Environmental Assessment of the Alaskan Continental Shelf

Volume 8. Effects of Contaminants

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for the Year Ending March 1976

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Environmental Assessment of the Alaskan Continental Shelf

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Fourth quarter and annual reports for the reporting period ending March 1976, from Principal Investigators participating in a multi-year program of environmental assessment related to petroleum development on the Alaskan Continental Shelf. The program is directed by the National Oceanic and Atmospheric Administration under the sponsorship of the Bureau of Land Management.

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ANNUAL REPORT

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Title: The Physiological Effect of Acute and Chronic Exposure to Hydrocarbons of Petroleum on the Near-Shore Fishes of the Bering Sea

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I. Summary of objectives, conclusions and implications with respect ot OCS oil and gas development--

This study is concerned with the physiological effects of acute and chronic exposure to the water soluble hydrocarbons of Alaskan crude oils on the near shore Bering Sea fishes. Much of our attention has been directed towards the aromatic hydrocarbons because they are extremely toxic, concentrations as low as 2 parts per million naphthalene killing some fishes in 48 hours. Special emphasis is being put on studies of the effects of the aromatic hydrocarbons on the antifreeze systems of these fishes because the aromatics such as the naphthalenes have been shown to concentrate in the liver where they are presumably detoxified. Because the liver is also the site of the synthesis of the peptide and glycopeptide antifreezes, accumulation could cause damage to the protein synthetic machinery. Such damage could lead to reduced levels of antifreeze causing these fishes to freeze during the winter.

II. Introduction

A. General nature and scope of study--

Some data exist on the effects of petroleum hydrocarbons on marine temperate invertebrates and vertebrates (1), however little is known about the effects of hydrocarbons on invertebrates and vertebrates in the northern oceans and especially their effects at low temperatures. Thus we feel that it is extremely important to examine the effects of petroleum hydrocarbons on some of the year-around fish fauna of the Bering Sea, especially at sub-zero temperatures.

B. Specific objectives--

The specific objectives of our studies are: 1) To determine the levels of water soluble hydrocarbons of Alaskan crude oil which will kill selected near shore Bering Sea fishes in two days. 2) To determine the levels of selected aromatic hydrocarbons (naphthalene) which will kill the same species in two days. 3) To determine the effects of sublethal doses of the water soluble fraction of crude oil and the naphthalenes on the survival and freezing resistance of these same species.

III Current state of knowledge--

Some data on the lethal effects of hydrocarbons on temperate invertebrates and fishes exist in the literature (1). However, so far we have been unable to find any data on hydrocarbon effects on polar fishes or Bering Sea fishes. One of the reasons for this is that there are no marine stations on the Bering Sea coast which have ready access to the fish fauna. Although research ships have recently been involved in studies of Bering Sea fishes, no hydrocarbon effects of the physiology of fishes have been attempted abord ship.

IV. Study area-- Shallow waters of the Bering Sea.

Thus far we have examined fishes from the shore area near Nome on Norton Sound. These same species also occur in the areas of the Bering Sea under consideration for oil exploration and leasing.

V. Materials and methods--

The fishes we have worked with so far are some of the flounders, cods and sculpins. The species are the arctic flounder, Liopsetta glacialis, the saffron cod, Eleginus gracilis, and the sculpins, Myoxocephalus scorpius and Megalocottus platycephalus. All of these fishes were collected from Safty Sound, a brackish lagoon 20 miles south of Nome. Specimens were also collected 3 miles off Nome in 60 feet of water in the Bering Sea. All fishes were collected by gill net or with a small (14 feet) otter trawl. Tows were short in order to collect fish which were in good shape. The fish were held for a few days and the best ones were flown to our laboratory at Scripps Institution of Oceanography (SIO). The remainder of the fish were flown to the National Marine Fisheries Laboratory at Auke Bay, Alaska where Dr. Stan Rice performed the acute bioassay experiments using the water soluble fractions of Cook Inlet crude oil. Acute bioassays were done on the saffron cod and the arctic flounder. The assays on saffron cod were done at +8°C and +3°C. The bioassay method and the statistical analyses of the lethal dose data used were the same as those outlined by Karinen and Rice (2).

Baseline measurements of various physiological parameter-- Our hydrocarbon effect study also called for establishing baseline data for whole organism oxygen consumption, tissue oxygen consumption and freezing resistance capabilities, and determining the effects of selected hydrocarbons of these physiological parameters.

The whole organism oxygen consumption measurements were done only on sculpin because of the lack of specimens of saffron cod. Measurements were made at temperatures between -1°C and +6°C. Measurements of oxygen uptake were made using a Rank oxygen electrode.

Tissue oxygen consumption was determined for muscle, gill and liver tissue taken from both saffron cod and sculpin using a Gilson respirometer. Measurements were made at +6°C and +0.7°C.

The degree of freezing resistance of the fish which were collected at Nome during September, the summer ice-free season was determined by measuring the freezing point of the blood serum. Upon collection the water temperature was +6°C to +7°C. The blood serum from flounder, saffron cod and sculpin was frozen and shipped to SIO where both freezing and melting

points were determined. Inorganic ion levels were also determined. Freezing point and melting points were measured in order to determine whether a peptide or glycopeptide antifreeze was present. A large difference (1°C) between the blood freezing point and melting point indicates the presence of an antifreeze. As these fishes were collected at +7°C during the summer, they would not be expected to possess an antifreeze because at this temperature no ice would be present and they would be in no danger of freezing.

Acclimation studies--The sculpins which were not used for tissue oxygen consumption studies were cold acclimated to determine the time course of antifreeze production. Fish were sampled as the temperature of water was lowered to -1.0°C, and the freezing and melting points of their blood serum was measured. Once the fish synthesized a full compliment of antifreeze, they were warm acclimated to determine the rate at which they lost their freezing resistance capability. These studies are being done in preparation for determining the effect of long term sublethal doses of naphthalene on the synthesis and time course of synthesis of the antifreeze.

Naphthalene uptake and toxicity experiments--In order to determine the toxic effects of naphthalene on the Bering Sea sculpin we performed a bioassay with pure naphthalene. Naphthalene was dissolved in a minimum volumn of ethanol then slowly infused into 9 liters of filtered seawater. Naphthalene concentration was monitored throughout the experiment by relating the absorbance at a wavelength of 276 mμ to concentration. To demonstrate that naphthalene was being taken up and concentrated in the liver we performed C^{14}-naphthalene uptake experiments. Usually one microcurie of C^{14}-naphthalene was dissolved in one liter of seawater and the uptake by a sculpin was determined by following the disappearance from the seawater using liquid scintillation counting techniques. After several hours the fish was sacraficed and the blood, white muscle, liver, brain, gill and gut analyzed for C^{14}-naphthalene accumulation. A comparison was made between the fraction of radioactivity that could be extracted with chloroform methanol and the total radioactivity in the tissue. The uptake studies were done to demonstrate that naphthalene is rapidly taken up from water and accumulates in the liver. Naphthalene uptake has been demonstrated in temperate fish but not in Bering Sea fish.

VI and VII. Results and discussion

Acute toxicity studies using water soluble fractions of Cook Inlet oil--The acute bioassays were done only on the saffron cod and arctic flounder because at the time the fish were captured it was impossible to identify the sculpin spicies. There is a taxonomic problem with the genus

Myoxocephalus which has not yet been resolved even by the best taxonomists at SIO.

Acute toxicity data are given in Table I for the saffron cod at +8 and +3°C, At the lower temperature the water soluble hydrocarbons have much less of an effect than at the higher temperature the reduced metabolic rate may result in a decrease in uptake of toxic hydrocarbons. Also of great interest is the high concentration of hydrocarbons needed to kill the arctic flounder. Thus it is clear that the different species of Bering Sea fishes show diffening responses to exposure to the water soluble fraction of Alaskan crude oils.

Baseline measurements of physiological parameters--The oxygen uptake of specimens of the sculpin Megalocottus are given in Table II. Values are in milliliters of oxygen consumed per gram per hour for temperatures of -1.0°C, +1.0°C and +6°C. Fish were always acclimated to the temperature of measurement for at least a week. Oxygen consumption for the tissues of these same fishes are given in Table III. Oxygen uptake is in microliters oxygen consumed per gram dry weight per hour. Oxygen consumption was determined at +6°C and +0.7°C for the sculpin, Megalocottus and at +6°C for the saffron cod. The rates of oxygen consumption of the sculpin at the various temperatures agree with those obtained for other cold water fishes (3). The rate of oxygen consumption of the various tissues of both the saffron cad and sculpin also agree with those obtained for polar cold adapted fishes (4,5). These baseline values for oxygen consumption rates will be used for comparison of oxygen consumption rates of Megalocottus exposed to long sublethal does of naphthalene.

Seasonal levels of freezing resistance--Analyses of the freezing-melting behavior of the blood serum collected from Bering Sea fishes in the summer showed no difference between these two parameters which indicated that fishes living at +7°C lacked antifreeze (Table IV). All of the freezing point depression could be accounted for by the inorganic ions in the serum when it was obvious that the summer Bering Sea fishes lacked antifreeze. Cold acclimation studies were immediately begun and it took from 3 to 4 weeks acclimation to -0.5°C to produce sufficient antifreeze to lower the freezing point of the blood to that of seawater (Table IV). This is a similar time response observed with the sculpin, Myoxocephalus scorpius which inhabits the coastal waters of Nova Scotia (6). Thus it is clear that the production of antifreeze is a seasonal phenomenon and presumably the control of its synthesis is partially influenced by temperature.

Once the sculpin had synthesized their full compliment of antifreeze, warm acclimation studies were begun. Acclimation for one month at +5°C resulted in the loss of only a small part of the compliment of antifreeze (Table IV). Another

two weeks at +10°C resulted in only a small loss. Not until the fish were held for another 33 days did they lose half of their antifreeze. The warm acclimation study period corresponded to the normal winter season (January, Feburary and March) of the fish. Despite the fact that a long photoperiod was employed during acclimation, loss of antifreeze was unexpectedly small and the rate of loss small. This is in sharp contrast to the results of similar experiments which were done on winter flounder from the freezing waters of Nova Scotia. In those studies long photoperiod and elevated temperature of +12°C produced a rapid decline in levels of antifreeze (6). Thus it appears that factors other than photoperiod and warm temperatures play a role in the regulation of the levels of antifreeze in the Bering Sea sculpin.

The slow disappearance of the antifreeze in response to warm acclimation has presented us with some difficulties. We had hoped to begin experiments to determine the effect of sublethal concentrations of naphthalene on the rate of appearance of antifreeze in warm acclimated Megalocottus when they were cold acclimated to induce antifreeze formation. We may have to wait until late summer to carry out this part of the experiment.

Determination of naphthalene uptake and sublethal levels-- In preparation for studying the effect of chronic naphthalene exposure on the antifreeze system of the sculpin we needed to determine concentrations of naphthalene which are sublethal for Megalocottus, and also to demonstrate that at sublethal concentrations naphthalene dissolved in seawater. We demonstrated that Megalocottus could live for several days at a concentration of 2 ppm. In our long term exposure we plan to use about 0.2 ppm.

To demonstrate that naphthalenes are taken up at low concentrations we exposed Megalocottus to low levels of C^{14}-naphthalene. C^{14}-naphthalene was rapidly taken up from seawater even when present at concentrations of only 0.035 ppm (Figure 1). Uptake is rapid and after three hours significant amounts are found in the blood and brain. Most of it, however, is concentrated in the liver (Table V). Large amounts of naphthalene have also been demonstrated to accumulate in the livers of temperate fish (1). There it is metabolized to more water soluble derivatives and excrete via the digestive track and urine. Presumably a similar detoxification mechanism exists in the cottid Megalocottus.

VIII. Conclusions

We have presented toxicity data for exposure to water soluble hydrocarbons on a few Bering Sea fishes. The results show that different species have different levels susceptibility to hydrocarbons. Also the temperature of exposure appears to have profound effect. At low temperatures the

hydrocarbons appear to be less toxic.

Baseline measurements of oxygen consumption both at the organismal and tissue level reveal that the Bering Sea fishes are similar to other cold water fishes in regards to their physiology. The effect of hydrocarbons on their oxygen consumption has not been investigated.

Studies of the levels of freezing resistance in sculpin indicated, as expected, that it is a seasonal phenomonen. The time course of the appearance and disappearance of freezing resistance indicates that this system will be a good model for studying the effect of naphthalene on antifreeze synthesis. Furthermore naphthalene uptake studies indicate large amounts of naphthalene are strongly concentrated in the liver even when it is present at low levels in the environment.

IX. Needs for further study-- Most of the emphasis will be put on utilizing the antifreeze system as a model to determine whether the extreme concentration and probable detoxification of naphthalene in the liver produce damage at that site. We should be able to gain some insight into this problem because the antifreezes are also synthesized in the liver and damage to the liver would be reflected in reduced levels of antifreeze in the blood serum after cold acclimation.

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Figure 1. Uptake of C^{14}-naphthalene by the sculpin Megalocottus from 1 liter of seawater containing 1 microcurrie of C^{14}-naphthalene. Counts per minute per milliliter of seawater being subjected to aeration is shown by (x) the top line. The solid circles represent counts per minute per milliliter when a 30 gram fish was introduced into the water.

FIG. 1. UPTAKE OF C^{14} NAPHTHALENE BY MEGALOCOTTUS PLATYCEPHALUS LATICEPS

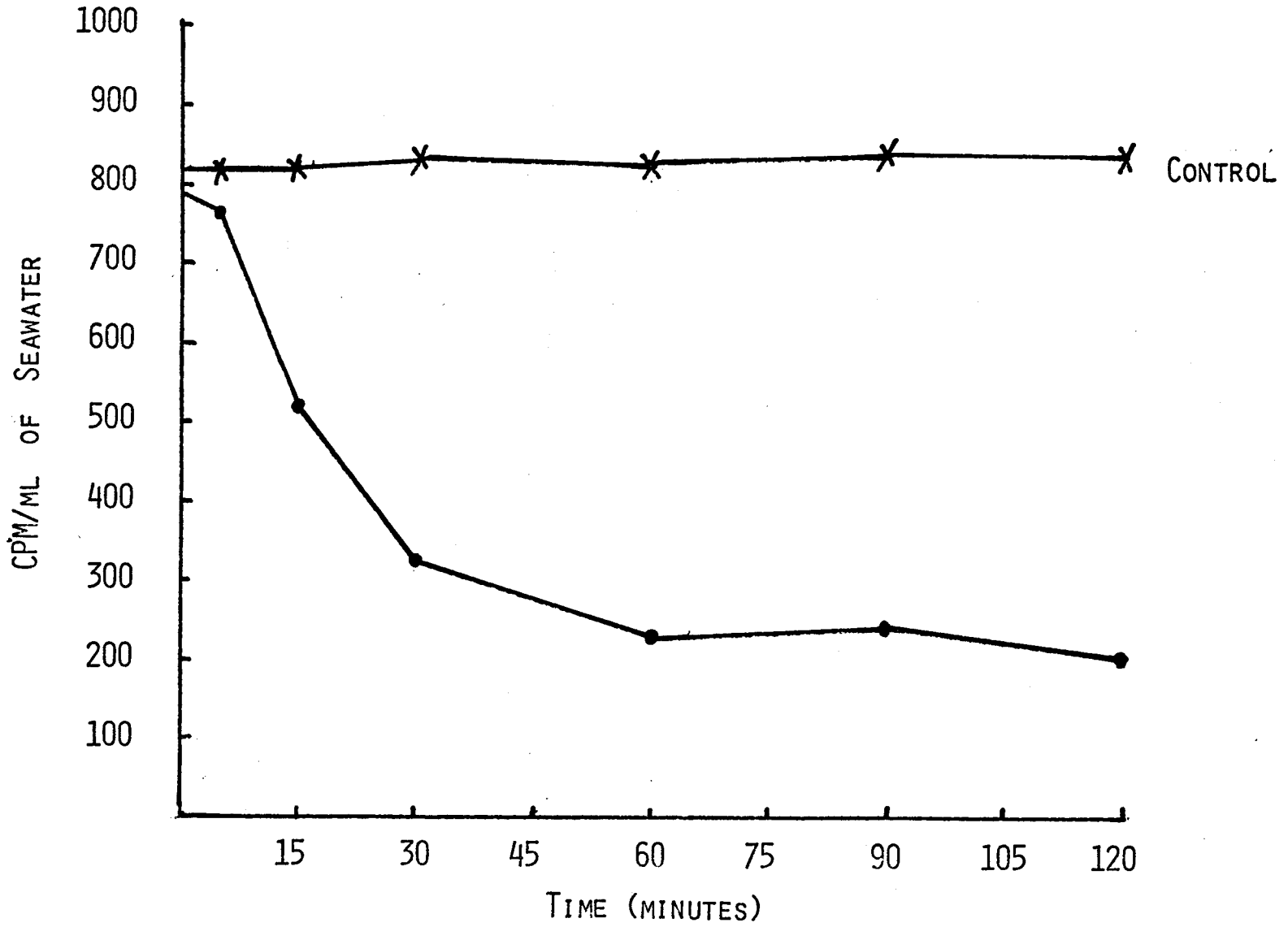

Table I. Toxic lethal dosage of water soluble fraction of Cook Inlet oil necessary to kill 50% of the experimental population of two Bering Sea fishes.

Species	Temperature	Type Oil	24 hr TL_{50}	96 hr TL_{50}
Eleginus gracilis (saffrin cod)	+8°C	Cook Inlet	1.83 ppm-IR* 0.043 UV O.D.**	1.029 ppm-IR 0.034 UV O.D.
Eleginus gracilis (saffron cod)	+3°C	Cook Inlet	2.48 ppm-IR 0.102 UV O.D.	2.28 ppm-IR 0.092 UV O.D.
Liopsetta glacialis (arctic flounder)	+8°C	Cook Inlet	>3.6 ppm-IR >0.228 UV O.D.	>3.6 ppm-IR >0.228 UV O.D.

* Parts per million parafins measured by infrared spectroscopy.

** Optical density at 210-240 mμ; gives a relative measurement of the amount of aromatic hydrocarbons present.

Table II. Oxygen consumption of the Bering Sea sculpin, Megalocottus platycephalus at several temperatures expressed as milliliters of oxygen consumed per gram (wet weight) per hour.

Number of Individuals	Temperature °C	[mean], ml of oxygen per gram per hour
5	-1	0.053
6	+1	0.066
6	+6	0.070

Table III. Oxygen consumption of tissues in microliter (STP) per gram (dry weight) per hour.

Species	Tissue	Mean Oxygen Consumption per gram per hour
Eleginus gracilis (saffron cod)	White muscle	276 (+6°C)
	Liver	492 (")
Megalocottus platycephalus (sculpin)	White muscle	431 (+0.7°C); 431 (+6°C)
	Liver	825 ("); 825 (")
	Gill	881 ("); 882 (")

Table IV. Summary of freezing and melting behavior of Bering Sea fishes collected during the summer, after cold acclimation and warm acclimation.

Species	Thermal History	Blood Freezing Point (°C)	Blood Melting Point (°C)	Difference (MP-FP) (°C)
Eleginus gracilis (saffron cod)	Summer, +7°C	-0.80	-0.70	0.10
Megalocottus platycephalus (sculpin)	+7°C	-0.80	-0.68	0.12
M. platycephalus	30 days at -1°C	-1.95	-1.0	0.95
M. platycephalus	30 days at +7°C	-1.60	-0.85	0.75
M. platycephalus	30 days at +7°C then, 30 days at 14°C	-1.07	-0.67	0.40

Table V. Accumulation of C^{14}-naphthalene in various tissues of a 30 gram Megalocottus after 3 hours exposure to one microcurrie of C^{14}-naphthalene in 1 liter of seawater. Comparisons were made between extractable radioactivity and total radioactivity in the tissue.

Tissue	cpm/100 µl of 2.5 ml chloroform-methanol extract of 100 mg tissue	cpm/100 mg of solubilized tissue
White muscle	16	1195
Gill	32	656
Kidney	30	865
Gut	70	1500
Brain	55	2329
Blood	30	2361
Liver	1314	29000

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Physiological Impact of Oil on Pinnipeds

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ANNUAL REPORT

RU 71 - PHYSIOLOGICAL IMPACT OF OIL ON PINNIPEDS

I. Summary of objectives, conclusions and implications with respect to OCS and gas development.

The objectives of this project are (1) to determine the effects of crude oil fouling that are measurable by changes in metabolic rate and diving ability in the northern fur seal, and (2) to determine the effect of crude oil fouling on heat transfer properties of the skin and pelage of the sea otter and as many pinnipeds as possible. Until the final tests and measurements have been made (July and August, 1976) we can draw no conclusions and predict no implications with respect to OCS oil and gas development.

II. Introduction

A. General nature and scope of study.

This study applies laboratory physiological measurements (metabolic rates and heat flux) to the field problem of pinnipeds and otters encountering an oil spill. It also makes direct measurements of diving behavior in unrestrained animals at sea. The rationale for these two approaches is that for fur bearers oil fouling is likely to directly effect the insulative properties of the fur which will result in altered metabolic costs and impaired diving performance. In non-fur bearers different effects are anticipated.

B. Specific objectives.

We will test the above hypothesis by measuring both metabolic rates and diving performance before and after exposure to oil in the northern fur seal, a fur bearer. These studies will be coupled with measurements of the heat flux across the pelt (a measure of insulative property), before and after oiling. As a comparison, we will also measure heat flux across the pelts of other fur bearers (specifically the sea otter) and non-fur bearers (such as the harbor seal).

C. Relevance to problems of petroleum development.

If this research shows that contact with oil has a seriously negative impact on fur bearers, then any oil development near large concentrations of fur bearers would be potentially harmful or destructive to these animal stocks, and the decision to develop such a field would have to be made with that knowledge. If this research does not show an adverse effect of oil on fur bearers or on non-fur bearers, we can conclude only that our measurements did not cover the spectrum of possible effects, and that more research is needed.

III. Current state of knowledge.

Previous studies on the effects of oil pollution on marine mammals are not extensive, and do not permit accurate prediction of impact to be made. In September, 1975, Smith

and Geraci completed their study on the effects of oil on ringed seals. They conclude that surface exposure to oil had relatively little effect on these non-fur bearers. However, some of their animals died soon after exposure to oil. The cause of their deaths is not known with certainty, but they suspect "stress" of an undetermined nature. Their study was preliminary and allows no more predictions than our present efforts on fur seals.

IV. Study area.

All equipment was developed at the Physiological Research Laboratory, Scripps Institution of Oceanography. Furthermore, preliminary metabolic measurements and all heat flux measurements will be made there. All final metabolic measurements, and all diving trials, will be made on the northern fur seal population at St. George Island, Alaska.

V. Sources, methods and rationale of data collection.

A. Thermal conductance.

The apparatus to measure thermal conductance was patterned after that of Kooyman, et al (1974) on penguin pelts.

B. Diving.

The present depth-time recorder is a modification of similar hardware developed by Kooyman (1968) for Weddell seals. The main changes were inclusion (a) of a Bourdon

tube as a pressure transducer, and (b) a pressure-sensitive paper strip transported past the transducer stylus by a motor-driven gear train. These modifications permitted continual recording for eight days rather than for a few hours as in the previous study on Weddell seals.

C. Metabolic rates.

The metabolic chamber was patterned after that of Kooyman, et al (1974). However, instead of a paramagnetic O_2 analyzer an AEI analyzer was obtained because it can be linked to a recorder. The methods used in this part of the study are modifications of standard metabolic procedures in wide usage today.

VI. Results.

Four lactating female fur seals were instrumented with depth-time recorders in September and October, 1975, at St. George Island. The females were recaptured on their next visit to the island and the instruments were recovered. Twenty-five days of consecutive diving records were thus obtained. The duration of absence for all four animals was considerably longer than for marked but un-instrumented females in the same period. Laboratory studies on a California sea lion showed that swimming effort (measured in strokes / meter) was nearly doubled when the animal was fitted with the same harness and depth-time recorder used

on female fur seals. Therefore, it is likely that increased swimming effort was responsible for the inordinately long time period that instrumented fur seals spent at sea. We do not know whether diving depths reported below were similarly affected. Until the 1976 measurements are made with an improved harness, the diving depths below should be considered minimum figures.

