reviewed according to subject (e.g., toxicity, sublethal effects, studies at Port Valdez). A bibliography and abstracts are also included.

Studies With Crude Oil, No. 2 Fuel Oil, and Their Components

Results from different studies should be compared with caution because the comparisons can be misleading if exposure methods, chemical analyses, test animals, or life stages are different. Temperature also influences results of studies by affecting evaporation and biodegradation of petroleum hydrocarbons. Some generalizations, however, can be made from the results of our studies. The water-soluble fraction (WSF) of No. 2 fuel oil is consistently more toxic than the WSF of crude oil even though the WSF's of fuel oil contain low concentrations of monoaromatic hydrocarbons. The total toxicity of individual aromatic hydrocarbons in the WSF's did not, however, account for all of the toxicity of the oils; thus, the aromatic hydrocarbons could be, in some cases, interacting synergistically.

Many biological and environmental variables affect sensitivity of Alaskan species to the WSF's of oils. For example, pelagic fish and invertebrates are more sensitive than intertidal species. There can be extreme differences in sensitivity between the life stages of one species. For example, eggs often seem as tolerant as adults to short-term exposures, but abnormalities can appear after the eggs hatch. Salmon (Oncorhynchus spp.) alevins become much more sensitive to oil as they develop and lose their yolk. Crustacean larvae are usually sensitive to WSF's and are affected within minutes of exposure. In fact, the lowest LC50 (concentration that killed 50% of the test animals) we have measured was 0.2 ppm aromatic hydrocarbons for Stage VI coonstripe shrimp (Pandalus hypsinotus) larvae exposed to Cook Inlet WSF. The effect of temperature on toxicity of hydrocarbons varies for each species and for the hydrocarbons tested. Low temperatures, however, increase the persistence of

hydrocarbons in water. Salinity consistently increases the toxicity of hydrocarbons to salmonids, and juvenile salmonids are about twice as sensitive in seawater as in freshwater.

The rate and quantities of hydrocarbons found in test organisms vary considerably and depend on the compounds tested, the life stages and species of animal tested, and the tissues analyzed. Naphthalene and methylated naphthalene, for example, reach higher concentrations in the test animals than other aromatic hydrocarbons. Concentrations of aromatic hydrocarbons in crustacean larvae usually equilibrate within minutes to concentrations of aromatic hydrocarbons in the test water, whereas salmon eggs required several days. Fish accumulate aromatic hydrocarbons rapidly, whereas blue mussels (Mytilus edulis) accumulate them more slowly.

Metabolism can be an important mechanism for ridding tissues of hydrocarbons. Of the animals tested, fish had the greatest ability to metabolize hydrocarbons. For invertebrates, however, metabolism does not appear to be important. Fish and invertebrates usually eliminate low molecular weight aromatic hydrocarbons and their metabolites via the gills.

Oil and its components have a variety of sublethal effects that can affect population size. Feeding rates of fish and invertebrates are frequently reduced during long-term exposures to crude oil at concentrations that are 15-30% of the short-term LC50; thus, growth and energy available for growth (scope-for-growth or energy budget) are decreased.

Sensitivity of an organism in a laboratory study is different from vulnerability after an oil spill. Laboratory tests isolate one variable at a time, whereas oil spills have many variables operating at the same time, and each spill is unique. Planktonic larvae are among the most vulnerable organisms after an oil spill because they are sensitive to oil, are affected after only minutes of exposure, and cannot avoid spilled oil. In fact, some larvae are killed within minutes by concentrations of crude oil that are <1 ppm. Other animals, like fish, may be nearly as sensitive as planktonic larvae but can probably avoid an oil-contaminated area. Although intertidal animals are not sensitive to oil spills and may require long-term exposures to about 20-25% of their LC50 before growth is affected, many are sessile and must rely solely on physiological tolerance to endure exposure to oil.

Studies at Port Valdez

Effluent from the ballast-water treatment plant at Port Valdez is rapidly diluted, and in the short term, is apparently not toxic. However, concentrations of aromatic hydrocarbons in the effluent were as high as 15 ppm, and dilutions of the effluent as low as 2% (vol/vol) were toxic to some crustacean larvae. Shrimp and fish were less sensitive to the effluent (LC50 of 19-43% dilutions) than larvae. Repeated tests with shrimp and fish suggest that the toxicity of the effluent may be caused by contaminants other than aromatic hydrocarbons.

A decrease in the population of Baltic clams (Macoma balthica) and the presence of hydrocarbons in sediments near the effluent-treatment facility suggest that a continuous discharge of effluent could cause long-term damage.

Drilling Muds

Because drilling muds are rapidly diluted and have low toxicity, they are probably not toxic to planktonic larvae. Unlike the WSF's of oil, crustacean larvae exposed to drilling muds do not immediately cease swimming or die, and most of the toxicity is apparently caused by physical stress of the particulates in suspension rather than chemical stress. The alkalinity of one mud was quite high, however, and its alkalinity was the primary cause of toxicity.

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INTRODUCTION

This report summarizes all the research on effects of oil on Alaskan organisms conducted by the Auke Bay Laboratory from the first study in 1970 through 1981. Most of the research was conducted in the laboratory. The report includes all the major findings from 62 studies, regardless of funding source.

Emphasis in earlier years was on short-term toxicity of crude oil to fish and invertebrates. More recently, most of the studies have been long term and have determined sublethal effects of crude oil and its components on fish and invertebrates. Several field studies were conducted at the Trans-Alaska Pipeline System terminal at Port Valdez, Alaska, to determine the effects of effluent from the ballast-water treatment plant on the marine environment. We also evaluated the toxicity of drilling muds to several species of crustacean larvae found in Alaska.

We review the research by categories, such as toxicity, uptake and metabolism, and effects of oil and its components on growth, and include abstracts for all 62 studies (Appendix A). Appendix B contains a list of all the species tested, including scientific names. Some of the results of our research have been published in individual reports, some have been included in earlier reviews (Evans and Rice 1974; Karinen 1977; Rice 1977; Rice et al. 1977a), and other results are unpublished. A comprehensive review of all our oil-effect studies, however, has never been included in one document.

Oil-effects research by the Auke Bay Laboratory began after enormous deposits of oil were discovered at Prudhoe Bay on Alaska's Arctic coast. The planning and construction of the Trans-Alaska Pipeline, through which nearly 20% of U.S. oil production now flows, aroused great concern in the fishing industry about the potential for catastrophic effects of oil pollution on fish and their habitat. Until that time, little information was available on effects of oil on Alaskan species under cold-water conditions. Baseline observations in Port Valdez (terminus of the pipeline) began in 1970 with funding from the U.S. Fish and Wildlife Service. The first Laboratory experiments on oil toxicity began in late 1971, and a study of pink salmon (Oncorhynchus gorbuscha) avoidance of oil was completed in 1972. In 1973, the Auke Bay Laboratory Environmental Physiology Section was formed with a staff of three biologists and funding from the National Marine Fisheries Service (NMFS).

A core research staff has been funded by NMFS continuously through 1982 at an annual level of $150,000-$200,000.

The first non-Government funding for toxicity studies at the Laboratory was from Shell Oil Company in 1974. Money from this contract provided basic analytical equipment and an increase in staff. Other contracts followed with the Outer Continental Shelf Environmental Assessment Program (OCSEAP) (1975 to present), Environmental Protection Agency (1979-80), and Office of Marine Pollution Assessment (1979 to 1981).

METHODS

Comparisons of sensitivities of different species or life stages can be misleading if there are differences in types of chemical analyses, exposure methods (static versus flow-through exposure), or response rates of the animals. The complex composition and physical behavior of crude oil test solutions requires sophisticated chemical analyses and complicated exposure apparatus. Standard gravimetric methods for analyzing crude oil are not suitable for determining concentrations of crude oil in water. These methods measure nonvolatile oil and grease by weight after volatile components are extracted; thus, volatile toxic compounds, such as the monoaromatic hydrocarbons, are not measured.

We used infrared (IR) and ultraviolet (UV) spectroscopy, UV fluorescence, and gas chromatography to analyze oil and the WSF's of oil. Spectrophotometric and fluorimetric methods, however, do not separate or quantify individual compounds. Gas chromatography is the most useful method for detailed analyses of components of crude oil because individual compounds can be separated and measured. Measurement of individual compounds in oil is important because the toxicity, persistence, and degradation of each compound are variable. Furthermore, temperature influences the stability of compounds in test solutions by affecting rates of evaporation and biodegradation.

We report the concentrations of WSF's in parts per million total monoaromatic and diaromatic hydrocarbons (benzene through methylated naphthalenes). Monoaromatic hydrocarbons are slightly water-soluble and predominate in water-soluble fractions (WSF's) of crude oil (Short et al. 1976). Other aromatic hydrocarbons are found only in trace quantities in the WSF's.

Comparisons between static and flow-through tests are not appropriate because hydrocarbons evaporate rapidly from solutions; therefore, static exposures are less toxic than flow-through exposures (Table 1; Brodersen and Rice in preparation). Furthermore, ratios of toxicities from static exposures to toxicities from flow-through exposures vary with the species tested because some species, such as salmon fry, respond rapidly whereas others, such as blue mussels, respond slowly.

Table 1.--Short-term (96-h) toxicity of Cook Inlet crude oil water-soluble fractions in static and flow-through exposures to pink salmon fry and kelp shrimp (Brodersen and Rice in preparation). LC50 is the concentration that killed 50% of the animals.

Species	Static 96-h LC50 (ppm)	Flow-through 96-h LC50 (ppm)
Pink salmon	2.5	1.0
Kelp shrimp	4.0	1.4

Flow-through exposures are superior to static exposures because flow-through exposures have constant concentrations of hydrocarbons, supply oxygen, and remove metabolic wastes. We have built two devices for producing stable concentrations of aromatic hydrocarbons for short- and long-term tests (Moles et al. in press). For tests with individual components of crude oil, a syringe pump injects hydrocarbons into a stream of water at a rate that does not exceed the solubility of the compound. To prepare WSF's, water is dripped through a 2-m long X 15-cm diameter glass column that has a 15-cm layer of constantly replenished oil at the top. Water flowing through the oil layer absorbs aromatic hydrocarbons and continues through the column into the exposure tanks.

Different species, or even life stages of the same species, respond to oil and its components at different rates; therefore, simple comparisons of sensitivities of animals over a standard time interval (such as a 4-d LC50) are inadequate. For example, salmon fry surviving the first 24 h of exposure to oil are unlikely to succumb to further exposure (Rice 1973; Rice et al. 1975; Moles and Rice in preparation). In contrast, adult coonstripe shrimp

(Pandalus hypsinotus) died each day in a test lasting 28 d (Brodersen and Carls in preparation). Other studies have shown that blue mussels did not die after exposure for 21 d to lethal concentrations but do die after exposure to the same concentrations for 28 d (Stickle et al. in press).

Sublethal or moribund responses are often detected in test organisms well before they die. In these cases, we calculated median effective concentrations (EC50's: concentrations at which 50% of the animals show a particular sublethal response). For example, crustacean larvae are unable to swim after a 20-min exposure to lethal concentrations of WSF but do not die until 7-10 d later (Brodersen in preparation). In nature, these larvae would probably be eaten or buried in sediments; therefore, they may be considered ecologically dead.

TOXICITY OF OILS AND THEIR COMPONENTS

Because oils are a mixture of many different compounds, toxicity tests with crude oil or refined oils require a different experimental approach than tests for other toxic substances, such as metals. Physical properties affect the rate and amount that a given compound in oil is incorporated into water. These properties include, but are not limited to, solubility of the compound, viscosity and composition of the oil, ease of mixing the oil with water, and the time that the compound and water remain mixed.

Once in water, the persistence of oil components is influenced by a variety of processes, such as biodegradation and evaporation, which in turn are affected by temperature. Biodegradation and evaporation are important even at low temperatures that characterize Alaskan waters. Cheatham and Rice (in preparation) demonstrate that losses of total aromatic hydrocarbons from WSF's of crude oil are significant; however, the rate of loss is reduced at lower temperatures (Fig. 1). Diaromatic hydrocarbons are lost primarily through biodegradation, whereas low molecular weight monoaromatic hydrocarbons, which have higher vapor pressures than diaromatic hydrocarbons, are primarily lost through evaporation. For example, at 12°C, up to 80% of the low molecular-weight monoaromatic hydrocarbons are lost from the WSF of Cook Inlet crude oil in static tests lasting 96 h. Oil and seawater mixtures at

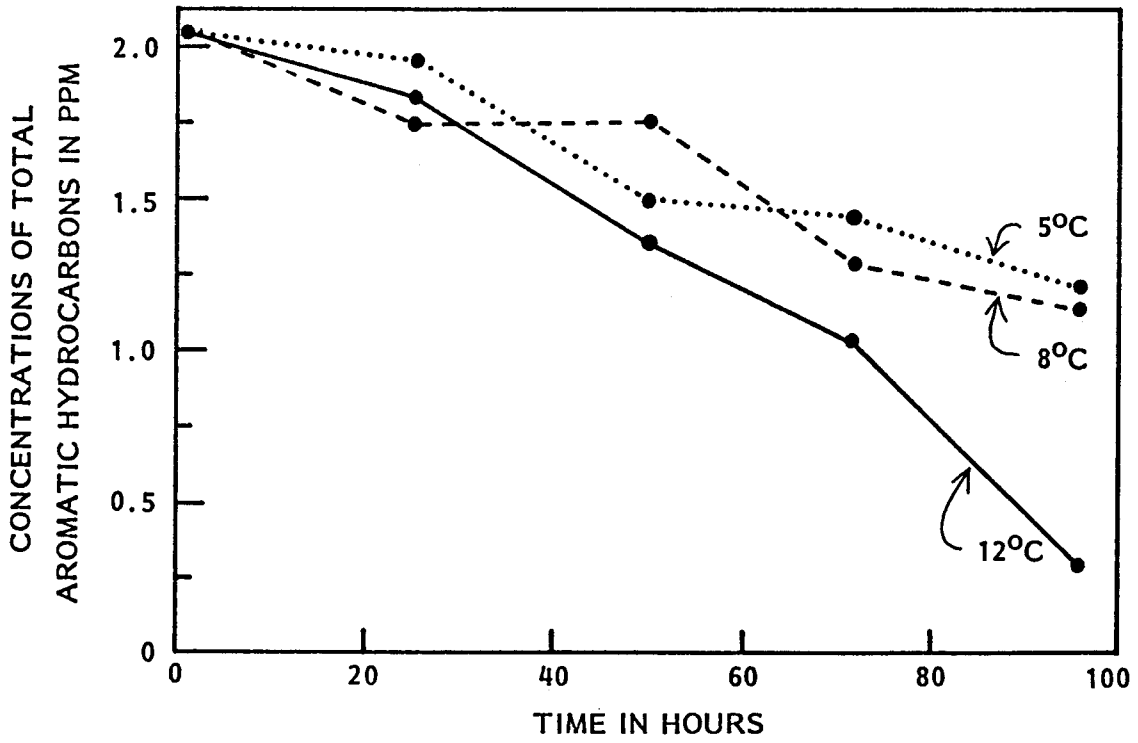

Figure 1.--Concentration of total aromatic hydrocarbons in water-soluble fractions of Cook Inlet crude oil as measured by gas chromatography at 24-h intervals. Solutions were kept at either 5°, 8°, or 12°C and were not aerated (from: Cheatham and Rice in preparation).

low temperatures are more toxic because aromatic hydrocarbons persist longer in cold water than in warm water.

The WSF's of Alaskan crude oils are similar (Table 2), but concentrations of different aromatic hydrocarbons in the WSF's are usually different. For example, the aromatic hydrocarbons in the WSF's of Prudhoe Bay crude oil, Cook Inlet crude oil, and effluent from the ballast-water treatment plant at Port Valdez are nearly identical and have a ratio of monoaromatic hydrocarbons to diaromatic hydrocarbons of about 20:1. The similarity in composition of WSF's of crude oils is influenced by the solubility of the individual aromatic hydrocarbons in water and their ability to be extracted into water. In contrast, the WSF of No. 2 fuel oil has a much lower concentration of monoaromatic hydrocarbons than the WSF of other oils (the ratio of monoaromatic hydrocarbons to diaromatic hydrocarbons is about 1:1) because most monoaromatic hydrocarbons are removed during refining.

Table 2.--Representative concentrations of aromatic hydrocarbons in effluent from the Port Valdez tanker ballast treatment plant and in water-soluble fraction (WSF) of Prudhoe Bay and Cook Inlet crude oil and No. 2 fuel oil.

Component	Concentration (ppm)			
	Prudhoe Bay[a] WSF	Cook Inlet[a] WSF	Ballast[a] effluent	Fuel[b] oil WSF
Benzene	1.8	3.2	3.2	0.11
Toluene	2.0	2.5	2.2	0.17
o-xylene	0.28	0.35	0.32	0.12
m- and p-xylene	0.58	0.78	0.68	0.17
Naphthalene	0.084	0.15	0.098	0.15
1-Methylnaphthalene	0.032	0.066	0.049	0.13
2-Methylnaphthalene	0.048	0.088	0.066	0.25
Ratio of all mono-aromatic hydrocarbons to diaromatic hydrocarbons	19.3:1	15.7:1	23.6:1	1.1:1

[a]From Rice et al. 1981.
[b]From Rice et al. 1979.

Although the ratio of monoaromatic to diaromatic hydrocarbons is different for the WSF's of No. 2 fuel oil and Cook Inlet crude oil, different animals have similar patterns of sensitivity to the WSF's of the two oils. For example, pelagic animals sensitive to crude oil are also sensitive to No. 2 fuel oil, and tolerant species are tolerant to both crude oil and No. 2 fuel oil. Number 2 fuel oil, however, is consistently more toxic than Cook Inlet crude oil (Table 3). The LC50's for No. 2 fuel oil (measured by gas chromatography) are 10-59% of the LC50's for crude oil, and the LC50's for fuel oil (measured by IR spectrophotometry) are 18-36% of the LC50's for crude oil.

Although monoaromatic hydrocarbons, which predominate in crude oil WSF, are only 10% as toxic as diaromatic hydrocarbons, such as naphthalene, monoaromatic hydrocarbons are more water soluble and have higher concentrations in the WSF's of crude oil than diaromatic hydrocarbons. The LC50's of crude oil, measured as total concentration of aromatic hydrocarbons, reflect concentrations of toxic compounds that consist of high percentages of the less toxic

Table 3.--Short-term toxicity of the water-soluble fractions (WSF's) of Cook Inlet crude oil and No. 2 fuel oil to Alaskan marine organisms (Rice et al. 1979). LC50 is the concentration that killed 50% of the test animals.

	96-h LC50 (ppm total aromatic hydrocarbons)	
Organism	Cook Inlet crude oil	No. 2 fuel oil
Salmonids	1.5-1.66	0.97
Benthic fish	3.96->6	1.31->2
Shrimp	0.87-1.86	0.36-1.1
Crab	3.6->10	1.02->3

monoaromatic hydrocarbons. In contrast, the high toxicity of No. 2 fuel oil is probably caused by the low concentrations of very toxic diaromatic hydrocarbons and the low viscosity of No. 2 fuel oil, which permits more diaromatic hydrocarbons to dissolve in water.

The contribution of each class of compounds to the toxicity of the WSF is difficult to isolate because a WSF is a complex mixture of paraffins and aromatic hydrocarbons. Furthermore, the interactions of aromatic hydrocarbons in WSF's appear to be complex and difficult to predict. Aromatic hydrocarbons have been presumed to be major contributors to the toxicity of WSF's because they are present and toxic, but quantitative studies on toxic interactions of aromatic or other compounds are lacking. Korn et al. (in press) concluded that phenolic compounds are not major contributors to the toxicity of WSF's because their concentrations in WSF's are too low. When a simulated WSF was made using the 10 aromatic hydrocarbons that predominate in the WSF of crude oil (see Table 2), the toxicity of the simulated WSF was only 20-30% of the toxicity of crude oil WSF, which was made by mixing oil into water, even though the proportions of individual aromatic hydrocarbons in both WSF's were the same (Rice and Andrews, unpublished data on file ABL). Other laboratory experiments testing pairs of aromatic hydrocarbons indicate that the toxicities of some aromatic hydrocarbons in the WSF are synergistic (Rice and Andrews, unpublished data on file ABL).

COMPARATIVE ANIMAL SENSITIVITIES

The effects of oil on the marine fauna in Alaska need to be assessed because many species of fish and shellfish support commercial fisheries or are the food sources for these valuable fisheries. Toxicity tests previous to our studies used warm-water species and crude oils not found in Alaska; therefore, the results from these tests cannot be used to predict sensitivities of species and life stages in Alaska. When assessing the potential effect of an oil spill, it is necessary to know whether some groups of animals are more sensitive than others and what factors affect this sensitivity. We have found that a variety of biological and environmental variables affect sensitivity of Alaskan species.

Effect of Habitat Adaptations on Sensitivity to Oil

Different phylogenetic groups and animals living in different habitats have different patterns of sensitivity to oil. In short-term exposures, sensitivity of animals generally increases from lower invertebrates to higher invertebrates to fish (Rice et al. 1976b; Rice et al. 1979). Variation in sensitivities is wide, however, and there are many exceptions. The most distinctive pattern is the correlation between sensitivity of test animals and their habitat (Table 4). Many species have specialized structures and adaptations for survival in different niches and habitats, such as exoskeletons or cryptic behavior. These adaptations can also affect the sensitivity of an animal to oil. Pelagic fish and invertebrates tend to be the most sensitive (LC50's of 1-5 ppm aromatic hydrocarbons), benthic species are moderately

Table 4.--Ranges of sensitivities (96-h LC50 in parts per million aromatic hydrocarbons) for different habitat groups exposed to Cook Inlet WSF (Rice et al. 1979).

	Habitat		
Organism	Pelagic	Benthic	Intertidal
Fish	1-3	4->5	>12
Crab and shrimp	1-5	3-5	8->10
Mollusc	--	4->8	8

sensitive (LC50's 3->8 ppm aromatic hydrocarbons), and intertidal fish and invertebrates are usually the least sensitive (LC50's of >8 ppm) (Table 4).

Pelagic animals are the most mobile, have the most uniform environment of the three habitat groups, and are most sensitive to stress. Intertidal animals, which inhabit a highly variable and stressful habitat, have little or no mobility or ability to escape but are well adapted to withstand natural stresses (Taylor and Karinen 1977; Rice et al. 1979). Because intertidal animals can withstand natural stresses, they also resist stress caused by petroleum hydrocarbons.