Table 1 shows the frequency with which dives were recorded to various depths. Depths are collated by 30 meter intervals for convenience. When more samples are available, a finer breakdown will be possible.

TABLE 1

Number of Dives to Various Depths by Four Northern Fur Seals

Fur Seal No.	No. Hrs. Recorded	0-20M	21-50M	51-80M	81-110M	111-140M	141-170M	171-200M	≤No. Dives
1	167	301	314	37	9	13	2	0	843
2	92	1148	1	10	9	49	1	2	1224
3	200	54	478	89	1	4	0	0	626
4	149	201	200	14	7	6	3	0	580

VII. Discussion

Since the continental shelf near the Pribilof Islands varies from 75 to 100 meters, it is clear that at least 3 of our 4 animals were diving off the shelf, in one case to nearly

twice the depth of the shelf.

The greatest number of dives extended to less than 20 meters. Through laboratory tests with a sea lion, we have determined that these dives are probably real and do not result from some instrument artifact. The relatively un-patterned occurrence of these dives suggests that they are not associated with feeding but may be part of the normal traveling pattern of seals at sea.

Aside from these extremely shallow excursions there were two identifiable types of dives; very deep dives occuring singly, and dives to intermediate depths occuring in clusters with marked regularity of duration, spacing and depth. We interpret the first type to be some form of exploratory dive, perhaps while the animal seeks the bottom or the deep scattering layer, and the second to be feeding dives.

We consider the depth-time recorder developed in this project to be a major breakthrough in technology and methodology for studying marine mammals at sea. Not only can physiological and behavioral interpretations be made from its simple trace, but ecological interpretation can as well. This instrument has potential uses far beyond its present application.

VIII. Conclusions.

It is not possible to draw firm conclusions from the existing data.

IX. Needs for further study.

Adequate funds, personnel, and time remain for obtaining the measurements proposed in the work statement. We cannot recommend further research on fur seals until these data have been obtained. It is clear that some investigation into the effects of oil pollution on sea otters is desirable for comparison with the present effort. Finally, we conclude from the results of Smith and Geraci that the most rational study to conduct on the effects of oil pollution on non-fur bearing species would involve toxic and possible "stress" effects.

X. Summary of 4th quarter operations.

A. Laboratory activities.

1. Trained captive fur seals to voluntarily enter the metabolic test chamber.

2. Constructed the metabolic test chamber and tested O_2 analyzer in presence of petroleum vapors.

3. Began construction of device for measuring heat flux of pelts (main component a Beckman-Whitney heat flow disc).

4. Collected seal pelts from various sources and stored them frozen for heat flux measurements.

5. Constructed a frequency distribution for 1975 diving data.

6. Began collecting materials and methods for cleaning oil from equipment and animals.

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ACUTE AND CHRONIC TOXICITY, UPTAKE AND DEPURATION, AND SUBLETHAL METABOLIC RESPONSE OF ALASKAN MARINE ORGANISMS TO PETROLEUM HYDROCARBONS

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I. Summary of Objectives and Conclusions

This study was designed to determine the acute and chronic toxicity of crude oil and its component fractions on physiological and behavioral mechanisms of selected arctic and subarctic organisms and to determine recovery rates of selected organisms in laboratory and field studies.

Conclusions to date: Bioassays with invertebrates require modification of standard bioassay procedures because they do not complete their response in 96 hours. Temperature has little effect on toxicity, so that data generated at 12°C can be extrapolated to colder climates. Oil exposures stimulate metabolism in fish, rather than depress metabolism, as in crabs.

It would be premature to relate these conclusions to oil and gas development activities. Most of our experiments are in various states of completion because this research effort did not start until May 1975 and full operation did not start until October 1975.

II. Introduction

The research was addressed to the general question, "What are the effects of hydrocarbons and associated contaminants on arctic and subarctic biota?" It involved physiological and bioassay tests of applied research on species indigenous to the Gulf of Alaska, Bering Sea, and Beaufort Sea. The major emphasis is the determination of acute toxicity of crude oil to a variety of organisms, the uptake-depuration of hydrocarbons, the effects of oil on metabolic rate, and other studies on sublethal physiological effects.

A. General Tasks

1. Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms.

2. Conduct laboratory and field studies to determine recovery rates of selected organisms and ecosystems from perturbations resulting from either contamination or other disturbances associated with petroleum development.

B. Specific Objectives and Studies

1. Determine acute toxicity of previously untested species such as amphipods, mysids, sandlances, and others.

2. Determine acute toxicity at different temperatures with several species such as scallops, pink salmon, and shrimp.

3. Determine the chronic toxicity to shrimp and herring eggs and the effects of oil on newly extruded eggs of crabs.

4. Determine the uptake and depuration of oil components for previously untested species.

5. Determine the effect of temperature on oil component uptake and depuration.

6. Determine the effect of oil on metabolic rate of fish and invertebrates.

7. Determine the effect of oil on scallop growth and behavior.

8. Determine the effect of oil on crab autotomy response.

9. Determine histopathology effects of oil using routine histology, enzyme histochemistry, and electron microscopy.

C. Relevance to Problems of Petroleum Development

The above objectives, when answered, will allow an evaluation of which Alaskan animals are more sensitive to oil exposure, the effect of colder temperature on toxicity, and the ability of different species to recover. This information is needed to predict possible effects from oil contamination. The predictive value of the data generated will be known at a later date, when laboratory and field experiments have been analyzed.

III. Current State of Knowledge

Previous to this research, information on acute and chronic toxicity to Alaskan organisms was limited to certain commercial species. Little was known about the effects of petroleum on noncommercial Alaskan species and nothing was known about the effects of temperature on toxicity. Essentially nothing is known about the quantitative ability of arctic and subarctic organisms to metabolize, eliminate, or recover from petroleum exposure. The effects of temperature on uptake and depuration are unknown.

IV. Study Area--Nonspecific (Physiological Effects)

V. Methods

A. General

All laboratory work was done at the Auke Bay facility. The majority of organisms for our studies were collected in the vicinity of Auke Bay, Alaska. Exceptions to this were (1) shrimp collected at Little Port Walter, Alaska, and (2) saffron cod collected in cooperation with Dr. Arthur De Vriés near Nome, Alaska.

Cook Inlet oil was obtained from Shell Oil Company in 55-gallon drums. No. 2 fuel oil was taken from the laboratory heating system fuel tanks.

1. Mixing.--Water-soluble fractions were prepared depending on the volume needed by mixing a 1% oil to water mixture in either an 18-liter bottle, a 100-liter glass aquarium, a 55-gallon polyethylene-lined oil barrel, or an 800-liter fiberglass tank. In each case, suitable electric motors with mixing paddles were adjusted to the proper mixing energy to allow the oil vortex to descend one-third of the depth of the container. The mixing continued for 20 hours followed by a 3-hour settling period. The WSF was then siphoned off from beneath the oil slick and diluted for use in exposures.

2. Exposure.--Most exposures were static, aerated, and at a tissue-to-volume ratio of 1 gram per liter or less. Oil concentrations are measured analytically at the beginning of the exposures. Organism response (mortality or some other parameter) are analyzed by computerized probit statistics. For several studies repetitive dosing was used. Periodically the oil concentration was monitored (UVOD) and brought up to the initial concentration by addition of 100% water-soluble fraction. The concentration deviation is minimized by frequent redosing.

3. Analytical methods.--Our oil-water solutions (WSF's) are routinely analyzed by infrared (IR) and ultraviolet (UV) spectroscopy, as well as by gas-liquid chromatography (GC).

The IR method is from Gruenfeld (1973), and involves determining the absorbance of light at 3412 nanometer wavelength by the oil-derived

hydrocarbons. Aliphatic compounds, which are not very toxic, absorb most strongly at this wavelength. The method does not measure toxic aromatic or polar compounds. We use this method as a general indication of relative differences between successive WSF preparations, and as a means of comparing our work with that of previous investigations. Despite its limitations, this method of determining oil in water is far superior to measuring only the volume of oil added to water, since the amount of oil that enters the water column is very dependent on the mixing energy and duration.

A second method that we routinely employ is a modified version of the UV method of Neff and Anderson (1975). This method involves determining the absorbance of oil-derived hydrocarbons at 221 nanometers. Naphthalene and methyl-substituted naphthalenes absorb most strongly at this wavelength, although high concentrations of mononuclear aromatics (such as benzene, toluene, xylene, etc.) can also cause appreciable absorption.

The naphthalenes have been implicated in several toxicity studies. We use this method principally as a means of predicting the toxicity of successive WSF preparations. Since the different methyl-substituted naphthalenes have slightly different molar absorptivities at 221 nanometers, we report results by this method as naphthalene equivalents. This is the amount of pure naphthalene that would account for most of the absorbance observed in a given sample at 221 nanometers.

The third method that we employ routinely involves gas-liquid chromatography (GLC). We used a column suggested by Supelco, Inc., which is especially suited for separating aromatic hydrocarbons. In this method we extract the WSF with two aliquots of methylene chloride, and then analyze from 1 to 10 microliters of the combined extracts immediately by GLC for mononuclear aromatics. Then we concentrate the extract to 500 microliters, and analyze from 1 to 10 microliters again by GLC for the higher aromatics.

We have established the identity of most of the aromatic peaks by comparing retention times with known standards, by spiking WSF's with known aromatics, and by mass spectroscopic (MS) identification of selected samples. The results from these different methods have always been in agreement. The MS study (conducted at the Northwest Fisheries Center in Seattle) also established that both normal and some branched paraffins elute from our column, and in some cases they elute simultaneously with some of the aromatic compounds. On the basis of this information we are now able to correct for this interference.

4. Bioassay Statistics.--When possible, all of our bioassay results are analyzed by a computerized probit analysis by Finney (1971). This statistical technique calculates a maximum likelihood estimation of the oil concentration that would cause 50% of the exposed animals to respond after exposure to the WSF for some given time.*

*We call this concentration of oil in the WSF the median tolerance limit, or TLm.

Usually the response is death, although we do note certain other behavioral responses as well.

In addition, the probit analysis estimates a 95% fiducial limit about this TLm, a slope function and 95% fiducial limit about this slope function. The slope function is the rate at which the proportion of animals responding changes with changing oil concentrations in the WSF. From it, one can estimate the most likely proportion of animals that would respond at WSF oil concentrations other than the TLm. It is related to the tolerance distribution of the species being tested.

In some cases our bioassay results are not amenable to probit analysis. Probit analysis requires at least two dose levels of WSF at which the proportion of animals responding is observed to be between zero and one. Occassionally our dose levels will be so distributed that none of the animals respond in some set of lower doses, and all of them respond in the next highest dose and all higher doses. Or, there will be only one dose at which the proportion of animals responding is observed to lie between zero and one. In the former case we estimate the TLm as the antilog of the sum of the log of the highest dose, where no animals respond, plus the log of the lowest dose where all of the animals respond divided by two. In the latter case we estimate the TLm by plotting the dose versus the percent of the animals responding, and noting the dose level that corresponds to 50% response. This is the method of Doudoroff et al. (1951).

All of our data are being stored on punched computer cards, as well as in our record books.

B. Methods of Specific Experiments

1. Static acute bioassays were conducted with several organisms and developmental stages at temperatures ranging from 4-8°C. Water-soluble fractions of crude oils were prepared by a standard method. Oil exposures were monitored by chemical analyses, i.e. IR, UV, and GC. Chemical dynamics and degradation of exposure solutions were investigated. The identity and concentrations of the major toxic components were analyzed by probits, and 95% fiducial intervals for 0-, 24-, 72-, and 96-hour TLm values were determined. Organism variability was tested in a standard bioassay using dodecylsodium sulphate (DSS) as a standard toxicant. Sublethal quantifiable behavioral responses were used when possible as an additional indicator of effect.

A special assay was run with Macoma balthica to determine effects of oil on the clam's burying activities (see attached manuscript).

An assay with scallops was run to determine limits of recovery for affected behavioral responses of the animal when removed from Cook Inlet WSF. Scallops were exposed to three concentrations for 10 exposure time periods (30 per treatment), and then put in clean flowing seawater where their condition was monitored for 3 months. This was repeated with scallops for a replicate run and will be duplicated (two replicates per animal) with hermit crabs and pink salmon alevins.

2. Temperature assays were run using acute assay methods at 4°, 8°, and 12°C with scallops and Eualus shrimp. Benzene and Cook Inlet WSF were used in these tests. The three temperatures were run simultaneously with the same initial mix to minimize variability. In a separate study, two types of Cook Inlet WSF (identical UVOD but different benzene, toluene, and xylene concentrations) were tested at 4°, 8°, and 12°C with Eualus shrimp.

3. Chronic shrimp assays (30-day exposure) with Eualus fabricii started in March 1976. Gravid shrimp were held individually and exposed to several doses of oil renewed every other day until hatching. Survival of females and hatching success will be monitored.

Preliminary experiments were conducted with newly extruded eggs of tanner and king crabs. Exposure of eggs on mature king crab females is logistically difficult because of the large biomass. Tanner crabs do not present as much difficulty. Experience from these preliminary experiments provided input which had contributed in experimental design for studies scheduled in the spring of 1976.

4 and 5. Uptake and depurtaion experiments with new species and at different temperatures are not started yet. Methodology is given in amended proposal starting April, 1976. A combination of isotope uptake and GLC analyses of hydrocarbons in tissues will be used.

6. Determination of effects of oil on salmon fry respiration was completed in the summer of 1975. Opercular breathing rates and coughing rates were measured by use of electrodes that were not attached

to the fish (see enclosed manuscript accepted for publication--Rice, Thomas, and Short 1976).

Determination of effects of Cook Inlet WSF, benzene, and naphthalene on the heart rates and metabolism of king crabs was completed. Heart rates in juvenile male and female king crabs were recorded by implanting small electordes in the pericardial space overlying the heart and monitoring the impulses on a sensitive recorder. Six to 13 crabs were used in each of seven short-term (22- 28-hour) experiments and one long-term (86-hour) experiment. Correlation of heart rates and respiration was demostrated in one experiment by placing crabs in flow-through respirometers and monitoring heart rate and oxygen uptake simultaneously during oil exposure.

The various water-soluble fractions used in the experiments were measured by UV spectrophotometry and GC.

7.--Determination of effects of oil on scallop growth is scheduled for spring 1976. Based on a preliminary scallop growth study of September 1975, a new experimental design was developed. Five groups of scallops will be exposed to different doses of oil for 10 weeks (March-May 1976).

8. Study of effects of oil on tanner crab autotomy is starting in March 1976. Premolt tanner crabs will be held unitl molting and then exposed to WSF at varying periods after molting. The effects of aged versus fresh WSF will be examined as well as certain single aromatic components on crab autotomy.

9. Scallops, shrimp, and juvenile king crabs were exposed to two doses of Cook Inlet WSF for 6-9 days to determine histological effects. Tissues were prepared for histological examination using standard methods. The mantle, gill, and gonad of scallops; gills, antennules, and antenna of shrimp; and antenna, antennules, gills, and heart tissues of crab are being examined; completion is due by the summer of 1976.

In a second experiment crabs used in the metabolism study (6b) were sampled with several tissues prepared and sent to Dr. Mary Ann Smith, University of Alaska, Fairbands, for electron microscopy.

VI. Results

1. Acute assays were run on 14 species (Table 1). Gas chromatographic analyses of the 100% WSF yeilds quantifiable values for benzene, toluene, xylene, naphthalene, methylnaphthalene, and dimethylnaphthalene.

The Macoma assay results are included in the enclosed manuscript by Taylor et al., which is being reviewed for publication.

All assay data have been keypunched and are ready for probit analyses. (These begin in March, 1976).

Preliminary results from scallops exposed to Cook Inlet oil (o.205 UVOD) for 6-48 hours and then held in clean seawater indicate a delayed response to oil. Scallops exhibiting mantle retraction, gaping, and response to a prodding stimulus (stage 4) continue to deteriorate and eventually die (1-3 weeks after exposure). This indicates that stage 4 scallops which are recorded as a "living" response is a 96-hour bioassay, are

actually moribund. These results are preliminary, but are commensurate with other observations that invertebrates are slow to die; for this reason, false estimates of high resistance to oil are observed in a standard 4-day test.

Bioassay tests in general are on schedule. More are programmed for spring and summer, with special attention on delayed responses.

2. The effects of temperature on the toxicity of Cook Inlet WSF's to Eualus shrimp and scallops are not significantly different (Table 2). In contrast, tests using the high aromatic mix at each temperature were more toxic than the test with the low aromatic mix at the same temperature (Table 3).

3. Preliminary experiments with early stages of crab eggs resulted in the following:

a. Egg samples were collected and preserved daily for the first 20 days of development from several control and exposed king and tanner crabs. Exposed crabs and control crabs had clutches of newly extruded eggs, which in the exposed were subjected to 24 hours of either a high or low dose of the WSF of Cook Inlet crude oil under static conditions.

b. Oil appeared to have little effect on the firming up and attachment of eggs to pleopodal setae, but effects on the development of eggs were suggested.

c. Eggs from these exposures are being tested with various staining techniques to facilitate examination to determine whether differences occur in development rates between exposed and controlled eggs.

d. Some success with a staining technique has been obtained, but it is not entirely satisfactory. Examination and recording of developmental stages of eggs prior to preservation may be necessary in final experiments planned for spring 1976.

e. Chronic exposure with shrimp eggs began in March 1976, but there are no results yet. Chronic exposures to herring eggs are scheduled for May.

4. All uptake-depuration experiments with new speceis have been rescheduled for April-October under the amended contract.

5. All uptake-depuration experiments at different temperatures have been rescheduled for April-October under the amended contract.

6. Meatbolic rate in Pink salmon fry increased with increasing oil exposure. Cook Inlet, Prudhoe Bay, and No. 2 fuel oil WSF's caused the same response. When a constant dose was maintained, the fish showed a constant elevated metabolic response (see attached manuscript by Rice et al.).

Heart rates and oxygen consumption in juvenile king crabs were both shown to decline during exposures to WSF's of Cook Inlet crude oil. In all but one of the six experiments (several crabs each) using crude oil, the heart rates showed partial recovery during the exposure and complete recovery when returned to noncontaminated seawater. In the exception, in which the WSF concentration was high (0.213 TVOD), the four crabs died, but the heart rates in the other four eventually returned to near control rates.

Exposure to benzene also resulted in heart rate depression, but the depression occurred much sooner, the slowest rate occurring by 30 minutes after exposure--as opposed to 3-7 hours for crude oil. Further, although the benzene concentrations in the exposure water declined rapidly, this decline did not result in the recovery of heart rates until the benzene concentration had declined by 90%.

High concentrations of naphthalene did not cause as great or as precipitous a decline in heart rates (maximum depression was 20% from control rates) as did either benzene (50% depression) or sublethal doses of crude oil (52% depression). The naphthalene concentrations were relatively stable in the exposure water, not declining as rapidly as benzene concentrations. The heart rates recovered when the naphthalene concentrations eventually declined.

In conclusion, for king crabs exposed to Cook Inlet crude oil or to benzene and naphthalene: (1) continuous monitoring of heart rates simultaneously with periodic measurements of the declining WSF concentrations is a sensitive method for detecting and describing sublethal toxicity; (2) the heart rate (and oxygen uptake) response to sublethal doses is one of depression, followed by recovery as WSF concentrations decline; (3) WSF's of benzene cause a more rapid heart rate depression, and slower recovery than do WSF's of either crude oil or naphthalene; (4) benzene causes more severe heart rate depression than does naphthalene; and (5) chronic exposure appears to produce an intensifying effect on heart rate depression.

A manuscript describing the king crab heart rate experiments is in preparation for publication by Mecklenburg et al.

7. A preliminary scallop growth study gave indications of a reduced growth rate due to repeated exposures to Cook Inlet WSF. The definitive scallop growth study will begin in April 1976 to coincide with the spring plankton bloom.

8. Experiments on the effect of oil on crab autotomy are scheduled for April.

9. Effects of oil on histopathology--no data available yet. Samples have been taken and are in various stages of processing.

VII. Discussion

The discussion of our results is mostly premature at this time. The bulk of the studies are scheduled for spring and summer, 1976, and only a few specific studies have been under way for more than a few months.

1. The probit analysis has not been run on the acute assays due to problems with the computer. Many invertebrates appear to be very resistant to Cook Inlet and No. 2 fuel oil WSF's with the TLm values >0.4 UVOD and >3 ppm by IR. However, the chronic reversibility study with scallops is demonstrating delayed mortality several weeks after exposure was completed. Other invertebrates are barely alive at the termination of the 96-hour assay, but are counted as live organisms using standard assay methods. To obtain a more meaningful assay value, animals must be held in clean water with normal feeding after the 96-hour exposure. When delayed mortality ceases or levels off, the 96-hour

TLm can be calculated. The delayed mortality response will vary with species.

We are now changing our bioassay methodology to include a holding period after the standard 96-hour exposure. This will result in fewer assays, but more meaningful values will result. As a further effort to improve our toxicity data, we are going to repeat the scallop reversibility study in the field (Auke Bay) to compare the laboratory and field experiments. The field study conducted in net-covered pens will also determine effects of predation by amphipods, zooplankton, etc., on scallops previously exposed to Cook Inlet crude oil WSF's.

2. The temperature assays are demonstrating a significant effect of the lower aromatics (benzene, toluene, and xylene) on toxicity. The low and high aromatic mixes used in this study have identical UVOD values, but differ in their benzene, toluene, and xylene content because of the mixing methods used. The lower aromatic mix is mixed in a 700-liter fiberglass tank which has a far greater surface-area-to-volume ratio than the 200-liter barrel mixes (high aromatic). This increased aeration effect has resulted in lower mononuclear aromatic levels. The question of which components in WSF's contribute to toxicity has yet to be definitely answered. The data we are collecting during temperature effect assays as well as gas chromatographic analyses of the WSF during the assay period will contribute toward this end. We are planning a detailed gas chromatographic study for April 1976 to determine effects of time, temperature, bacterial action, and aeration on component concentrations in the Cook Inlet crude oil WSF's.

3. The study determining the effects of Cook Inlet crude oil WSF's on king crab heart rates has yielded a sensitive method that correlates with metabolism. Analysis of the data indicate the heart beat rate correlates with dose. Opercular rate increases in pink salmon fry indicate an increased metabolic rate for oil-exposed fish. Vertebrate and invertebrate responses are dramatically different.

Determination of the effects of oil exposure on metabolism is very significant because of the obvious potential impact on growth and reproductive potential in marine organisms. Further metabolic studies are planned in shrimp and salmonids. The next logical step is correlating metabolic change with uptake and depuration. If there is a metabolic "cost" caused by oil component depuration and metabolism, then the uptake of oil components gains a new significance. Studies of this type with juvenile king crabs and pink salmon fry are scheduled for the summer of 1976.

VII. Conclusions

1. Some invertebrates exhibit delayed mortality after completion of 96-hour assays. The previous observations of high resistance in static 96-hour tests may be in error. Modification of standard procedures is needed.

2. While oil did not appear very toxic to Macoma clams in standard 96-hour tests, there were significant effects of oil on Macoma burying activities. The clams would surface when contaminated sediments settled on top of the substrate. See Taylor et al. for effects on Macoma balthica.

3. Preliminary evidence suggests little effect of temperature on Eualus shrimp toxicity to Cook Inlet crude oil WSF's. A high aromatic mix (more benzene, toluene, and xylene, but identical UVOD) is more toxic to Eualus than a low aromatic mix.

4. Heart rate was a sensitive parameter to determine effects of oil on juvenile king crabs. Heart rates were depressed during exposures to Cook Inlet crude oil, benzene, and naphthalene WSF's, but recovery usually occurred. Heart rate depression correlated well with the dose and depuration of the compounds. Benzene caused a faster, more severe depression and slower recovery than naphthalene.

Opercular rates in salmon fry are stimulated when exposed to oil, indicating an increase in metabolism. This indicates that vertebrates and invertebrates may respond in very different ways to oil exposures. See Rice et al. (1976) for effects on breathing rates in pink salmon.

IX. Needs for Further Study

1. Expanded acute assays are needed that include a recovery phase to calculate the true TLm for each species. Determination of TLm's for a wide ecological range of organisms will be made. Determination of chronic toxicity to herring, crab, and shrimp eggs will be made in this study, but other life stages and species are needed.

2. Effects of time, bacterial degradation, aeration, and temperature on oil component concentration in a dosed water sample need to be determined.

3. The toxicity of key oil components in the WSF needs to be determined in order to ascertain the relative toxicity contribution of important components.

4. Investigation into the mechanics of acute toxicity and chronic toxicity are needed, with the assumption that physiological and behavioral responses from present studies will supply clues.

5. The uptake, distribution, persistence, and depuration of oil components in eggs, larvae, and untested invertebrates are needed.

6. Determination of the effects of oil on organisms under laboratory and field conditions need to be made. These experiments should include exposure, marking, release, and recapture.

7. The determination of oil concentrations that can be achieved after an environmental spill are severely needed. Initially, a survey team should be sent to spills for quantitative determination of oil concentrations in water rather than quantitating the volume spilled.

X. Summary of Fourth Quarter Operations

1. Seventeen acute assays were run this quarter (Table 1).

2. Assays at different temperatures were conducted on shrimp and scallops (Table 2).

3. The effect of Cook Inlet crude oil, benzene, and naphthalene on king crab heart rates was determined.

4. The delayed effect of oil exposure to scallops was started. This requires lengthy holding of the animals after exposure.

5. Animal collection effort was considerable.

Table 1.--Acute toxicity tests completed with WSF of Cook Inlet crude oil (CI), WSF of No. 2 fuel oil (FO), and dodecylsodium sulfate (DSS).

Scientific name	Common name	Toxicant
Eleginis gracilis	Saffron cod	CI, FO
Idothea wosnesenskii	Isopod	CI, FO
Pandalus danae	Dock shrimp	CI, FO, DSS
Pandalus hypsinotus	Coonstripe shrimp	CI, FO
Thais lamellosa	whelk	CI, FO
Pagurus hirsutiusculus	Hermit crab	CI, FO
Pandalus goniurus	Humpback shrimp	CI, FO, DSS
Pandalus borealis	Pink shrimp	CI, FO
Acmaea scutum	Limpet	FO
Eupentacta quinquisemita	Sea cucumber	
Orchomene pinquis	Amphipod	CI, FO
Acanthomysis pseudomarcopsis	Mysid	CI, FO
Chlamys sp.	Pink scallop	CI, DSS

Table 2.--96-hour TLm's as measured by ultraviolet (UVOD) spectrometry on scallops and shrimp exposed to Cook Inlet WSF and/or benzene at 4°, 8°, and 12° C.

Species and temperature (° C)	96-hour TLm		
	Cook Inlet crude		
	High aromatic[1]	Low aromatic[1]	Benzene
Shrimp (Eualus)			
4°	0.11	0.206 (184-0.229)	--
8°	0.135 (0.121-0.151)	0.247 (0.229-0.266)	--
12°	0.115 (0.104-0.127)	0.183 (0.161-0.209)	--
Scallops (Chlamys)			
4.4°-5.1°	NA[2]	NA	NA
7.1°-8.2°	NA	NA	NA
11.7°-12.8°	NA	NA	NA

[1]Low aromatic and high aromatic mixes had identical UVOD values but differed by an order of magnitude in their benzene, toluene, and xylene contents because of the mixing method used. See Table 3.

[2]NA = Experiment tested, but data not analyzed by probits yet.

Table 3.--Results of chemical analyses of high aromatic and low aromatic mixes of Cook Inlet crude oil WSF's used in Eualus shrimp temperature assay. The two WSF's were analyzed by ultraviolet (UV) and infrared (IR) spectrophotometry and gas chromatography (GC).[1]

Methods and compound concentrations	Cook Inlet crude oil WSF	
	High aromatic mix	Low aromatic mix
UV (given as UVOD--ultraviolet optical density)	0.405	0.390
IR (given as ppm of oil)	8.03	7.42
GC (aromatic concentrations given in ppm of--)		
Benzene	2.00	0.142
Toluene	2.15	0.178
o-xylene	0.377	0.184
m-, o-, p-xylene[2]	0.782	0.203
Naphthalene	0.115	0.169
1-methylnaphthalene[2]	0.0706	0.848
2-methylnaphthalene	0.0579	0.090
2-, 6-dimethylnaphthalene[2]	0.028	0.0417

[1]The high aromatic mix was made in a 200-liter oil drum with polyethylene liner and cover. The low aromatic mix was made in a 700-liter fiberglass tank with no cover.

[2]Concentration value includes some contribution from undetermined compounds.

RESPONSE OF THE CLAM, *MACOMA BALTHICA* (LINNAEUS), EXPOSED TO PRUDHOE BAY CRUDE OIL AS UNMIXED OIL, WATER-SOLUBLE FRACTION, AND SEDIMENT-ADSORBED FRACTION IN THE LABORATORY

CONTENTS

ABSTRACT

The small clam, Macoma balthica (Linnaeus, 1758), occurs throughout the coastal areas of Alaska in the upper 4-8 cm of intertidal mudflats. Because it is both a deposit and suspension feeder, M. balthica is potentially susceptible to oil slicks layered on the mud and to water-soluble or sediment-adsorbed fractions of crude oil. Settling of unmixed Prudhoe Bay crude oil on the mud during five simulated low tides (2-3 h each) had negligible effects on buried adult M. balthica observed for 2 months. However, the water-soluble fraction (WSF) of Prudhoe Bay crude oil had an effect on M. balthica both in static and flow-through bioassays. In static bioassays, WSF's prepared from a 1% oil-water mixture in concentrations of 11% and 87% of the saturated WSF, (naphthalene equivalents, 0.036 and 0.331 ppm respectively) caused many buried clams to come to the surface. The greatest response occurred within 3 days at the high concentration (0.331 ppm) and 9 days at the low concentration (0.036 ppm). Although at the lower concentration the response took longer to occur, more clams came to the surface. In flow-through bioassays WSF's prepared from 1% oil-water mixture in concentrations ranging from 7% to 80% of the saturated WSF (naphthalene equivalents, 0.019 to 0.302 ppm) inhibited burrowing of some unburied clams and caused other buried clams to come to the surface. The ECm is 0.233 and 0.222 ppm naphthalene equivalents respectively for ability of unburied clams to burrow into the sediment within 60 and 170 min from start of exposure. The ECm is 0.361 ppm naphthalene equivalents for response of buried clams to move to the surface within 3 days from start of exposure. Oil adsorbed on

sediment and allowed to settle over buried M. balthica also stimulated movement to the surface. The proportion of clams that moved to the surface increased as the depth of oil-contaminated sediment increased. We calculated that under conditions of our laboratory experiment it would take a layer of oil-contaminated sediment 0.668 cm deep to cause 50% of the buried clams to move to the surface within 1 day. In our tests many of the clams recovered from exposure, but in nature they might have fallen to predators or adverse environmental conditions. Data on the response of M. balthica to oil can be used in the evaluation of the organism as an indicator of the effect of oil in the sediment environment.

Response of the Clam, Macoma balthica (Linnaeus), Exposed to Prudhoe Bay Crude Oil as Unmixed Oil, Water-Soluble Fraction, and Sediment-Adsorbed Fraction in the Laboratory

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INTRODUCTION

When the trans-Alaska oil pipeline is completed, the port of Valdez in Prince William Sound will become the staging area for loading crude oil into tankers for transport to refineries. It is during oil transporting activities here that the highest risk of oil contamination will occur.

Several scientists have been involved in projects near the terminal site which are aimed at determining how such activities might affect the marine resources in the area. Since the fall of 1968, Dr. Richard T. Myren and the Environmental Impact Investigation at ABFL (Auke Bay Fisheries Laboratory) have been gathering quantitative data on the intertidal communities near Valdez (unpublished). John Karinen, Dr. Stanley Rice, and the Physiology-Bioassay Section at ABFL have run a series of experiments on key marine species to determine the effects of oil under varying conditions. Dr. Howard M. Feder, Institute of Marine Science, University of Alaska, Fairbanks, has conducted a project funded by the Environmental Protection Agency to determine the effects of oil on the sediment environment of Port Valdez and Galena Bay (Feder, this report).

Emphasis in the studies by Myren and Feder, has been on intertidal organisms of the sediment environment, especially on the tiny clam, Macoma balthica (Fig. 1), which is abundant on the low-gradient mudflat at Dayville, 1 mile east of the tanker terminal in Valdez and on other suitable mudflats throughout Alaska. M. balthica buries itself in soft sediments just below the surface and reaches out with separate siphons to feed and respire at the surface (Brafield 1961, 81-82; Rasmussen 1973, 308-9). Since it feeds on both deposited and suspended matter, it is likely to be a good indicator of the effect of oil in a sediment environment.

This paper is a summary of the laboratory work done with M. balthica in 1975 at ABFL. The objective was to measure the response of the clam to exposure to Prudhoe Bay crude oil.

The effect of oil on the clams was tested three ways, which involved three methods of mixing Prudhoe Bay crude oil into the environment of M. balthica. Experiment 1, representing a low level of mixing energy, was designed to simulate a crude oil spill stranded on a tideflat under calm conditions. Experiment 2, representing a moderate level of mixing energy, consisted of exposing clams to water-soluble fractions (WSF) of oil. Experiment 3, representing a high level of mixing energy, consisted of exposing clams to oil-contaminated sediments. Mortality and behavior were observed and recorded in all three experiments; burrowing was the primary response observed in the second and third experiments.

Organization of the report is as follows: methods common to all experiments are presented first, then each type of experiment is described separately, followed by a general discussion and evaluation of M. balthica as a bioassay organism and baseline indicator of the effect of oil in the sediment environment.

sediment and allowed to settle over buried M. balthica also stimulated movement to the surface. The proportion of clams that moved to the surface increased as the depth of oil-contaminated sediment increased. We calculated that under conditions of our laboratory experiment it would take a layer of oil-contaminated sediment 0.668 cm deep to cause 50% of the buried clams to move to the surface within 1 day. In our tests many of the clams recovered from exposure, but in nature they might have fallen to predators or adverse environmental conditions. Data on the response of M. balthica to oil can be used in the evaluation of the organism as an indicator of the effect of oil in the sediment environment.

3

METHODS COMMON TO ALL EXPERIMENTS

The M. balthica and marine mud used in the experiments were collected from the mudflat 200 yards south of the public launching ramp at Amalga Harbor near Eagle River northwest of Juneau. The area has had only limited use and is regarded as relatively free of oil contamination.

Techniques of chemical analyses of the water and tissue samples were the same for all experiments. Water samples were analyzed for hydrocarbons by IR (infrared) and UV (ultraviolet) spectrophotometric procedures. Infrared water analysis to determine paraffinic hydrocarbons followed the technique of Gruenfeld (1973) using tricholorotrifluoroethane (Freon 113) as a solvent and reading at a wave number of 2930 cm^{-1}. This method detects paraffins in concentrations greater than 0.25 ppm (Loren Cheatham, personal communication). Water analysis was also accomplished by an ultraviolet spectrophotometric technique using hexane as the extracting solvent and estimating naphthalene concentration by reading OD (optical density) at 221 nm (Neff and Anderson, 1975, p 122-128). This method is accurate for concentrations greater than 0.005 ppm equivalents of naphthalene (Loren Cheatham, personal communication). Efficiency of naphthalene extraction ranged from 91% to 95%. Concentrations were expressed as naphthalene equivalents, relating them to a naphthalene standard. Oil contamination in clam tissues was measured with UV by a similar method but modified to include tissue digestion with papain (Neff and Anderson, 1975, p 122-128).

UNMIXED CRUDE OIL SPILL--EXPERIMENT 1

This experiment is similar to an earlier experiment conducted in Port Valdez in which tidal stranding of an oil slick on a mudflat was simulated (Shaw et al. in press). Shaw et al. put crude oil in aluminum frames (topless and bottomless boxes) placed on the Valdez mudflat and sampled the enclosed M. balthica regularly to determine how many were alive or dead and how much hydrocarbon they had in their tissue. The hydrocarbon content of living clams and percentage of empty valves indicated that oil killed M. balthica, but more information regarding the response of the clams to the oil was needed. We designed laboratory experiments with provisions for gradual draining and refilling of tanks to simulate the tidal ebb and flow on a mudflat. We determined survival, behavior, and uptake of oil by M. balthica.

Apparatus and Experimental Procedure

Our experiment was conducted in four rectangular tanks (10,000 cm^2 each). The tanks were constructed of marine plywood painted with two coats of Woolsey Caulux marine paint and the seams sealed with silicone caulk. Six cm of mud was placed on the bottom of each tank except in the areas of the inflow and outflow, which were kept clear of mud by wooden partitions flush with the mud surface (Fig. 2). The tanks were inclined at a slight angle (5°) to allow water drainage.

The mud used in the experiment was collected in three steps designed to preserve natural conditions as much as possible. First, an area the size of the tanks was marked on the mudflat. Next, the top 3 cm of mud was removed from the area and put into buckets. Last, the next 3 cm of mud was removed and held separately. The mud was not screened nor were any organisms removed from it. The two layers were placed in their original order in the tanks and leveled. The mud contained many M. balthica and other organisms, especially the polychaete worm, Arenicola sp. Several hundred M. balthica were later added so that each tank contained approximately one clam to every 5 cm^2 or about 2,000 clams--a density comparable to that found in Port Valdez (Feder and Myren unpublished data).

Fresh seawater from Auke Bay flowed continually into the tanks and was maintained 13 cm deep over the mud by a removable standpipe in the drain. To simulate a low tide the standpipes were removed, the water turned off and the tanks allowed to drain. The water level fell at the rate of 0.1 cm min^{-1}. The mud was exposed for 2-4 h and then the standpipes were replaced and the tanks refilled with a gentle flow of water (1.2 l min^{-1}), which did not disturb any sediment. The water was calm and clear throughout the experiment. Water temperatures gradually increased during the course of the experiment and ranged from 7° C in May to a maximum of 12° C in later summer.

Description of Simulated Oil Spill

The experimental set-up was put into operation on May 1, 1975--4 weeks before the first exposure to oil to allow time for the clams to become acclimated to the apparatus. The first oil was added on May 27 and continued daily for 5 successive days. Three tanks were treated with oil, and the fourth untreated tank was maintained as a control. Three doses of oil (1.2, 2.4, and 5 µl cm^{-2} day^{-1}) were added as the "tide" was falling when the water was about 5 cm deep. The oil was gently poured onto the water. It spread unevenly over the water and as the water receded settled unevenly on the mud, where it remained during low tide. As the water returned on the incoming tide, it lifted the oil from the mud and carried it out through the overflow. A small amount of oil remained on the sides of the tanks, making visible sheens for a few days during subsequent tide manipulations. There were no visible signs that oil had adhered to the surface or had been incorporated into the sediments.

Sampling Method

The effect of the oil on M. balthica was measured in two ways: (1) by counting live and dead clams and (2) by measuring the amount of naphthalene in clam tissues. In addition, water analyses were conducted to determine oil content in the water.

Several pieces of apparatus were used to obtain samples. To collect clams for determining number live and dead and as a source of tissue, a hollow drill (10 cm inside diameter and beveled at the end) was used to cut mud. The drill was removed and a glass cylinder (6 cm high by 10 cm inside diameter) was twisted into the space left by the drill and sediment spooned out from inside the cylinder. The cylinders were left in place for the duration of the experiment to prevent mud from caving in and changing the water flow patterns.

The live and dead clams were counted and tissue samples taken beginning 2 days after the oil exposure ended. Three replicate samples of clams were taken from each tank once a week for 4 weeks; the tanks were visually monitored for 2 months longer. Water samples were collected from each tank once a day during the oil additions and once a week thereafter for 4 weeks.

The samples taken for the live and dead counts and tissue were obtained by washing the mud from a 160-cm^2 area (two adjacent glass spacers) through a 1.68-mm screen. Live and dead clams were then segregated from the samples. Clams were considered dead from oil exposure if they were gaping and contained tissue or if they contained no tissue but the valves were intact and the hinge elastic. The live clams were counted and placed in running seawater for 24 h to clear their digestive tracts. They were then frozen to await tissue analysis. Two grams of whole clams (wet weight) were used in tissue analyses.

Results and Discussion

No significant mortality of control or exposed clams occurred during 2 months after exposure to oil and the only indication that the exposed clams experienced any stress was reduced siphon activity which was noticed during the time the oil slicks were on the mud. The quantity of oil in the water and naphthalene in the clam tissues was below the detection limits of our methods. However, water samples were collected after most of the water had drained and what was left was slowly percolating through the mud under the end board into the effluent end drain. Since this water was essentially filtered through the mud, any small amount of oil in the water may have been adsorbed as the water passed through the sediment. No sediment analyses were done to verify this, however.

Our results with respect to clam mortality and accumulation of hydrocarbons within the clams are contrary to the results of a field application of oil reported by Shaw et al. (in press). In our experiment oil did not measurably affect the clams, but Shaw found significant mortality at the oiling rate of 5 μl oil cm^{-2}.

Differences in the behavior of oil under field conditions versus our aquarium situation may help to explain the different results. In contrast to what occurs in a field situation, virtually no mixing energy was applied to the oil/water/sediment mixtures in the aquaria. Therefore one would expect a minimum amount of oil to dissolve into the water phase. A second difference noted in the aquaria which contrasts to a field situation was that because of the slight incline of the tanks, most of the water drained slowly across the surface of the sediment rather than moving down through it. Only a small amount of the water percolated through the sediment and under the endboards as the water level in the outflow buffer zone dropped below the sediment surface. Movement of water under the endboards was restricted enough that all of the water did not drain completely from the sediment surface and therefore the oil simply settled on the elevated portions and rested on a thin layer of water in the depressions of the sediment surface. Close contact of the oil with the sediment was not uniformly achieved in the aquaria.

The discrepancy between our experiment and Shaw's is attributed to the fact that virtually no mixing energy was applied to our system; thus, little of the oil was dissolved in the water or adsorbed to the sediments. Analysis of oil in the sediment is needed to verify the later point, but lack of clam response supports the idea that little or no oil remained in the sediment of our aquaria. Although Shaw et al. did not attempt to quantify mixing energy on the Valdez mudflat during their tests, calm weather prevailed (Feder, personal communication)--yet it is a fact that the water in that area carries a heavy sediment load (R. Myren and N. Calvin, personal communication) which would have mixed with the oil during normal tidal and surf action and resulted in transport of the adsorbed oil to the sediment surface. A study by Clark and Finley (1975) gives evidence that direct contact of Mytilus edulis with oil causes higher mortality and greater uptake of hydrocarbons than contact with the dissolved fractions.

ACUTE BIOASSAY WITH WATER-SOLUBLE FRACTION--EXPERIMENT 2

Experiments with the WSF and M. balthica were conducted to measure the effect of dissolved oil on the clams. The response of the clams to the WSF was measured in two ways. First, clams already buried in sediment were exposed to WSF and observed for burrowing activity. Second, clams were placed on top of the sediment and observed as they burrowed. We had two basic types of experimental design with our WSF tests. The first was a static situation in which water temperature and oxygen content were not controlled but were essentially the same for control and exposed clams within tests. The second type of design was a flow-through system where recycled seawater flowed continually, was aerated, and was cooled to a constant temperature to reduce experimental variables. WSF for use in exposures were always prepared in the same manner.

Preparation of the WSF For Use in Exposures

One-percent Prudhoe Bay crude oil in seawater (1 l oil:100 l seawater) was mixed slowly and nonviolently at about 200 R min^{-1} for 20 h at ambient water temperatures (10°-12° C). The mixture was allowed to separate for 20 h before the virtually saturated WSF was siphoned from below the slick (Anderson et al. 1974, p 79). Since the naphthalene equivalents of the WSF vary from mix to mix, we analyzed the initial mixture using the ultraviolet spectrophotometric technique and diluted it with seawater to concentrations that correspond to percentages of saturated solution (designated 100% solution) containing 0.379 naphthalene equivalents. After the dilutions are made the water is analyzed by IR and UV to verify the concentrations to which the clams are actually exposed.

Design of Static Water System Experiment

Clam exposure in the static water system experiment which tested the response of buried clams to WSF's was conducted in two stainless steel trays (26 cm wide by 40 cm long by 3 cm deep) completely filled with screened mud and submerged in a larger seawater tank (160 cm long by 37 cm wide by 8 cm deep). Water temperature was 8° C at the time of its addition and gradually warmed to a maximum of 18° C. No attempt was made to circulate or aerate the water during exposure, but it was oxygenated at the time of introduction.

There was a seawater control and two WSF dose levels in the experiment. The sample size in each case was 400 initially buried clams. The number used to identify the strength of WSF dose is the average of the ppm of naphthalene equivalents measured on day 0 and day 2 when the water in the tanks was changed. The average for the lower dose is 0.036 ppm and for the higher dose is 0.331 ppm, which is 11.7% and 87% respectively of the 100% WSF containing 0.379 ppm naphthalene equivalents. Concentrations of n-paraffins determined by IR for these same doses were 1.24 and 7.76 ppm.

Design of Flow-through Water System Experiment

The setup for the flow-through water system experiment which tested the response of both buried and unburied clams to WSF's was somewhat more elaborate than the static water system experiment because it was designed to accommodate greater water capacity, continued water circulation, aeration, and cooling. Each exposure was conducted in a glass tray (25 cm wide by 45 cm long by 3 cm deep) completely filled with screened mud and submerged in a seawater tank (37 cm wide by 53 cm long by 8 cm deep) similar to the trays and tanks used in the static system test. In addition, there was a separate water-holding tank in association with each seawater tank. The holding tank was filled with 100 l of seawater or WSF which was pumped at the rate of 1.2 l min^{-1} via a submersible pump into the tank containing the clam trays which overflowed through a standpipe into a drain tube leading back into the holding tank. Water aeration occurred at this point. Each holding tank was equipped with a cooling coil which kept the water temperature between 7° and 9° C.

There were a seawater control and five WSF dose levels in this experiment. The sample size in each case was 200 initially buried clams and 40 initially unburied clams. The number used to identify the strength of WSF dose is the average of the naphthalene equivalents in ppm measured on days 0, 2, and 4 when the water in the tanks was changed. The average is 0.019, 0.036, 0.081, 0.160, and 0.302 ppm which is 7.5%, 11.6%. 23%, 44%, and 80% respectively of a 100% WSF containing 0.379 ppm naphthalene equivalents. Concentrations of n-paraffins in these doses as measured by IR were 0.378, 1.040, 1.661, 2.480, and 5.809 ppm.

Experimental Methods

The method for measuring response of buried clams to WSF's was different from the method for unburied clams. To test the response of buried clams, trays of mud were held in plain seawater and seeded with M. balthica which buried themselves prior to later introduction of WSF's into the same tanks. To test the response of unburied clams marked clams were held in similar mud trays in fresh seawater separate from the experimental tanks until just before their exposure. At that time the clams were gently screened out of the mud and moved to the surface of mud trays in WSF exposure and control tanks. Time of response for both exposures was measured from the time that oil exposure started.

Doses of the WSF were replaced at various intervals within the experiments in an attempt to compensate for the natural loss of the aromatics (mostly from bacterial growth or volatility) in the WSF over time. There is evidence that after 48 h the loss is rapid and varies from one dose to another even under the same conditions (Jeffrey W. Short, personal communication).

The static water system experiment began Oct. 6, 1975, and lasted 11 days. The water-changing schedule in the experiment was as follows: (1) To start the exposure the plain seawater was drained from the tanks and refilled with 50 l of WSF dose for the exposures and seawater for the control. This water was left in the tanks 48 h. (2) At 48 h the water was drained from the tanks and the exposure tanks were refilled with the same amounts of newly prepared WSF's of the same approximate concentration; the control tank was refilled with seawater. This water was left in the tanks 144 h. (3) At 192 h into the experiment, the water was drained from the tanks and they were all refilled with seawater. This water was left in the tanks for 3 days to constitute the recovery period.

The flow-through water system experiment began Nov. 10, 1975, and lasted 10 days. The water-changing schedule was as follows: (1) To start the exposure the plain seawater was drained from the tanks and the holding tanks refilled with 100 l of WSF dose for the exposures and clean seawater for the control. This water was left in the tanks 48 h. (2) At 48 h the water was drained from the tanks and the exposure holding tanks refilled with the same amounts of newly prepared WSF of the same approximate oil concentration as was initially applied, the control holding tank was refilled with seawater. This water was left 48 h. (3) At 96 h step 2 was repeated. This water was left for the duration of the 10-day period.