Sensitivity of Different Life Stages to Oil

Sensitivities to oil are different for each life stage of a species and for the same life stages of different species. Extrapolation of the sensitivity of eggs or larvae to other untested species and groups is, therefore, not warranted. Extreme tolerances and extreme sensitivities have been found in the early life stages of fish and invertebrates. For example, salmon eggs and alevins are more tolerant to short-term (96-h) exposures of petroleum hydrocarbons than fry or juvenile salmon. Eggs of pink salmon and coho salmon (Oncorhynchus kisutch) exposed to benzene had 96-h LC50's of 340 ppm and 540 ppm, respectively; whereas, pink salmon and coho salmon fry had LC50's of 15.3 ppm and 9.8 ppm, respectively (Moles et al. 1979; Fig. 2).

Salmon eggs are more sensitive to long-term exposures than short-term exposures for at least two reasons: 1) Hydrocarbons move slowly across egg membranes (Korn and Rice 1981), and 2) large quantities of hydrocarbons are accumulated and sequestered in the lipid-rich yolk rather than in developing tissues. Thirty percent of coho salmon eggs exposed to toluene and 60% of those exposed to naphthalene for 17 d died on hatching even though the exposure levels (1.8 ppm toluene and 0.11 ppm naphthalene) were <1% of the 96-h LC50 for eggs (Korn and Rice 1981).

The early life stages of most salmon are spent in freshwater; however, pink salmon often deposit their eggs in the intertidal reaches of streams, and salinity can modify the sensitivity of the early-life stages of this species. For example, Moles et al. (in preparation) found that at WSF concentrations of

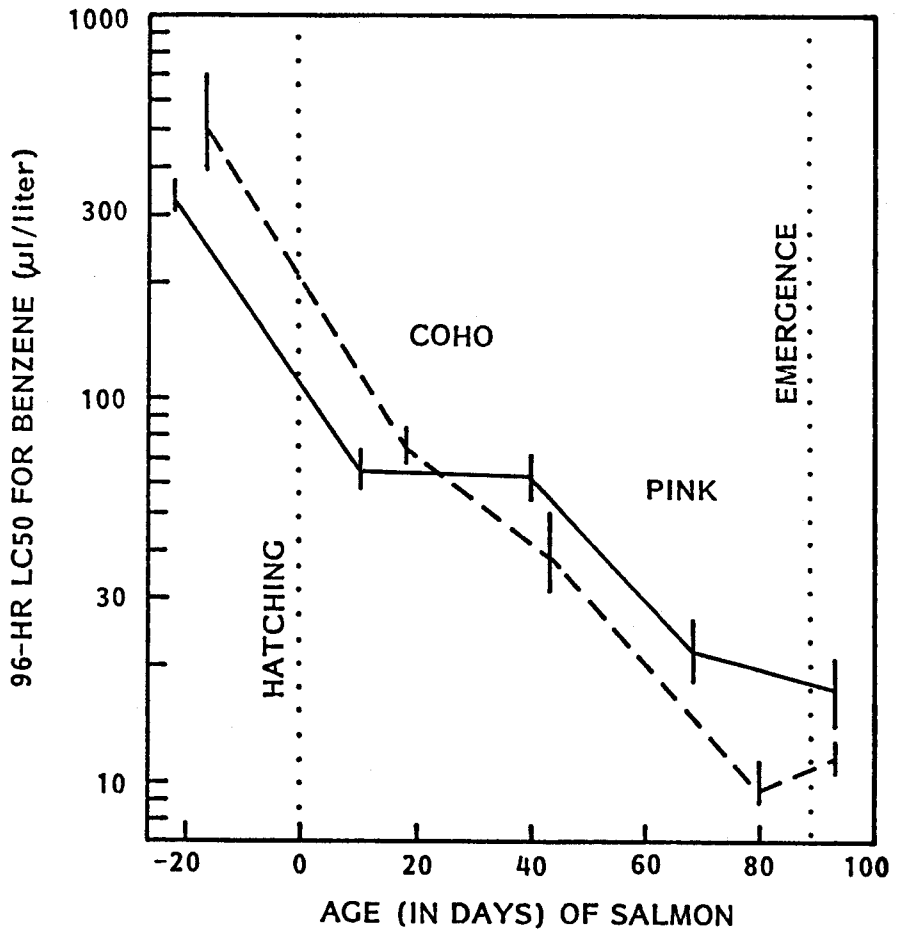

Figure 2.--Sensitivity (96-h LC50's) of early life stages of pink salmon and coho salmon to benzene. Vertical bars indicate 95% confidence intervals (Moles et al. 1979).

2.4 ppm aromatic hydrocarbons, pink salmon eggs in fresh water died in 16 d, whereas eggs in intermittent seawater died within 9 d.

Carls and Rice (in preparation) exposed walleye pollock (Theragra chalcogramma) eggs and larvae to the WSF of Cook Inlet crude oil. Eggs exposed for 20 d to concentrations of 2.9 ppm WSF were abnormal, and larvae were affected by even lower concentrations than eggs. The LC50 for larvae, based on initial concentrations, was approximately 1.3 ppm aromatic hydrocarbons.

Shrimp embryos were more tolerant to the WSF of oil than were adults, even in exposures lasting 30 d (Table 5), but eggs are carried by the female throughout development and cannot survive if she does not. Brodersen and Carls (in preparation) exposed gravid coonstripe shrimp and kelp shrimp (Eualus

suckleyi) to several concentrations of crude oil WSF for 28 d (flow-through exposures). In both species, the eggs survived if the female did. Even female shrimp that were weak and apparently near death released swimming larvae indistinguishable from the larvae of control shrimp.

Table 5.--Sensitivities (LC50's, parts per million aromatic hydrocarbons) of coonstripe shrimp exposed to Cook Inlet crude oil WSF for 96 h and 28 d (Brodersen and Carls in preparation).

Life stage	LC50 in ppm	
	96-h	28-d
Egg	>1.4	>0.5
Larva (Stage I-III)	1.0	1.0
Adult	1.4	0.5

Crustacean larvae are generally more sensitive to crude oil WSF than the adults (Brodersen et al. 1977). For four species of adult shrimp and juvenile king crab (Paralithodes camtschatica), 96-h LC50's (static exposures) were between 1.9 ppm and 4.3 ppm of oil in the WSF (measured by infrared spectrophotometry). The LC50's for the Stage I larvae of the same species were between 0.95 ppm and 1.8 ppm of oil. Differences between sensitivities of early- and late-stage larvae can be large. For example, Stage I (96-h) larvae of coonstripe shrimp had an LC50 of 0.2 ppm, whereas Stage VI larvae had a 96-h LC50 of 1.8 ppm (Brodersen et al. 1977). Molting could be impaired during exposure to crude oil (Karinen and Rice 1974; Mecklenburg et al. 1977).

Larval coonstripe shrimp take up hydrocarbons extremely fast: in <10 min, tissue concentrations of hydrocarbons can reach several times the concentration of hydrocarbons in the water column (Short et al. in preparation). Rapid uptake of hydrocarbons results in a rapid response for larval shrimp and crabs. Larvae in lethal concentrations of hydrocarbons stop swimming in <20 min, but they may live for 10 d before they die, at least in the laboratory (Brodersen in preparation). In the marine environment, however, planktonic larvae that fail to swim probably die.

Brief exposures to oil WSF are almost as toxic to shrimp larvae as long exposures (Brodersen and Carls in preparation). Both 24-h and 28-d flow-

through LC50's for coonstripe shrimp larvae were 1 ppm aromatic hydrocarbons (Table 5).

Effect of Temperature on Sensitivity to Oil

The low temperature of Alaskan waters has two effects on the toxicity of aromatic hydrocarbons, effects that are difficult to separate. Low temperature increases the toxicity of oil by increasing the persistence of aromatic hydrocarbons in water and modifies the physiological sensitivity of test animals (Rice et al. 1977a). The LC50's for fish and invertebrates from cold environments are generally lower than the LC50's for similar species from warmer climates (Rice et al. 1977a). Although differences in LC50's are not large, they are consistent and probably related more to differences in persistence of toxicants at different exposure temperatures than to differences in sensitivities of the species.

The effects of temperature on the sensitivity of Alaskan species are quite variable. For example, in tests where persistence of the toxicant was not a factor, sensitivity of kelp shrimp increases with higher temperature, but pink salmon fry are more sensitive at lower temperatures (Korn et al. 1979; Table 6). In another test with five circumpolar species of fish and amphipods, there was no general relationship between sensitivity to naphthalene and exposure at temperatures of 1° and 10°C (Carls and Korn in press). Like intertidal animals, these circumpolar species are adapted to a very harsh environment and are tolerant to temperatures far higher than those they usually encounter. Unpredictability of the effect of temperature is probably caused by the inseparable effects of physiological response of animals at each temperature and persistence of the toxicants.

Table 6.--Sensitivities (96-h LC50's, parts per million aromatic hydrocarbons) of pink salmon fry and kelp shrimp exposed to Cook Inlet WSF at three temperatures (Korn et al. 1979).

	Crude oil			Toluene		
Species	4°C	8°C	12°C	4°C	8°C	12°C
Pink salmon	1.5	1.7	1.8	6.4	7.6	8.1
Kelp shrimp	1.7	1.9	1.6	21.4	20.2	14.7

Effect of Salinity on Sensitivity to Oil

Several salmonids are twice as sensitive to oil and aromatic hydrocarbons in seawater as they are to the same compounds in fresh water. Dolly Varden (Salvelinus malma), sockeye salmon (Oncorhynchus nerka), and pink salmon fry, for example, are more sensitive to benzene or the WSF of Prudhoe Bay crude oil in seawater (30°/$_{oo}$) than to these compounds in fresh water (Table 7; Moles et al. 1979). The changes in sensitivity were caused by the fish being in seawater rather than differences in composition of the oil in fresh water and seawater. The LC50's of coho salmon smolts exposed to toluene and naphthalene in water with 0, 10, 20, or 30°/$_{oo}$ salinity increased linearly with salinity, and acclimation to seawater for 12, 22, and 42 d did not change the sensitivity (Stickle et al. 1982).

Table 7.--Sensitivity of three salmonids to the water-soluble fraction (WSF) of Prudhoe Bay crude oil and aromatic hydrocarbons at different salinities.

Species	Hydrocarbon	0°/$_{oo}$	30°/$_{oo}$
Sockeye salmon[a]	WSF	2.2	1.0
	Benzene	10.8	5.6
Pink salmon[a]	WSF	8.0	3.7
	Benzene	17.1	8.5
Coho salmon[b]	Toluene	8.7	4.7
	Naphthalene	1.6	0.9

[a]From Moles et al. 1979.
[b]From Stickle et al. 1982.

Accumulation and metabolism of aromatic hydrocarbons are probably affected by the physiological adjustments that some fish make after moving from fresh water to seawater. For example, Dolly Varden consistently accumulate higher concentrations of toluene and naphthalene (and lower percentages of metabolites) in their tissues when exposed in seawater than when exposed in fresh water (Thomas and Rice 1981).

Effect of Parasitism on Sensitivity to Oil

Diseased or parasitized organisms are more susceptible to pollutant stress than healthy organisms. Conversely, an organism that has been weakened by exposure to a toxicant is more susceptible to disease. Moles (1980) experimentally controlled the level of parasitism of larval freshwater mussel Anodonta oregonensis on coho salmon fry. Sensitivity of the fry to toluene, naphthalene, and the WSF of crude oil increased as the number of parasites on each fry increased (Fig. 3). Parasitized fry are probably more sensitive to

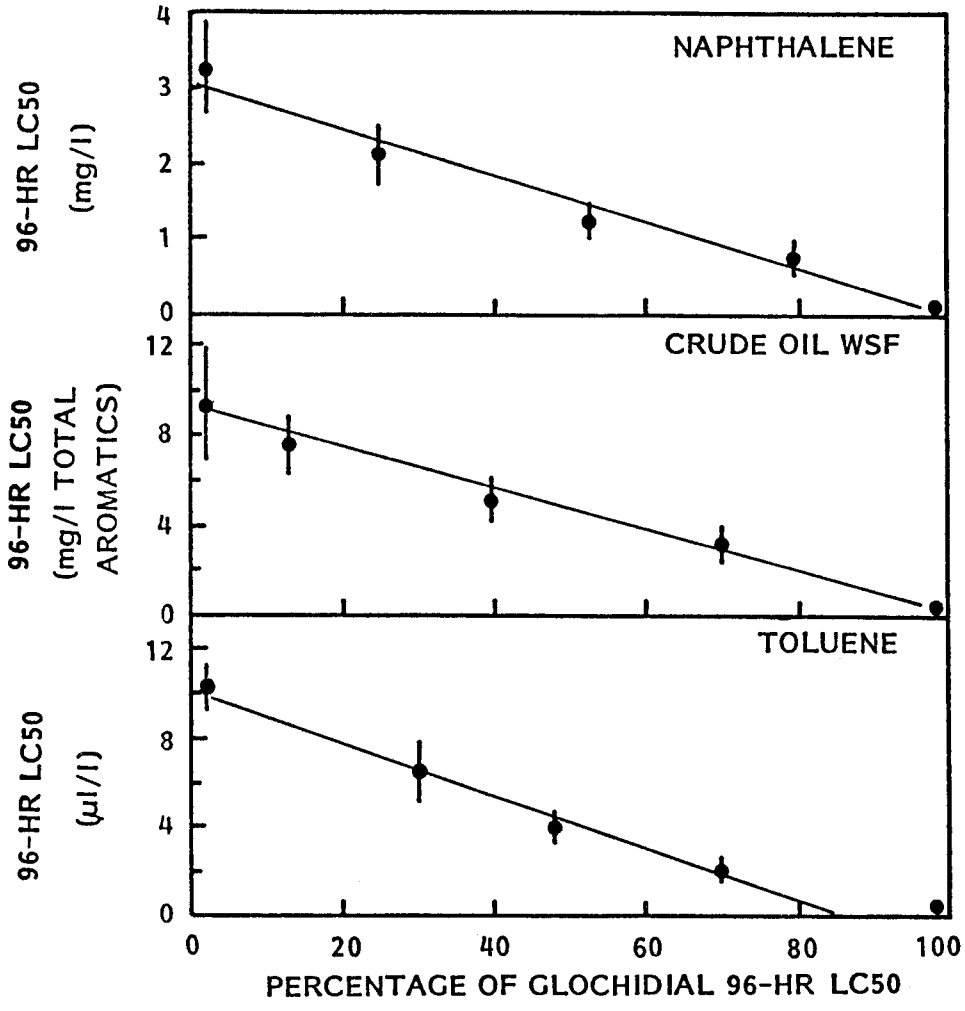

Figure 3.--The effect of infestation by Anodonta oregonensis glochidia on coho salmon fry sensitivity to naphthalene, the water-soluble fraction (WSF) of Prudhoe Bay crude oil, and toluene. The number of glochidia per fish is expressed as a percentage of the glochidial 96-h LC50. The 96-h LC50's of glochidia alone were 115 glochidia per fry (Moles 1980).

these toxicants because parasites reduce the amount of energy available to the fish, and the fish use energy for repair of wounds rather than metabolism of toxicants.

HYDROCARBON UPTAKE AND METABOLISM BY ORGANISMS

Concentrations of hydrocarbons that accumulate in animal tissues can vary considerably depending on tissue, life stage of the animal, and species. Furthermore, several physical, biological, and environmental factors can influence the rate of hydrocarbon uptake, the level of accumulation in tissues, distribution of hydrocarbons between organs, and the ability of the organ to depurate hydrocarbons. Differences in tissue concentration of parent hydrocarbon or metabolite often correlate with differences in survival, severity of sublethal effects, or rate of appearance of sublethal effects.

Physical and Biological Factors Affecting Hydrocarbon Uptake

Hydrocarbons can diffuse into the circulatory system from the stomach (food) or integument, but the primary organ is the gills, which have a large surface area exposed to the environment. The structure, molecular weight, polarity, and methylation of petroleum hydrocarbons affect both the solubility of the compound in the WSF and the rate the hydrocarbons are accumulated in and depurated by animal tissues. Aromatic hydrocarbons accumulate in tissues more readily than aliphatic hydrocarbons (Rice et al. 1977b). Larger compounds, such as methylnaphthalenes, accumulate slowly but to higher concentrations and are retained longer in tissues than the smaller, more polar monoaromatic hydrocarbons (Table 8). Because monoaromatic hydrocarbons are more water soluble than polynuclear aromatic hydrocarbons, they cross membranes more easily than polynuclear aromatic hydrocarbons and are not sequestered in the lipid portions of tissues or organelles as tightly as larger aromatic hydrocarbons (Rice et al. 1976a; Rice et al. 1977a; Korn and Rice 1981; Thomas and Rice 1981, 1982; Short and Rice in preparation). For example, early life stages of coho salmon accumulate 2-3 times more 2-methylnaphthalene than naphthalene (Korn and Rice 1981). Snails, crabs, and adult shrimp accumulate 10 times more naphthalene than toluene (Gharrett and Rice in preparation-a).

Table 8.--The effect of size and methylation on the accumulation factor of petroleum aromatic hydrocarbons by pink salmon after 3 h of exposure to the WSF of Cook Inlet crude oil. Accumulation factor is the ratio of tissue to water concentration.

Oil component	Accumulation factor
Toluene	3[a]
Naphthalene	64[b]
Methylnaphthalene	139[b]
Dimethylnaphthalene	198[b]
Trimethylnaphthalene	380[b]

[a]From Korn and Rice 1981.
[b]From Short and Rice in preparation.

Larger hydrocarbons are sequestered into lipid-rich tissues where they are tightly held in the lipid matrix (Lauren and Rice in preparation). Tissues with high lipid content tend to attract hydrocarbons both from the circulatory system and from less lipophilic tissues (Table 9). Liver and gall bladder in fish, digestive gland in invertebrates, and egg yolk typically accumulate hydrocarbons to concentrations that may be 10 times higher than those in muscle, gill, or embryo (Rice et al. 1977b; Thomas and Rice 1982; Korn et al. in preparation; Gharrett and Rice in preparation-b). Purple shore

Table 9.--Effect of tissue type on the accumulation of naphthalene in pink salmon and purple shore crab. Length of exposure (in hours) is in parentheses. Accumulation factor is the ratio of tissue to water concentration.

Species	Tissue	Maximum accumulation factor
Pink salmon[a]	Viscera	180 (72)
	Muscle	160 (72)
Purple shore crab[b]	Digestive gland	104 (12)
	Thoracic ganglion	15 (12)
	Hemolymph	4.2 (12)

[a]From Rice et al. 1977a.
[b]From Lauren and Rice in preparation.

crabs (Hemigrapsus nudus) exposed to naphthalene had virtually no naphthalene in gill tissue; whereas viscera contained 1,200 times the concentration of naphthalene in the exposure water (Lauren and Rice in preparation).

Different species have different rates of hydrocarbon uptake and accumulation because of morphological and physiological differences. Fish have high metabolic rates and accumulate hydrocarbons rapidly; shrimp are intermediate in both factors; and some lower invertebrates, such as pink scallops (Chlamys hericius) and other bivalves, have low metabolic rates, accumulate hydrocarbons slowly, and incompletely depurate hydrocarbons over weeks or months (Fig. 4).

Different life stages of the same species can have different rates of hydrocarbon uptake and concentrations of accumulated hydrocarbons because of differences in anatomy, activity, and lipid content. For example, coho salmon eggs accumulated hydrocarbons very slowly; however, after long exposures,

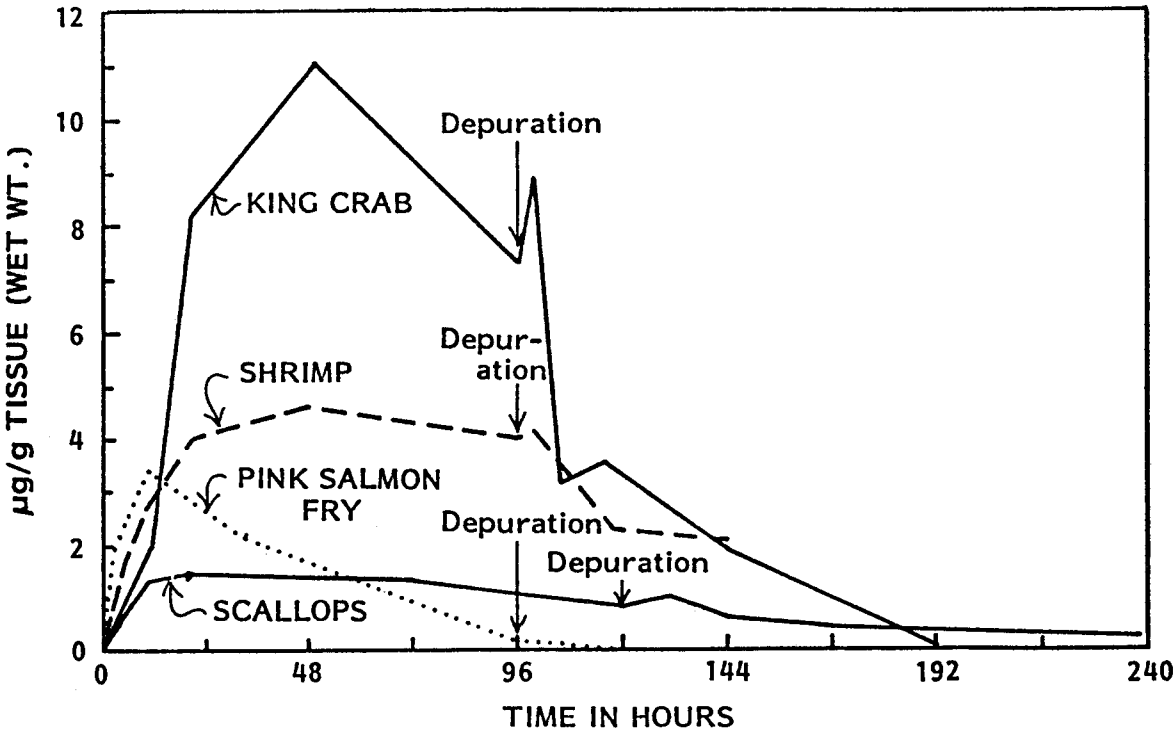

Figure 4.--Concentrations of aromatic hydrocarbons in pink shrimp, pink scallops, pink salmon fry, and king crab during exposure to the water-soluble fraction of Cook Inlet crude oil and during subsequent depuration. Points on the graph represent the sum of all aromatic hydrocarbon concentrations found in groups of five individuals determined at various time intervals (Short and Rice in preparation).

concentrations of accumulated hydrocarbons were high (Korn and Rice 1981). The rates of accumulation and depuration increased as salmon eggs developed into alevins and fry (Fig. 5). The time required for tissue concentrations of hydrocarbons to reach equilibrium with concentrations of hydrocarbons in toxicant baths was over 10 times longer for eggs than for alevins and fry. Salmon fry rapidly absorb hydrocarbons across their gills. Salmon eggs, on the other hand, have a large lipid pool, take up hydrocarbons slowly across the egg membrane, and have higher concentrations of hydrocarbons. Concentrations of aromatic hydrocarbons in shrimp eggs reach equilibrium with concentrations of aromatic hydrocarbons in the toxicant bath in 48 h, and 50% of the total aromatic hydrocarbons are lost from the eggs after 48 h in clean seawater (Korn et al. in preparation). Shrimp larvae accumulate and lose hydrocarbons faster than shrimp eggs. Larval coonstripe shrimp reach equilibrium with naphthalene solutions after only 30 min of exposure, and 70% of the naphthalene is lost 1 h after the shrimp are returned to clean water (Short et al. in preparation).

Processes of Metabolism and Elimination

Hydrocarbons often diffuse from tissues and are slowly extracted from lipid-rich structures and pass to the external environment if the gradient is favorable. Metabolism enhances depuration of hydrocarbons because the metabolites are usually more polar, more water soluble, and, therefore, more easily eliminated from the tissues.

Many fish and some invertebrates, such as crabs, metabolize hydrocarbons (Rice et al. 1977b; Short and Rice in preparation). The specific activity of enzyme systems that metabolize hydrocarbons is much higher in fish than in invertebrates (Lauren and Rice in preparation). In fish, hydrocarbons are primarily metabolized in the liver although some metabolism can occur in many tissues (Thomas and Rice 1981, 1982). In crabs, there is little evidence that hydrocarbons are metabolized in the digestive gland; however, antennal glands have some metabolizing capacity (Lauren and Rice in preparation). Most hydrocarbons are discharged by the gills rather than metabolized by any crab tissue (Lauren and Rice in preparation).

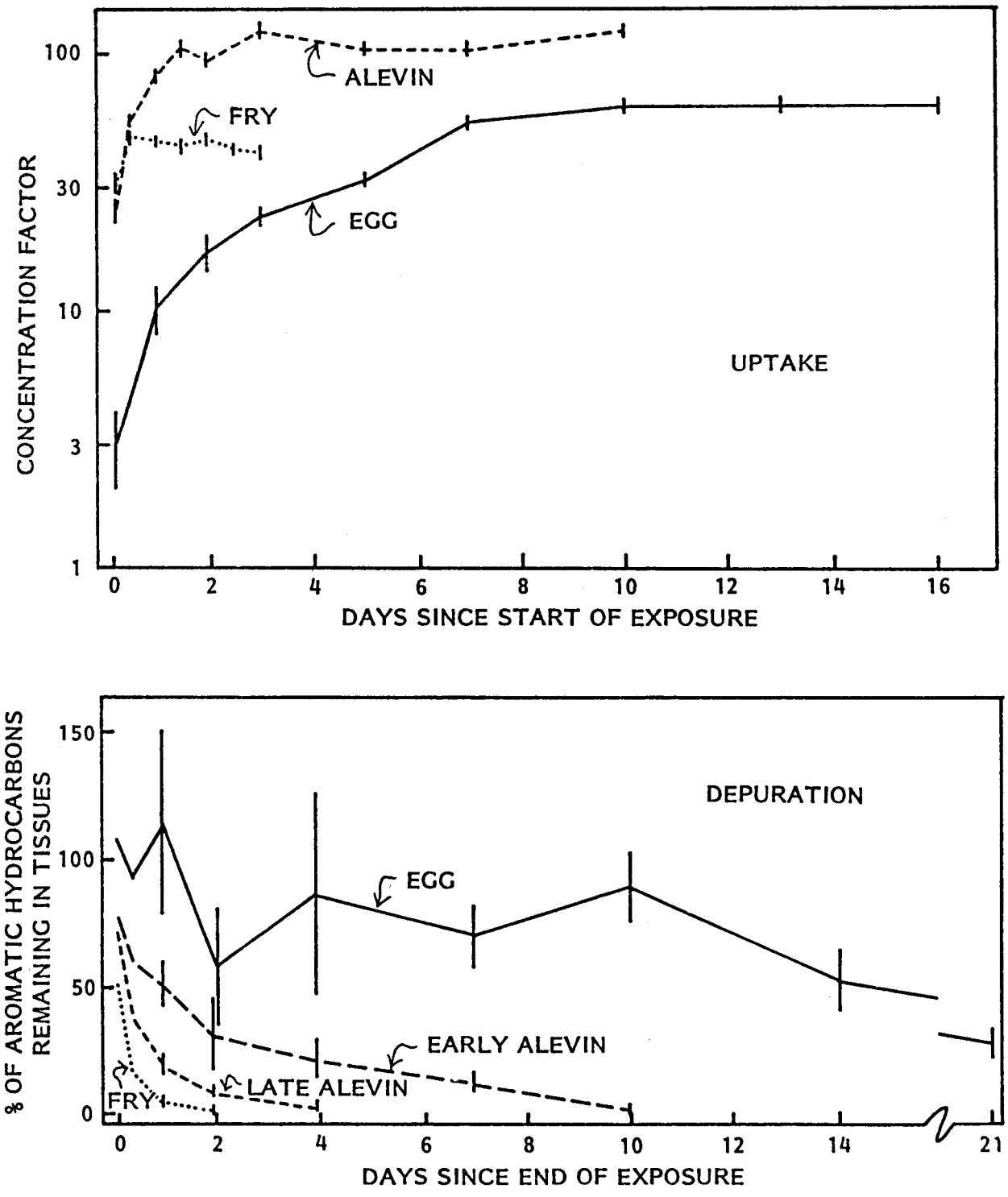

Figure 5.--Accumulation and depuration of naphthalene in early life stages of coho salmon. Fish were exposed to 100 mg/L naphthalene then placed in flowing seawater for depuration (Korn and Rice 1981).

Compounds excreted through the gills, the primary organ for the elimination of many hydrocarbons (Table 10), vary with species. Unmetabolized phenols and other monoaromatic compounds are rapidly eliminated from the gills of fish (Thomas and Rice 1981). Only small amounts of naphthalene and no compounds larger than naphthalene are released from the gills (Thomas and Rice 1982). Gills of crabs are, however, the primary organ for elimination of unmetabolized naphthalenes (Lauren and Rice in preparation). Crabs with blocked nephropores and anus had the same excretion rates as crabs with open nephropores and anus. In crabs, elimination via the urine is unimportant (Lauren and Rice in preparation).

Table 10.--Importance of different excretory pathways of Dolly Varden fed toluene and naphthalene (Thomas and Rice 1981).

Excretory pathway	Carbon-14 excreted during 24 h, as a percentage of administered carbon-14	
	Toluene	Naphthalene
Gill	19.2	12.6
Cloaca	3.4	1.1
Urine	0.008	0.003

Fish slowly excrete high molecular weight hydrocarbons because these compounds are metabolized in the liver and excreted through the bile rather than diffused across gill membranes (Thomas and Rice 1981, 1982). The gall bladder usually has the highest concentration of metabolites; however, the volume of the gall bladder is small and seldom accounts for large quantities of metabolites from mono- and dicyclic aromatic hydrocarbons. Metabolites of toluene were found in all tissues because these metabolites are highly mobile and most tissues have some metabolizing capacity. Elimination of metabolites via the urine in fish (Table 10) is not an important depuration pathway (Thomas and Rice 1981).

Environmental Factors Affecting Hydrocarbon Uptake, Metabolism, and Elimination

Temperature, salinity, and previous exposure to crude oil affect hydrocarbon uptake and depuration by affecting the physiological ability of animals to respond to absorbed hydrocarbons. The effect of temperature is complex, and higher temperatures increase rates of uptake, metabolism, and excretion. At higher temperatures, the rate toluene and naphthalene are accumulated and metabolized in tissues is increased. These compounds are absorbed faster from the stomachs of Dolly Varden at 12°C than at 4°C, and recovery of metabolites of ^{14}C-labeled naphthalene is always greater at the higher temperature (Table 11) (Thomas and Rice in preparation-a). Decreased sensitivity at higher temperatures probably results from increased metabolism of aromatic hydrocarbons (Thomas and Rice in preparation-a).

Table 11.--Effect of temperature on the recovery of ^{14}C-naphthalene metabolites 24 h after Dolly Varden (in seawater) were force-fed ^{14}C-naphthalene (Thomas and Rice in preparation-a).

Temperature	Gall bladder	Liver	Brain	Muscle
4°C	66.5	5.1	6.2	17.2
12°C	95.2	15.6	8.6	15.1

Salmonids are much more sensitive to aromatic hydrocarbons in seawater than in fresh water (Moles et al. 1979; Stickle et al. 1982). Higher salinity increases the uptake of hydrocarbons and reduces metabolism of aromatic hydrocarbons. In one experiment, Dolly Varden acclimated to either seawater or fresh water were force-fed toluene or naphthalene. Dolly Varden in seawater absorbed more of these hydrocarbons and had fewer metabolites than those in fresh water (Table 12; Thomas and Rice in preparation-b). Pink salmon fry exposed to WSF absorbed more hydrocarbons during alternating freshwater and seawater exposures than pink salmon fry exposed to the same oil concentrations in fresh water only (Moles et al. in preparation). Hydrocarbons had greater toxicity when the fry were in seawater because the fry did not metabolize hydrocarbons to more readily excreted forms.

Table 12.--Effect of salinity on the percentage of ^{14}C-labeled naphthalene recovered as ^{14}C-labeled metabolites. Dolly Varden were fed ^{14}C-labeled naphthalene at 12°C, and metabolites were measured 48 h later (Thomas and Rice in preparation-b).

Salinity (°/oo)	Gall bladder	Liver	Brain	Muscle
0	99.9	93.9	99.4	98.9
30	98.0	29.1	13.4	34.9

The rate fish metabolize aromatic hydrocarbons can be increased by exposing fish to sublethal concentrations of hydrocarbons in the aquarium water before force-feeding them ^{14}C-labeled hydrocarbons (Thomas and Rice in press). The rate dietary naphthalene is converted to tissue metabolites depends on the concentration of naphthalene in the previous exposure (Table 13), the length of the previous exposure, and time lapsed between the previous exposure and subsequent gastric exposure (depuration time). Fish previously exposed for 48 h to naphthalene in the aquarium water had higher concentrations of metabolites in tissues after the second exposure than those that had no previous exposure. A previous exposure of only 24 h, however, did not affect the concentrations of metabolites in tissues after the fish were fed naphthalene. As depuration time increased, the percentages of carbon-14 recovered in the metabolite fraction from the tissues was greatly decreased. In fact, after

Table 13.--Effect of previous exposure concentration on metabolism of ^{14}C-naphthalene fed intragastrically to Dolly Varden in seawater (Thomas and Rice in preparation-a).

Previous exposure concentration (% 96-h LC50)	Percent of ^{14}C-labeled naphthalene recovered as metabolites in tissues			
	Gall bladder	Liver	Brain	Muscle
0	82.3	3.0	0.8	2.3
25	84.5	3.2	0.9	2.6
50	86.8	4.5	1.7	5.6
75	87.5	10.1	12.8	34.3

24 h of depuration, concentrations of tissue metabolites were similar to those in fish with no previous exposure.

SUBLETHAL EFFECTS OF OIL ON FISH AND INVERTEBRATES

Petroleum hydrocarbons have several different sublethal effects, ranging from behavioral (e.g., avoidance of hydrocarbons: Rice 1973) to transitory physiological effects (e.g., increase in respiration: Thomas and Rice 1979) and long-term physiological effects (e.g., decreased growth: Moles and Rice 1983).

Measurable sublethal effects usually appear at about 25% of the LC50; however, LC50's vary widely between species (Table 14). For example, growth

Table 14.--Sublethal effects of the water-soluble fraction (WSF) and individual components of crude oil on Alaskan marine organisms.

Species	Toxicant	Length of exposure (days)	Parameters affected	Percent of LC50 that affected parameters
Pink salmon[a,b] (fry)	WSF	40	Growth	30
	Naphthalene	40	Growth	28
	WSF	4	Opercular rhythm	20
Pink salmon[c] (alevins)	WSF	10	Growth	10
Coho salmon[d]	Toluene	40	Growth	50
	Naphthalene	40	Growth	32
Seastar[e] (*Evasterias troschelii*)	WSF	28	Feeding rate	28
	WSF	28	Growth	24
File periwinkle[f] (*Thais lima*) (now called *Nucella lima*)	WSF	28	Scope for growth	18
Blue mussel[g,h] (adult)	WSF	28	Scope for growth	19
		28	Byssal thread extrusion	15
Blue mussel[i]	WSF	40	Growth, development	61

[a] Moles and Rice 1983.
[b] Thomas and Rice 1979.
[c] Rice et al. 1975.
[d] Moles et al. 1981.
[e] O'Clair and Rice in press.
[f] Stickle et al. 1984.
[g] Stickle et al. in press.
[h] Babcock et al. in preparation.
[i] O'Clair and Rice in preparation.

of coho salmon fry in fresh water and pink salmon fry in seawater was decreased at naphthalene exposures as low as 32% of the 4-d LC50. At about 30% of the 4-d LC50, metabolic rates of pink salmon fry increase (Thomas and Rice 1979). The increase in energy demand during hydrocarbon exposures at ≥30% of the LC50 probably causes energy reserves to be shunted from growth to hydrocarbon metabolism and excretion. Sublethal concentrations that measurably reduce growth range from 10% of the LC50 for pink salmon (Rice et al. 1975) to 61% of the LC50 for blue mussels (O'Clair and Rice in press).

Measurement of energy utilization and partitioning of energy to different biological functions can be used to detect sublethal responses. The energy available for growth (scope-for-growth) is determined from caloric intake less the body-maintenance costs. Scope-for-growth of the carnivorous periwinkle Thais lima declined to zero when these snails were exposed to 18% of the 28-d LC50 (Stickle et al. 1984). Similarly, scope-for-growth in blue mussels was greatly reduced in the latter part of a 28-d experiment at 20% of the LC50 (Stickle et al. in press).

Declining scope-for-growth is the net effect of hydrocarbons on several processes, but the primary cause of reduced scope-for-growth is reduced feeding rates (Stickle et al. 1984, in press). Concentrations that affected feeding rates were much lower than concentrations that affected the other factors (Stickle et al. 1984, in press). A similar pattern was found with the starfish Evasterias troschelii (Fig. 6; O'Clair and Rice in preparation-a). Feeding rates were significantly reduced at 34% of the LC50, whereas gonad and hepatic indices were affected at higher concentrations.

Growth beyond a critical size is important for survival in some organisms such as juvenile salmonids. Fish that are smaller because of sublethal exposures to petroleum hydrocarbons (Fig. 7) would likely suffer increased predation, and recruitment into the fishery would be reduced (Rice et al. 1975).

Stress caused by exposure to sublethal concentrations of hydrocarbons also changes respiration and metabolic rates. The breathing rate of pink salmon fry exposed to the WSF of Prudhoe Bay crude oil increased at approximately 20% of the 96-h LC50 (Thomas and Rice 1975) and was matched by similar increases in oxygen consumption (Thomas and Rice 1979). Both breathing rate and oxygen consumption of pink salmon fry depend on the concentration of toxicant and reflect increased energy demands resulting from increased tissue concentrations of toxicants.

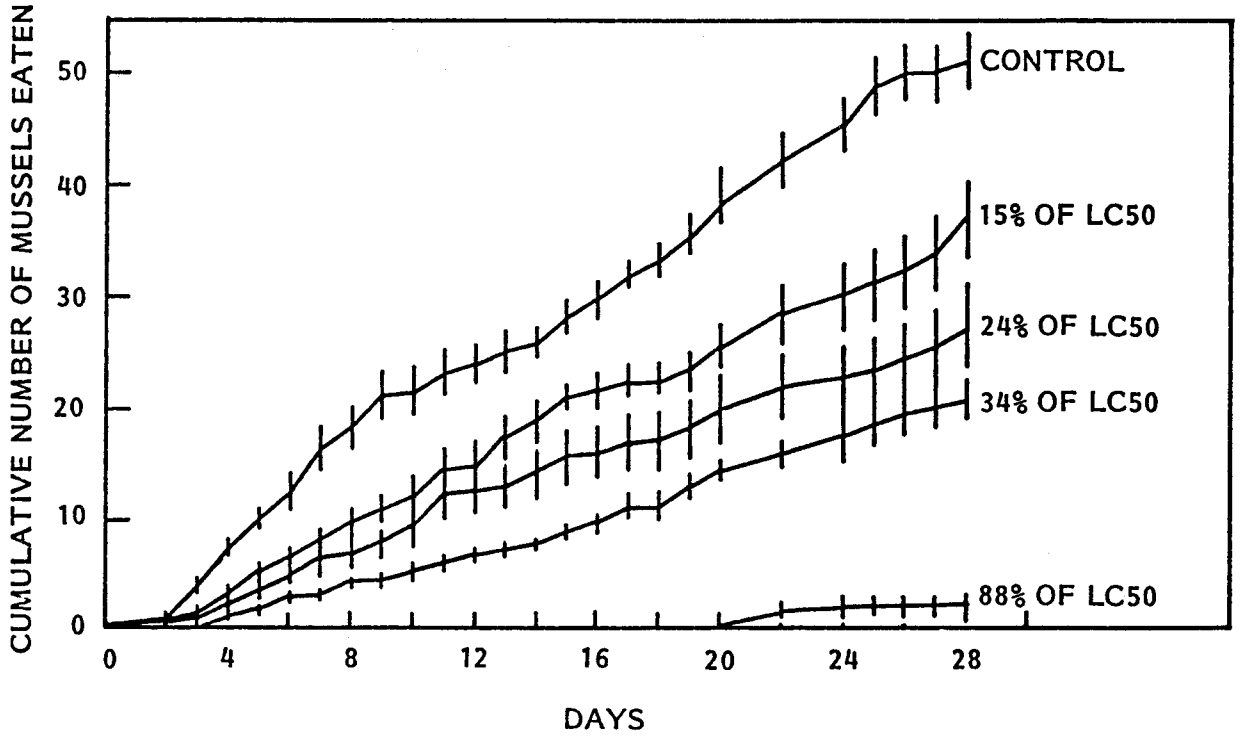

Figure 6.--Cumulative number of mussels eaten by Evasterias troschelii exposed to four concentrations of the water-soluble fraction of Cook Inlet crude oil. Numbers to the right of the curves are percentages of the LC50 for Evasterias troschelii exposed to the water-soluble fraction of Cook Inlet crude oil. Error bars are one standard error of the mean (O'Clair and Rice in press).

Unlike fish, some invertebrates have decreased vital functions when exposed to petroleum hydrocarbons. For example, king crabs exposed to benzene, naphthalene, and the WSF of Cook Inlet crude oil have decreased oxygen consumption and reduced heart rates (Mecklenburg et al. in preparation).

Changes in behavior can be used to detect effects of sublethal concentrations of hydrocarbon. For example, crustacean larvae are unable to swim after a 20-min exposure to WSF but may not die for many days (Brodersen in preparation). Mobile species may avoid petroleum hydrocarbons. Pink salmon fry detected and avoided very low concentrations of Prudhoe Bay crude oil in laboratory conditions (Rice 1973); however, in the natural environment, some fish, such as salmon, may be genetically motivated to migrate along a specific path, even if the path goes through a polluted area. Furthermore, pink salmon fry exposed to benzene had damaged olfactory epithelium (Babcock in press).

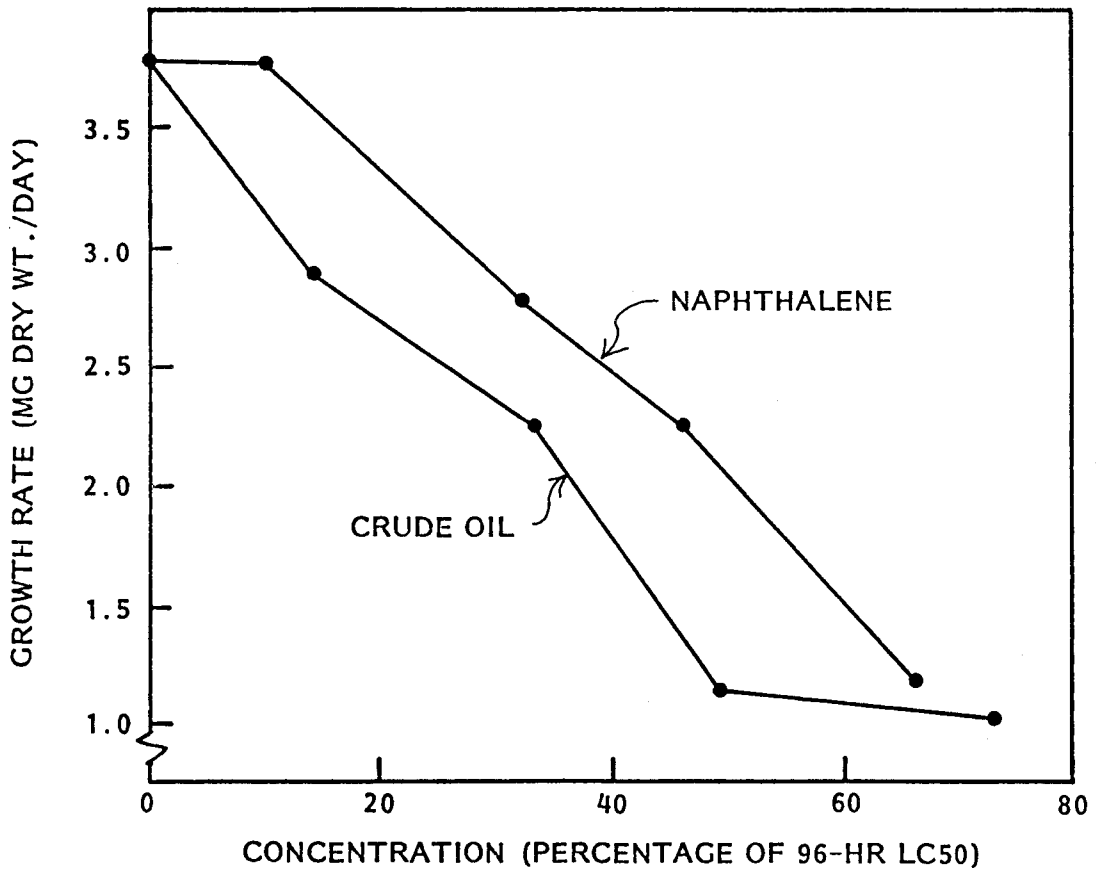

Figure 7.--Growth of pink salmon juveniles per day during a 40-d exposure to concentrations of naphthalene and the water-soluble fraction of Cook Inlet crude oil. Concentrations are expressed as a percentage of the 96-h LC50 (Moles and Rice 1983).