In addition to testing the response of buried clams to WSF's this experiment included provisions for testing the response of unburied clams to WSF's. The marked clams for this test were placed on the surface of the mud immediately after the introduction of the first dose of WSF. Response was defined as clams burying themselves.

Water analyses by UV and IR techniques were conducted at each WSF dose change to verify the actual dose applied. Water samples were taken from each tank within 10 minutes of the dose change to obtain data of hydrocarbon content at its highest concentration.

Responses to WSF's are recorded in numbers dead and unburied clams. A dead clam was defined as a clam that was gaping and did not close in response to probing. Dead clams were removed from the exposures at least every other day. Unburied clams were counted as a half if they were partly visible yet vertical in position and partly buried and as a whole if they were lying flat on the surface and totally visible.

In the static water system experiment counts of clams that had responded by burrowing to the surface or dying were made on days 1, 2, 3, 8, 9 , 10, and 11.

In the flow-through water system experiment, initially unburied clams were counted for number still unburied at 10-min intervals from the time of introduction through 170 min, then daily for the remainder of the 10-day experimental period. Counts of initially buried clams that had responded by burrowing to the surface were made daily throughout the 10-day experimental period.

Response statistics were analyzed by a computerized probit analysis program (Finney 1971). Results of the initially unburied clam test are expressed as the calculated dose (naphthalene equivalents) at which 50% of the clams will fail to burrow within a specified period of time (ECm) together with the 95% confidence interval of that dose level. In addition, the slope function predicted by the probit analysis program is used to calculate the dose (naphthalene equivalents) with a 95% confidence interval at which the burrowing rates of 10% of exposed clams would be significantly reduced from the normal rate of control clams at 60 min. Results of the initially buried clam tests are expressed as the calculated dose (naphthalene equivalents) at which 50% of the clams will come to the surface (ECm) within a specified period of time together with the 95% confidence interval of that dose level. The data were adjusted through Abbott's formula (Finney 1971, p 125) to correct for partial response from the control clams.

Results and Discussion of WSF Exposures

The major observation in the experiments testing the response of initially buried clams to WSF's was that it caused some of the clams to come out of the sediment.

The data from the static water system test indicate that at an average concentration of 0.331 ppm naphthalene equivalents (87% of saturated WSF) the greatest response occurs within 72 h and involves 35% of the individuals (Fig. 3c). At an average concentration of 0.036 ppm naphthalene equivalents (11% of the saturated WSF) the greatest response is delayed to 9 days and involves 57% of the individuals (Fig. 3b). In contrast to the oil exposed clams, 98% of the control clams remain buried during the 11-day monitoring period (Fig. 3a). The calculated dose (ppm of naphthalene equivalents) at which 50% of the clams would respond by burrowing to the surface within 3 days under static water system conditions (ECm) is 0.436, with 95% confidence intervals of 0.484 and 0.392.

The data from the flow-through water system test show response proportional to dose clearly for the higher doses (Fig. 4). The control clams remain 100% buried throughout the exposure while all of the lower doses show some response. The calculated dose naphthalene equivalents at which 50% of the clams would respond by burrowing to the surface within 3 days under flow-through water system conditions (ECm) is 0.367, with 95% confidence intervals of 0.411 and 0.317. Under the same conditions the ECm for response within 5 days is 0.323, with 95% confidence intervals of 0.363 and 0.288.

Death as a result of exposure involved 22% of the clams exposed to 11% WSF in the static water system test (Fig. 3b). Surprisingly, few of the clams exposed to the higher dose had died at the end of the 11-day observation period. The reason for this unproportional response is not clear; one possibility is that components of the WSF enhanced the growth of bacteria, which may be pathogens to the clams. Higher concentrations of WSF might inhibit such bacterial growth. Another possibility is that various oil doses in connection with enhanced growth of microorganisms may also differentially affect aeration of the sediment and thereby cause toxic conditions to develop. Apparently no such toxic conditions developed with the flow-through water system experiment since there were no actual deaths at any dose. Stegeman and Teal (1973, p 39) have data for oysters which suggest that for concentrations up to 450 µg hydrocarbon l^{-1} there is a direct relationship between the hydrocarbon concentration in the water and uptake rate, while at higher concentrations the rate of uptake falls. The oysters remained tightly closed when exposed to 900 µg hydrocarbon l^{-1}; thus, they concluded the observed drop in uptake rate at that concentration was probably the result of the oysters avoiding contact with the oil. It is possible that our high dose of WSF caused the clams to "close up" and isolate themselves from toxins.

The major observation in the flow-through system experiment testing the burrowing response of initially unburied clams to the WSF was that it inhibited the rate of burrowing of some clams. Burrowing rate decreased in proportion to WSF concentration for the higher two doses (Fig. 5). There was a decrease at the lower concentrations, although it was not clearly in proportion to dose. The calculated dose (naphthalene equivalents) at which the rate of burrowing of 10% of exposed clams would be significantly reduced from the rate of the control clams at 60 min is 0.044, with 95% confidence intervals of 0.088 and 0.010. The calculated dose at which 50% of the initially unburied clams will fail to burrow within 60 min (ECm) is 0.234, with 95% confidence intervals of 0.310 and 0.175. At the end of the observation period (170 min) the calculated dose at which 50% of the initially unburied clams will fail to burrow (ECm) is 0.222, with 95% confidence intervals of 0.272 and 0.181.

By day 7 in the experiment, at least 97% of all the clams in all the doses were buried. None died within the 10-day period.

In both static and flow-through water system experiments with initially unburied clams, the clams show trends of recovering from exposure and reburying themselves (Figs. 3 and 4). We attribute this recovery to loss of toxicants by the WSF and relief from stress for the clams. Recovery occurred without transfer into clean seawater except in the case of the 11% concentration in the static water system test where response was delayed. The depression in the curves at day 4 of the flow-through test (Fig. 4) is related to the decrease in potency of the WSF concentration prior to replenishment of a fresh dose of the WSF later that same day.

Preparation of Oil-Contaminated Sediment For Use in Exposures

Oil-Contaminated sediment was prepared by mixing 1 part Prudhoe Bay crude oil with 2 parts dry sediment (collected at Amalga Harbor, the source of experimental clams) and 10 parts seawater (1/2 l oil: 1 sediment:5 l sw) in 1-gallon bottles and mixing in an oscillating shaker for 1 h. The mixture was allowed to separate for 1 h and the liquid decanted and discarded. The containers with the retained sediment were refilled with seawater and mixed for an additional 30 min and then allowed to separate for 1 h, at which time the liquid was again decanted and discarded. Uncontaminated sediment for control was made in the same manner, with the exception that no oil was put in the mix. The mixture was made just prior to its use in exposure.

Experimental Methods

The trays of untreated mud were set up 3 days prior to the start of the experiment in the seawater tanks and seeded with 200 clams. Clams that did not bury themselves within 3 days were removed, which reduced the sample size to about 190.

On day 0 in the experiment (September 3, 1975), the water in the tanks was turned off and drained. An appropriate amount of sediment was shaken with seawater into suspension and poured into the tanks. It was allowed to settle 24 h and then the water flow was continued. Temperature did not exceed 12° C at the end of the 24 h period, but oxygen concentrations were not determined.

OIL-CONTAMINATED SEDIMENT TEST--EXPERIMENT 3

The intertidal zone receives energy from wind, waves, and tidal action, and surface sediments and detritus are raised and held in suspension. If oil is present in the intertidal zone, it will probably mix with suspended particles and adsorb to them. The particles of sediment and adsorbed oil will later settle, forming a surface layer of contaminated sediment. We tested the effect of such sediments on M. balthica. In our experiment oil-contaminated sediment is suspended in seawater and later allowed to settle over the surface of an established clam bed.

Experimental Design

Each exposure in this experiment was conducted in a stainless steel tray (26 cm wide by 40 cm long by 8 cm deep) completely filled with non-oiled screened mud and submerged in a larger seawater tank (37 cm wide by 53 cm long by 8 cm deep). Water temperature ranged between 9° and 12° C. Fresh seawater from Auke Bay flowed continually at the rate of 1.2 l min^{-1} throughout the experiment except on day 0 when the water flow was interrupted for 24 h while sediment was added.

Oil-contaminated sediment for exposures or uncontaminated sediment for controls was allowed to settle over the trays of clams on day 0 to constitute exposure. The depth of the contaminated sediment allowed to settle over the trays was varied experimentally to form three different doses: 0.1 cm, 0.25 cm, and 0.5 cm. There was a control of the same depth of uncontaminated sediment corresponding to each of the three oil-contaminated sediment doses. The sample size was about 200 clams for each exposure.

Before sediment was added, a small Petri dish was placed in each tank beside the clam trays to catch an equivalent layer of sediment and confirm the actual depth of sediment added.

Counts of clams that had responded by burrowing to the surface or dying were made on days 1, 2, 3, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 19, 20, 21, 23, 26, 29, and 30.

Response statistics were analyzed by computerized probit analysis (Finney 1971) and a comparison of two observed proportions analysis (Natrella 1966, p ORDP 20-111). Results of the probit program are expressed as the calculated depth of sediment (cm) at which 50% of the clams will move to the surface within a specified period of time (ECm) together with the 95% confidence interval of that dose level. These data were adjusted through Abbott's formula (Finney 1971, p 125) to correct for partial response from the control clams.

Results and Discussion

Death as a result of oil-contaminated sediment exposure was significant (Natrella 1966) in the 0.5-cm dose of oil-contaminated sediment (Fig. 6), but death of clams at the 0.25-cm dose was only slightly greater than death in the controls. Death of clams in the 0.1-cm dose was similar to that of the controls. Most clams came to the surface before dying and only a small portion of dead clams were found buried (Fig. 6).

In both control and exposure doses, increasing numbers of clams came to the surface and died over the 30-day experimental period. The gradual increases in both the controls and exposures indicate to us that there is stress either from the addition of sediment, experimental setup, or initial condition of the clams. Responses occurring in the three control doses of non-oiled sediment were approximately equal and therefore not dependent on depth of sediment added.

The major observation in oil-contaminated sediment tests was that many clams moved to the surface after exposure but did not die. Numbers of individuals at the surface were proportional to the depth of the oil-sediment film added (Fig. 6). Response after 24 h was linear with respect to sediment depth squared. (Fig. 7) In our heaviest dose (oil-contaminated sediment approximately 0.5 cm deep) 29% moved to the surface within 24 h (Fig. 8). In the intermediate dose (oil-contaminated sediment approximately 0.25 cm deep) 10% moved to the surface within 24 h, while 6% of the clams at the lowest dose (0.10 cm) surfaced. Less than 2% of the control clams came to the surface within this period of time.

The depth of sediment calculated by probit, that it would take under conditions of the experiment to stimulate 50% of the clams to move to the surface within 1 day is 0.668 cm, with 95% confidence intervals of 0.758 and 0.579.

OVERALL DISCUSSION

From the results of these three types of experiments, it seems apparent that the impact of an oil spill on M. balthica depends on the amount and location of mixing energy applied to the sediments and/or seawater. If there is essentially no mixing energy associated with a spill, such as we had in our unmixed crude oil spill, effects will probably be negligible. If there is enough mixing energy offshore to form WSF's of oil, these may move in over the clam beds and if concentrated enough affect the burrowing activities of clams. If there is mixing energy in the intertidal zone, both WSF and oil-contaminated sediment may form. Such contaminants will result in inhibited clam burrowing activity, movement to the surface, and presumably death of exposed clams either from toxicity of the oil, exposure to adverse environmental conditions, or increased predation.

In our experiments there was a trend of clams first coming to the surface and a portion of them later dying (Figs. 3 and 6). A very small percentage of the clams that died were not immediately visible at the surface but were later discovered when the mud was screened at the end of the experiment. If this trend is the same under natural conditions, it is possible that there was a much greater effect of the oil on the clams in the Valdez field experiment of Shaw et al. (in press) than is indicated by their data, since the aluminum containment frames used in their study were not designed to retain clams that might come to the surface. Many clams may have come to the surface after oil exposure and could have died, floated away, or been taken by predators while still living but exposed on the sediment surface.

Throughout our work with M. balthica we observed deposit-feeding activities only during April, May, and June, when Experiment 1 was underway. In late summer, fall, and winter no deposit-feeding was observed in either control or exposed clams. Because we conducted our WSF and sediment experiments during this later period and still got a response to oil, we conclude response is not only dependent on direct ingestion, but also hydrocarbons must enter or affect the clams through respiration or direct transport through membranes.

Although several questions regarding the responses of M. balthica to hydrocarbon remain to be answered, the results of this study lead us to agree with Shaw et al. (in press) that M. balthica shows potential as an indicator of oil pollution. Our results suggest that the actual and ecological death of M. balthica upon exposure to oil-contaminated sediments and dissolved oils may be even greater than is indicated by the mortality reported by Shaw et al. (in press). The responses of clams proportional to oil dose as observed in our study over both short- and long-term exposures suggest that these small clams are a good bioassay organism and well suited for use in baseline studies. Even though few of the clams die upon short-term exposure to the water-soluble fractions of crude oil or oil-contaminated sediments, their immediate behavioral response to oil in their environment may result in ecological death.

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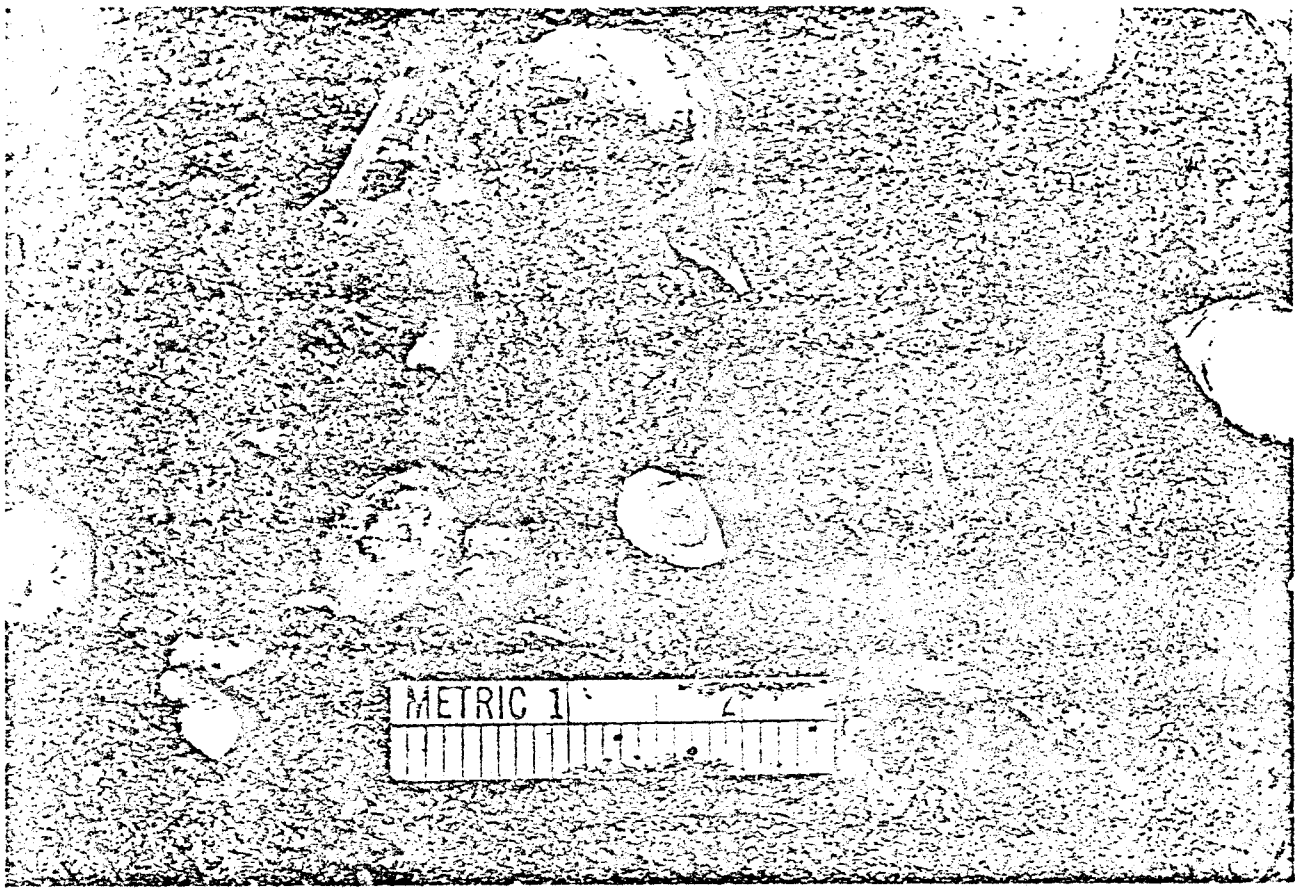
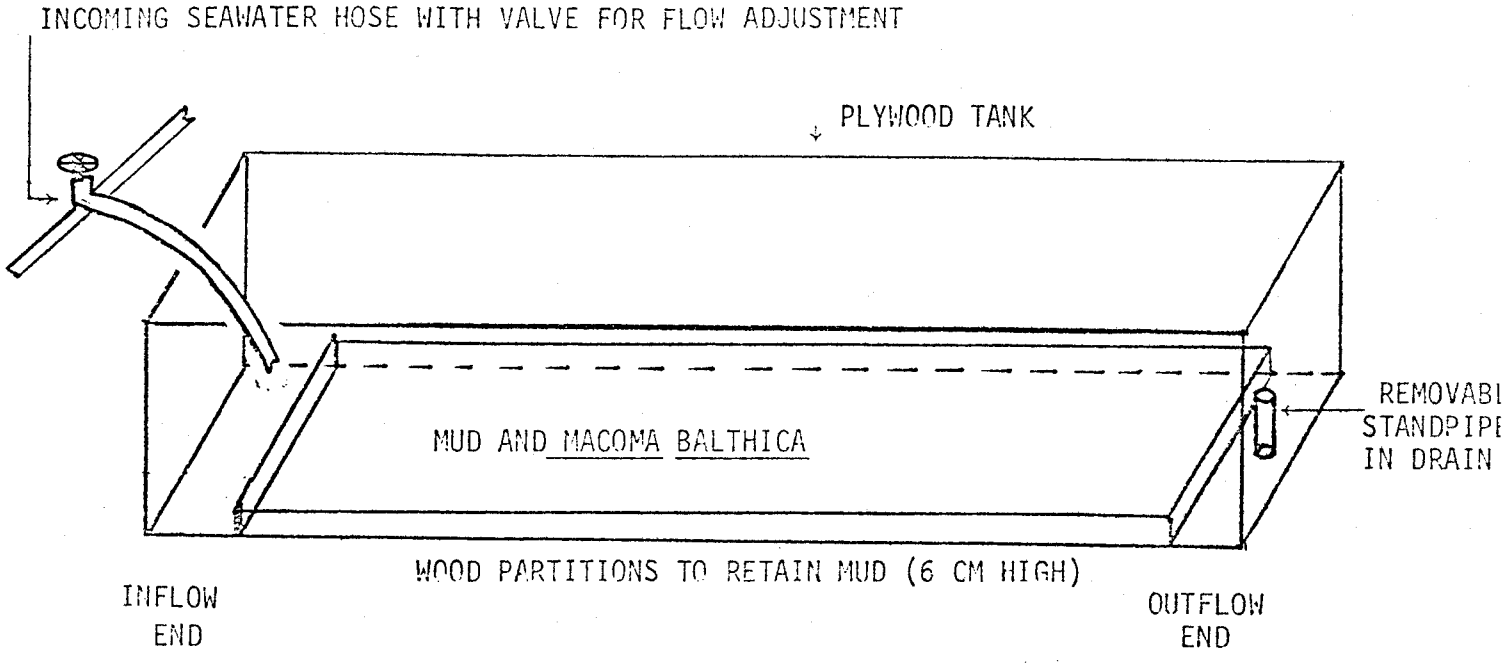


Figure 1.--Photograph of experimental animal, Macoma balthica. Note the separate siphons. The incurrent is the longer and more frequently seen of the two; the excurrent is shorter and normally held beneath the sediment surface.



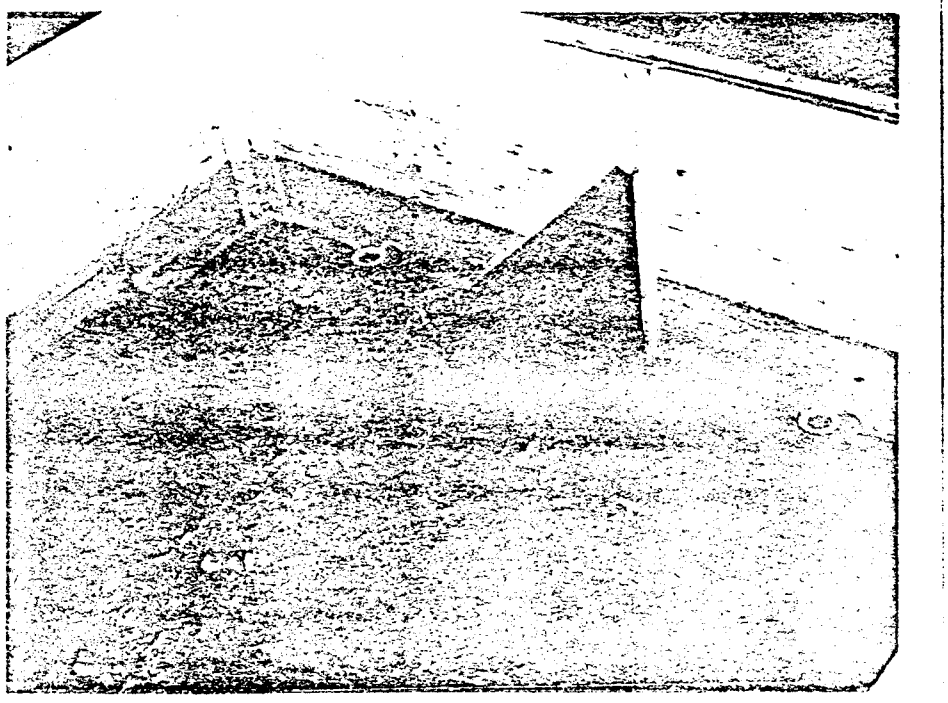


Fig. 2.--Diagram and photograph of outflow tanks used in simulated oil spill. Note 6 CM mud layer centered in the bottom of the tank held by the wood partitions, with the water flowing over the mud and draining via the standpipe in the drain. Water depth over mud was approximately 7 cm.

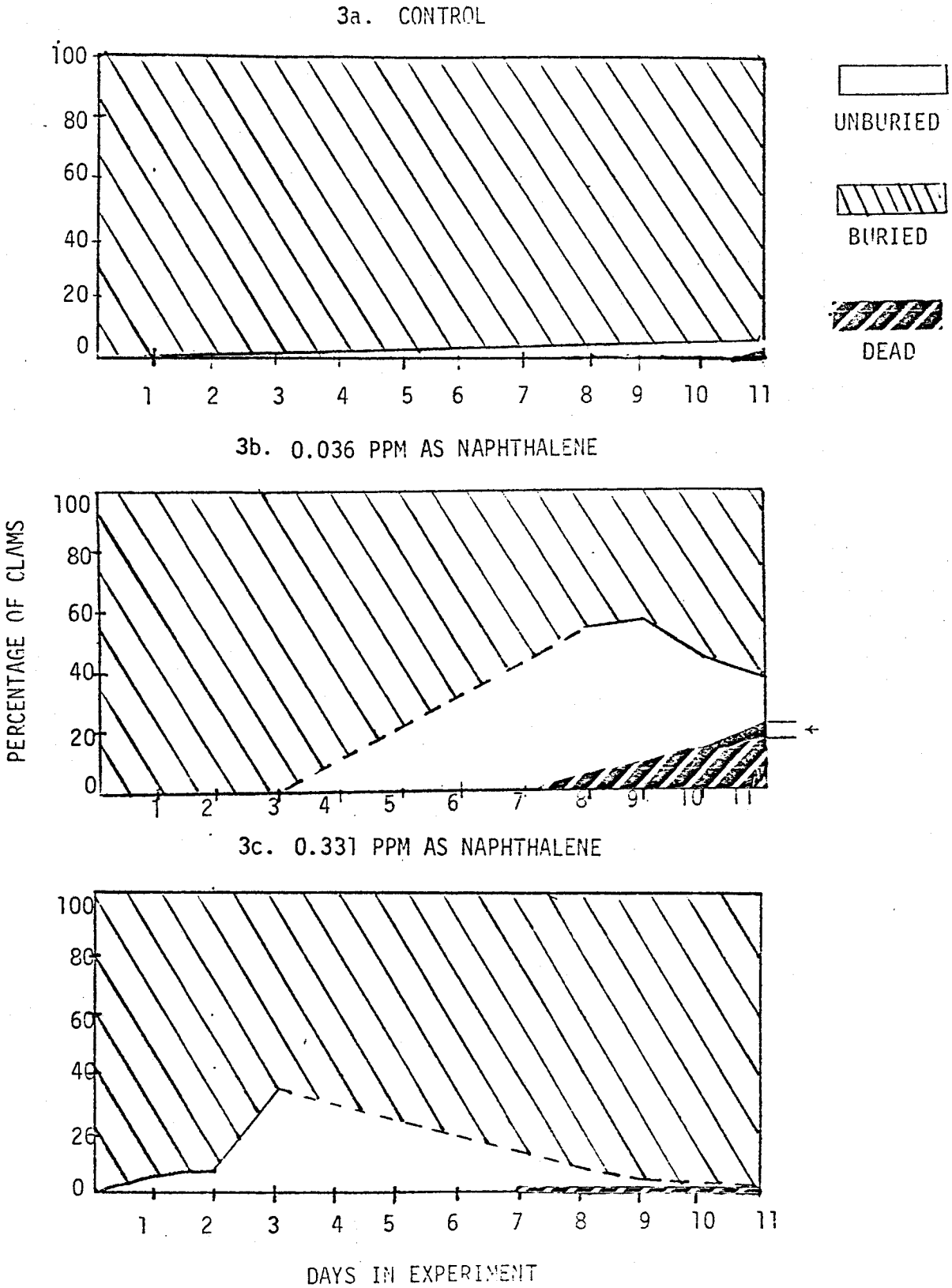


Fig. 3.--Response of buried M. balthica to low (3b) and high (3a) concentration of the WSF of Prudhoe Bay crude oil under static flow condition. The graphs are plotted so that the percentage of clams buried, unburied, or dead are accounted for. Observations were made on days 1, 2, 3, 8, 9, 10, and 11. The dotted line between days 3 and 8 is connecting known point 3 to known point 8 and is not necessarily representative of how many clams were unburied. The arrow in Fig. 3b indicates the area of solid shading that represents the percentage of dead clams not visible at the surface. Control clams made virtually no response.

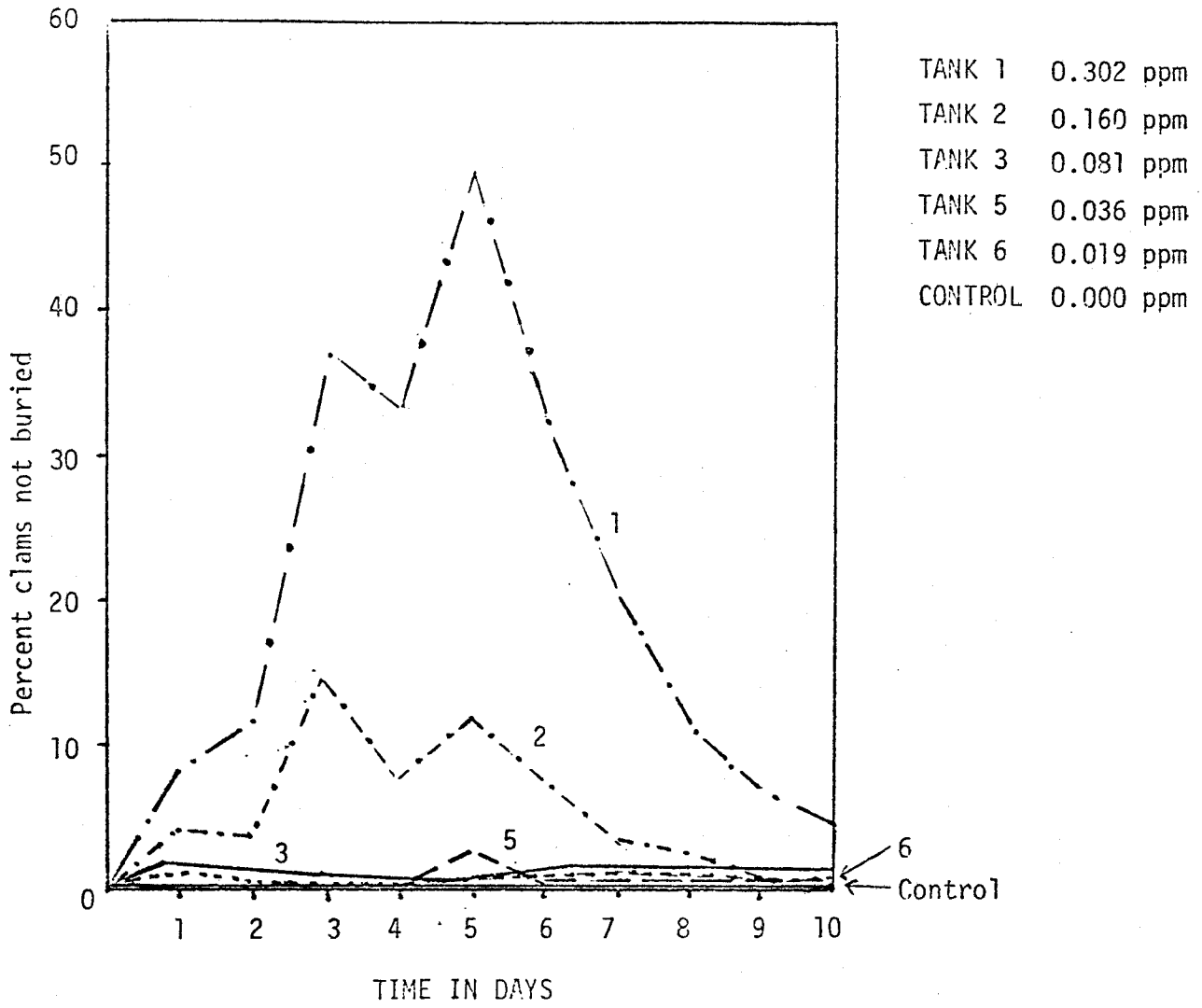


Fig. 4.--Response of buried M. balthica to exposure to WSF of Prudhoe Bay crude oil in flow-through water system type setup. The control clams made 0 response throughout. The percentage of clams that responded by coming to the surface is graphed; the area above each line would correspond to the percentage of clams still buried at any time. Concentrations of oil in WSF is expressed as equivalents of naphthalene.

Fig. 5.--Response of unburied clams put in WSF of Prudhoe Bay crude oil at time 0. The points after time 0 record the progress of each group of clams in burrowing into the sediment. The experiment was conducted in flow-through system with marked clams. Concentrations are expressed as naphthalene equivalents.

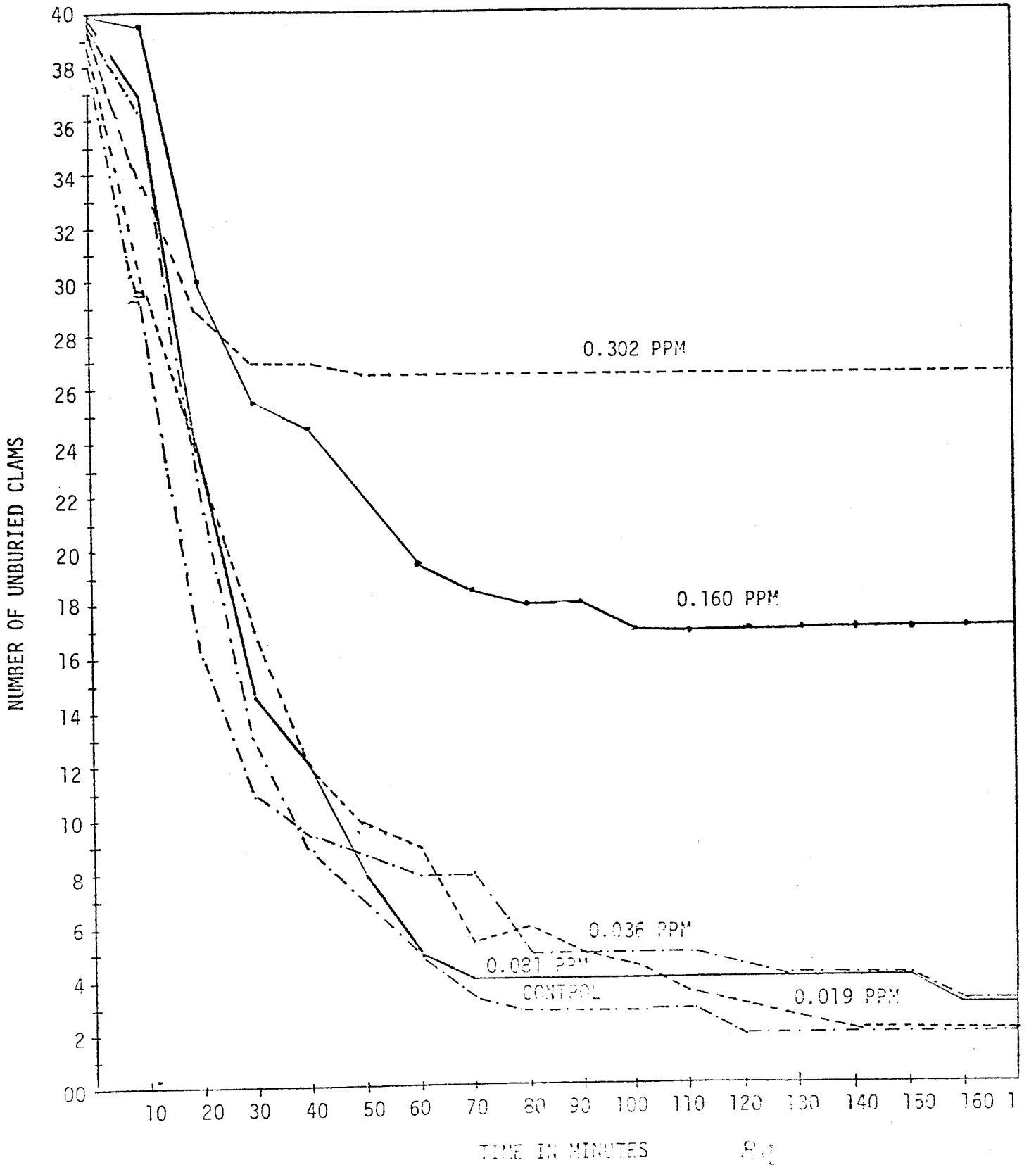
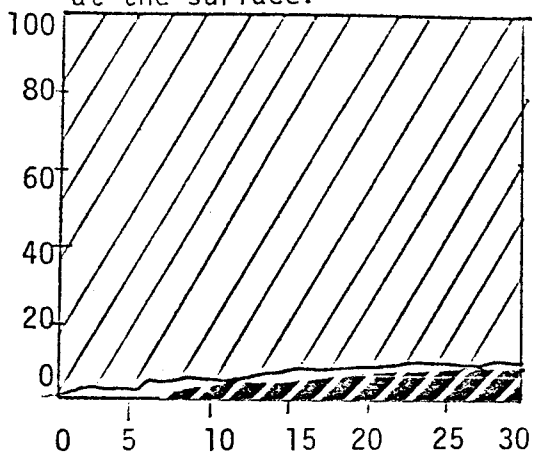
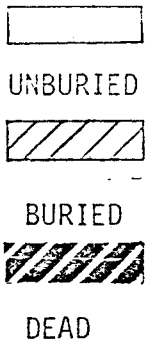
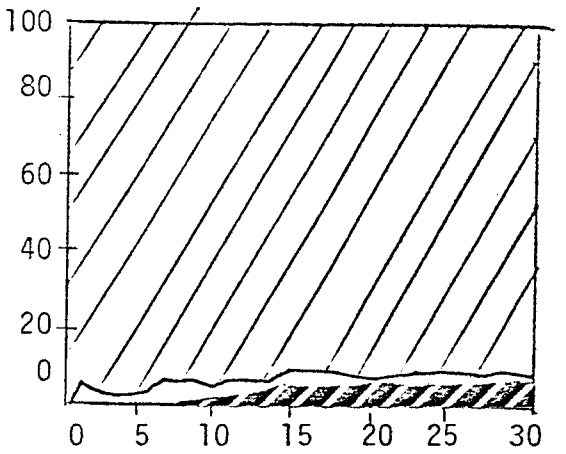


Fig. 6.--Results of oil-contaminated sediment experiment. Each graph below represents a control or exposure dose; each dose has a corresponding control: low level exposure-low level control. The graphs are plotted so that the percentage of clams buried, unburied, or dead are accounted for. Observations were taken on days 1, 2, 3, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 19, 20, 21, 23, 26, 29, and 30. The arrows in the lower graphs indicate the areas of solid shading that represent the percentage of dead clams not visible at the surface.

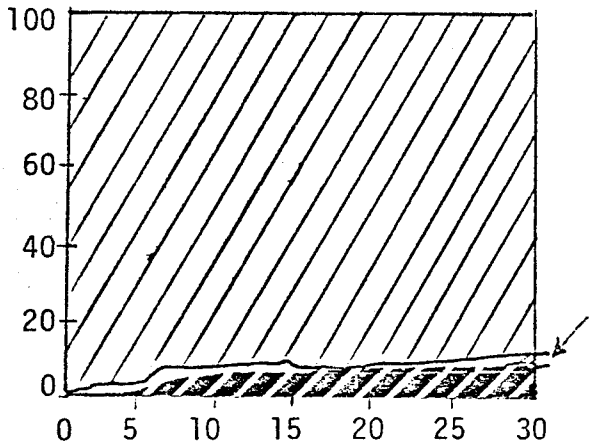


CONTROL LOW-LEVEL RECEIVED 1/10 CM LAYER OF PLAIN MUD

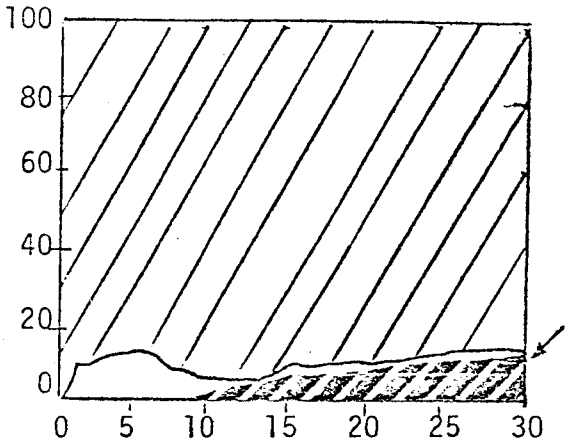




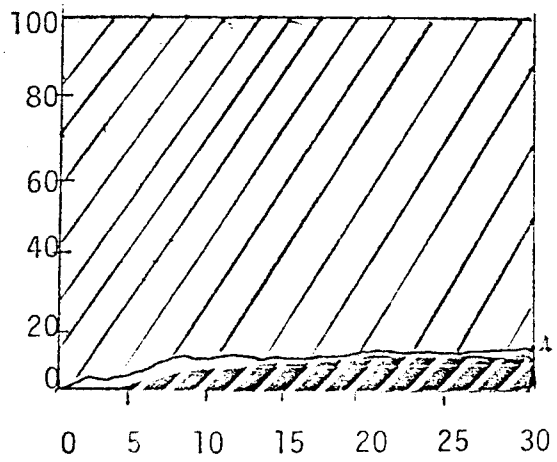
EXPOSURE LOW-LEVEL RECEIVED 1/10 CM LAYER OF OIL-CONTAMINATED MUD



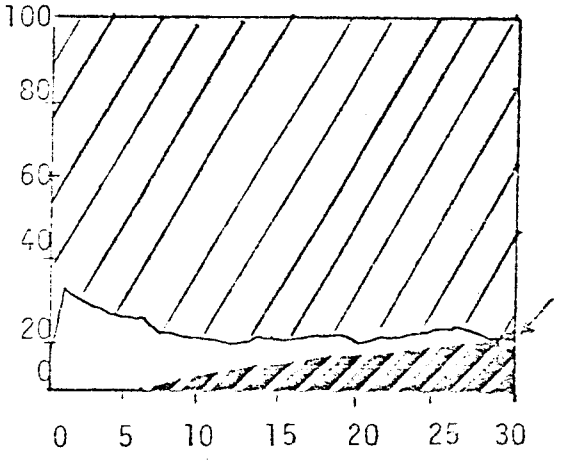
CONTROL INTERMEDIATE-LEVEL RECEIVED 1/4 CM LAYER OF PLAIN MUD



EXPOSURE INTERMEDIATE LEVEL RECEIVED 1/4 CM LAYER OF OIL-CONTAMINATED MUD



CONTROL HIGH-LEVEL RECEIVED 1/2 CM LAYER OF PLAIN MUD



EXPOSURE HIGH-LEVEL RECEIVED 1/2

←DAYS IN EXPERIMENT→

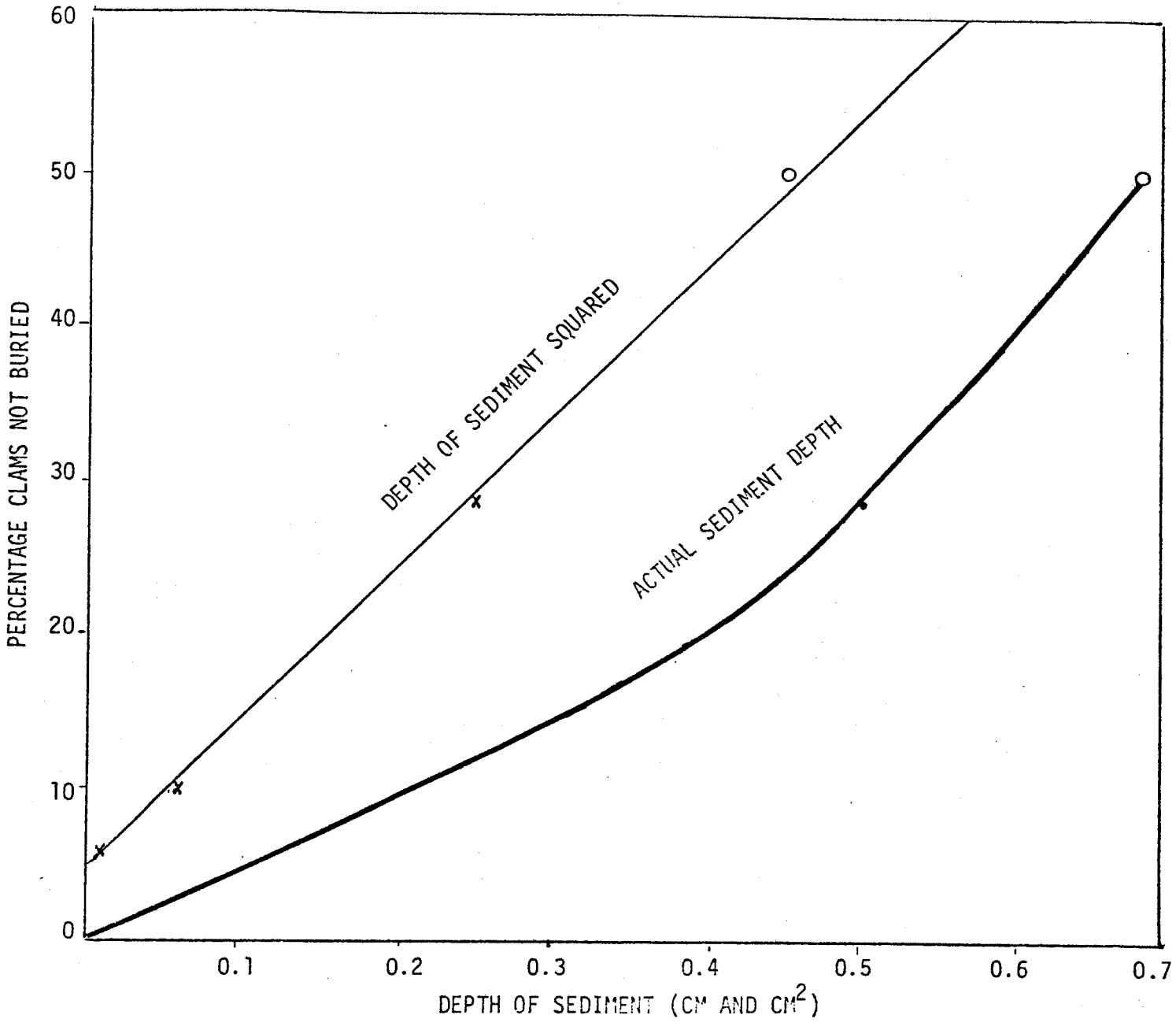


Fig. 7.--Percentage of clams responding to oil-contaminated sediment by coming to the surface versus depth of sediment (solid circles) and depth of sediment squared (X's). Open circles represent ECm values calculated by probit.

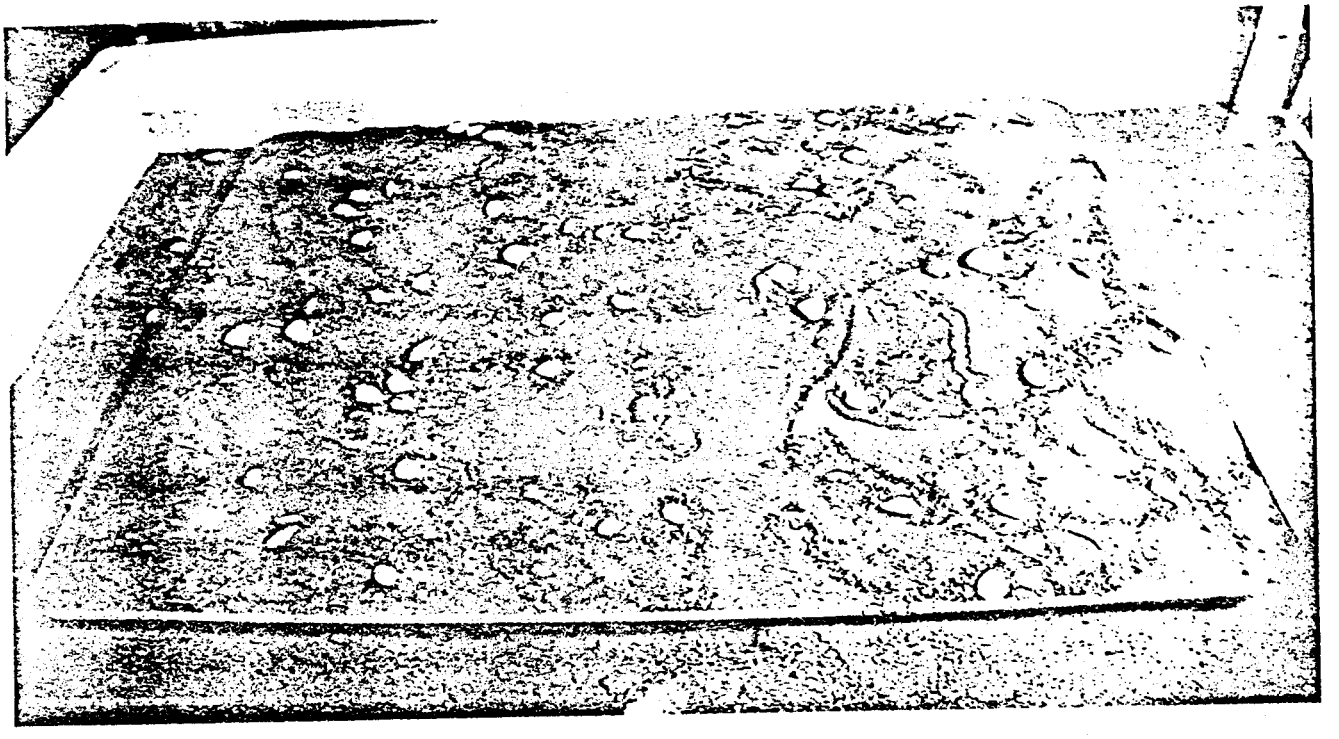


Figure 8.--Photograph of clams in high level exposure (1/2 cm) to oil-contaminated mud, taken 24 h after stard of exposure. No clams appeared on the surface of control sediments.