The rate at which blue mussels produce byssal threads is immediately reduced after they are exposed to sublethal concentrations of hydrocarbons (Table 15; Faris et al. in preparation). Byssal thread production, however, has only limited value as a monitoring tool because affected animals start extruding byssal threads immediately after they are returned to clean water. The rate byssal threads are produced thus reflects only current concentrations of hydrocarbons (Babcock et al. in preparation).

Low levels of crude-oil contamination reduce fish and invertebrate populations by reducing reproduction either by causing malformed gonads and gametes or by decreasing the energy available to the organism to contribute to growth or gamete production. Reproduction, like other growth processes, would

Table 15.--Cumulative number of byssal threads produced by blue mussels exposed to the water-soluble fraction of Cook Inlet crude oil (Faris et al. in preparation).

Exposure concentration	Number of threads			
	24 h	48 h	72 h	96 h
Control	16	24	29	37
0.19 ppm	12	19	23	27
0.63 ppm	3	9	15	18
2.34 ppm	0	1	5	12

be affected at hydrocarbon concentrations well below those that affect long-term survival of the animals. At the Auke Bay Laboratory, we are currently determining the effects of petroleum hydrocarbons on the maturation success of gametes in crab and shrimp and the effects of these compounds on the transition of male shrimp into female shrimp.

In conclusion, long-term exposures (30-40 d) to sublethal concentrations of petroleum hydrocarbons (20-30% of the long-term LC50) affect the physiology of most species, reduce growth and reproduction, and change behavior. Exposures longer than 30-40 d to concentrations lower than 20-30% of the long-term LC50 may also have deleterious effects.

STUDIES OF TREATED BALLAST WATER AT PORT VALDEZ

Ballast water containing crude oil is pumped ashore from tankers at Port Valdez where it is treated to remove hydrocarbons before being discharged into Port Valdez. The treatment process is extremely effective and removes more than 99% of the hydrocarbons, but because of the enormous volume of effluent (10-20 million gal/d [38-75 million L/d]), substantial quantities of hydrocarbons are discharged into Port Valdez. The effluent contains 8-15 ppm oil, mostly aromatic hydrocarbons. Research to determine the effects of the effluent on the marine environment of Port Valdez and Prince William Sound consisted of several separate but related studies.

Onsite Laboratory Tests of Treated Tanker-Ballast Water

The toxicity of effluent to several species of fish and invertebrates was studied in a mobile laboratory at the Port Valdez tanker terminal (Rice et al. 1981). Continuous samples of treated ballast water were obtained for toxicity tests by tapping directly into the pipe discharging effluent from the treatment plant. Different larval stages of king crab, Dungeness crab (Cancer magister), coonstripe shrimp, and Pacific herring (Clupea harengus pallasi) were used for the tests, as well as pink salmon fry and adult kelp shrimp.

During the tests, the concentrations of aromatic hydrocarbons in the effluent varied considerably daily; however, they generally declined from >15 ppm to <2 ppm between April and July 1980 (Fig. 8).

Larval stages of shrimp and crab were more sensitive to the effluent than juveniles or adults. All larvae tested in 96-h static tests had LC50's at concentrations between 2% and 26% dilution of the effluent, and affected larvae ceased swimming within 10 min of exposure.

The LC50's for pink salmon fry and adult kelp shrimp in repeated, continuous-flow 48-h and 96-h tests were consistently at 19-43% dilutions of the effluent (Fig. 8). Although the concentration of aromatic hydrocarbons decreased during the study, the toxicity of the effluent remained fairly constant. The 8-h LC50 did not change (Fig. 8); therefore, contaminants other than aromatic hydrocarbons contributed to the toxicity of the effluent. Oxidation products of aromatic hydrocarbons, heavy metals, or hydrogen sulfide are likely possibilities.

In Situ Studies: Survival of Caged Animals in Port Valdez

Survival of caged animals was determined at various distances from the treated ballast-water diffuser in Port Valdez (Karinen et al. in prep.-a). Blue mussels, pink salmon fry, and kelp shrimp were suspended in cages 50 m from the surface and 2 m off the bottom at sites either in the plume of effluent extending from the diffuser or at two control sites. All animals were exposed for 8 d; some blue mussels were exposed for 30 and 90 d. Tissues from test animals were sampled to determine hydrocarbon concentrations.

Less than 10% of the animals died, and their deaths were not attributable to hydrocarbons. After 8 d, low concentrations of aromatic hydrocarbons were detected only in mussels at the two stations nearest the diffuser. Apparently,

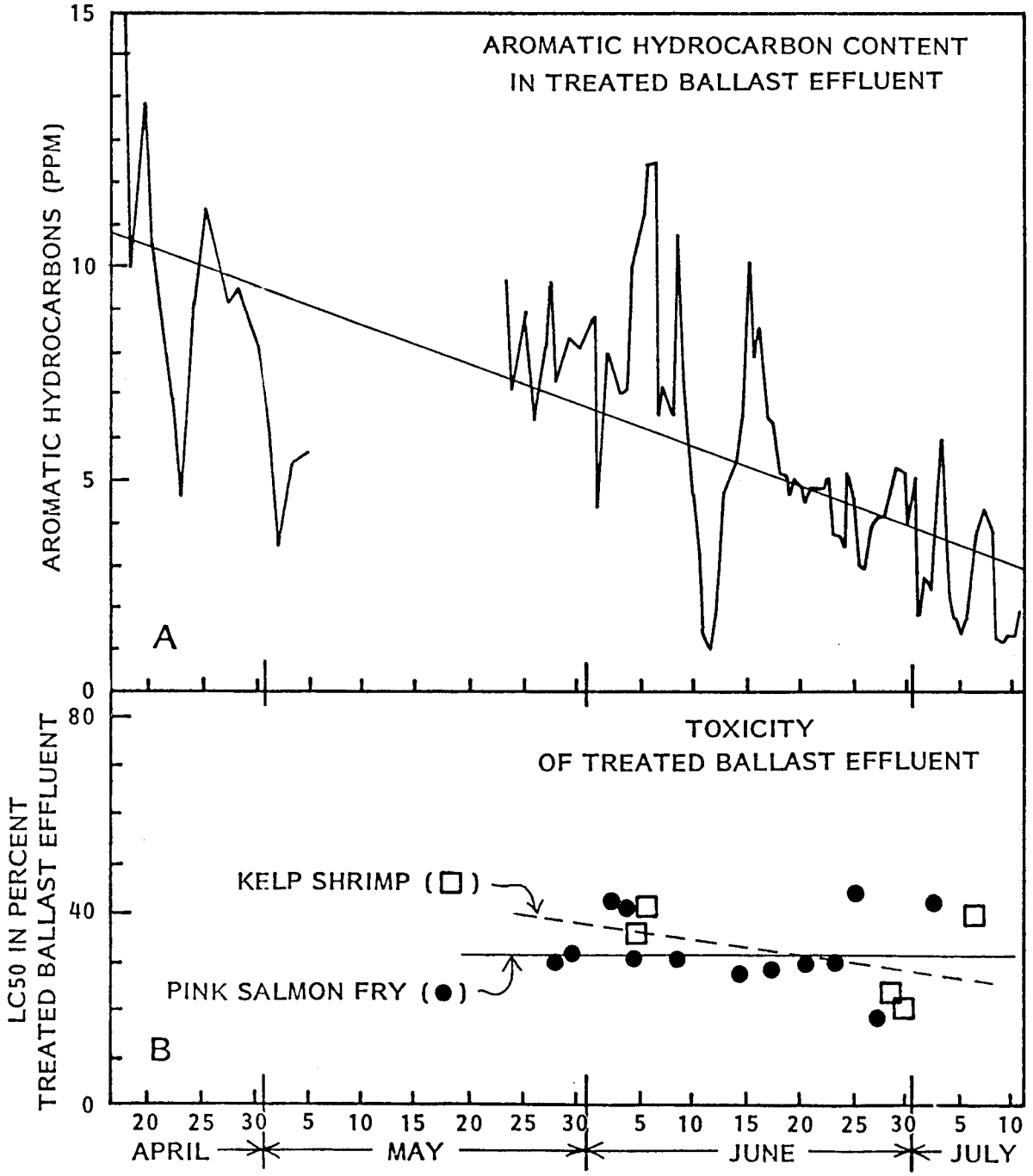

Figure 8.--(A) Daily concentration of aromatic hydrocarbons (monoaromatic and diaromatic) in the effluent from the ballast-water treatment plant at Port Valdez (17 April through 11 July 1980) as determined by gas chromatography. (B) Toxicity of treated ballast water to kelp shrimp and pink salmon fry. Tests were repeated periodically, and 96-h LC50's are expressed as a percent dilution of the treated ballast water (Rice et al. 1981.)

the effluent is diluted at middepths as it extends horizontally from the diffuser.

Hydrocarbons in the Intertidal Environment of Port Valdez

Hydrocarbon concentrations in sediment, water, and tissues of blue mussels and yellowfin sole (Limanda aspera) were monitored annually from 1977 to 1980 (Karinen et al. in preparation-b). Two sites in Port Valdez and eight sites in Prince William Sound were sampled. Intertidal sediments at four sites (Mineral Creek, Dayville Flats, Constantine Harbor, and Rocky Bay) contained detectable but low concentrations of polyaromatic hydrocarbons; however, there is no conclusive evidence that the treated ballast water was the source of hydrocarbons at any of the sites.

Long-Term Monitoring of Intertidal Clam Populations in Port Valdez

The population of Baltic clams on the Dayville Flats, a mud flat 4 km from the tanker terminal, was measured quantitatively for 12 yr, 1971-82 (Myren and Pella 1977; Myren and Perkins in preparation). During the first 9 yr of the study, 6 yr before and 3 yr after the tanker terminal began operation, the Baltic clam population was remarkably constant. During the most recent 3 yr, however, the number of Baltic clams declined (Fig. 9).

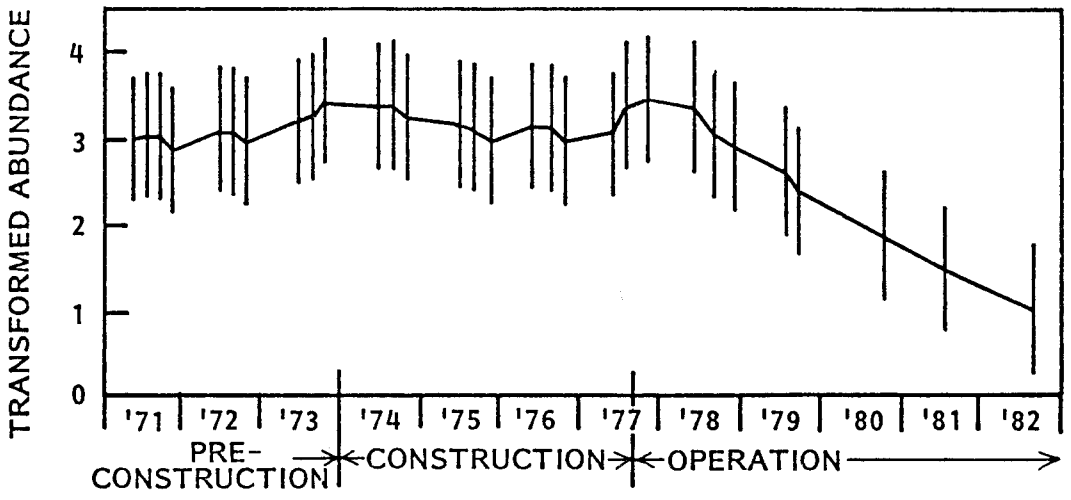

Figure 9.--Abundance of large Baltic clams from the Dayville mudflats of Port Valdez (average of square-root transformed numbers per 100 cm² sample area) (Myren and Pella 1977; Myren and Perkins in preparation).

A slight increase in the concentrations of aromatic hydrocarbons in sediments at Dayville Flats (Karinen et al. in preparation-b) has been measured coincident with the decline in the Baltic clam population. The increase in aromatic hydrocarbons is, therefore, suspected to be associated with the decrease in Baltic clam population.

TOXICITY OF DRILLING MUDS

The mud used during drilling of oil wells is a potential source of pollution because large quantities of drilling muds are discharged into the ocean from offshore drilling rigs. Carls and Rice (1984) determined the tolerances of Stage I planktonic larvae of three shrimp and three crab species to six water-based drilling muds. Five of these muds contained ferrochrome lignosulfonate. Because toxicity of drilling muds is a combination of physical and chemical toxicities, the toxicity of some of its components (the WSF of the muds, ferrochrome lignosulfonate, and the particulates barite and bentonite) was also determined.

The toxicity of the drilling muds depends on the composition of the mud and the species tested, and LC50's ranged from 0.9% to 38% (vol/vol). The mud in which particulates remained suspended throughout the test period (Cook Inlet mud) was the most toxic. In general, particulates quickly settled out of suspension, and toxicity was usually low because larvae were only briefly exposed to physical stress. The WSF's of the muds are toxic but much less toxic than whole muds (Table 16), and ferrochrome lignosulfonate accounts for most (46-100%) of the toxicity of the WSF. The toxicity of barite and benton-

Table 16.--Tolerance of shrimp and crab larvae to suspensions and water-soluble fractions (WSF) of Cook Inlet mud (Carls and Rice 1984).

	144-h LC50 (% vol/vol)	
Species	Complete mud	Mud WSF
King crab	0.48	3.34
Dungeness crab	0.20	1.41
Kelp shrimp	0.44	0.47
Dock shrimp	0.05	0.3

ite is low, and alkalinity of only one mud was beyond the pH tolerance limits of the larvae.

Mud solutions inhibit larval swimming only after 1-2 d of exposure; thus, the toxicity of drilling muds is probably more physical than chemical (Fig. 10). In contrast, petroleum hydrocarbons inhibit larval swimming of the same species within minutes after exposure (Rice et al. 1981). Drilling muds contaminated with petroleum hydrocarbons from oil-bearing formations might be more toxic than the muds we tested. Conversely, small quantities of hydrocarbons could be adsorbed on mud particulates and would not be as toxic as comparable concentrations of hydrocarbons in the WSF of crude oil.

Because the toxicity of drilling muds to crustacean larvae is primarily physical, drilling muds discharged into the marine environment probably would not damage planktonic and nektonic communities under most conditions. Toxic

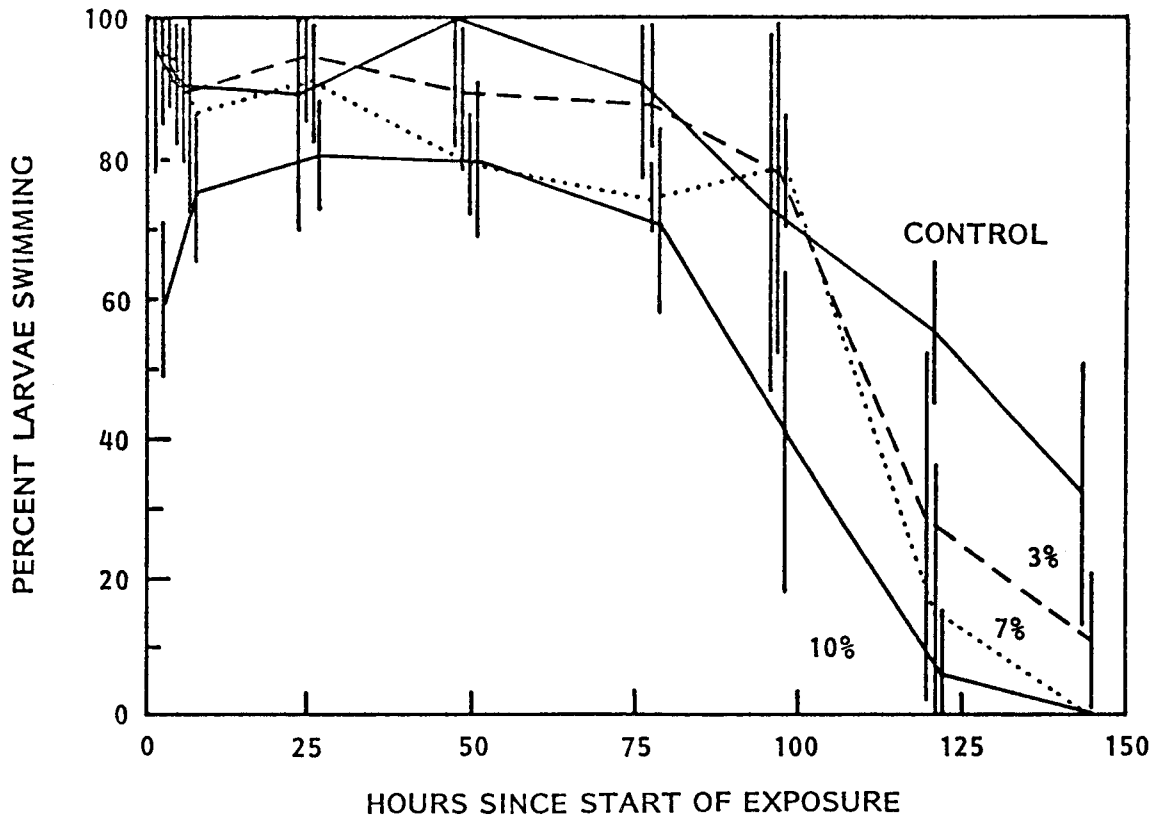

Figure 10.--Inhibition of swimming for larval king crab exposed to different concentrations of used Homer drilling mud. Vertical bars indicate the 95% confidence intervals for the sample means (Carls and Rice 1984).

concentrations of drilling muds are only briefly in the water column and are limited to the immediate area of discharge.

ECOLOGICAL IMPLICATIONS OF THE STUDIES

What will happen if oil is spilled into the marine environment? This question is frequently asked by managers but is seldom answered because the answer is complex, requires massive funding if the research is to be done at the site of an oil spill, and then, unfortunately, may not be useful because each oil spill is unique.

Laboratory studies are cost effective and have contributed to the data base that supports guesses and predictions. Laboratory studies on the effects of oil have identified species, life stages, and biological processes (e.g., growth and molting) that are sensitive, as well as identified environmental factors that affect the toxicity of oil (e.g., temperature) or the sensitivity of organisms (e.g., salinity).

In laboratory studies, one or more variables are tested and the effects of these variables determined. Some of the effects found in these studies, however, may never be observed after an oil spill. For example, in laboratory experiments sublethal concentrations of oil can decrease growth; however, the same concentrations in an actual oil spill would result in the animals being eaten or cause them to migrate to areas of lower oil concentrations. In either case, reduced growth would not be observed.

Although a test animal is sensitive to aromatic hydrocarbons in oil, it may not be vulnerable to an oil spill. For example, fish are sensitive to oil but can swim away from an oil spill and are not vulnerable. In contrast, shrimp larvae are vulnerable because they are very sensitive to oil and cannot move from a spill area. Intertidal species are less sensitive to oil than shrimp larvae and fish, but if crude oil comes ashore, most cannot move out of their contaminated habitat. Consequently, intertidal species are trapped but could survive through adaptive tolerance.

Some habitats, like some species, are more vulnerable than others to spilled oil. Oil spills offshore are rapidly diluted and dispersed, and no apparent lasting effects can be measured. In contrast, spills within confined bays or spills that wash ashore onto marshes or wetlands can have long-term

effects. Marshes and wetlands may erode or the contamination may last for several years and make the habitat unsuitable. Benthos may accumulate hydrocarbons over many years so that even the ocean bottom is vulnerable (Karinen 1980).

Effects of many interacting variables in nature are not quantitatively known. Some pairs of variables (salinity and concentration of oil or effects of two oil components) have been measured in the laboratory, but the complex interaction of many variables is poorly understood. Generally, we know whether a variable will increase or decrease the toxicity of oil, but not the magnitude, especially when the variable is interacting with other variables.

Oil spills can damage organisms if oil concentrations are high enough and the organisms are quickly affected. We have observed short-term toxicity of oil at about 1-3 ppm, concentrations that would briefly occur after oil is spilled and mixed in confining bays.

Fish and crustacean larvae would most likely be affected by an oil spill. Fish respond quickly and are sensitive to concentrations of oil and its components in the low parts per millions. Crustacean larvae are even more sensitive to oil and its components than fish: EC50's and LC50's are between 0.2 and 0.7 ppm. Furthermore, crustacean larvae are more quickly affected (10-30 min) by oil exposure than fish and cannot move away from a spill area.

Long-term exposures at about 20% of the short-term LC50 can affect respiration, feeding rate, and growth. If an organism stays in the contaminated area, exposure to 0.1-0.2 ppm crude oil for 30 to 40 d could be harmful, particularly to invertebrates. Oil concentrations at 0.1-0.2 ppm can be expected after an oil spill but are not sustained unless oil is being continuously added to the environment. Longer exposures to even lower concentrations of oil could affect growth, maturation, and reproduction; however, the length of time and the oil concentrations required are unknown.

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THOMAS, R. E., and S. D. RICE. In preparation-a. Effect of environmental temperature on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden, Salvelinus malma.

THOMAS, R. E., and S. D. RICE. In preparation-b. Effect of salinity on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden char, Salvelinus malma.

APPENDIX A.
ABSTRACTS OF ALL PAPERS ON OIL-EFFECTS RESEARCH AT THE AUKE BAY LABORATORY, 1970-81

Babcock, M. M.

In press. Morphology of olfactory epithelium of pink salmon, Oncorhynchus gorbuscha, and changes following exposure to benzene: a scanning electron microscopy study. In J. S. Gray and M. E. Christiansen (editors), Marine biology of polar regions and effects of stress on marine organisms. John Wiley & Sons, London.

The pink salmon fishery is the most valuable fishery in Prince William Sound, Alaska (USA), and there is great concern that this resource may be damaged by oil pollution from tankers or discharges from a ballast-water treatment plant near Valdez. Because juvenile pink salmon school along shallow estuarine shorelines of Prince William Sound for several weeks before migrating to oceanic feeding grounds, they are vulnerable to oil pollutants from these sources.