EFFECT OF PETROLEUM HYDROCARBONS ON BREATHING AND COUGHING RATES, AND HYDROCARBON UPTAKE-DEPURATION IN PINK SALMON FRY

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SYMPOSIUM ON POLLUTION AND PHYSIOLOGY OF MARINE ORGANISMS

EFFECT OF PETROLEUM HYDROCARBONS ON BREATHING AND COUGHING RATES, AND HYDROCARBON UPTAKE-DEPURATION IN PINK SALMON FRY

Stanley D. Rice, [1/] Robert E. Thomas, [2/] and Jeffrey W. Short [1/]

ABSTRACT

Pink salmon fry, Oncorhynchus gorbuscha, were exposed to the water-soluble fraction of Cook Inlet and Prudhoe Bay crude oils, and No. 2 fuel oil. Concentrations of oil in water were measured by ultraviolet and infrared spectroscopy. Breathing and coughing rates were measured in free swimming fry without anesthesia or surgery. During 22 h exposures, breathing and coughing rates initially increased as the dose increased but then decreased after several hours. Breathing and coughing rates increased significantly during exposures to oil concentrations as low as 30% of the 96 h median tolerance limit as determined by ultraviolet spectroscopy.

The breathing and coughing rates of fry exposed for both 72 h and 22 h to a constant dose were similar. However, after the initial 24 h exposure to a constant dose, the breathing and coughing rates decreased but were higher than control levels for the remaining 48 h.

Tissues of fry exposed for up to 96 h to the water-soluble fraction of Cook Inlet crude oil were analyzed by gas chromatography for nonpolar hydrocarbons. Toxic aromatic hydrocarbons were accumulated but were apparently being eliminated from the tissues during the exposures. We speculate that the increased respiration rate reflects an increased energy demand for enzyme synthesis. Chronic exposure requiring elevated energy demands may be detrimental to the survival of a population.

INTRODUCTION

Changes in respiratory activity have been used as sensitive indicators of stress in fish exposed to pollutants such as DDT, kraft pulp mill effluent (Schaumberg et al., 1967), bleached kraft mill effluent (Davis 1973; Walden et al., 1970), zinc (Sparks et al., 1972), copper (Drummond et al., 1973), combinations of copper and zinc (Sellers et al., 1975), benzene (Brocksen and Bailey, 1973), and refined crude oils (Anderson et al., 1974a; Thomas and Rice, 1975). Anderson et al. (1974a) observed increased oxygen consumption rates in sheepshead minnows, Cyprinodon variegatus, after 24 h exposure to refined and crude oils. Thomas and Rice (1975), observed an immediate increase in opercular breathing rates of pink salmon fry exposed to Prudhoe Bay crude oil. The increased breathing rate response observed by Thomas and Rice was related to the dosage level but dropped to near control levels during a 24-h exposure. Because of the absence of precise analytical measurement of oil concentrations during the exposures, Thomas and Rice could not explain the reduction of breathing rates to near control levels. The decrease in breathing rates during oil exposure may have been due to a change in the effective concentration of oil or to a physiological response of the pink salmon fry such as adaptation or narcosis.

In this paper we report the changes in breathing rates during the extended exposure of pink salmon, Oncorhynchus gorbuscha, fry to oil, and examine the reasons for return to near normal rates during extended exposure. We determined: (1) the acute toxicity of Cook Inlet and Prudhoe Bay crude oils and No. 2 fuel oil to pink salmon fry, so that sublethal dosage exposures and responses could be compared to a lethal dosage exposure; (2) changes in opercular breathing and coughing rates of fish exposed to a variety of sublethal concentrations of WSF's (water-soluble fractions) of the three oils; (3) the breathing and coughing rates of fish during exposures to a constant oil concentration for three days; and (4) the hydrocarbon uptake and depuration by fish exposed to the WSF of Cook Inlet crude oil for varying periods.

MATERIALS AND METHODS

We conducted the experiments at the Northwest Fisheries Center Auke Bay Fisheries Laboratory using pink salmon fry raised in gravel incubators (Bailey and Taylor, 1974). The fry emerged in April 1975 and were kept in running seawater aquaria until used in the study. Temperatures in the aquaria during the experiments, June-August 1975, ranged from 10-12.5°C. The fry were fed Oregon Moist Pellets daily and appeared normal in every respect.

Preparation of the Water-Soluble Fraction

Water-soluble fractions were prepared with Prudhoe Bay and Cook Inlet crude oils, and No. 2 fuel oil. One percent oil in seawater (1 liter oil/100 liters seawater) was mixed slowly for 20 h at ambient water temperatures (10-12°C). The mixture was allowed to separate for

3 h before the WSF was siphoned from under the slick. We determined the concentration of oil in the water by UV (ultraviolet) spectroscopy, and diluted the WSF to the desired concentrations used in the experiments. Detailed analyses of the WSF by GC (gas chromatography) are available.[3/]

Oil Analyses

We determined the concentrations of the WSF's several times during the exposures by both UV and IR (infrared) spectroscopy, so that we would know what changes occurred. We used the IR method of Gruenfeld (1973), and the UV method of Neff and Anderson (1975). For IR analysis, samples of WSF's were extracted with trichlorotriflouroethane (Freon 112),[4/] and the absorbance of the extract at 2930 cm^{-1} was measured on a Beckman Acculab 1 IR spectrophotometer.[4/] This method is particularly sensitive to paraffins, since absorbance at 2930 cm^{-1} is due mainly to methyl and methylene CH stretch (Silverstein and Bassler, 1966). For UV analysis, water samples were extracted with n-hexane, and the absorbance of the extract was measured at 221 nm on a Beckman model 25 scanning UV spectrophotometer.[4/] Absorbance at 221 nm is mainly due to naphthalene and aliphatic substituted naphthalenes (Neff and Anderson, 1975). The absorbance measurement was adjusted to correct for the extraction step where unequal proportions of water sample to extracting solvent were used. The results are expressed as the equivalent absorbance that would have occurred in a 1-cm path length of the WSF sample. These equivalent absorbances at 221 nm are referred to as corrected UVOD (ultraviolet optical density) hereafter.

Bioassays

We conducted static bioassays for 96 h. The aquaria (19-liter glass jars) were aerated at approximately 100 bubbles per minute and maintained at ambient seawater temperatures (10-12°C). Tissue-to-volume ratios in the aquaria never exceeded 1 g tissue (wet weight) per liter of seawater. Ten fish were exposed to each dose level for 96 h and the response statistics were analyzed by a computerized probit analysis.

Measurements of Opercular Breathing and Coughing Rates

We measured breathing and coughing rates by electronically recording the opercular movements of individual fish swimming free in small compartments (Thomas and Rice 1975). The fish had not undergone any anesthesia or surgery. The breathing and coughing rate experiments were conducted in a heat and sound insulated room to protect the fry from extraneous stimuli--no one was in the room and the door was never opened during the recording periods.

One fish (50 ± 5 mm long) was placed in each of 38 chambers at 1430, and the breathing rate was first recorded at 0630 the following day. This first recorded rate was averaged for each group of fry and was used as the basal or normal breathing rate. The use of the initial recorded rate as the basal rate was validated in preliminary studies where no significant diurnal rhythm was detected in nonexposed fry and no significant changes occurred in breathing rates during 72 h of confinement. The average breathing rates were determined for each fry during a 3 min segment of each recording period. In some cases, a fish may have been against an electrode, actively swimming, or in a position within the

chamber where 3 consecutive minutes of clear recordings could not be obtained. When this happened, that fish was not used for that particular recording period, so sample size at different recording times varies within a group of fry. After the basal breathing rates were recorded, the test solution was introduced to 31 of the fish chambers at 0800; breathing rates were recorded at that time and 3, 6, 9, 12, and 22 h later. The breathing rates of the unexposed fry (controls) in the other seven chambers were recorded at the same time intervals. Coughing, a brief reversal of water flow in the opercular cavity, was detected in some fish as a spike superimposed on the recordings of opercular breathing movements. Our recording was not sensitive enough to pick up low intensity coughs in most of these small fish. However, when the coughs could be detected in individual fish, the rates were constant during that recording period. Coughs could be detected in exposed fish more frequently than in controls, apparently because the intensity of the cough was greater. The differences in breathing and cough rates were tested by analysis of variance and Student's t test.

During each test, the water in the test chamber was nearly constant in oxygen, water flow, and temperature. We monitored the temperature continuously as it entered the test aquarium, which was 11.5 ± 0.8°C for all tests. Oxygen (measured by a Yellow Springs Instruments Polarographic Probe[4/]) never dropped below 8.2 ppm. The flow rate was approximately 1 liter per hour through each of the recording chambers. This flow was maintained by pumping water from reservoirs of stock solution into small head tanks. The use of head tanks produced a constant hydrostatic

pressure to the manifold which fed the individual chambers. Most breathing rate tests lasted 22 h and each used one stock of WSF. The WSF was passed through the recording chambers and discarded. Although the test water was not recirculated, oil concentrations in the stock reservoir decreased during the 22 h exposures. We required a constant dose for a 72 h experiment and acheived it by adding the WSF to the large reservoir of test water every three hours (concentrations were measured by UV each time).

Measurement of Tissue Hydrocarbon Uptake and Depuration

We measured hydrocarbon uptake and depuration in one lot of fish exposed to WSF in a large tank. To measure uptake, we sampled fish at 0, 3, 10, 33, and 96 h after the initial exposure. The oil concentration decreased continuously (as measured both by UV and IR) and was only about 20% of the initial concentration. After 96 h the exposure tank was converted to running seawater for depuration. To measure depuration, we sampled fish after 3, 10, 72, and 240 h in clean water--gill, viscera (minus heart and kidney), and muscle were dissected out of 5 fish each period. The tissues of 5 individuals were pooled to form one sample for gill, one sample for viscera, and one for muscle. The samples were then frozen in glass jars with Teflon[4/] lined caps. Each pooled sample was extracted and analyzed by GC for nonpolar paraffins and aromatic hydrocarbons. GC analysis was done by Dr. J. Scott Warner of Battelle Memorial Laboratories, Columbus, Ohio. Dr. Warner analyzed one tissue sample by GC-MS (mass spectrometry) for positive identification of major components.

RESULTS AND DISCUSSION

Toxicity

The three oils differed in toxicity to pink salmon fry, although none of the three oils killed many animals after the initial 24 h (Table 1). The ranking of relative toxicities depended on the method used to measure the WSF concentrations. Measured by UVOD, the WSF of No. 2 fuel oil was much less toxic than the WSF's of the crude oils, which were about equally toxic. Measured by IR ppm, the WSF of No. 2 fuel oil was more toxic than either of the crude oils, and of the crude oils, Prudhoe Bay crude oil was more toxic than Cook Inlet crude oil. Rice et al.[3/] determined TLm's of Cook Inlet and Prudhoe Bay crude oils and No. 2 fuel oil with several marine invertebrates and fish and found that toxicity was more closely associated with oil concentrations measured by UVOD than by IR. Since naphthalene and substituted naphthalenes are detected by UV, it appears that toxicity is more closely associated with naphthalene concentration. Anderson et al. (1974b) previously observed that the toxicity of WSF's of oils is a function of their diaromatic and triaromatic hydrocarbon contents. UVOD measurements are apparently more meaningful than IR in explaining toxicity since UVOD reflects naphthalene concentrations, but we include IR measurements for comparison with other studies.

The UVOD measurements of TLm's on the WSF of No. 2 fuel oil are much higher than UVOD measurements of toxicity for the two crude oils. Like many processed (cracked) oils, No. 2 fuel oil contains significant quantities of olefinic (unsaturated) compounds. Olefinic compounds absorb generally between 220 to 230 nm with extinction coefficients roughly one-tenth those of the naphthalenes (Willard et al.,1965). Olefinic compounds are more water-soluble than the corresponding n-paraffin compounds (McAuliffe, 1966, and 1969). Thus, it may be that the presence of more olefinic compounds in No. 2 fuel than in the crude oils oil accounts for the fuel oil being less toxic than the crude oils (all WSF's measured by UVOD). This assumes that the olefinic compounds are much less toxic than the aromatic compounds. Although it may not be appropriate to compare UVOD measurements of oil concentrations from crude oils with those of fuel oil, the UVOD of WSF's from different concentrations of a particular oil can be compared. Thus the oil exposures measured by UVOD in the breathing rate experiments can be compared with the oil exposures measured by UVOD in the 96-h TLm's.

Effects on Breathing Rates in 22 h Oil Exposures

The breathing rate response during a 22-h exposure is similar for all three oils (Figure 1). In all cases, the highest breathing rate was between 3 and 6 h, the first measurements during exposure. For all the oils, the breathing rate subsequently declined, and only at the higher concentrations did breathing rates continue above normal after 22 h. The

increase in breathing rate was linear with increasing oil concentrations. This is evident in Figure 2 where breathing rates at 3 h increased linearly as exposure dose increased (expressed as percents of the 96-h TLm). There appears to be little difference between the oils when oil concentrations are expressed as percents of the 96-h TLm. The lowest concentration that causes a significant response is estimated at about 30% of the 96-h TLm from Figure 2.

The oil concentrations during the 22-h exposures decreased significantly for all three oils (as measured by UV and IR). The average decrease in UVOD during the 22-h exposures was 42.1%, 53.0% and 42.7% for No. 2 fuel oil, Cook Inlet and Prudhoe Bay crude oils respectively. In many cases the final concentrations of oil measured by UVOD was below the threshold concentration (30% of the 96h TLm) that would cause a significant increase in breathing rates.

The increased breathing rates are more closely related to increased oil concentrations in the WSF as measured by UVOD than measured by IR. This is best seen in results of exposures to the two highest concentrations of Cook Inlet crude oil--the breathing rate increased as the UVOD values increased and the IR values decreased. The close association of toxicity and breathing rate with UVOD values may best be explained by the fact that naphthalenes are measured by UV at 221 nm and are quite toxic, while paraffins are measured by IR and are not very toxic. This again agrees with observations by Rice et al.[3/] and Anderson et al. (1974b) who found toxicity more closely related to concentrations of aromatic hydrocarbons than to paraffins.

Cough Response

The pattern of cough response was the same as that of breathing rate changes--but detected in relatively fewer fish (Figure 3). Sellers et al. (1975) criticize the use of either breathing rate or cough frequency alone to indicate pollution stress because of individual variability. They measured cough frequency, ventilation frequency, and ventilation intensity (buccal and opercular cavity pressures) in large trout (225g) exposed to copper and zinc. Increased response was usually observed with increased dose. They suggested that all three measurements were needed for each fish, since individual variability was large. It was not feasible to monitor ventilation intensity in our experiments with small pink salmon fry (2g), but we were able to get statistically significant results at the $p = 0.01$ level with a sample size of approximately 20 per dose. Our data on cough response of sample sizes of fish confirm our observations on breathing rate from larger samples of fish. Although a rather large sample is needed to obtain significant data on breathing rate responses large samples are easy to obtain with the system we used.

Concentrations of Hydrocarbons in Tissues

Concentrations of both paraffinic (nonpolar) and aromatic hydrocarbons were measured in samples of gill, viscera (minus heart and kidney), and muscle taken from fish exposed for 96 h to the WSF of Cook Inlet crude (25% of 96h TLm) and depurated in noncontaminated seawater up to 240 h (Figures 4 and 5). Only n-paraffins with carbon numbers 15-19 were consistently present in all three tissues. Muscle tissue showed no consistent pattern for any of these paraffins. In the viscera paraffins generally decreased from a high level in control samples before the

exposure started to low concentrations at the end of the depuration period. In gill tissue, 4 of the 5 paraffins reached their greatest concentration after 10 h of exposure, while C_{17} decreased through the exposure and depuration periods. Paraffins C_{12}-C_{30} were all detected in gill tissue after 10 h exposure, but were not detected at other times in gill tissue or in the other tissues at any time.

The peak of paraffin concentrations in the gill tissue after 10 h of exposure suggests that paraffins are moving into at least the gills of the fish. The general decrease of paraffins in the gut tissue is probably due to the lack of feeding during the 96-h oil exposure and the 240-h of depuration. Paraffins of non-oil origin are normally present in the guts of feeding fish. The paraffin concentrations in muscle are little affected by the exposure, suggesting few of the paraffins reach the muscle tissue. However, in all three tissues, paraffin concentrations changed (increased or decreased) during the first 10 h of exposure and the first 10 h of depuration, suggesting that the fish are affected physiologically by changes in their environment. Paraffins are not toxic and can be metabolized. The amount of stress, if any, caused by higher paraffin concentrations in some tissues cannot be evaluated by this data.

Monoaromatic and diaromatic hydrocarbons were found in the tissue samples. Methyl-naphthalene was the most abundant aromatic in the tissues, and like all other aromatics found was most abundant after 10 h of exposure (Figure 5). The highest concentrations of all aromatics were found in the gut. Methyl and dimethylnaphthalenes were the slowest to be removed from the gut tissues. All the aromatics were below detectable levels from the gill and muscle tissues by the fourth day of exposure. Phenanthrenes were not detected in any of the tissues.

The greatest concentration of aromatics in the tissues occurred at 10 h and the concentrations of aromatics had declined by 20 h. These changes in the tissues correlate with the results in our other experiments: (1) few deaths occurred after 24 h of exposure and (2) the breathing rates declined to near control levels by 22 h. The stress caused by high concentrations of aromatics in the tissues should diminish after 10 h since these compounds are disappearing from the tissues. We cannot determine whether the compounds are being excreted directly or transformed into metabolites. The presence and persistance of high concentrations of aromatic hydrocarbons in the gut suggests that the liver is metabolising the oil and excreting the products to the gallbladder and gut. This is consistent with Pedersen and Hershberger (1974) who demonstrated that trout have the capability to metabolize benzopyrene and with Lee et al. (1972) who demonstrated metabolism and excretion of naphthalene by 3 species of marine fish. Both Lee et al. (1972) and Neff (1975) found higher concentrations of naphthalenes in the gallbladder than in any other tissue.

The return of breathing rates to near control levels during the 22-h exposure can be explained by one of three hypotheses: (1) The fish acclimate to the stress and decrease their adrenal response; (2) the fish decrease their response to the stress because the oil concentrations drop during the exposure; (3) the fish acclimate to the stress by making physiological adjustments that increase their ability to cope with the stress.

The first hypothesis, adrenal response to stress by the fish does not explain our data. We tested the duration of the adrenal response to stress by banging violently on the recording chambers. Breathing rate was immediately elevated, but dropped to near control levels within 2 h. The same stress repeated during the day elicited immediate elevated breathing rates, but the return to near control levels became quicker.

The second hypothesis, a decreased response because of decreased oil concentrations, does explain the observations. Even though the fish were exposed to a flow-through oil exposure for 22 h, the oil concentrations decreased continuously, apparently because of microbial degradation. After 22 h, only the highest doses had oil concentrations greater than the threshold concentration. The stress to the fish during the 22-h exposure was continuously lowered; both externally in the water and internally as shown by reduction of aromatic hydrocarbons in the tissues.

The third hypothesis, acclimation by physiological adjustment was tested by exposing fry to a constant oil concentration for 72 h. The oil concentration was kept at a relatively constant 65% of the 96-h TLm for Cook Inlet crude oil--enough (freshly mixed) WSF was added every 3 h to restore the UVOD to the desired level. Both breathing and coughing rates peaked during the first 24 h then dropped somewhat but remained above the original baseline rates for another 48 h (Figure 6). The elevated and sustained respiratory demand in response to continued oil exposure suggests that a new steady state of metabolism is required to cope with continued hydrocarbon stress. We believe that both the second and third hypotheses are true--that is, the fish respond to dropping oil concentration by decreasing their respiratory response, and the fish will make physiological adjustments to cope with a continued stress of oil.

We conclude that the increased oxygen consumption is needed to support increased physiological activities in metabolism and excretion of the hydrocarbons. We suspect that part of this increased oxygen consumption is used to synthesize enzymes since (1) the disappearance of aromatic hydrocarbons suggests metabolism, (2) metabolism of naphthalene and benzopyrene has been demonstrated in 3 species of marine fish (Lee et al., 1972) and metabolism of benzopyrene by rainbow trout (Pedersen and Hershberger 1974), (3) induction of enzymes (aryl hydrocarbon hydroxylases) capable of breaking down aromatic hydrocarbons has been demonstrated in trout exposed to petroleum (Payne and Penrose, 1975), and (4) synthesizing these enzymes requires energy. Thus, we speculate that much energy is

required initially, probably to synthesize large quantities of enzymes needed to metabolize hydrocarbons into forms that can be excreted. The somewhat reduced but still elevated breathing rates continuing after the initial response suggests greater than normal quantities of energy are still needed to maintain enzyme synthesis and oxidation of the hydrocarbons.

If not overwhelmed by the initial exposure to oil, the fish can rid themselves of toxic compounds and suffer little apparent damage. Higher concentrations of oil are lethal, but sublethal concentrations may have substantial effects on survival. Continued exposure to sublethal concentrations results in continued elevated metabolism and energy demands. This increased energy demand requires increased food intake which puts the fish at a disadvantage in the struggle for survival. Survival rates would be reduced for a group of fish subjected to this stress for significant periods.

CONCLUSIONS

1. WSF's from Cook Inlet and Prudhoe Bay crude oils and No. 2 fuel oil all cause 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 UVOD but not with measurements by IR ppm. 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. Paraffins and monoaromatic and diaromatic hydrocarbons were found in tissues of fish exposed to the WSF of Cook Inlet oil. The fish started apparent depuration of the aromatics during the first 24 h of exposure, which indicates that they can cope with the stress physiologically. Our data supports the concept of excretion through the liver-gallbladder-gut.

5. High breathing rates during the first 24 h of exposure, elimination of most aromatics by 20 h, and the continued high breathing rates during the constant dose exposure for 72 h indicates 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.

ACKNOWLEDGMENTS

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FOOTNOTES

1/Northwest Fisheries Center Auke Bay Fisheries Laboratory, National Marine Fisheries Service, NOAA, Post Office Box 155, Auke Bay, AK 99821.

2/Chico State University, Chico, CA 95926.

3/S. D. Rice, et al. [1975] "Final report to Shell Oil Company, Standard Oil of Calif., Union Oil of Calif., Texaco Inc., Marathon Oil Co., and Phillips Petroleum Corp. on the toxicity and uptake-depuration of Cook Inlet Crude Oil to Alaska Marine Organisms." On file at Northwest Fisheries Center Auke Bay Fisheries Laboratory, National Marine Fisheries Center, NOAA, P. O. Box 155, Auke Bay, AK 99821.

4/Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

Table 1.--Median tolerance levels of pink salmon fry exposed to water-soluble fractions of Cook Inlet and Prudhoe Bay crude oils and No. 2 fuel oil. Concentrations of oil were determined by ultraviolet and infrared spectroscopy (ultraviolet optical density equals the optical density measured at 221 nm in 1 cm path length cell, infrared measurements are given in ppm).

Test length and confidence interval	Cook Inlet crude oil	Prudhoe Bay crude oil	No. 2 fuel oil
	Ultraviolet Optical Densities		
24 hour	0.1439	0.1008	0.2253
95% confidence interval	0.1229-0.1684	0.0916-0.1109	0.2063-0.2461
96 hour	0.1012	0.1008	0.2087
95% confidence interval	0.0917-0.1116	0.0916-0.1109	0.1896-0.2305
	Infrared Spectroscopic Measurement		
24 hour	4.13	1.56	0.89
95% confidence interval	3.51-4.84	1.41-1.73	0.82-0.97
96 hour	2.92	1.56	0.81
95% confidence interval	2.65-3.22	1.41-1.73	0.72-0.92

FIGURE LEGENDS

Figure 1.--The mean opercular breathing rate (± 95% confidence interval) of pink salmon fry exposed to water-soluble fractions of 3 oils. Individual doses are given in UVOD, percent of the 96-h TLm (as measured by UVOD), and in ppm measured by IR. Asterisk indicates significant differences at 0.01 level between that mean and mean at 0 h. Mortality was noted at the highest oil exposures at 22 h (fuel oil--8 fry in 0.171 UVOD, 18 fry in 0.240 UVOD; Cook Inlet--17 fry in 0.107 UVOD; Prudhoe Bay--10 fry in 0.075 UVOD) Sample sizes ranged 15 to 32 except at 22 h in the above doses because some fish died.