Benzene, a major component of crude oil and the effluent from the treatment plant, is water soluble and relatively toxic to fish. To determine the histopathological effects of benzene on olfactory rosettes of pink salmon, I exposed juveniles to sublethal concentrations of benzene in seawater. Fish were exposed in seawater to 4.3 ppm benzene for 12 days or to one of four concentrations ranging from 0.15 to 4.40 ppm benzene for 29 days. (The concentration that killed half the fish in 96-h was 8.47 ppm).

After the exposures, olfactory rosettes from the fish were examined with scanning electron microscopy. Rosettes from all fry exposed to benzene had exhausted mucous cells. The olfactory lamellae of fry exposed to ≥0.51 ppm benzene had altered distribution of cilia. Olfactory lamellae of fish exposed to 4.3 ppm benzene for 12 days had patchy losses of cilia; olfactory lamellae of fish exposed for 29 days to concentrations of benzene ≥0.51 ppm had a generalized loss of cilia. These differences in cilia loss may indicate regeneration of cilia or different individual responses to benzene.

Exhausted mucous cells and loss of cilia on the olfactory lamellar surfaces could change circulation of water through the olfactory rosettes or otherwise interfere with normal chemosensory reception and consequently affect homing, traditional migratory patterns, feeding activity, and avoidance of predators.

Babcock, M. M., S. D. Rice, and P. J. Arasmith.

In preparation. Reduced byssal thread extrusion and recovery following long-term exposure of mussels Mytilus edulis to crude oil.

We conducted tests to determine whether byssal thread extrusion by Mytilus edulis is a sensitive indicator of exposure to pollutants. Blue mussels (16.3-25.1 mm) were glued to plates (four per plate), and the plates were stacked in tanks (six plates per tank). Mussels in the tanks were exposed for 28 days or 48 days to seven concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil. Concentrations of aromatic hydrocarbons in the WSF were 0-2.1 ppm. The 28-day LC50 was 1.4 ± 0.3 ppm (mean ± 95% confidence interval). Byssal thread production for each 24-h or 48-h period was inversely proportional to concentration of aromatic hydrocarbons. Byssal thread extrusion rates were reduced at 15% of the 7-day LC50. Mussels exposed to higher concentrations of aromatic hydrocarbons produced only one byssal thread in 24 h and two byssal threads in 48 h. The controls produced 16-21 byssal threads after 24 h or 48 h.

After 28 days, mussels exposed to higher concentrations of the WSF were returned to clean seawater. After 24 h or 48 h in clean seawater, the number of byssal threads was near or greater than number of byssal threads produced by controls even for mussels exposed to concentrations as high as 90% of the 28-day LC50.

Byssal thread production is a very sensitive indicator of exposure to crude oil, but mussels returned to clean water for 1 day after 28-day exposures to the oil WSF produce the same number of byssal threads as controls.

Brodersen, C. C.

In preparation. Rapid narcosis and delayed mortality in larvae of king crab and kelp shrimp exposed to the water-soluble fraction of crude oil.

Larvae of king crab (Paralithodes camtschatica) and kelp shrimp (Eualus suckleyi) were exposed to the water-soluble fraction of crude oil to determine the length of exposure required to kill them. Static bioassay exposure times were from 20 min to 96 h. For larvae of both species, narcosis severe enough to prevent swimming occurred almost immediately, even at low exposure concentrations. Half of the shrimp larvae exposed to 1 ppm of aromatic hydrocarbons for 20 min and half of the crab larvae exposed to 0.5 ppm for 20 min ceased swimming by the end of the exposure. Larvae in the ocean that stop swimming and sink to the bottom are likely to be eaten, injured, or buried; therefore, exposures causing narcosis are likely to be as fatal as longer or stronger exposures that cause death directly. Half of the larvae of either species exposed to 8 ppm of aromatic hydrocarbons for 6 h died, as did half of the larvae exposed to 1-3 ppm for 24 h. In contrast to narcosis, however, death occurred very slowly for exposed larvae. Most larvae did not die until after the exposure had ended.

Brodersen, C. C., and M. G. Carls.

In preparation. Sensitivity of egg, larval, and adult coonstripe shrimp (Pandalus hypsinotus) to long-term exposure to the water-soluble fraction of Cook Inlet crude oil.

Several life stages of coonstripe shrimp (Pandalus hypsinotus) were exposed to the water-soluble fraction (WSF) of Cook Inlet crude oil in continuous-flow exposures. The LC50's of the WSF's were measured in parts per million aromatic hydrocarbons. Gravid females had a 96-h LC50 of 1.4 ppm (range, 1.2-1.5 ppm) and a 24-day LC50 of 0.54 ppm (range, 0.40-0.68 ppm). Adult female shrimp died continuously throughout the exposure period; however, none died when returned to clean water.

Late-stage eggs were exposed during the month before hatching while they were still being carried by the females. The eggs of all surviving females hatched into swimming larvae, and the larvae were physiologically more resistant than females to the WSF. However, the eggs must be considered to have the same LC50's as the females because eggs cannot survive without the females.

Larvae at different stages of development were exposed to a variety of concentrations and exposure periods. Exposures ranged from a minimum of 24 h (timed to fall between molts) to a 30-day exposure that covered three molts. Unlike adults, larvae seemed unaffected by the length of exposure. All tests with larvae gave similar results: 1.3 ppm of aromatic hydrocarbons killed them; 0.6 ppm did not.

Brodersen, C. C., and S. D. Rice.

In preparation. Comparison of static and continuous-flow exposure methods for exposing marine animals to toluene, naphthalene, and the water-soluble fraction of crude oil.

Several species were exposed for 96 h to toxicants in either static or continuous-flow tests. Dead animals were counted after each exposure and after the animals were held in clean seawater. Results from the two types of exposures were compared.

Different species responded differently to the toxicants. Pink salmon fry and kelp shrimp were quickly affected and soon died. Purple shore crabs and Hall's colus (Colus halli) were affected quickly but did not die immediately after being damaged; however, longer exposure periods increased the toxicity of the compounds tested. Blue mussels and tarspot cucumbers (Cucumaria vegae) resisted exposure for the first day and then responded very slowly. Once the mussels and cucumbers were affected, they were even slower to die.

Concentrations of toxicants decreased rapidly in static solutions but remained constant in continuous-flow solutions. In assays with individual hydrocarbons, animals that were quickly affected (pink salmon fry and kelp shrimp) had similar results in the two types of tests, whether dead animals were counted at the end of the exposure or after being held in clean seawater. Results of static tests with the WSF and animals resistant to the toxicants were different from results of flow-through tests with the same toxicants and animals. The proportions of crude oil WSF delivered by the static method and by the continuous-flow method were too different to give similar results even in assays of pink salmon fry and kelp shrimp.

Brodersen, C. C., S. D. Rice, J. W. Short, T. A. Mecklenburg, and J. F. Karinen.

1977. Sensitivity of larval and adult Alaskan shrimp and crabs to acute exposures of the water-soluble fraction of Cook Inlet crude oil. In Proceedings 1977 oil spill conference (prevention, behavior, control, cleanup), p. 575-578. American Petroleum Institute, Washington D.C.

The sensitivity of adult and larval Alaskan shrimp and crabs to the water-soluble fraction (WSF) of Cook Inlet crude oil was measured by tests using 96-h static tests at the water temperatures that these animals normally encounter. Larval crustaceans were found to die more slowly than adults, making it necessary to measure sensitivity in terms of concentrations causing moribundity (death imminent) instead of in terms of concentrations causing death during exposure. The cessation of all motion and reaction indicated moribundity in adults, and the cessation of swimming indicated moribundity in larvae exposed for 96 h. The 96-h LC50's for moribundity for Stage I larvae ranged from 0.95 to 1.8 ppm, depending on species, whereas 96-h LC50's for adults ranged from 1.9 to 4.2 ppm oil. Sensitivities for Stages I-VI larvae of coonstripe shrimp ranged between 0.24 and 1.9 ppm.

Larvae were more sensitive to oil than adults. The sensitivity of larvae depended on species and developmental stage. Larvae are probably more vulnerable than adults to oil exposure because of greater sensitivity to oil and greater susceptibility to predation. Coldwater species may be particularly vulnerable because of increased time spent as developing larvae.

Carls, M. G., and S. Korn.

In press. Sensitivity of arctic marine amphipods and fish to petroleum hydrocarbons. Can. Fish. Aquat. Sci. Tech. Rep.

We tested the sensitivities of several arctic marine species to petroleum hydrocarbons and compared them to the sensitivities of temperate species previously tested using the same flow-through procedures and toxicants. We restricted our comparisons between arctic and temperate species to experimental data collected within our laboratory in order to avoid the problems caused by variations in techniques and toxicants which plague oil toxicity research.

We examined two alternative hypotheses: 1) marine arctic animals, adapted to a wide range of environmental parameters, are unusually resistant to unaccustomed stresses such as petroleum hydrocarbons; or 2) marine arctic animals are unusually sensitive to hydrocarbon stress because they are already stressed to their limits by the environment in which they live.

We determined the sensitivities to WSF's of Cook Inlet crude oil or naphthalene for six circumpolar benthic species: The amphipods Anonyx nugax, Boeckosimus nanseni, and Gammaracanthus loricatus; a mysid, Mysis relicta; Arctic cod, Boreogadus saida; and a sculpin, Oncottus hexacornis. Exposures were flow-through and lasted up to 40 days. Median lethal concentrations (LC50's) of the WSF ranged from 1.6 to 3.8 ppm total aromatics. Naphthalene assays were conducted at several different temperatures (ranging from 1.5 to 9.6°C) to study temperature effects on sensitivity to hydrocarbons. Upper lethal temperatures for the amphipods and mysid were surprisingly high: 17-24°C, suggesting the assay temperatures in themselves were not particularly stressful. Naphthalene LC50's ranged from 1.35 to 3.35 ppm. General relationships between exposure temperatures and LC50's were not found.

We conclude that arctic species are about equal in sensitivity to temperate species. However, their habitat is more vulnerable to the effects of petroleum hydrocarbon pollution than temperate habitats because low temperatures lead to slower losses of hydrocarbons from volatilization and biodegradation, and oil entrapment under sea ice can result in very lengthy exposures. Once physical or chemical perturbations have caused damage to habitat and decreases in animal populations, recovery and re-establishment of communities may be slow because of low productivity, low species diversities, and slow growth rates (Dunbar 1968; Grainger 1975; Wacasey 1975).

Carls, M. G., and S. D. Rice.

1984. Toxic contributions of specific drilling mud components to larval shrimp and crabs. Mar. Environ. Res. 12:45-62

We investigated the toxicities of six drilling muds, toxicities of mud fractions (supernatants and suspensions), and the toxicities of common mud components--barite and bentonite (particulates) and ferrochrome lignosulfonate (soluble)--to the stage I larvae of six species of shrimp and crab. The drilling muds we tested were not very toxic to these larvae: LC50's for supernatants ranged from 0.6 to 82% (vol/vol). Shrimp larvae were slightly more sensitive than crab larvae.

Drilling muds were not rapidly toxic, in contrast to toxicants such as the water-soluble fractions of oil. Supernatants, prepared by centrifuging whole muds, were mildly toxic. Suspensions were more toxic than supernatants, and toxicity was greatest when particulates remained suspended: for example, used Cook Inlet mud suspensions were about seven times more toxic than supernatants. The toxicity of used Cook Inlet mud was therefore primarily due to suspended solids (88%) rather than chemical toxicity: ferrochrome lignosulfonate was relatively toxic alone but accounted for only about 6% of the toxicity of used Cook Inlet mud suspensions. Contributions of particulates to mud toxicities varied considerably. Barite and bentonite were not very toxic when tested alone. The toxicity of one mud was caused by its high alkalinity.

Carls, M. G., and S. D. Rice.

In preparation. Comparative stage sensitivities of walleye pollock, Theragra chalcogramma, to external hydrocarbon stressors.

Fish larvae are often more sensitive to petroleum hydrocarbons than eggs, yet sensitivity is usually very early in development when damage to a few precursor cells results in extensive damage as the embryo develops. In our studies with walleye pollock (Theragra chalcogramma) embryos, concentrations of petroleum hydrocarbons in tissues were lower before hatching than after hatching. With concentrations adjusted to tissue levels, the developing embryos were more sensitive to hydrocarbons before hatching if concentrations of hydrocarbons were measured as tissue concentrations.

Eggs exposed to hydrocarbons had slower development, greater mortality, more variable hatching time, and the resulting larvae were shorter and had more morphological abnormalities than controls. Larvae exposed to the hydrocarbons did not have morphological abnormalities; however, the concentration that killed half of the larvae was the same as the concentration that produced abnormalities when embryos were exposed. Surprisingly, exposures that began at fertilization and lasted for the 2-h water-hardening period had no discernible effects.

The deformed larvae would probably have lower survival in nature because they would have difficulty feeding and avoiding predators.

Cheatham, D. L., and S. D. Rice.

In preparation. The relative importance of evaporation and biodegradation temperature on the loss of some mononuclear and dinuclear aromatic hydrocarbons from seawater.

The seawater-soluble fraction (WSF) of Cook Inlet crude oil was held at 5°, 8°, and 12°C for 96 h. During the 96 h, samples of the WSF were analyzed by gas chromatography to determine the effect of temperature on evaporation and biodegradation of mononuclear and dinuclear aromatic hydrocarbons. We separated losses from evaporation and biodegradation by aerating some solutions and by killing microorganisms in others.

At lower temperatures, mononuclear aromatic hydrocarbons were primarily lost through evaporation rather than biodegradation. Biodegradation, however, was important in the loss of dinuclear aromatic hydrocarbons, particularly naphthalene. Although aromatic hydrocarbons evaporate or are biodegraded at low temperatures, oil and seawater mixtures could be more toxic longer at lower temperatures because aromatic hydrocarbons would persist longer.

Evans, D. R., and S. D. Rice.

1974. Effects of oil on marine ecosystems: a review for administrators and policy makers. U.S. Natl. Mar. Fish. Serv., Fish. Bull. 72:625-638.

A broad selection of recent literature on the effects of oil on marine ecosystems is reviewed. The focus is on studies on crude oil, and the results are discussed with the purpose of providing a summary of findings that will be a useful reference for administrators and policy makers involved in decisions concerning petroleum developments and related activities. The characteristics of crude oil and factors modifying its impacts on the marine environment are discussed. Most research on the toxicity of oil has dealt with acute effects, and data on long-term impacts at the community level are inconclusive. It is concluded that chronic low-level pollution is potentially more damaging to ecosystems than isolated catastrophic spills. Decision makers are forced to rely on interpretative judgments rather than conclusive data.

Faris, T. L., S. D. Rice, and M. M. Babcock.

In preparation. Reduced byssal thread extrusion by the mussel Mytilus edulis during short-term exposure to toluene, naphthalene, and the water-soluble fraction of crude oil.

To determine whether byssal thread production by blue mussels is an indicator of sublethal response to hydrocarbons, we exposed these animals to five concentrations each of naphthalene, toluene, and the water-soluble fraction of Cook Inlet crude oil and No. 2 diesel fuel in a series of 96-h exposures. In previous studies, byssal thread production was enhanced at concentrations lower than those that were inhibitory. At 24, 48, 72, and 96 h, cumulative number of byssal threads produced was significantly depressed in all but the lowest doses of toxicant. After 24 h of exposure, however, the number of new byssal threads extruded during a 24-h period was the similar to the number extruded by controls. By 72 h, toxicant concentrations decreased because of evaporation, and the rate byssal threads were extruded increased and sometimes exceeded the control rate.

Gharrett, J. A., and S. D. Rice.

In preparation-a. Temperature modification of uptake and depuration of two petroleum hydrocarbons in four marine species.

Acute toxicity, accumulation, and depuration of naphthalene and toluene at 4° and 12°C were determined for pink salmon fry, the purple shore crab, a subtidal snail Colus jordani, and kelp shrimp. All tests were continuous flow, and tissues or whole animals were sampled as appropriate.

The effect of temperature on sensitivity of animals varied with species and toxicant. Snails and kelp shrimp were more sensitive to both toxicants at 12° than at 4°C. Purple shore crabs, however, were more sensitive to naphthalene at 4°C than at 12°C. Sensitivity to toluene was similar at both temperatures. Pink salmon fry were more sensitive to both toxicants at 4°C than at 12°C.

Temperature did not affect the amount of toluene accumulated in any tissues, except crab gills, or the amount depurated by any species. Three to four times more toluene was accumulated in crab gills at 12° than at 4°C.

In some cases, temperature affected the uptake of naphthalene. Tissues of snails and salmon fry initially took up naphthalene more rapidly at 12°C, but the total concentration accumulated was higher at 4° than at 12°C. The reverse was true for purple shore crabs and kelp shrimp, which accumulated more naphthalene at 12°C than at 4°C. Temperature did not affect depuration of naphthalene by any species or tissue.

Regardless of temperature, naphthalene was always more toxic than toluene and accumulated to higher concentrations. The rates of depuration were also similar for the two toxicants, regardless of temperature.

Gharrett, J. A., and S. D. Rice.

In preparation-b. Intermittent aerial exposure and the toxicity, uptake, and depuration of aromatic hydrocarbons in the purple shore crab Hemigrapsus nudus.

The purple shore crab was used to determine the relationship between intermittent aerial exposure and toxicity, accumulation, and depuration of two aromatic hydrocarbons, toluene and naphthalene. Three tidal regimes were studied in which crabs spent 0, 33, or 66% of the time aerially exposed and the remainder of time in different concentrations of toxicant solutions.

Aerial exposure significantly affected sensitivity, bioaccumulation, and depuration of toluene and naphthalene. Because the crab only accumulated toxicants during immersion, sensitivity was greatest for crabs that were immersed longest.

Naphthalene was more toxic than toluene in all exposure regimes. Tissue concentrations of naphthalene increased faster and, at the end of the exposures, were 10 times higher than tissue concentrations of toluene. The LC50 for naphthalene was one tenth that for toluene whether effects were measured by the number of dead crabs, the number of crabs with locomotor abnormalities, or exposure time before death. Depuration was greatest in crabs immersed longest in clean water; therefore, recovery potential was reduced in aerially exposed crabs that already had high concentrations of hydrocarbons in their tissues.

Significantly higher respiration rates of crabs in air (as opposed to rates for the same crabs in water) indicated that reduced accumulation and depuration with aerial exposure was not due to a general metabolic quiescence.

Karinen, J. F.

1977. Assessing oil impacts with laboratory data application, limitations, and needs. In B. Melteff (editor), Oil and aquatic ecosystems, tanker safety and oil pollution liability, Proceedings of the Cordova Fisheries Institute, p. 99-110. University of Alaska, Fairbanks, Sea Grant Rep. 77-8.

This paper outlines some of the problems associated with the utilization of laboratory data to assess the impact of oil in the environment and some of the considerations which we should make in trying to apply the toxicity and sublethal effects data. In applying these results to the environment to determine what the actual effects of oil exposure will be, there are both biological as well as chemical considerations which we must take into account. This paper deals more with the chemical aspects rather than the biological aspects (although I fully realize the great importance of the biological relationships) and is limited to a consideration of the behavior of oil in water and some chemical factors influencing the application of laboratory effects data toward assessing oil impacts.

Karinen, J. F.

1980. Petroleum in the deep sea environment: potential for damage to biota. Environ. Int. 3:135-144.

Information on the fate, persistence, and biological impact of petroleum hydrocarbons in shallow marine environments, coupled with recent data on hydrocarbons in offshore sediments and the biology of deep-sea organisms, have provided new perspectives on the potential impact of oil on the deep-sea environment. A review of literature on petroleum hydrocarbons in deep-sea sediments, mechanisms for transport of petroleum to the deep-sea floor, interaction of petroleum hydrocarbons and particulate matter, and physiology and metabolism of deep-sea fish and crustaceans has resulted in the following conclusions:

1. Hydrocarbons of apparent anthropogenic origin are accumulating in bottom sediments of coastal margins and in deeper offshore waters at unknown rates.

2. Several mechanisms exist for the rapid transport of petroleum hydrocarbons to the deep-sea floor.

3. Petroleum hydrocarbons are intimately associated with particulate matter in the sea and behave much the same as natural biogenic material and have the potential to modify or interrupt natural processes.

4. The unique physiology of deep-water life forms increases the potential for adverse impact of petroleum hydrocarbons in the deep-sea environment.

5. There is a need to determine trends of temporal and spatial deposition of hydrocarbons in deep-sea sediments and evaluate the biological impact of this introduction of xenobiotic compounds on the largest environment on earth.

Karinen, J. F., and S. D. Rice.

1974. Effects of Prudhoe Bay crude oil on molting Tanner crabs, Chionoecetes bairdi. U.S. Natl. Mar. Fish. Serv., Mar. Fish. Rev. 36(7):31-37.

Pre- and post-molt juvenile male Tanner crabs, Chionoecetes bairdi, from Alaskan waters were exposed to Prudhoe Bay crude oil in static tests in the laboratory. Crabs in both stages were similarly susceptible to crude oil; the estimated 48-h median tolerance limits values were 0.56 ml oil/liter. Molting success decreased with increasing exposure of crabs to oil, and newly molted crabs autotomized limbs during exposure to oil. Relating the results of our study to the known behavior of crabs and the documented behavior of oil spills in the ocean suggests that oil spilled in Alaskan waters would harm the Tanner crab resources. The impact on all crab resources of chronic low-level oil pollution from the ballast water discharged into Prince William Sound is unknown. This study further illustrates our present state of ignorance concerning the biological effects of oil in the marine environment.

Karinen, J. F., G. Perkins, R. T. Myren, and W. D. MacLeod, Jr.

In preparation-a. Survival and uptake of hydrocarbons by mussels, pink salmon fry, and kelp shrimp caged near the treated-ballast water diffuser, Port Valdez, Alaska.

We determined the survival of blue mussels, pink salmon fry, and juvenile kelp shrimp that were caged and suspended in the plume of treated ballast water extending from the diffuser of the ballast-treatment facility at Port Valdez, the terminus of the trans-Alaska pipeline. Salmon fry and shrimp were exposed for 8 days; mussels were exposed for up to 30 days.

Hydrocarbon content of the exposed animals was determined to identify the major toxicants in the effluent and to correlate toxicity with the amount of hydrocarbons in the tissues. We also determined the concentration of aromatic hydrocarbons adsorbed on suspended sediments within and outside the plume by collecting sediment samples from several stations in Port Valdez.

Although some of the caged animals died, no deaths were attributable to hydrocarbons. Concentrations of hydrocarbons greater than background levels were found only in blue mussels from cages closest to the diffuser.