Figure 2.--The linear relationship between mean opercular breathing rates and exposure dose expressed as a percent of the 96-h TLm (as measured by UVOD). Mean breathing rate ± 95% confidence interval and regression line for breathing rates of pink salmon fry to water-soluble fractions of No. 2 fuel oil, Cook Inlet crude oil, and Prudhoe Bay crude oil (correlation coefficients of 0.970, 0.997, and 0.999 respectively).

Figure 3.--Mean cough rate of pink salmon fry exposed up to 22 h to water-soluble fractions of 3 oils. Sample sizes ranged from 1-14, and ± 95% interval is indicated where sample sizes were 3 or more. Asterisk indicates significant differences at 0.05 level between that mean and the mean at 0 h. Exposure doses are expressed in UVOD, percent of the 96-h TLm (measured by UVOD), and in ppm by IR.

Figure 4.--Concentrations of saturated paraffinic hydrocarbons as measured by GC in gut, gill, and muscle tissue of pink salmon fry exposed up to 4 days to the WSF of Cook Inlet crude and depurated up to 10 days. Only C_{15} through C_{19} paraffins were consistantly detected in all tissues, except that C_{12} through C_{30} were found in gill tissue after 10 hours of exposure. Limits of detectability were 0.05 ug/g wet weight in gill and gut and 0.02 ug/g in muscle.

Figure 5.--Concentrations of individual aromatic hydrocarbons as measured by GC in gut, gill, and muscle tissue of pink salmon fry exposed up to 4 days to the WSF of Cook Inlet crude and depurated 10 days. Samples were taken at 0, 3, 10, 33, and 96 h after exposure began and after 3, 10, 20, and 72 h and 10 days of depuration. Missing data points represent samples that were below the limits of detectability (0.04 ug/g in muscle, and 0.1 ug/g in gut and gill). B = sum of 4 mononuclear aromatics, N = naphthalene, M = methylnaphthalene, D = dimethylnaphthalene and T = trimethylnaphthalenes.

Figure 6.--Mean breathing and coughing rates of pink salmon exposed to a constant dose of Cook Inlet crude oil, water-soluble fraction for 72 h. Exposure dose was held "constant" at an UVOD averaging 0.064 by the additions of fresh water-soluble fraction every 3 h. Significant differences from the 0 h mean are indicated (asterisk) for breathing rate (0.01 level) and coughing rate (0.05 level). 95% confidence intervals are given for both breathing rate (all sample sizes greater than 16 individuals) and for coughing rate (sample sizes varied between 3 and 9).

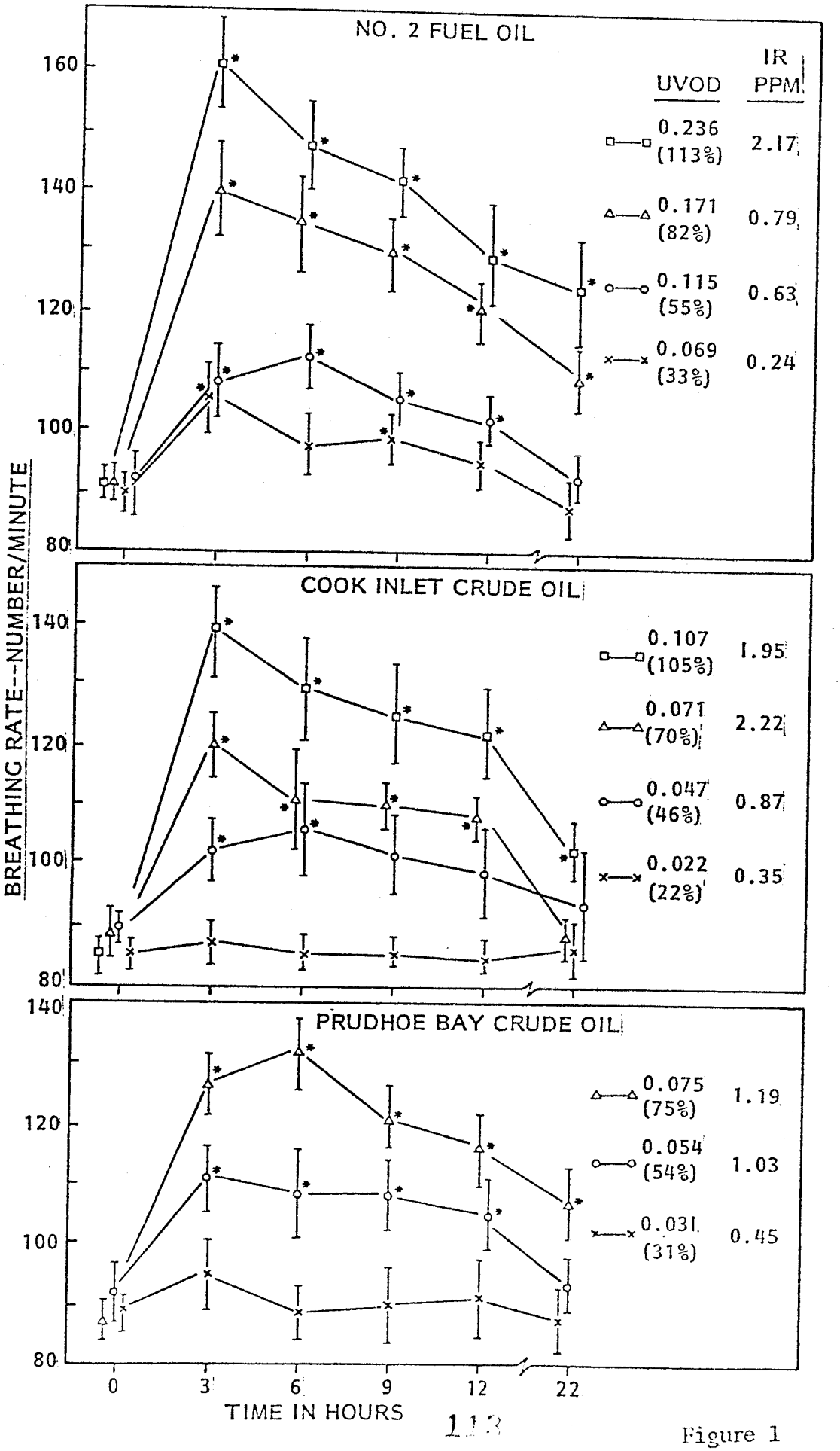
NO. 2 FUEL OIL
UVOD
IR PPM
0.236 (113%) 2.17
0.171 (82%) 0.79
0.115 (55%) 0.63
0.069 (33%) 0.24
COOK INLET CRUDE OIL
0.107 (105%) 1.95
0.071 (70%) 2.22
0.047 (46%) 0.87
0.022 (22%) 0.35
PRUDHOE BAY CRUDE OIL
0.075 (75%) 1.19
0.054 (54%) 1.03
0.031 (31%) 0.45
BREATHING RATE--NUMBER/MINUTE
TIME IN HOURS
0
3
6
9
12
22
80
100
120
140
160

Figure 1

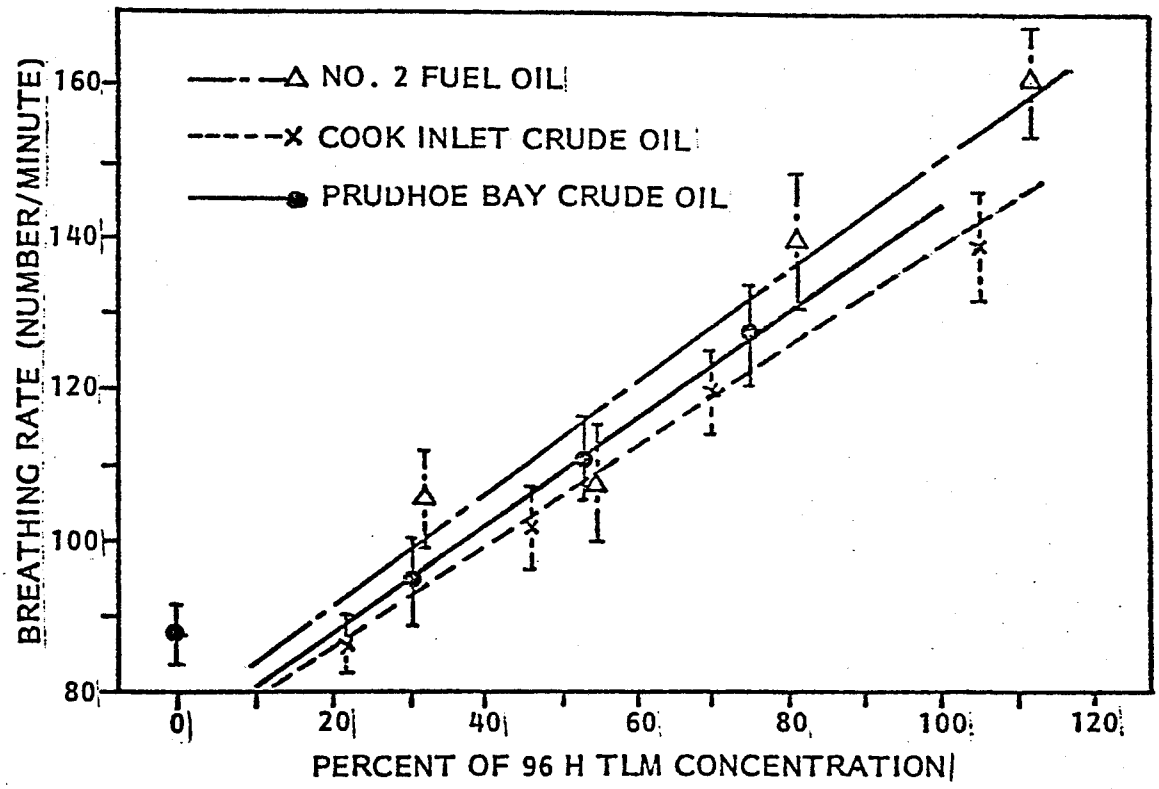
NO. 2 FUEL OIL
COOK INLET CRUDE OIL
PRUDHOE BAY CRUDE OIL
BREATHING RATE (NUMBER/MINUTE)
160
140
120
100
80
0
20
40
60
80
100
120
PERCENT OF 96 H TLM CONCENTRATION

Figure 2

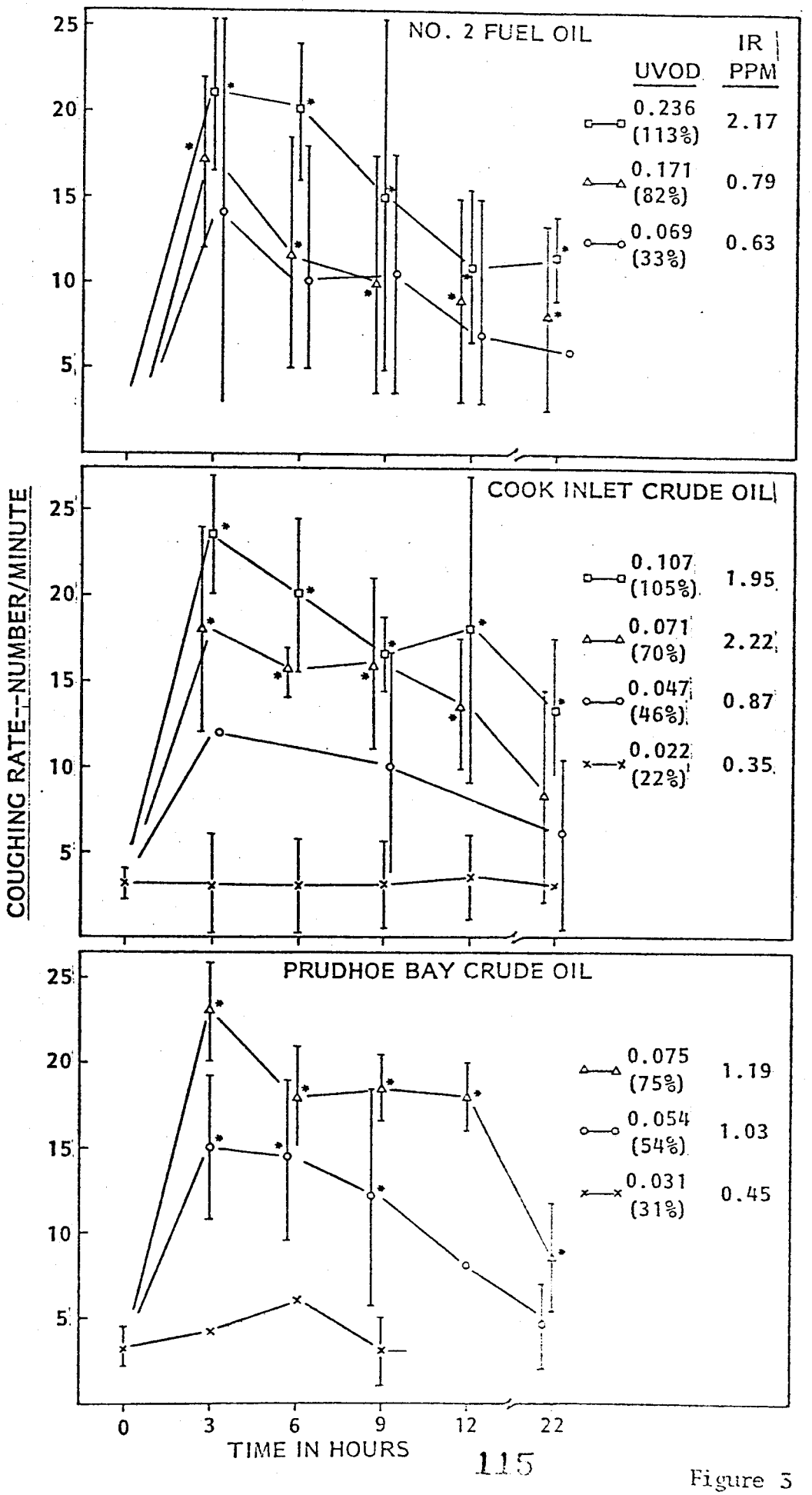
NO. 2 FUEL OIL
UVOD
IR PPM
0.236 (113%) 2.17
0.171 (82%) 0.79
0.069 (33%) 0.63
COOK INLET CRUDE OIL
0.107 (105%) 1.95
0.071 (70%) 2.22
0.047 (46%) 0.87
0.022 (22%) 0.35
PRUDHOE BAY CRUDE OIL
0.075 (75%) 1.19
0.054 (54%) 1.03
0.031 (31%) 0.45
COUGHING RATE--NUMBER/MINUTE
25
20
15
10
5
0
3
6
9
12
22
TIME IN HOURS

Figure 5

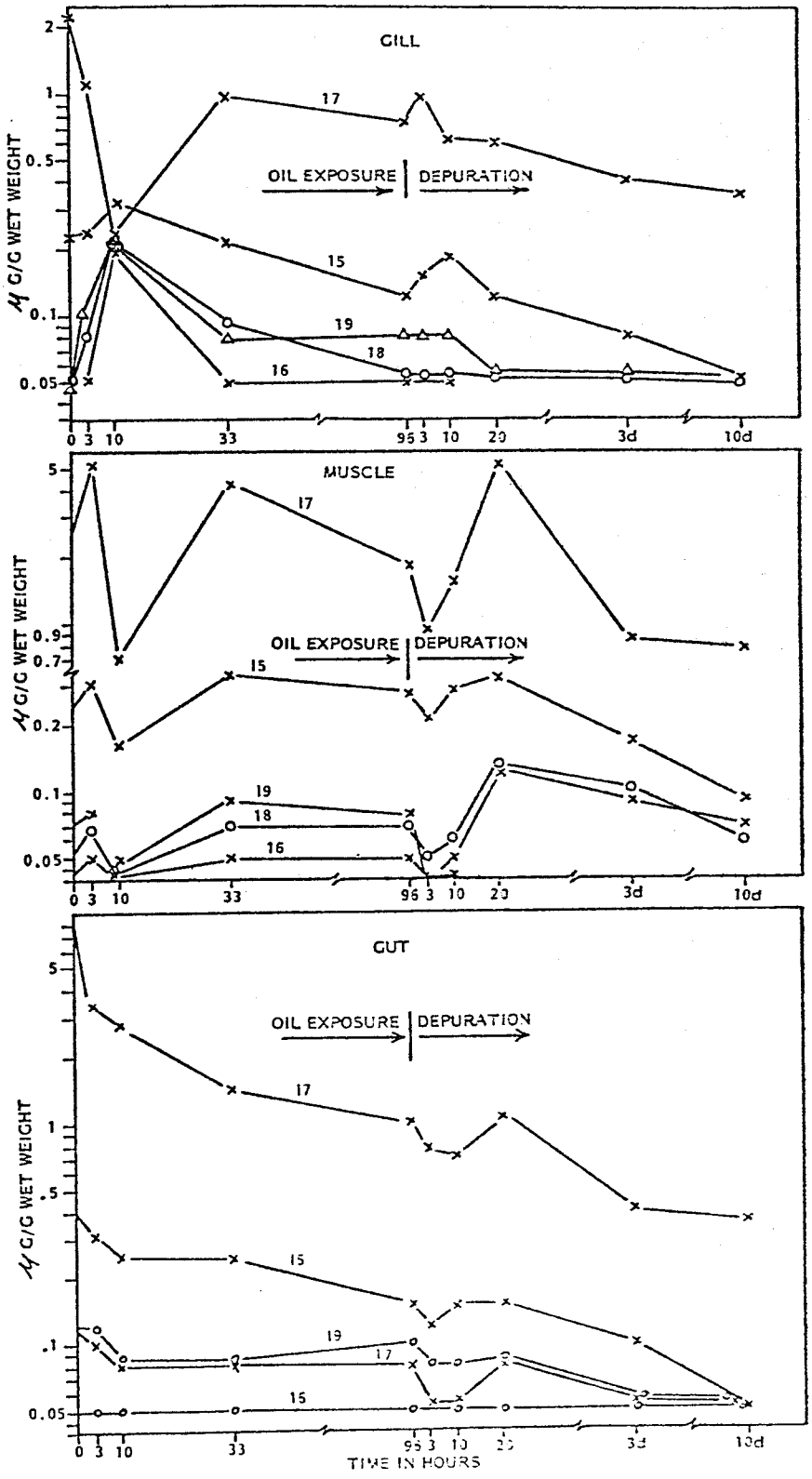
GILL
MUSCLE
GUT
OIL EXPOSURE
DEPURATION
μg C/G WET WEIGHT
TIME IN HOURS
0 3 10 33 96 3 10 20 3d 10d
15
16
17
18
19

Figure 4

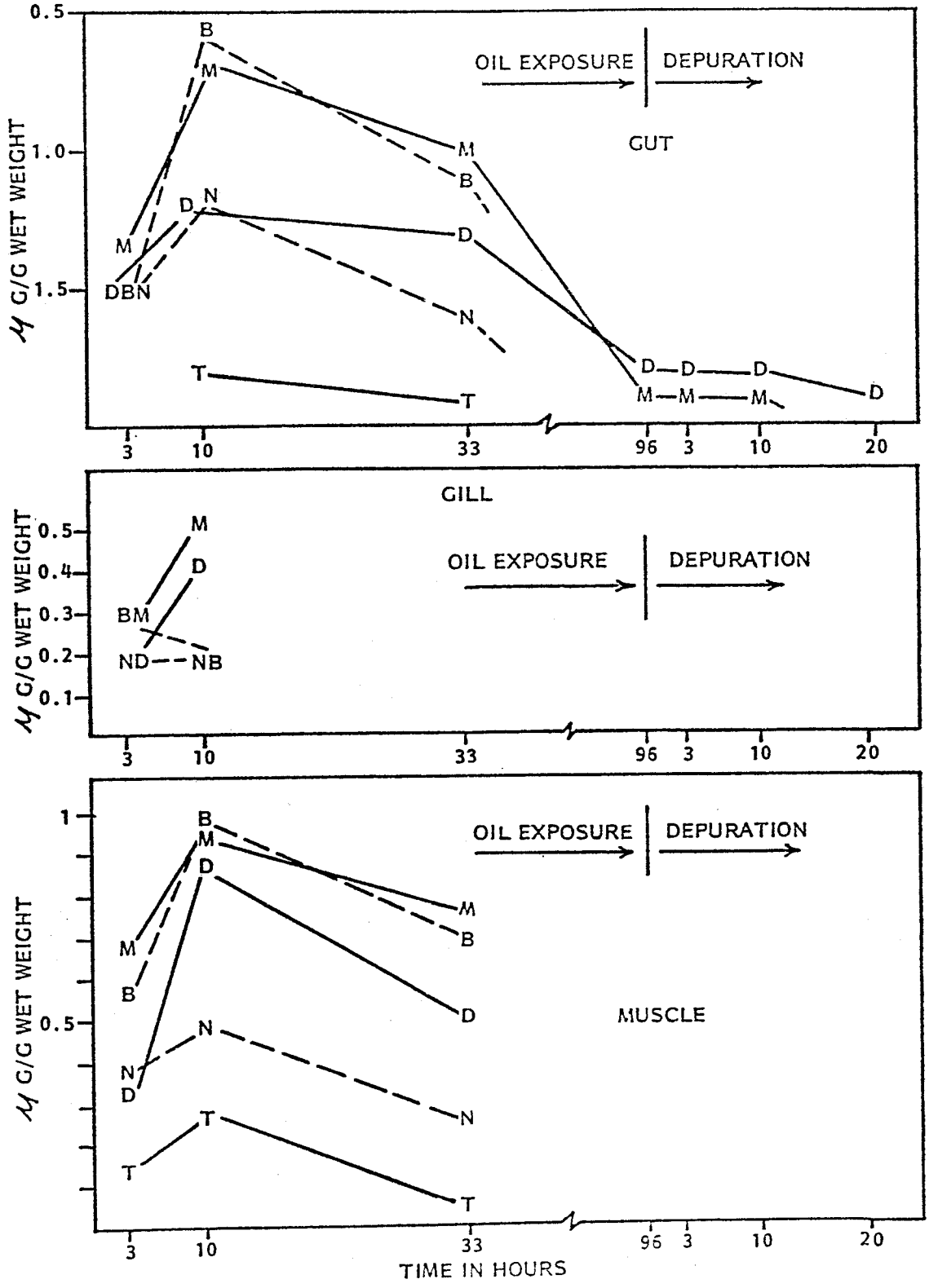
0.5
1.0
1.5
μ G/G WET WEIGHT
OIL EXPOSURE
DEPURATION
GUT
B
M
M
B
M
D
N
D
M
DBN
D
N
T
T
D
D
D
D
M
M
M
3
10
33
96
3
10
20
GILL
0.5
0.4
0.3
0.2
0.1
M
D
BM
ND
NB
1
0.5
B
M
D
M
B
M
B
D
N
N
D
T
T
MUSCLE
TIME IN HOURS

Figure 5

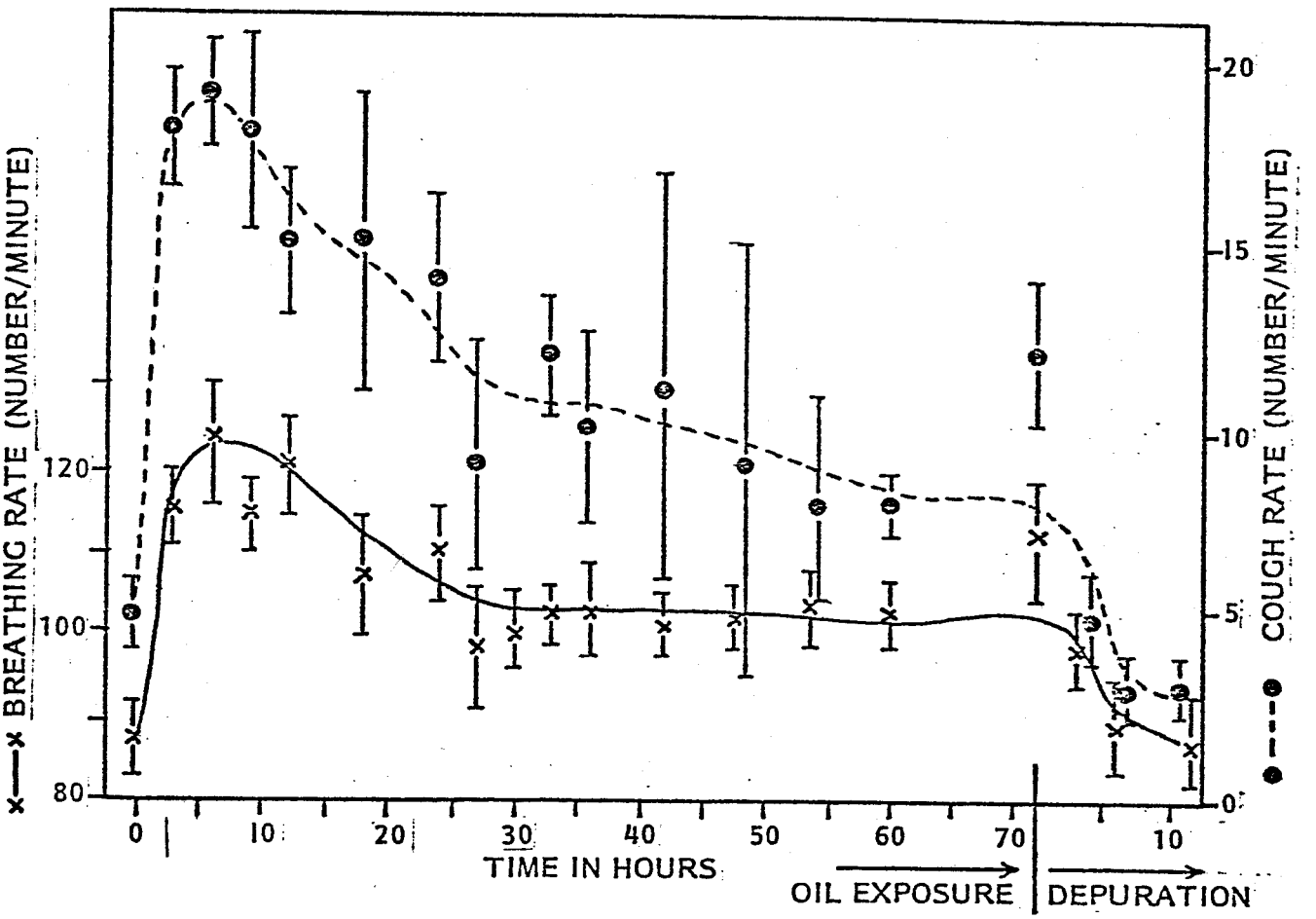
BREATHING RATE (NUMBER/MINUTE)
COUGH RATE (NUMBER/MINUTE)
120
100
80
20
15
10
5
0
0
10
20
30
40
50
60
70
10
TIME IN HOURS
OIL EXPOSURE
DEPURATION

Figure 6

ANNUAL REPORT

TITLE: Research Unit 73. Sublethal effects as reflected by morphological, chemical, physiological and behavioral indices

PRINCIPAL INVESTIGATORS:

Drs. Donald C. Malins, Harold O. Hodgins and Mr. Douglas D. Weber

National Marine Fisheries Service, Northwest Fisheries Center,

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April 1976

ANNUAL REPORT FOR R.U. 73

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

The objective of these studies is to identify and evaluate in selected marine organisms the effects of chronic exposure to petroleum hydrocarbons and trace metals. Particular emphasis is on changes in tissue structure and ultrastructure, chemosensory perturbations and related behavior, disruptions in larval development, and alterations in physicochemical properties of mucus.