Karinen, J. F., L. S. Ramos, and W. D. MacLeod, Jr.

In preparation-b. Hydrocarbon distribution in the intertidal marine environment of Port Valdez and Prince William Sound, Alaska.

Prince William Sound, in the northern part of the Gulf of Alaska, is an important fishing area of about 2,500 square miles. Two months before the first crude oil was shipped from Port Valdez, the terminus of the Trans-Alaska Pipeline, we began monitoring 10 intertidal sites in Prince William Sound and Port Valdez to determine annual fluctuations in petroleum hydrocarbon content of blue mussels, yellowfin sole, and sediments.

The hydrocarbon content varied depending on the site, the date of collection, and the type of sample. Sediments and biota usually had very low concentrations of petroleum hydrocarbons, except for one harbor site in Port Valdez, and typified the low hydrocarbon concentrations expected in a pristine environment.

Korn, S., and S. Rice.

1981. Sensitivity to, and accumulation and depuration of, aromatic petroleum components by early life stages of coho salmon (Oncorhynchus kisutch). Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178:87-92.

Coho salmon eggs, alevins, and fry were exposed to toluene, naphthalene, and 1-methylnaphthalene (aromatic hydrocarbons found in crude oil) in a series of short-term toxicity and hydrocarbon-uptake studies to determine whether acute toxicity is related to uptake-depuration patterns. Uptake studies used radio-labeled compounds and radiometric analyses. The time to reach maximum tissue concentrations of these hydrocarbons was determined from long-term exposures.

Sensitivity to the aromatic hydrocarbons increased from egg to fry with the greatest increase in sensitivity between the egg and early alevin. The rates of uptake and depuration of the aromatic hydrocarbons also increased during the development from egg to fry. Eggs had the slowest rates of uptake and depuration.

Eggs required 10 days to accumulate stable tissue concentrations of toluene and naphthalene; alevins required 36 h (both toxicants); fry required 3 h (toluene) and 10 h (naphthalene). The rate of uptake and toxicity was higher with increased ring size and increased substitution (2-methylnaphthalene > naphthalene > toluene).

Eggs were more tolerant than alevins and fry to short-term exposures of aromatic hydrocarbons probably because the chorion prevented rapid uptake. The amount of yolk also influenced sensitivity because aromatic hydrocarbons were selectively partitioned into the yolk thus reducing availability of the hydrocarbons to the embryo and resulting in lower toxicity.

Although eggs take up hydrocarbons at a slow rate, they may accumulate lethal levels of hydrocarbons during long-term exposures and, therefore, would be more sensitive to hydrocarbons than indicated by short-term experiments.

Korn, S., S. A. Lindsay, and S. D. Rice.

In preparation. Accumulation and depuration of the water-soluble fraction of Cook Inlet crude oil in eggs and muscle tissue of adult kelp shrimp.

Gravid kelp shrimp were continuously exposed to the water-soluble fraction of Cook Inlet for 10 days. Muscle tissue and eggs were sampled periodically and analyzed for aromatic hydrocarbons by gas chromatography. Muscles and eggs rapidly accumulated hydrocarbons, and maximum concentrations were reached after 3-24 h in muscle and after 48 h in eggs. Concentrations of aromatic hydrocarbons were 10 times greater in the eggs than in muscle. Of the aromatic hydrocarbons found in the eggs and muscle, substituted benzenes had the highest concentration followed by xylenes, toluene, and 2-methylnaphthalene. Phenanthrene was also found but accumulated steadily only after 4 days.

The rate hydrocarbons were depurated varied. Toluene persisted in eggs and muscle, and small amounts were depurated only after 4 days. Other monoaromatic hydrocarbons and naphthalene were eliminated rapidly, and less than 30% remained after 4 days. Methylnaphthalene and methylated benzenes had intermediate rates of depuration.

Exposure to oil probably affects fertilization, development, and hatching of shrimp eggs due to rapid accumulation and slow depuration of certain aromatic hydrocarbons.

Korn, S., D. A. Moles, and S. D. Rice.

1979. Effects of temperature on the median tolerance limit of pink salmon and shrimp exposed to toluene, naphthalene, and Cook Inlet crude oil. Bull. Environ. Contam. Toxicol. 21:521-525.

A series of static 96-h tests at different temperatures was run with toluene, naphthalene, and the water-soluble fraction of Cook Inlet crude oil using pink salmon and Eualus shrimp. The 96-h median tolerance limit (TLm) of pink salmon exposed to toluene was significantly lower at 4° than at 12°C. The 96-h TLm's for shrimp exposed to toluene and naphthalene were significantly higher at 4°C than at 12°C. Other tests were not significant. We concluded that temperature affects the sensitivity of oil components to pink salmon and shrimp in a nonuniform way, and this effect should be tested for each component with each organism.

Korn, S., S. D. Rice, D. L. Cheatham, and D. W. Brown.

In press. Contribution of phenol, and p-cresol to the toxicity of crude oil to (Oncorhynchus gorbuscha) fry and kelp shrimp (Eualus suckleyi). In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Symposium on pollution and physiology of marine organisms, Mystic, Conn., Nov. 1983. University of South Carolina Press.

Although aromatic hydrocarbons are presumed to be the major contributors to the toxicity of oil-water solutions, the quantitative contribution of nonaromatic and aromatic compounds is undocumented. The objective of this research is to measure the contribution of the highly water-soluble phenolic compounds to toxicity of water-soluble fractions (WSF) of oil.

Phenol and p-cresol do not contribute greatly to the toxicity of the WSF probably because concentrations of phenols in the WSF are low, their toxicity is relatively low, and the relatively low accumulation and rapid depuration of phenol and cresol compared with the important oil aromatic hydrocarbons, toluene and naphthalene.

Phenol and phenolic compounds were found in the WSF of Cook Inlet crude oil at concentrations of 0.013-0.092 ppm. The acute toxicity of phenol and cresol to pink salmon was 3.73 and 3.36 ppm, respectively, and with shrimp, 10.31 and 7.36 ppm, respectively. Salmon and shrimp accumulated 11-22 times the initial exposure concentration of phenol and cresol in 24 h. Both compounds were eliminated rapidly with residues being undetectable after 7 days or less.

Lauren, D. J., and S. D. Rice.

In preparation. Uptake, depuration, metabolism, and excretion of naphthalene by the purple shore crab, Hemigrapsus nudus.

Adult male shore crabs were exposed statically in seawater to ^{14}C-labeled naphthalene for 12 h, followed by up to 156 h of depuration. Hemolymph, heart, cardiac and pyloric stomachs, muscle, thoracic ganglion, digestive gland, and gills were removed at intervals and analyzed for the accumulation of ^{14}C-labeled naphthalene and its metabolites. Biomagnification was rapid, and by 12 h, the digestive gland had 105 times the seawater concentrations of carbon-14, whereas other tissues had less than 15 times the seawater concentration. Depuration was rapid at first but slowed by 12 h after exposure. After 156 h of depuration, the parent naphthalene that remained was located primarily in the digestive gland and muscle; the highest percentages of naphthalene metabolites were found in the gills, muscle, and hemolymph. Because no significant difference in depuration rate was found between control crabs injected with ^{14}C-labeled naphthalene and those with their nephropores and anus blocked, it was concluded that the gills are the major route of naphthalene elimination. Thin-layer chromatography of extracts of depurated ^{14}C-labeled naphthalene indicated that <10% of the total naphthalene depurated was metabolites. In vitro mixed function oxygenase activity (MFO) was assayed on centrifuged (15,000 g) tissue homogenates using dipheyloxazole as the terminal electron acceptor. The specific activity of the gills was 2-4 times that of the antennal glands, and no activity was detected in either muscle, digestive glands, or cardiac and pyloric stomachs. Furthermore, MFO activities were very low compared to fish or mammals. Thus, it was concluded that metabolism of naphthalene plays a minor role in the reduction of toxic body burden, the major role being played by simple diffusion of unmetabolized naphthalene down a concentration gradient. This occurs across the tissue with the largest body-to-water surface area, the gill.

Mecklenburg, T. A., J. F. Karinen, and S. D. Rice.

In preparation. Decrease in heart rates of the Alaskan king crab (Paralithodes camtschatica) during exposure to Cook Inlet crude oil, benzene, and naphthalene.

Heart rates of king crab were depressed during exposure to seawater-soluble fractions (WSF's) of Cook Inlet crude oil, benzene, and naphthalene. As concentrations of crude oil or aromatic concentrations in the seawater declined, the heart rate increased; thus, heart rate of king crabs can be a sensitive indicator of hydrocarbon stress. In one of the experiments with crude oil, changes in respiration closely paralleled changes in heart rate. Benzene produced quicker, more severe, and longer-lasting depression of the heart rate than naphthalene or crude oil. The longlasting sublethal effect of benzene was evident even though the benzene degraded more rapidly in the water than either crude oil or naphthalene.

Mecklenburg, T. A., S. D. Rice, and J. F. Karinen.

1977. Molting and survival of king crab (Paralithodes camtschatica) and coonstripe shrimp (Pandalus hypsinotus) larvae exposed to Cook Inlet crude oil water-soluble fraction. In D. A. Wolfe (editor), Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, Proceedings of a symposium, 10-12 Nov. 1976, Seattle, Wash., p. 221-228. Pergamon Press, New York.

Larvae of coonstripe shrimp and king crab were exposed to solutions of the water-soluble fraction (WSF) of Cook Inlet crude oil in a series of tests on intermolt Stages I and II and the molt period from Stage I to Stage II. Molting larvae were more sensitive than intermolt larvae to the WSF, and molting coonstripe shrimp were more sensitive than molting king crab larvae. When molting larvae were exposed to high concentrations of the WSF (1.15-1.87 ppm total hydrocarbons) for as little as 6 h, molting success was reduced by 10-30%, and some deaths occurred. When larvae were exposed to these high concentrations for 24 h or longer, molting declined 90-100%, and the larvae usually died. The lowest concentrations tested (0.15-0.55 ppm total hydrocarbons) did not inhibit molting at any length of exposure, but many larvae died after molting. Median lethal concentrations (LC50's) based on 144 h of observation for molting coonstripe shrimp and 120 h for molting king crab were much lower than the 96-h LC50's, showing that the standard 96-h LC50 is not always sufficient for determining acute oil toxicity. Although our LC50's for intermolt larvae are higher than levels of petroleum hydrocarbons reported for chronic and spill situations, some of our LC50's for molting larvae exposed 24 h and longer are similar to or below these environmental levels. Comparisons of sensitivity to oil between different crustacean species or life stages should be based on animals tested in the same stage of the molt cycle, such as intermolt.

Moles, A.

1980. Sensitivity of parasitized coho salmon fry to crude oil, toluene, and naphthalene. Trans. Am. Fish. Soc. 109:293-297.

The effect of parasitism by glochidia of Anodonta oregonensis (a freshwater mussel) on the sensitivity of coho salmon fry, Oncorhynchus kisutch, to oil was determined by exposing fry with different levels of parasitism to several concentrations of either the water-soluble fraction of Prudhoe Bay crude oil or the aromatic hydrocarbons toluene and naphthalene. Fry infested with 20-35 glochidia were significantly ($P < 0.05$) more sensitive to each of the toxicants than uninfested fish. Sensitivity increased linearly with increased parasite numbers. Interpretation and application of results of toxicity tests should take into account the kinds and intensities of parasitism found both in the test animals and in the wild populations of fish.

Moles, A. and S. D. Rice.

1983. Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater. Trans. Am. Fish. Soc. 112:205-211.

Juvenile pink salmon, Oncorhynchus gorbuscha, were exposed for 40 days to stable, sublethal concentrations of naphthalene (<0.80 mg/l) and the water-soluble fraction (WSF) of Cook Inlet crude oil (<0.87 mg/l total aromatic hydrocarbons). All fish were fed equal daily rations of Oregon Moist Pellets Formula II. Concentrations (percentage of the 96-h LC50, median lethal concentration) of 10% naphthalene and 14% WSF of crude oil did not affect weight or length of exposed fish; however, at higher concentrations, growth per day, determined from dry weight, decreased with increased toxicant concentration. Fish exposed for 40 days to concentrations of toxicants as low as 33% of the 96-h LC50 were significantly smaller in dry weight, wet weight, and length than control fish (P <0.05). Juveniles exposed to the WSF of crude oil had slower growth rates than those exposed to the same concentration (percentage of the LC50) of naphthalene. Fish exposed to either naphthalene or the WSF of crude oil had decreased caloric content; however, fat content of the fish was not affected. Chronic marine oil pollution at a concentration as low as 0.40 mg/l total aromatic hydrocarbons could be detrimental to juvenile pink salmon growth.

Moles, D. A., and S. D. Rice.

In preparation. The sensitivity of early life stages of coho salmon to long-term exposures to crude oil, toluene, and naphthalene.

Length of exposure affected the concentration of toluene, naphthalene, or crude oil that killed coho salmon eggs, alevins, and fry. The differences are most pronounced in early alevins and least pronounced in fry, and test animals continued to die after they were placed in clean water. The number that died after the exposures was related to length of previous exposure and amount of yolk present during the exposure.

Moles, D. A., S. D. Rice, and S. A. Andrews.

In press. Continuous-flow devices for exposing marine organisms to the water-soluble fraction of crude oil and its components. Can. Fish. Aquat. Sci. Tech. Rep.

The devices produce stable concentrations of aromatic hydrocarbons that can be used in continuous-flow toxicity tests. The crude-oil mixing device produces a stable (<5% deviation) water-soluble fraction of 2.5 mg/l total aromatic hydrocarbons for 30-40 days. The device uses a gentle flow of water to dissolve aromatic components in a layer of crude oil floating on a 2-m column of seawater. Because the water does not pick up oil droplets as it passes through the column, a water-soluble fraction is produced rather than a dispersion. The other device, a syringe pump, introduces compounds directly into a water stream and produces a stable (<1% deviation for toluene) solution of monoaromatic hydrocarbons of any desired concentration or mixture up to the maximum solubility of the compounds. Both devices give reproducible results, are inexpensive, easily maintained, safe, and adaptable to many toxicants.

Moles, D. A., M. M. Babcock, and S. D. Rice.

In preparation. Effects of simulated intertidal crude oil exposures on the survival, development, and uptake in pink salmon (Oncorhynchus gorbuscha) alevins.

Pink salmon (Oncorhynchus gorbuscha) often spawn intertidally in the lower reaches of freshwater streams where developing eggs and embryos of these fish are vulnerable to the possible interaction of varying salinity and oil pollution. We exposed 5-day- and 60-day-old alevins to concentrations of Cook Inlet crude oil in fresh water and in a simulated tidal cycle. Only the higher concentrations (1.6-2.4 mg/l total aromatic hydrocarbons) in the intertidal regime reduced survival. Size was reduced by both changing salinity and exposure to oil, and 60-day-old alevins were more severely affected than the 5-day-old alevins. Even a few hours of daily exposure to oil reduced size. The 60-day-old alevins in the simulated tidal cycle accumulated the highest concentrations of petroleum hydrocarbons.

Alevins exposed intermittently to oil, regardless of salinity, sequestered few hydrocarbons in their tissues. In conclusion, pink salmon alevins in intertidal substrates would be more adversely affected by exposure to oil than their freshwater counterparts.

Moles, D. A., S. Bates, S. D. Rice, and S. Korn.

1981. Reduced growth of coho salmon fry exposed to two petroleum components, toluene and naphthalene, in fresh water. Trans. Am. Fish. Soc. 110:430-436.

Coho salmon, Oncorhynchus gorbuscha, fry were exposed 40 days to stable, sublethal concentrations of toluene (0.4, 0.8, 1.6, 3.2, 5.8 ml/l) and naphthalene (0.2, 0.4, 0.7, 1.4 mg/l) in fresh water. All fry were fed equal daily rations of Oregon Moist Pellet Formula II. Dry weights, wet weights, and lengths of fry exposed to the two highest concentrations of each toxicant for 40 days were significantly less than controls ($P < 0.01$). Growth per day, determined from weights and lengths, decreased linearly with increased concentrations. Fry exposed to naphthalene had a slower growth rate than fry exposed to equivalent concentrations (percentage of the 96-h median lethal concentration of LC50) of toluene. Concentrations 18% of the LC50 of naphthalene and 26% of the LC50 of toluene had no effect on dry weight, wet weight, or length of exposed fry.

Moles, D. A., S. D. Rice, and S. Korn.

1979. Sensitivity of Alaskan freshwater and anadromous fishes to Prudhoe Bay crude oil and benzene. Trans. Am. Fish. Soc. 108:408-414.

The sensitivity of various species and life stages of Alaskan freshwater and anadromous fishes to benzene and the water-soluble fraction of Prudhoe Bay crude oil was determined with 96-h toxicity tests. Freshwater juveniles of the six salmonid species tested had similar sensitivities. Median tolerance limits (TLm's) of these salmonids for crude oil ranged from 2.7 to 4.4 mg/l; TLm's of benzene ranged from 11.7 to 14.7 ml/l. Threespine sticklebacks and, to a lesser extent, slimy sculpins were more tolerant than salmonids and had larger TLm's: threespine sticklebacks had a crude-oil TLm of 10.4 mg/l and a benzene TLm of 24.8 ml/l; slimy sculpins had a crude-oil TLm of 6.44 mg/l and a benzene TLm of 15.4 ml/l. Eggs of pink salmon and coho salmon were quite tolerant to crude oil (TLm = >12 mg/l) and benzene (TLm = 339-542 ml/l). Emergent fry were the most sensitive freshwater stage (crude-oil TLm = 8.0 mg/l; benzene TLm = 12.3-17.1 ml/l). Outmigrant salmonids tested in seawater were twice as sensitive as outmigrant salmonids tested in fresh water apparently because of the additional stress of entering seawater and the physiological changes associated with this transition. Freshwater TLm's were 2.3-8.0 mg/l for crude oil and 10.8-17.1 ml/l for benzene. Corresponding seawater sensitivities were 1.1-3.6 mg/l for crude oil and 5.5-8.5 ml/l for benzene.

Myren, R. T., and J. J. Pella.

1977. Natural variability in distribution of an intertidal population of Macoma balthica subject to potential oil pollution at Port Valdez, Alaska. Mar. Biol. (Berl.) 41:371-382.

Natural variability in the abundance of an intertidal population of the lamellibranch Macoma balthica (Linnaeus 1758) was measured during 1971 and 1972 in a study area near the proposed oil storage and tankship loading facility at the southern terminus of the Trans-Alaska Pipeline in Port Valdez, Alaska. Macoma balthica were divided for analysis into a large- and a small-size category. Small temporal changes in population densities throughout the entire study area were detected for both size categories over several of the seven sampling times of the 2-yr period. Large and persistent differences in density were found among elevation contour intervals for either size category; however, variations in the density profiles on elevation occurred among sampling times. Large M. balthica became more equitably distributed and the small category less equitably distributed among elevation contours over the 2-yr period. Densities of both size categories were more stable at the higher elevations of the study site. Large M. balthica were more homogeneously distributed along a given elevation contour interval than the small category. Mobility and time available to redistribute at a horizontal location would explain the more homogeneous distribution of large M. balthica if competition for food resources exists.

Myren, R. T., and G. Perkins.

In preparation. Decline in Macoma balthica (L.) abundance at Port Valdez, Alaska, 1971-80.

Annual and seasonal changes in abundance of Macoma balthica clams on an intertidal mudflat at Port Valdez, Alaska, were measured over a 10-yr period, 1971-80. Variability in densities of large (>6.4 mm) and small (3.2-6.4 mm) M. balthica changed during highway and oil-terminal construction, and operation of the ballast-water treatment plant and trans-Alaska oil pipeline compared to a 1971-73 baseline observation period. Densities of large M. balthica decreased after the ballast-treatment plant at Port Valdez began operating in 1977, and the lowest densities were recorded in 1980. Effluent from the ballast-water treatment plant is the main source of petroleum hydrocarbons in Port Valdez. A causal relationship, however, was not demonstrated between decreased density of M. balthica and operation of the oil terminal and ballast-water treatment plant. A model was developed to explain variability of M. balthica density during the 10-yr period.

O'Clair, C. E., and J. F. Karinen.

In preparation. Rates of colonization in sublittoral communities near the oil tanker ballast facility at Valdez, Alaska.

Field experiments were conducted for 14 months to determine whether the effluent from the ballast-water treatment plant at Port Valdez, Alaska, affected the rate marine invertebrates form colonies in Port Valdez. Settling plates of compressed asbestos and pans containing defaunated sediment were bolted to an aluminum frame, which was placed at depths of about 90 m and 150 m. The sediments were protected from disturbance when the frames were lowered to depth or retrieved. Two frames were placed near the effluent diffuser of the ballast treatment plant; two other frames were placed in areas not affected by the effluent.

Rates of colonization on the settling plates were markedly different with depth: shallow stations had higher rates of colonization. Serpulid polychaetes were the most abundant early (at 3 months) colonists on the settling plates. Ectoprocts began appearing at 6 months and predominated at the end of the experiment. Effluent from the ballast treatment plant evidently did not affect the rate of colonization.

Sediments were colonized more slowly than plates. Paraonid and syllid polychaetes began colonizing sediments after the plates were immersed for 3 months. Gnathiid isopods and harpacticoid copepods began appearing at 6 months. Preliminary results show no clear trends in rate of colonization on sediments with respect to depth or effluent from the ballast treatment plant.

O'Clair, C. E., and S. D. Rice.

In press. Inhibition of a predator-prey interaction by crude oil: survival, feeding, growth, and condition of Evasterias troschelii preying on Mytilus edulis (L.). Mar. Biol. (Berl.).

Predation by the starfish Evasterias troschelii (Stimpson, 1862) on the mussel Mytilus edulis (L.) can be a strong biological interaction (sensu Paine, 1980) in the inner marine waters of Alaska. To test the effect of petroleum hydrocarbons on this interaction, we exposed E. troschelii with M. edulis to six concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil in a flow-through bioassay system for 28 days. The starfish were more sensitive than the mussels to the WSF; the LC50 for E. troschelii was 0.82 ppm at day 19. Although no mussels were exposed to WSF for more than 12 days, none died.

Daily feeding rates of Evasterias troschelii decreased with increasing concentration of the WSF, whether the rates were measured as the number of mussels·starfish^{-1}·day^{-1} or dry tissue weight of mussels·starfish^{-1}·day^{-1} (mg/sfd). Feeding rates were significantly reduced at all concentrations ≥0.2 ppm; starfish in the two highest concentrations did not feed. At concentrations of 0.20, 0.28, and 0.72 ppm WSF, starfish fed at rates (mg/sfd) that were, respectively, 53, 37, and 5% of the control rates.

Evasterias troschelii exposed to concentrations of WSF ≥0.20 ppm grew slower than control starfish and those exposed to 0.12 ppm combined. The condition of the pyloric caeca and gonads of the starfish expressed as fresh weight or hepatic and gonadal indices was not significantly affected by exposure to WSF. Chronic low level oil pollution can thus inhibit a strong biological interaction and favor Mytilus edulis, a tolerant prey that is a superior competitor for space in the intertidal region.

O'Clair, C. E., and S. D. Rice.

In preparation. Survival, growth, and movement in plantigrades of Mytilus edulis exposed to the water-soluble fraction of Cook Inlet crude oil.

Blue mussel plantigrades collected in nature on nylon ropes were brought to the laboratory and exposed to six concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil. The tests were made in a flow-through system and lasted for 40 days. Control animals were held in fresh, running seawater. Mortality was estimated from dead M. edulis that had unbroken shells. Growth was measured as the change in mean shell length of a sample of the plantigrades on each rope, measured at the beginning and end of the experiment.

Plantigrades in the highest concentration of the WSF (1.40 ppm) had the highest mortality (18.5%), and the 40-day LC50 was 1-7 ppm. At concentrations <1.04 ppm, mortality did not consistently decrease with decreasing concentration. Few plantigrades exposed to ≤0.78 ppm of the WSF died. A layer of bacteria that covered the plantigrades exposed to the WSF, especially concentrations ≥0.78 ppm, may have shielded the plantigrades from some of the toxic effects of the WSF. Plantigrades grew the most in 0.78 ppm and 0.38 ppm of WSF, and plantigrades in 1.40 and 1.04 ppm of WSF (61% of the 40-day LC50) decreased in shell length by the end of the experiment.

Rice, S. D.

1973. Toxicity and avoidance tests with Prudhoe Bay oil and pink salmon fry. In Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills, p. 667-670. American Petroleum Institute, Washington, D.C.

With the potential of oil pollution harming Alaska's marine resources, experiments were conducted at the National Marine Fisheries Service Auke Bay Laboratory to determine the concentrations of Prudhoe Bay crude oil that are acutely toxic to pink salmon fry in fresh water and seawater and also the concentrations of this oil that the fry would avoid. Observed 96-h TLm values were 88 mg/l of oil in fresh water, 213 mg/l in seawater in June, and 110 mg/l in seawater in August. Among fry held in seawater, older fry were more susceptible to oil toxicity than younger fry, and older fry were also more sensitive in their detection and avoidance of oil; older fry in seawater avoided oil concentrations as low as 1.6 mg of oil/l of water. The avoidance of oil by salmon fry was quite apparent and suggests that there is potential for oil pollution to change their migration behavior.

Rice, S. D.

1977. A review of oil toxicity studies conducted at the Auke Bay Laboratory. In B. Melteff (editor), Oil and aquatic ecosystems, tanker safety and oil pollution liability, Proceedings of the Cordova Fisheries Institute, 1-3 April 1977, p. 111-113. University of Alaska, Fairbanks, Sea Grant Rep. 77-8.

Although each of our publications has specific conclusions, the two most significant general conclusions are:

1. We have generally found crustacean larvae to be the most sensitive life stage, especially when molting.

2. Alaskan species may be more vulnerable to oil than species from warmer waters since lower temperatures cause toxic aromatic hydrocarbons to persist longer. Temperature effects on oil toxicity and animal sensitivity are complex and warrant further study.

Rice, S. D., S. Korn, C. C. Brodersen, S. A. Lindsay, and S. A. Andrews.

1981. Toxicity of ballast-water treatment effluent to marine organisms at Port Valdez, Alaska. In Proceedings, 1981 Oil Spill Conference, Atlanta, Georgia, 2-5 March, p. 55-61. American Petroleum Institute, Washington, D.C.

Approximately 12 million gallons of oily ballast water is taken ashore and treated daily at the Alyeska treatment plant, where tankers take on crude oil at the terminus of the Trans-Alaska Pipeline System near Valdez, Alaska. Most oil is removed, but some light aromatic hydrocarbons (1-16 ppm) remain in the large volume of discharged effluent. Between May and July, the concentration of aromatic hydrocarbons in the treated effluent (measured by gas chromatography) generally declined as the seasonal temperatures increased. We measured the toxicity of the effluent on site at Valdez. For the larvae of crustaceans and of fish, the median lethal concentration (LC50) was between 10 and 20% of treated effluent in 96-h static tests. For salmon fry and shrimp in repeated acute flow-through assays, the LC50 was quite consistent, between 20 and 40% of treated effluent. Because the concentration of aromatic hydrocarbons was much lower in the later tests, but the toxicity of the effluent was not lower, toxicants other than aromatic hydrocarbons must contribute significantly to the toxicity of the effluent from the ballast-water treatment plant.

Rice, S. D., D. A. Moles, and J. W. Short.

1975. The effect of Prudhoe Bay crude oil on survival and growth of eggs, alevins, and fry of pink salmon, Oncorhynchus gorbuscha. In 1975 Conference on prevention and control of oil pollution, Proceedings, p. 503-507. American Petroleum Institute, Washington, D.C.

Standard 96-h tests with "total" oil solutions in fresh water and seawater determined differences in sensitivity of the developing life stages of pink salmon (Oncorhynchus gorbuscha). Eggs were the most resistant and emergent fry (yolk sac absorbed), the most sensitive to acute 4-day exposures. In fresh water, the 96-h median tolerance limit (TLm) of fry was 0.4 ml oil/liter mixed mechanically (12 ppm as measured in subsurface water by infrared spectrophotometry). In seawater, it was 0.04 ml oil/liter mixed mechanically (6 ppm as measured in subsurface water by infrared spectrophotometry).

Three life stages of alevins were exposed to 10-day sublethal exposures of the water-soluble fraction to determine what doses might affect growth. Growth was affected most severely in alevins exposed during later developmental stages. Decreased growth was observed in fry after 10-day exposures at the lowest dose tests--0.075 ml oil/liter mixed by water agitation (0.73 ppm in subsurface water by infrared spectrophotometry--less than 10% of the 96-h TLm limit for that life stage).

In fresh water, susceptibility of early life history stages of pink salmon to oil pollution is great at the time of emergence (completion of yolk absorption). Susceptibility is even greater in seawater after fry migration.

Rice, S. D., A. Moles, T. L. Taylor, and J. F. Karinen.

1979. Sensitivity of 39 Alaskan marine species to Cook Inlet crude oil and No. 2 fuel oil. In Proceedings 1979 Oil Spill Conference (Prevention, Behavior, Control, Cleanup), p. 549-554. American Petroleum Institute, Washington, D.C.

The sensitivities of 39 subarctic Alaskan species of marine fish and invertebrates to water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil were determined. This is the largest group of animals ever tested under similar test conditions with the same petroleum oils and analytical methods. Organisms tested represent several habitats, 6 phyla, and 39 species, including fish (9), arthropods (9), molluscs (13), echinoderms (4), annelids (2), and nemerteans (2). Sensitivities were determined by 96-h static tests. Concentrations of selected aromatic hydrocarbons were determined by gas chromatography; concentrations of paraffins were determined by infrared spectrophotometry.

Although sensitivity generally increased from lower invertebrates to higher invertebrates, and from higher invertebrates to fish, sensitivity was better correlated to habitat. Pelagic fish and shrimp were the most sensitive animals to Cook Inlet crude oil with 96-h median tolerance limits (TLm's) of 1-3 mg/l total aromatic hydrocarbons. Benthic animals, including fish, crabs, and scallops were moderately tolerant (TLm's to Cook Inlet crude oil of 3-8 mg/l total aromatic hydrocarbons). Intertidal animals, including fish, crabs, starfish, and many molluscs, were the most tolerant forms to the water-soluble fraction of petroleum (TLm's greater than 8-12 mg/l of total aromatic hydrocarbons). Most of the intertidal animals were not killed by static oil exposures. Number 2 fuel oil was more toxic to most species than Cook Inlet crude oil.

Sensitive pelagic animals are not necessarily more vulnerable to oil spills than tolerant forms. Oil may damage intertidal environments more easily, and adverse effects may persist longer than in damaged pelagic environments.

Rice, S. D., J. W. Short, C. C. Brodersen, T. A. Mecklenburg, D. A. Moles, C. J. Misch, D. L. Cheatham, and J. F. Karinen.

1976a. Acute toxicity and uptake-depuration studies with Cook Inlet crude oil, Prudhoe Bay crude oil, No. 2 fuel oil, and several subarctic marine organisms. NWFC Processed Report, 90 p. Auke Bay Laboratory, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, P.O. Box 210155, Auke Bay, AK 99821.

1. Our studies do not suggest that major differences exist between the responses of the Alaskan marine animals tested and the responses of marine animals from other areas as reported in the literature.

2. The concentration of oil-in-water dispersions and water-soluble fractions of oil in solutions is dependent on a number of factors including oil volume, confinement of the oil, mixing duration, and mixing energy. As a consequence, we do not attempt to transfer the results of our study to a field situation (including Kachemak Bay) or to establish the potential effects of oil contamination on the environment. If oil pollution did occur, the oil concentrations that would occur in the water column are difficult to predict since the oil volume, mixing duration, mixing intensity, and confinement of the spill are all important but unknown variables that would depend on the specific conditions prevailing at the time of the spill. Further, there are too few quantitative studies on the effects of an oil spill in arctic and subarctic waters that include measurements of oil in the water column for us to state that our laboratory exposure concentrations might be encountered under a field spill situation.

Rice, S. D., J. W. Short, and J. F. Karinen.

1976b. Toxicity of Cook Inlet crude oil and No. 2 fuel oil to several Alaskan marine fishes and invertebrates. In Sources, effects and sinks of hydrocarbons in the aquatic environment, Proceedings of the symposium, American University, Wash., D.C., 9-11 Aug. 1976, p. 395-406. American Institute of Biological Sciences, Washington, D.C.

We used a 96-h static-test method to determine the median tolerance levels (TLm's) of 27 different invertebrate and vertebrate Alaskan marine species exposed to water-soluble fractions (WSF's) of Cook Inlet crude oil and No. 2 fuel oil. Concentrations of oil in the exposure doses of the WSF's were determined by infrared spectrophotometry.

The two different oils were about equally toxic; No. 2 fuel oil was somewhat more toxic than the Cook Inlet crude oil to some of the species. Fish were consistently among the more sensitive species with 96-h TLm's from 0.81 tc 2.94 ppm. Some invertebrates were as sensitive as fish, whereas others were quite resistant. Intertidal invertebrates were consistently among the most resistant species.

It appears that Alaskan marine species may be slightly more sensitive than similar species residing in more temperate regions. However, the differences in observed sensitivity may be due to the greater toxicity of oil at lower temperatures (because of greater persistence of hydrocarbons) rather than to actual increases in the sensitivity of the animals.

Rice, S. D., J. W. Short, and J. F. Karinen.

1977a. Comparative oil toxicity and comparative animal sensitivity. In D. A. Wolfe (editor), Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, Proceedings of a symposium, 10-12 Nov. 1976, Seattle, Wash., p. 78-94. Pergamon Press, New York.

The scope of this review is limited to studies dealing with the ability of crude and refined oils to kill marine animals. Emphasis is on the more recent quantitative studies that were not available to earlier reviewers (Evans and Rice 1973; Moore and Dwyer 1974; National Academy of Sciences 1975). This review covers (1) the behavior of oil in water; (2) the methodology problems associated with tests; (3) the comparative toxicity of oil-water mixtures, oils, and components of oils; and (4) the comparative sensitivity of different life stages and species.

Rice, S. D., R. E. Thomas, and J. W. Short.

1977b. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon fry. In F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg (editors), Physiological responses of marine biota to pollutants, Proceedings, p. 259-277. Academic Press, New York.

1. The water-soluble fraction (WSF) from Cook Inlet and Prudhoe Bay crude oils and No. 2 fuel oil causes similar increases in breathing and coughing rates in pink salmon fry.

2. Breathing and coughing rates increase in proportion to oil concentrations, as measured by ultraviolet but not by infrared spectrophotometry. This suggests that naphthalenes rather than paraffins are responsible for this effect. Significant responses were detected at about 30% of the 96-h TLm.

3. Breathing and coughing rates of pink salmon fry remained above normal during exposure to a constant dose of oil for 72 h.

4. Paraffinic, monoaromatic, and diaromatic hydrocarbons were found in tissues of fish exposed to the WSF of Cook Inlet crude oil. The fish started apparent depuration of the aromatic hydrocarbons during the first 24 h of exposure; this indicates that they can cope with the stress physiologically. Our data support the concept of excretion through the liver-gall bladder-gut.

5. High breathing rates during the first 14 h of exposure, elimination of most aromatic hydrocarbons by 20 h, and the continued high breathing rates during the constant-dose exposure for 72 h indicate that salmon fry can cope with a sublethal exposure to hydrocarbons, but at the cost of an increased metabolic rate. Increased metabolic rates may be detrimental to survival if the stress persists for long periods of time.

Rice, S. D., and S. A. Andrews.

Unpublished manuscript-a. Quantitative comparison between the toxicity of water-soluble fraction of crude oil and a similar mixture of aromatic hydrocarbons to pink salmon and kelp shrimp.

Aromatic hydrocarbons in the water-soluble fraction (WSF) of Cook Inlet crude oil are thought to cause most of the toxicity of the parent oil. To test this hypothesis, we compared the toxicity of the WSF of Cook Inlet crude oil with the toxicity of a mixture of aromatic compounds combined in the same concentrations as those found in the WSF. Pink salmon fry and kelp shrimp were exposed to several concentrations of the oil WSF and the simulated WSF. The oil WSF was three times more toxic to salmon fry and five times more toxic to kelp shrimp than the simulated WSF. Components other than aromatic hydrocarbons in the oil WSF contribute considerably to the toxicity of crude oil.

Rice, S. D., and S. A. Andrews.

Unpublished manuscript-b. Additive and synergistic toxicity of pairs of aromatic hydrocarbons to pink salmon fry.

Aromatic hydrocarbons are generally thought to be the primary contributors to the toxicity of crude oil in tests with marine organisms. Some laboratory evidence, however, indicates that the water-soluble fraction (WSF) of crude oil is more toxic than predicted by the individual toxicities of its component aromatic hydrocarbons. Because synergistic effects between aromatic hydrocarbons are possible, we tested whether there were synergistic effects between benzene and other monoaromatic hydrocarbons in the WSF of Cook Inlet crude oil. Pink salmon fry were exposed for 96 h to several concentrations of each of the following compounds mixed in different ratios with benzene: Toluene; o-, m-, and p-xylene; 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene; ethylbenzene; and isopropylbenzene. Many of the mixtures were more toxic than an additive model would predict. The greatest interaction was between benzene and p-xylene in the ratio of 1:1.3. This mixture was three times more toxic, as determined from LC50's, than predicted from the additive model. In conclusion, the toxicity of mixtures of monoaromatic hydrocarbons is unpredictable, unless individual combinations are tested, because the total toxicity of mixtures may be greater than the effects of any of them singly.

Short, J. W., and S. D. Rice.

In preparation. Accumulation, depuration, and retention of petroleum-derived aromatic hydrocarbons by four commercially important Alaskan marine animals (pink scallops, pink shrimp, king crab, and pink salmon).

We exposed pink scallops, pink shrimp, king crabs, and pink salmon fry to the water-soluble fraction (WSF) of Cook Inlet crude oil for up to 5 days and determined concentrations of aromatic hydrocarbons in each species. At the end of the exposures, live animals were transferred to clean seawater, and concentrations of aromatic hydrocarbon concentrations in each species were again determined after various intervals of up to 10 days.

During the exposures, naphthalene and methyl-substituted naphthalenes were consistently found in all four species; phenanthrene and methyl-substituted phenanthrenes were found in scallops; and three- and four- carbon aliphatic-substituted benzenes were found in scallops and fry.

Scallops, fry, and shrimp rapidly accumulated aromatic hydrocarbons during exposure to the WSF. After an initial delay of about 10 h, crabs rapidly accumulated aromatic hydrocarbons during exposure to the WSF. All four species had maximum accumulations of aromatic hydrocarbons after 10-48 h of exposure to WSF, and concentrations of aromatic hydrocarbons in the animals were between 33 times (naphthalene in scallops) and 1,024 times (methylnaphthalene in king crabs) the concentration of aromatic hydrocarbon in the WSF.

Each species had a unique pattern for the elimination of aromatic hydrocarbons. The concentrations of aromatic hydrocarbons in scallops declined slowly and incompletely during the 5 days that scallops were in clean seawater. Concentrations of aromatic hydrocarbons in pink shrimp declined rapidly within 24 h after transfer to clean seawater and more slowly thereafter. Concentrations of aromatic hydrocarbons in king crabs rapidly declined to control levels within 4 days after transfer to clean seawater. Concentrations of aromatic hydrocarbons appeared to decline dramatically in pink salmon fry while they were still being exposed to the WSF. The ability of these species to rapidly accumulate aromatic hydrocarbons at low temperatures indicates that their marketability could be impaired if they were exposed to sufficiently oil-polluted water for more than a few hours.

Short, J. W., S. D. Rice, and D. L. Cheatham.

1976. Comparison of two methods of oil and grease determination. In D. W. Hood and D. C. Burrell (editors), Assessment of the arctic marine environment, selected topics, p. 451-462. University of Alaska, Fairbanks, Institute of Marine Science, Occasional Publication No. 4.

A gravimetric method is used by government regulatory agencies for determining levels of oil pollutants in discharge waters. This method involves extraction with an organic solvent, evaporation at elevated temperatures, and gravimetric determination of the residue. The authors compare oil content determined by the gravimetric method with oil content determined by infrared spectrophotometry for toxic water-soluble fractions of Prudhoe Bay and Cook Inlet crude oils and a No. 2 fuel oil.

The gravimetric method is adequate for grease but not for the oils. Recovery of a synthetic grease standard was 98%, whereas the recovery of the three pure oils ranged from 52 to 65% by the gravimetric method. Recovery of all the oils and the grease standard was essentially 100% by the infrared method. The differences between the two methods are ascribed to significant losses of volatile compounds from the oils during the evaporation step of the gravimetric method.

Gravimetric estimates of oil in toxic concentrations of water-soluble fractions (WSF's) ranged from 0 to 36% of those determined by the infrared method. Oil content of the No. 2 fuel oil WSF's was below detectable limits of the gravimetric method (1.5 mg/liter). Four-day median tolerance limits of shrimp (Eualus fabricii) and scallops (Chlamys rubida), as evaluated by the infrared WSF's for the three oils, were between 0.25 mg/liter (No. 2 fuel oil) and 3.82 mg/liter (crude oils).

It is concluded that the gravimetric method is sensitive to heavier compounds, but these have only a casual relationship to acute toxicity. Concentrations of oil in water known to have adverse effects are much lower than can be detected by the standard gravimetric method. When oil concentrations in water are to be measured and correlated with chemical toxicity, the gravimetric procedure should be supplemented with a method specific for the more soluble and volatile components.

Short, J. W., J. A. Gharrett, S. Korn, and S. D. Rice.

In preparation. Rapid uptake and depuration of aromatic hydrocarbons in coonstripe shrimp larvae.

Coonstripe shrimp larvae were exposed to ^{14}C-labeled toluene, ^{14}C-labeled naphthalene, and ^{3}H-labeled 2-methylnaphthalene for 3 h. Larvae were kept in glass tubes with screen bottoms. The tubes were then placed in static seawater solutions of aromatic hydrocarbons (either toluene alone or the two naphthalenes together). At regular intervals, groups of larvae were removed, rinsed, and burned in an oxidizer. The residues were collected, and radioactivity was measured by liquid scintillation to determine the amount of aromatic hydrocarbons taken up by the larvae. All unsampled larvae were moved to uncontaminated seawater after 3 h, and sampling continued to trace the depuration of aromatic hydrocarbons.

In <10 min, the larvae accumulated many times the water concentrations of the aromatic hydrocarbons. About 1 h after the start of exposures, concentrations of aromatic hydrocarbons in the animals stabilized. The concentration of toluene (1.4 ppm) in the animals was 10 times the exposure concentration; the concentration of naphthalene (0.075 ppm) was nearly 100 times the exposure concentration; and the concentration of 2-methylnaphthalene was nearly 500-1,000 times the exposure concentration.

The larvae depurated hydrocarbons rapidly for the first 9 h after being moved to clean seawater but only slowly thereafter. Twenty-four hours after depuration began, all larvae still contained concentrations of hydrocarbons that were higher than the original exposure concentrations. After 48 h in clean seawater, they depurated about 50% of the hydrocarbons remaining after 24 h of depuration in clean seawater.

In similar but less extensive tests, Stage I and Stage II shrimp larvae took up similar amounts of hydrocarbons, and dead larvae took up nearly as much hydrocarbon as live ones. The uptake mechanisms for hydrocarbons in shrimp must, therefore, be passive.

Stickle, W. B., Jr., S. D. Rice, and D. A. Moles.

1984. Bioenergetics and survival of the marine snail, Thais lima during long-term oil exposure. Mar. Biol. (Berl.) 80:281-289.

The carnivorous snail Thais lima was fed Mytilus edulis during a 28-d exposure to the water soluble fraction (WSF) of Cook Inlet crude oil. The LC50 of T. lima declined from >3,000 ppb aromatic hydrocarbons on day 7 to 818 ± 118 ppb aromatic hydrocarbons on day 28. The LC50 of M. edulis declined from >3,000 ppb aromatic hydrocarbons on day 7 to 1,686 ± 42 ppb on day 28. Predation rate declined linearly with increasing aromatic hydrocarbon concentration up to 302 ppb; little predation occurred at 538 ppb and none at 1,160 or 1,761 ppb. Snail absorption efficiency averaged 93.5% and did not vary as a function of WSF dose. Total energy expenditure (R + U) increased at 44 ppb aromatics and declined at lethal WSF exposures. At sublethal WSF exposures, percentages of total energy expenditure were: respiration (87%), ammonia excretion (9%) and primary amine loss (4%). These percentages did not vary as a function of WSF dose or time. Oxygen:nitrogen ratios were not affected by WSF concentration or time and indicated that T. lima derived most of its energy from protein catabolism. The uptake of aromatic hydrocarbons into the soft tissues of snails and mussels was directly related to the WSF concentration. Naphthalenes accounted for 67 to 78% of the aromatic hydrocarbons in T. lima and 56 to 71% in M. edulis. The scope for growth was negative above 150 ppb WSF aromatic hydrocarbons and above 1,204 ppb soft-body aromatic hydrocarbons. These snails were physiologically stressed at an aromatic hydrocarbon concentration that was 19% of the 28-d WSF LC50 (818 ± 118 ppb) and/or 48% of the 28-d LC50 of soft tissue aromatics (2,502 ppb).

Stickle, W. B., Jr., S. D. Rice, C. Villars, and W. Metcalf.

In press. Bioenergetics and survival of the marine mussel, Mytilus edulis, during long-term exposure to the water-soluble fraction of Cook Inlet crude oil. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Symposium on pollution and physiology of marine organisms, Mystic, Conn., Nov. 1983. University of South Carolina Press.

The survival, energy budget (scope-for-growth), and oxygen:nitrogen ratios were determined in the filter-feeding mussel Mytilus edulis during exposures (flow through) to the seawater-soluble fraction (WSF) of Cook Inlet crude oil (30°/$_{oo}$ salinity and 10°C). Mussels were exposed for up to 28 days to concentrations of WSF as high as 2.1 ppm aromatic hydrocarbons. Scope-for-growth was calculated from feeding rate of M. edulis on Phaeodactylum tricornutum at 1.2 x 10^4 cells ml^{-1}, absorption efficiency, oxygen consumption, and ammonia excretion rates of six groups of large (2-5 cm) mussels that were exposed to 0, 0.09, 0.21, 0.38, 0.60, and 1.1 ppm aromatic hydrocarbons. Survival and tissue concentrations of aromatic hydrocarbons were determined on days 0, 7, 14, 21, and 28 of the experimental period.

The LC50 of mussels declined during the 28-day experiment from >2.5 ppm of aromatic hydrocarbons on day 7 to 2.5 ppm ± 0.71 (mean and 95% C.I.) on day 14, 1.9 ppm ± 0.48 on day 21, and 1.4 ppm ± 0.30 on day 28. Feeding rate was unaffected at 0, 0.09, and 0.21 ppm aromatic hydrocarbons and varied between 408 ml ± 105 ml (SE) and 939 ml ± 126 ml·(g dry weight of algae eaten)$^{-1}$·h^{-1}. The feeding rate of mussels exposed to 0.38 ppm aromatic hydrocarbons declined significantly after 14 days of exposure, whereas the feeding rates of mussels exposed at 0.60 and 1.1 ppm aromatic hydrocarbons were significantly lower than the control rate at all sampling times. Absorption efficiency varied between 51% and 84% and was unaffected by exposure time at concentrations ≤0.38 ppm aromatic hydrocarbons. Absorption efficiency declined to 43% on day 28 in mussels exposed to 0.60 ppm aromatic hydrocarbons and to 41% and 48% on days 21 and 28 in mussels exposed at 1.1 ppm. Caloric consumption due to respiration and ammonia excretion varied between 0.52 calories·mussel^{-1}·h^{-1} and 1.10 calories·mussel^{-1}·h^{-1} and did not fluctuate as a function of exposure time at any dose. Respiration accounted for more than 87% of caloric expenditure at all times. Oxygen:nitrogen ratios did not vary as a function of

exposure time or exposure concentration and ranged between 13.6 ± 1.3 (mean and 95% C.I.) and 50.0 ± 26.1 and indicated increased reliance on protein catabolism. Mussels had spawned just before the start of our experiment, however, and were presumably reabsorbing gametes during the experiment. Bioaccumulation of aromatic hydrocarbons was dose related.

Scope-for-growth was unaffected by exposure time at 0, 0.09, and 0.21 ppm aromatic hydrocarbons and varied between 1.21 and 3.87 $cal \cdot mussel^{-1} \cdot h^{-1}$. Scope-for-growth of mussels exposed to 0.38 ppm aromatic hydrocarbons declined significantly from earlier values on days 21 and 28, whereas scope-for-growth of mussels exposed to 0.60 and 1.1 ppm was significantly lower than the day-0 value on all sampling dates during exposure. The lowest aromatic hydrocarbon concentration that reduced scope-for-growth was 0.38 ppm on day 21 of exposure; this concentration represents 19.7% of the 21-day LC50.

Stickle, W. B., T. D. Sabourin, and S. D. Rice.

1982. Sensitivity and osmoregulation of coho salmon, Oncorhynchus kisutch exposed to toluene and naphthalene at different salinities. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Physiological mechanisms of marine pollutant toxicity, p. 331-348. Academic Press, New York.

Coho salmon (Oncorhynchus kisutch) smolts were more sensitive to toluene and naphthalene in seawater than in fresh water. Tolerance dropped linearly from 0 through 10, 20, and 30°/$_{oo}$ S. Smolt tolerances at 30°/$_{oo}$ were 54% and 63% of the 48-h TLm in fresh water for toluene and naphthalene, respectively. Smolt tolerances to toluene and naphthalene were the same after 12, 22, and 42 days of acclimation to seawater as they were after only 1 day of acclimation. The increase in sensitivity was not transient nor did it appear related to acclimation-stress because the smolts gained 30% in weight in 42 days.

Toluene and naphthalene affected serum osmolality and ions but only at the lethal concentrations of 100 and 130% of the 48-h TLm. At those exposure concentrations, osmolality, Na^+, and Cl drifted with the diffusion gradient, decreased in freshwater smolts, and increased in seawater smolts. At the same concentration, K^+ concentrations in the serum increased, even in freshwater smolts, indicating cellular damage. Exposures of 70% of the 48-h TLm had no effect on serum osmolality or ions. Consequently, we conclude that the increase in sensitivity of smolts in seawater is not related to a failure in ion-regulating ability, but rather the loss of ion-regulating ability at lethal exposures is symptomatic of toxic actions elsewhere. The cause of increased sensitivity of smolts in seawater is not transient and remains unknown.

Taylor, T. L., and J. F. Karinen.

1977. Response of the clam, Macoma balthica (Linnaeus), exposed to Prudhoe Bay crude oil as unmixed oil, water-soluble fraction, and oil-contaminated sediment in the laboratory. In D. A. Wolfe (editor), Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, Proceedings of a symposium, 10-12 Nov. 1976, Seattle, Wash., p. 229-237. Pergamon Press, New York.

The small clam, Macoma balthica (Linnaeus 1758), will likely be subjected to oil slicks layered on the mud and to water-soluble fractions (WSF's) of crude oil or oil-contaminated sediment. Groups of adult clams in or on their natural sediment were exposed in flow-through aquaria at 7-12°C to various concentrations of Prudhoe Bay crude oil layered on the mud surface, the WSF of the crude oil, and oil-treated sediment (OTS).

Gentle settling of crude oil over clam beds had negligible effects on clams observed for 2 months. The WSF and OTS of Prudhoe Bay crude oil inhibited burrowing and caused clams to move to the sediment surface. Responses were directly proportional to concentrations of WSF or amount of OTS. The 1-h and 72-h effective median concentrations of the WSF for the responses of burrowing by unburied clams and surfacing by buried clams were 0.234 and 0.367 ppm naphthalene equivalents, respectively. The interpolated amount of OTS needed for a 50% surfacing response within 24 h was 0.67 g OTS cm^{-2}.

Although short-term exposures of clams to the WSF of crude oil and OTS caused few deaths, behavioral responses of clams to oil may be of great importance to their survival in the natural environment. In these laboratory tests, many of the clams recovered, but in nature, clams that come to the sediment surface may be eaten by predators or die from exposure.

Thomas, R. E., and S. D. Rice.

1975. Increased opercular rates of pink salmon (Oncorhynchus gorbuscha) fry after exposure to the water-soluble fraction of Prudhoe Bay crude oil. J. Fish. Res. Board Can. 32:2221-2224.

The opercular rates of pink salmon (Oncorhynchus gorbuscha) fry were measured during 24-h exposure to sublethal concentrations of the water-soluble fraction (WSF) of Prudhoe Bay crude oil. Opercular rates increased significantly for as long as 9 and 12 h after exposure to WSF's prepared from oil-water solutions of 2.83 and 3.46 ppm. The increases in rates were proportional to increases in dose. Recording changes in opercular rates appears to be a suitable method for detecting sublethal physiological effects of hydrocarbon stress because the observed changes occurred at approximately 20% of the 96-h LC50.

Thomas, R. E., and S. D. Rice.

1979. The effect of exposure temperatures on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, naphthalene, and water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and F. J. Vernberg (editors), Marine pollution: functional responses, p. 39-52. Academic Press, New York.

Oxygen consumption and breathing rates of fry exposed to toluene and naphthalene began to increase immediately upon exposure and declined in later hours during exposure. Breathing rate reached maximum response values at 2 or 4 h, whereas oxygen consumption rates were greatest at 6 or 8 h of exposure.

All three concentrations of naphthalene (107%, 70%, and 45% of the 24-h median tolerance limit) resulted in significant increases in the opercular breathing rate of pink salmon fry ($P < 0.01$); whereas, of the four toluene concentrations (94%, 69%, 45%, and 30% of the 24-h TLm), only the two highest resulted in a significant increase in the breathing rate. Breathing rate response was linear with dose.

Although relatively few fish were used in the studies of oxygen consumption, the pattern of increased oxygen consumption along with increased breathing rates in each of the exposures indicates that increased breathing rate of pink salmon fry reflects increased energy demands. Oxygen consumption was greatest at about 6 h of exposure, about 4 h after the occurrence of maximum breathing rates. Apparently, as the fry became physiologically acclimated to the stress of the toxicant, they increased the efficiency of oxygen extraction, thus decreasing the need to move water across the gills and subsequent expenditure of energy.

The increase in breathing rates of naphthalene-exposed fry over control fry were much greater at the low temperature of 4° than 12°C. Control fry at 4°C had lower metabolic rates. However, the breathing rate response to hydrocarbons, as a percent of controls, was much larger at 4° than at 12°C, indicating that hydrocarbon exposures at 4°C are more stressful than equivalent exposures at 12°C.

Thomas, R. E., and S. D. Rice.

1981. Excretion of aromatic hydrocarbons and their metabolites by freshwater and seawater Dolly Varden char. In J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg (editors), Biological monitoring of marine pollutants, p. 425-448. Academic Press, New York.

The gills were the most important pathway for excretion of carbon-14 from ^{14}C-labeled naphthalene. Most of the carbon-14 excreted by the gills was still attached to the parent compound. About 10% of the excreted carbon-14 appeared in the cloacal chamber, mostly as metabolites. Less than 1% of the total carbon-14 was excreted in the urine, predominantly as metabolites.

Tissues retained a significant amount of carbon-14 at 24 h. Although muscle contained large amounts of carbon-14 because of its mass, the gall bladder had the highest specific activity. The brain also retained significant quantities of carbon-14.

Although more ^{14}C-labeled toluene was excreted and metabolized than ^{14}C-labeled naphthalene, more ^{14}C-labeled naphthalene was retained in the tissues. A lower percentage of the carbon-14 was recovered in ^{14}C-labeled naphthalene metabolites than in ^{14}C-labeled toluene metabolites.

Seawater and freshwater Dolly Varden char excreted similar amounts of carbon-14; however, the percentage of metabolites in the excretions and tissues of seawater fish was lower than the percentage of metabolites in excretions and tissues of freshwater fish. For example, we recovered greater amounts of carbon-14 with a lower percentage of metabolites from the brain-spinal cord of seawater fish than from the brain-spinal cord of freshwater fish--possibly explaining why seawater Dolly Varden char are more sensitive to aromatic hydrocarbons and the water-soluble fraction of oil than freshwater Dolly Varden.

Thomas, R. E., and S. D. Rice.

1982. Metabolism and clearance of phenolic and mono-, di-, and polynuclear aromatic hydrocarbons by Dolly Varden char. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Physiological mechanisms of marine pollutant toxicity, p. 161-176. Academic Press, New York.

Although the pathways of elimination of hydrocarbons have generally been identified, the relative importance of each route for compounds of different molecular weight and polarity is not known. In this study, we compared and determined the effects of molecular weight and polarity on the clearance of several phenolic and aromatic hydrocarbons from gills and other excretory pathways of Dolly Varden char (Salvelinus malma) in seawater. We also examined the distribution of parent hydrocarbons and metabolites in tissues after 24 h of exposure. Dolly Varden char were force-fed gelatin capsules containing a ^{14}C-labeled hydrocarbon and then placed in a split-chamber box for 24 h to separate gill excretions from cloacal excretions.

Size of the hydrocarbon appeared to be the most critical factor in excretion of hydrocarbons by the gills. The gills of fish can easily excrete phenolic and mononuclear aromatic compounds. Some naphthalene was excreted from the gills, but virtually none of the polynuclear aromatic hydrocarbons were excreted from the gills. Even though the partition coefficients (log of octanol/water partition) of phenol and cresol are about 1/10 of toluene, these similar-sized hydrocarbons were excreted from the gills in approximately equal amounts.

Polar phenolic compounds were excreted into the cloacal chamber but not toluene or the large polynuclear aromatic hydrocarbons. Partition coefficient is apparently a more important factor than size in excretion of hydrocarbons into the cloacal chamber because the excretion of phenol and cresol into the cloacal chamber was more than 10 times that of similar-sized toluene.

The excretion of the largest hydrocarbons that were tested, anthracene and benzo[a]pyrene, was minimal in 24 h (3.2% and 1.2%, respectively). These compounds were slowly absorbed from the gut probably because they are relatively nonpolar and have a high partition coefficient (octanol/water), therefore, are more difficult to remove from a lipid matrix (membrane). The mobility of these compounds between tissues is limited, and they probably have

to be metabolized before excretion. Consequently, metabolism in the liver and secretion into the bile is probably the most important pathway for excretion of large molecular weight hydrocarbons; however, this is a relatively slow process that takes much longer than 24 h.

Thomas, R. E., and S. D. Rice.

In press. Effect of previous exposure to naphthalene in the water column on the subsequent metabolism of dietary naphthalene by Dolly Varden char, Salvelinus malma. Symposium on pollution and physiology of marine organisms, Mystic, Conn., Nov. 1983.

Dolly Varden (Salvelinus malma) acclimated to seawater (30°/$_{oo}$) at 4°C were exposed to naphthalene in the water of their holding tank. The fish were fed ^{14}C-labeled naphthalene in a gelatin capsule that was placed in their stomach. Concentrations of parent hydrocarbon and metabolites in the gall bladder (bile), liver, central nervous system, and muscle were determined 24 h after administration of the capsule.

The conversion of dietary naphthalene to tissue metabolites was dependent on the concentration of naphthalene in the exposure water. Generally, the percent of carbon-14 in the metabolite fraction of the tissues increased after 48-h exposures as the concentration of the naphthalene in the water exposure increased from 25% of the 24-h LC50 to 75% of the 24-h LC50.

Groups of fish were also exposed for 48 h to naphthalene at 75% of the 24-h LC50, and then transferred to clean water for 12 or 24 h periods before ^{14}C-labeled naphthalene was placed in their stomachs. Decreased percentages of carbon-14 were recovered in the metabolite fraction from the tissues as the time in clean water increased. After 24 h in clean water, the percent radioactivity in the metabolite fraction of animals previously exposed to naphthalene was similar to that of animals that were not exposed to naphthalene.

Length of the previous exposure to naphthalene also influenced the amount of carbon-14 recovered in the metabolite fraction. Although a previous exposure of 48 h resulted in a significant increase in tissue metabolites, an exposure of only 24 h did not. The significance of enzyme induction resulting from a previous exposure to petroleum hydrocarbons is discussed.

Thomas, R. E. and S. D. Rice.

In preparation-a. Effect of environmental temperature on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden, Salvelinus malma.

Dolly Varden (Salvelinus malma) acclimated to seawater (30°/$_{oo}$) or fresh water at 4° or 12°C were force-fed gelatin capsules containing ^{14}C-labeled toluene or ^{14}C-labeled naphthalene. Concentrations of parent hydrocarbons and metabolites in the gall bladder (bile), liver, central nervous system, and muscle were determined 12, 24, and 48 h after initiation of exposure.

In fresh water, the temperature of 12°C promoted the absorption of both ^{14}C-labeled hydrocarbons from the stomach into tissues, and fish had more of the administered radioactivity recovered in the tissues than fish exposed at 4°C. Liver was the only tissue that did not follow this trend. More carbon-14 was recovered from the liver of fish exposed to naphthalene or toluene at 4° than at 12°C but only at the 12-h exposure. A similar pattern was observed for fish acclimated to seawater. More carbon-14 was recovered from the tissues at 12°C than at 4°C. However, more carbon-14 was recovered from fish acclimated to seawater than from fish acclimated to fresh water, regardless of temperature.

Significantly (P <0.01) more carbon-14 was recovered in the metabolite fraction after 12°C exposures to naphthalene in fresh water than after 4°C exposures. This was true for all tissues at 12, 24, and 48 h after exposure in fresh water. More carbon-14 was found in the metabolite fraction after 12°C exposures to toluene in fresh water than after 4°C exposures although the differences were not as pronounced as in naphthalene exposures. Temperature had a similar effect on the percent carbon-14 recovered in the metabolite fraction after fish were acclimated to seawater; however, the effect was not as pronounced as in fish exposed in fresh water. Liver, nerve, and muscle tissues removed from the fish exposed to toluene in seawater at 12 and 24 h contained significantly (P <0.01) more carbon-14 in the metabolite fraction at 12°C than at 4°C. For animals exposed to naphthalene, however, concentrations of tissue metabolites were similar for 4° and 12°C exposures until after 48 h of exposure.

Generally, compared to fish acclimated to 12°C, fish acclimated to 4°C had lower concentrations of parent hydrocarbon and its metabolites in the

bile, liver, central nervous system, and muscle, regardless of salinity or toxicant. The effect of temperature, however, was less pronounced in fish acclimated to seawater. The differences in the effect of temperature are undoubtedly caused by many temperature-sensitive processes, such as absorption, metabolism, retention, and excretion. The role of temperature in altering the rates of these processes is discussed.

Thomas, R. E., and S. D. Rice.

In preparation-b. Effect of salinity on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden char, Salvelinus malma.

Dolly Varden char maintained at 12°C in seawater (30°/$_{oo}$) or fresh water were force-fed gelatin capsules containing ^{14}C-labeled toluene or ^{14}C-labeled naphthalene. Tissue concentrations of parent hydrocarbon and metabolites were determined at 12, 24, or 48 h after initiation of the exposure. Tissues sampled included gall bladder (bile), liver, central nervous system, and muscle.

Consistently higher concentrations of toluene or naphthalene were recovered in the tissues of animals exposed in seawater than in the tissues of animals exposed in fresh water. Generally, the amount of labeled hydrocarbon recovered from the tissues increased 48 h after exposure; however, the magnitude of the increase was dependent on the tissue sampled, the compound, and whether the fish were exposed in seawater or fresh water.

Significantly ($P < 0.01$) more carbon-14 was recovered as polar metabolites from the animals exposed to naphthalene in fresh water than from animals exposed to naphthalene in seawater. More metabolites of toluene were also found in tissues of fish exposed in fresh water than in fish exposed in seawater; however, the differences were not nearly as great as in the naphthalene exposures. Generally, the percentage of carbon-14 recovered as metabolites increased with time for both compounds in both the freshwater and seawater exposures. Again, the magnitude of increase was dependent on type of tissue, the hydrocarbon administered, and freshwater or seawater exposure. The salinity of exposure does affect the accumulation and metabolism of aromatic hydrocarbons in fish and may explain why salmonids in seawater are more sensitive to aromatic hydrocarbons than salmonids in fresh water.

APPENDIX B.
SCIENTIFIC AND COMMON NAMES OF 64 ALASKAN SPECIES THAT HAVE BEEN EXPOSED TO HYDROCARBONS BY RESEARCHERS AT THE AUKE BAY LABORATORY

Annelids

Harmothoe imbricata	scale worm
Nereis vexillosa	mussel worm

Nemerteans

Lineus vegetus	brown ribbon worm
Paranemertes peregrina	purple ribbon worm

Crustaceans

Acanthomysis pseudomacropsis	mysid
Balanus glandula	barnacle
Idothea wosnesenskii	isopod
Anonyx nugax	amphipod
Boeckosimus nanseni	amphipod
Gammaracanthus loricatus	amphipod
Orchomene pinquis	amphipod
Pandalus borealis	pink shrimp
Pandalus danae	dock shrimp
Pandalus goniurus	humpy shrimp
Pandalus hypsinotus	coonstripe shrimp
Eualus fabricii	scooter shrimp
Eualus suckleyi	kelp shrimp
Crangon alaskensis	grass shrimp
Pagurus hirsutiusculus	hairy hermit crab
Paralithodes camtschatica	red king crab
Cancer magister	Dungeness crab
Chionoecetes bairdi	Tanner crab
Hemigrapsus nudus	purple shore crab

Molluscs

Colus halli	Hall's colus
Colus jordani	
Littorina sitkana	Sitka periwinkle
Margarites pupillus	purple margarite
Neptunea lyrata	ridged whelk
Thais lima	file periwinkle
Collisella (*Notoacmaea*) *pelta*	shield limpet
Collisella (*Notoacmaea*) *scutum*	plate limpet
Ischnochiton stelleri	giant chiton, gumboot
Katharina tunicata	leather chiton
Mopalia ciliata	ciliated chiton
Tonicella lineata	lined chiton
Chlamys hericius	pink scallop
Macoma balthica	Baltic clam
Protothaca staminea	littleneck clam
Mytilus edulis	blue mussel

Echinoderms

Leptasterias hexactis	six-armed starfish
Evasterias troschelii	seastar
Eupentacta quinquesemita	white cucumber
Cucumaria vegae	tarspot cucumber
Strongylocentrotus droebachiensis	green sea urchin

Fishes

Anoplarchus purpurescens	cockscomb prickleback
Aulorhynchus flavidus	tube snout
Boreogadus saida	Arctic cod
Clupea harengus pallasi	Pacific herring
Cottus cognatus	slimy sculpin
Eleginus gracilis	saffron cod
Gasterosteus aculeatus	threespine stickleback
Limanda aspera	yellowfin sole
Myoxocephalus polyacanthocephalus	great sculpin
Oncorhynchus gorbuscha	pink salmon
Oncorhynchus kisutch	coho salmon
Oncorhynchus nerka	sockeye salmon
Oncorhynchus tshawytscha	chinook salmon
Oncottus hexacornis	sculpin
Salvelinus alpinus	Arctic char
Salvelinus malma	Dolly Varden
Theragra chalcogramma	walleye pollock
Thymallus arcticus	Arctic grayling
Pholis laeta	crescent gunnel
Platichthys stellatus	starry flounder

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