Progress during this first annual reporting period has been concerned with development and preliminary assessment of: (1) effect of chronic ingestion of whole crude oil on reproductive success and internal cellular changes in trout; (2) surface structure modification in salmon and flatfish following immersion in sublethal levels of water-soluble fractions (WSF) of oil; (3) selection and/or acquisition of appropriate marine organisms, stimuli, and control data on behavioral and neurophysiological responses of these organisms to chemical compounds; (4) establishment of experimental conditions for the exposure of salmonids to metals (Pb, Cd, V) to elucidate chemical and physicochemical changes in epidermal mucus; and (5) development of facilities for holding, rearing, exposure, and analysis of organisms subjected to petroleum.

Preliminary experiments in cell biology indicated that the liver of trout (Salmo gairdneri) is a primary site of alteration in biochemical activity when the fish ingest crude oil. The liver showed two major changes: a depletion of energy storage products, glycogen and lipid, and an increase in the endoplasmic reticulum, a component of cells that is instrumental in protein synthesis. When coho salmon (Oncorhynchus kisutch)

and English sole (Parophrys vetulus) were immersed in a sublethal concentration of water-soluble fraction of crude oil there appeared to be a depletion of mucus from the producing cells at the skin surface. These cellular changes are considered to be symptomatic of deleterious effects of petroleum oil on all three species of fish. In contrast, in related experiments, the feeding of intentionally high levels (one part/1000 oil in food) of crude oil to sexually maturing trout for six months did not result in mortality or grossly detectable damage prior to spawning; nor did it appear to impair the viability of their eggs and sperm.

To date, the effort with larval invertebrates and organisms selected for behavioral assay has involved the testing of methods for producing and holding larval forms, gathering of baseline data, and the design and testing of facilities for flow-through bioassay. We have concluded this phase and will commence testing the animals by mid-April, 1976.

Studies on epidermal mucus have involved installment of specially designed tanks for exposure of salmonids to radioactive metals. Analytical methods to examine alterations in properties of mucus of metal-exposed fish were tested on several control fish and the procedures were streamlined for maximum efficiency.

It is premature to draw conclusions from the data acquired thus far, as much of the accomplishment is related to the establishment and standardization of experimental protocols. Significant data relevant to our objectives will, by design, mostly be obtained in the latter part of the contract period.

II. INTRODUCTION

A. General nature and scope of study

The responses of marine organisms to environmental contaminants are reflected in a number of changes at both the organismal and at the cellular and subcellular levels. The general scope of this investigation is to evaluate physiological, physicochemical, behavioral, and functional changes in animals exposed to petroleum hydrocarbons and trace metals.

B. Specific objectives

One objective of this study is to assess the cell structural effects and chemosensory responses of selected marine organisms to sublethal concentrations of water-soluble oil fractions. Of notable interest are the threshold concentrations at which structural and behavioral modification occur, time require to induce modifications, and the duration of an effect following removal of the organisms to non-contaminated waters.

Studies with larval, juvenile and adult invertebrates are concerned with: effects of total water-soluble oil and selected aromatic hydrocarbons on (a) sperm and egg viability in molluscs, (b) hatching time of shrimp, (c) metamorphosis of shrimp and molluscs, (d) settling success of molluscs; effect of ingestion of whole crude oil on (a) feeding rate of shrimp and molluscs, (b) growth of shrimp and molluscs; effect of surface coating and weathered whole crude oil on (a) sperm and egg viability of molluscs, (b) hatching time of shrimp, (c) metamorphosis of shrimp and molluscs.

The trace metal-mucus study will define and evaluate alterations in chemical and physicochemical properties of body mucus and skin surface produced by exposure of fish to water-borne trace metals.

Levels of metals (Pb, Cd, V) in the body mucus of fish (salmonids and flatfish) will be determined after they are exposed to sublethal concentrations (5 to 500 ppb) of trace metals for a relatively long period (30 days). These experiments will be carried out at 4° and 10°C to simulate arctic and subarctic conditions. At the end of each exposure, fish will be placed in a"metal-free"environment to assess the rate of depuration. The data on accumulation of metals in the mucus will be correlated with resultant changes in physicochemical properties of mucus using various specialized techniques.

C. Relevance to problems of petroleum development

In marine organisms the chemosensory system plays a major role in activities related to feeding, avoidance and escape responses, reproduction, settlement site selection, and homing. These responses can be induced in laboratory animals by specific compounds at levels of parts/trillion (ppt) (Kittredge, Terry and Takahashi, 1971) and have been abolished by water-soluble oil fractions at levels of 1 ppb (Jacobson and Boylan, 1973). Cellular damage (disruption of gill filaments) was shown to occur in fish taken in an oil spill area (Blanton and Robinson, 1973) and under sub-lethal exposure in laboratory experiments (Wolf and Strand, 1973).

The planktonic existence of invertebrate larval forms makes them particularly vulnerable to contaminant spills due to their inability to avoid the contaminants and because of their presence near the water surface. Studies of petroleum effects on species of these organisms found in the arctic and subarctic are therefore of vital importance in predicting effects of increased petroleum contamination.

Trace metals are components of petroleum. Moreover, very small amounts of these metals are known to be extremely toxic to many organisms. The metals are accumulated to much greater levels in various tissues in fish, including mucus, in comparison to the environment (Chow et al., 1974; McKone et al., 1971; Varanasi et al., 1975). Environmental levels of trace metals are expected to increase substantially from drilling operations; therefore, it is important to determine the effect of these metals on marine organisms.

III. CURRENT STATE OF KNOWLEDGE

As expressed by Laverack (1974),

> "Human beings tend to underestimate the value and significance of the chemical sense to other animals. This is probably due to a comparative lack of sensitivity in human chemoreceptors, and the overriding importance of visual cues. Amongst animal groups at large, chemical sensitivity in many cases far outweighs in information content any other single physical attribute of the environment."

Research in the field of chemical ecology has not been extensive until this decade. Information in this field is rapidly increasing, but is still minimal on the effect of sublethal levels of petroleum hydrocarbons on behavior of marine animals. There is clear evidence, however, that oil products interfere with chemosensory modulated behavior (Atema, Jacobson and Todd, 1973), and that polynuclear aromatic hydrocarbons in particular are probably the most active petroleum components in this regard (Kittredge, Takahashi and Sarinana, 1974).

In respect to cell biology, there are few reports on the effects of hydrocarbons on the morphology of arctic and subarctic marine species. However, several papers provide relevant information. Sabo et al. (1975), working with a field population of Fundulus heteroclitus from oil-

contaminated waters, found decreased glycogen and lipid stores and an increase in the rough endoplasmic reticulum (RER) in liver cells. Tissue from bream (Abramis brama) liver was examined histologically after the fish were treated with phenol. Gross changes, such as color differences and loss of delineation of the hepatic cells, were noted and the cytoplasm of the liver cells became a "granular mass" as observed with the light microscope (Waluga, 1966). No electron microscopy was conducted so it can only be speculated that the granularity was related to RER proliferation. Gardner (1975) immersed adult marine Atlantic silversides (Menida menida) in sea water containing whole crude oil, and olfactory lesions were observed with the light microscope. It is clear, therefore, that petroleum hydrocarbons affect certain fish tissues, but the precise nature and extent of damage await further investigation.

Studies on the effects of oil and oil fractions on larval invertebrates are few. Studies of contaminant effects on larval molluscs usually involve a 96-hour bioassay of the oyster Crassostrea gigas. At this stage the oyster forms a shell and begins to eat. Due to problems of food production, little work has been undertaken on forms beyond this stage. Larval shrimp have been used in studies of acute toxicity under static conditions; however, the biochemistry, histology, and much of the physiology remains unknown.

Previous studies with rainbow trout (Salmo gairdneri)(Varanasi et al., 1975) have shown that water-borne lead and mercury (0.1 to 1 ppm) accumulate to a much higher level (4 to 7.9 ppm) in the epidermal mucus. It appears that at lower levels of exposure, magnification of metal

concentrations in the mucus was even greater. These experiments were carried out for a maximum of four days. There is no information available on the uptake and accumulation of trace metals in body mucus or skin surface over long periods of time. It was shown with rainbow trout (Varanasi et al., 1975), using electron spin resonance techniques, that the body mucus of metal-exposed fish was "fluidized." Furthermore, this change was not reversed when the exposed fish were kept in a "metal-free" environment for a period of 24 hours.

Very little is known about the effect of long-term, chronic exposure of low levels of trace metals on the mucus and skin surface of fish. Neither is there any information on the levels of contaminants that may persist in the mucus after fish are placed in "metal-free" waters for extended periods. No data is available on the effects of low temperature on the uptake and accumulation of metals. Moreover, no studies have been carried out to discern metal-induced changes in hydrodynamic properties, or immunological properties of mucus.

IV. STUDY AREA

All experiments were conducted in laboratories on arctic and subarctic marine species.

V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

A survey was conducted on a number of Pacific subarctic marine organisms to determine through behavioral and electrophysiological observations which species demonstrated consistent chemoreceptive responses to specific stimuli. Of the animals surveyed, four representative species from a broad range of taxonomic groups were chosen for study on the basis of: distinct, reproducible, response to available

biogenic chemical stimuli; practicality of maintenance under laboratory conditions; and availability or adaptability of techniques for observing and quantifying their response. The marine organisms and chemosensory assays selected as a result of the survey were:

1. Avoidance behavior of the benthic holothurian Parastichopus californicus elicited by compounds present in the predatory asteroid Pycnopodia helianthoides.

2. Reproductive behavior of males of the pelagic copepod Calanus pacificus in response to pheromones released by females.

3. Feeding behavior of the shrimp Pandalus platyceros in response to tissue extracts and specific amino acids.

4. Discrimination response of the coho salmon Oncorhynchus kisutch in response to natural waters and specific amino acids.

Experiments in cell biology concerning the effects of immersion in WSF of crude oil were conducted on coho salmon and English sole. Long-term exposure to whole crude oil in food, used steelhead or rainbow trout as the experimental animal.

Two methods were used to expose the fish to oil: adding the WSF of crude oil to the water or adding whole crude oil to the food. To prepare the WSF, 10 ml of Prudhoe Bay crude oil per liter of salt water was stirred for 20 hours, allowed to settle for 3 hours, and the bottom fraction removed. The fish were exposed to a 13% solution of the WSF in aerated tanks and maintained at 10°C. Fish and water samples were taken 2 hours, 24 hours, and 5 days after addition of the WSF. The concentration of WSF was measured spectrofluorometrically and data stored for subsequent analysis.

In the feeding experiments, 2.5 ml of crude oil was dissolved in 10 ml of Freon and applied to 1.0 kg of Oregon moist pellet. Freon was evaporated and the pellets were then fed to the fish. The total food supply was 2% of body weight of the fish per day, fed five days per week for 2-1/2 months. A total of 4 replicates of both experimental and control fish were sampled. The controls were treated exactly the same as the experimental fish except that crude oil was omitted from the Freon-treated food.

In both WSF immersion and feeding studies, samples of skin (1 cm^2) from the head, mid-dorsal, and ventral body and from the dorsal and ventral tail and the gill were fixed overnight in a trialdehyde solution, rinsed in buffer, and dehydrated in an ethanol series. The ethanol was replaced with Freon and the tissues were subsequently critical point dried, coated with gold palladium, and examined by scanning electron microscopy (SEM). Additional samples prepared for future transmission electron microscopy (TEM) included head and mid-dorsal skin, gill, intestine, liver, spleen, and kidney. Initial fixation with the same trialdehyde fixative used for SEM was followed by a buffer wash and post-fixation in osmium tetroxide, and dehydration in ethanol was followed by infiltration and embedding in Spurr plastic. Thick sections (0.5μ - 1.0μ) were stained with toluidine blue and a special polychrome for plastic sections.

Another group of sexually maturing rainbow trout were fed Prudhoe Bay crude oil at one-half the level and prepared in the same manner as described above. Each fish was fed an average of 3.57 g of oil over a 6-month period. At spawning 40 crosses were made between control and

oil fed fish and within each group. Samples of skin, liver, kidney, intestine, muscle, egg, sperm, and embryo were collected for histological examination.

Gravid female shrimp (Pandalus platyceros) are held in the laboratory until hatching takes place. The larvae are then removed for immediate testing or reared until reaching the desired stage. Molluscs (Mytilus edulis and Crassostrea gigas) are conditioned in the laboratory and induced to spawn. The resulting gametes and/or embryos will be tested or reared to more advanced larval stages for testing. The testing regime will include flow-through conditions at several WSF concentrations and temperatures of 4° to 10°C. Animals will be sampled for histological and biochemical examinations at predetermined periods.

In the mucus studies the metal-exposure experiments are conducted with coho salmon in cooperation with the staff of R.U. 74. Test tanks will contain 5 to 500 ppb of radioactive $Pb(NO_3)_2$ in initial experiments. Each experiment will be carried out at 4° and 10°C to assess the effect of temperature. Test fish will be exposed at different levels of metals for 30 days. At ten-day intervals, control and test fish will be taken for analyses of mucus and skin. The amount of metal in mucus and skin will be determined by liquid scintillation spectrophotometry and by atomic absorption spectrometry. At the end of the 30-day exposure period, the remaining test fish will be placed in "metal-free" water to determine depuration rates.

In addition to uptake and depuration data, physicochemical properties of mucus will be determined. Protein content, sialic acid content, fluorescence spectra, electron spin resonance spectra and rheological

data will be obtained to determine perturbation in mucus structure. Studies will also be performed to detect differences in immunological properties of mucus and in resistance of fish to disease as a consequence of trace metal exposures.

VI. RESULTS

A. Oil ingestion studies (fish)

Steelhead trout sampled after 2 weeks of exposure to oil in food revealed a difference in glycogen reserves in the liver. The liver cells of the control fish contained high glycogen levels, whereas those of the experimental fish had virtually none. These changes were evident in the 0.5μ sections stained with toluidine blue. The polychrome method, which stains mucopolysaccharide moieties bright red when the cytoplasm is blue was used on 1.0μ sections to differentiate glycogen deposits in the cells.

All fish gained weight and no mortalities were observed at 2-1/2 months. The control fish increased in weight by 95.5%, the oil-fed fish 70.5%. After 2-1/2 months of oil feeding, stored glycogen in the liver showed a depletion similar to that observed after 2-weeks exposure. The glycogen stores were so small that only a rare cell showed differential staining with the polychrome method. In addition, lipid reserves decreased in the oil-fed fish. Thick sections of gut, skin, spleen, kidney and gill were examined; no major differences were apparent between control and experimental animals. Tissue samples from the 6-month oil feeding study on rainbow trout have not been analyzed. Reproductive capabilities, based on egg fertilization and initial embryo development, do not appear to be reduced.

B. Immersion studies (fish): petroleum WSF

The skin and gills of coho salmon and flatfish were evaluated for changes after exposure to WSF. The findings indicated that surface mucus glands had discharged their contents after WSF exposure; however, the numbers and degree of discharge varied considerably in both experimental and control groups. Skin from five locations on the coho and three on flatfish were prepared for scanning electron microscopy and transmission electron microscopy. Analyses of this material are in progress.

Sections of liver taken from both salmon and flatfish (22 in all), at each sampling time, were evaluated by light microscopy. No gross changes, no massive damage, and no mortalities were observed. Particular attention was paid to the distribution of glycogen and lipid in liver cells. As anticipated, the amounts of these substances varied in the controls because none of the fish were fed during the experiment and the flatfish, which had been captured a week before the experiment, did not eat in captivity. Although the number of glycogen-containing cells in the control groups varied considerably, there was a consistent lack of glycogen in the oil-treated animals.

C. Chemosensory studies

In acquiring baseline data for the caridean shrimp Pandalus platyceros, 1400 replicates of feeding behavioral response in control animals, as a function of hunger (9-day starvation period with testing at 3-day intervals), circadian rhythm, and 22 chemical

stimulants were tested. A squid tissue homogenate, an artificial mixture of compounds representative of squid tissue, and one amino acid were chosen as stimuli for eliciting standard behavioral responses.

Avoidance response in Parastichopus to the starfish predator Pycnopodia were selected following crossmatching responses of 16 prey species to extracts from 5 predator species. To elicit reproductive response in the male copepod, Calanus, β-ecdysterone at a concentration of 2.5 ppb, will be used as a female conspecific pheromone. Electrophysiological techniques for assessing olfactory activity in Oncorhynchus have previously been developed and accumulation of baseline response data is in progress.

D. Oil ingestion and WSF-immersion studies (invertebrates)

To date progress involved the design and testing of techniques for holding larval invertebrates and testing of equipment for providing reproducible, stable flow-through conditions. The work described under R.U. 74 details the studies with radioactive naphthalene. These same techniques will be employed for some of the tests to be conducted in this research unit (R.U. 73).

E. Mucus studies

Preliminary work focused on the experimental design for collection and examination of the epidermal mucus from control fish. We explored several methods for immobilizing fish without damaging the skin for mucus collection, such as asphyxiation, use of anaesthetic (tricaine methanesulfonate) and sudden chilling.

Both asphyxiation, and sudden chilling took 12-15 minutes before the fish could be handled, during this time fish sloughed off large quantities of mucus. Fish were rapidly immobilized by anaesthetic, however, the anaesthetic interferes in the measurement of fluorescent spectra of the mucus. Ultimately, a mechanical device was used which very quickly and efficiently stunned the fish without breaking the skin or allowing sloughing of mucus.

The method of mucus collection was standardized. It was found that the collection of mucus by gentle scraping with spatula is quick and efficient. The amounts and properties of mucus collected by this method were reproducible. We also tried to collect mucus by a jet of air. We found that mucus collected from two sides of the same fish either by the air-jet or by spatula, had the same concentrations of protein and had comparable fluorescent spectra.

The epidermal mucus of both rainbow trout and coho salmon yielded a workable fluorescence spectra (excitation, 277 nm; and emission, 335 nm). Rheological properties (ability to reduce friction) were measured at 5% and 50% dilution. The 50% mucus solutions gave rise to the most reproducible results in comparison to sea water standards.

VII. DISCUSSION

The reduction and, in some cases, complete loss of glycogen storage from the livers of steelhead trout exposed to oil was consistent in initial experiments. Field studies of Fundulus taken from oil-polluted waters showed a similar decrease in glycogen (Sabo et al., 1975). In Fundulus, the increased activity of glucose-6-phosphate dehydrogenase in the liver of oil-contaminated fish indicated increased depletion of "energy stores." The lipolysis found in Fundulus corroborates our findings of a decrease in lipid reserves in oil-fed fish.

Our work has shown a definitive metabolic response of fish livers to crude oil and implies a need for additional studies. Comparable studies on other organs have yet to be completed; the tissues are prepared and await examination.

One of the most obvious revelations of this study is the requirement for additional background information on "normal" fish and on the range of variability within the norm. Accordingly, a study of healthy fish was begun at the histological level. Two different fixatives and five staining methods were used on paraffin sections of gill, liver, intestine, spleen, and kidney. Serial sections of an entire liver were stained with trichrome, which differentiates connective tissue from cellular components. These sections are being studied to trace bile ducts in the hepatic vascular system.

It is anticipated that chronic exposure of fish, shellfish and selected marine organisms to water-soluble fractions of Prudhoe Bay crude oil will commence by mid-April, 1976. For cellular and sensory physiology studies, responses in animals not exposed to petroleum hydrocarbons will be compared to those from organisms living in flow-through chambers with water-soluble hydrocarbon concentrations of 1 ppm; 500,100, 50,10, and 5 ppb. Emphasis will be on threshold concentrations and specific oil fractions which induce cellular, behavioral and neural modifications.

The tests with oil WSF will be conducted at levels that might occur during oil and gas development. The effects of these concentrations on viability of larval molluscs and shrimp will be evaluated.

Epidermal mucus of fish, the primary site of contact with water-borne contaminants, also plays an important role in regulating swimming speed (Rosen and Cornford, 1971), and may serve as a defense against pathogenic microorganisms. Accordingly, physical and chemical integrity of the mucus may be of vital importance to the well-being of fish. Studies with rainbow trout (Varanasi et al., 1975) demonstrated that body mucus will accumulate ten times as much lead (1 ppm) as that in the surrounding water (0.1 ppm) in a relatively short period of time. Moreover, certain structural changes occurred, as observed by electron spin resonance spectroscopy. Alterations with mucus were not reversed when the fish were placed in a "metal-free" environment. These results indicate a need for further studies on chronic exposure of fish to trace metals.

Our present investigations involve 30-day exposure of salmonids and flatfish to low levels (5 to 500 ppb) of trace metals (Pb, Cd, V) at 4° and 10°C. The first exposure on salmonids will begin in the next quarter. Results will be evaluated in terms of exposure parameters, accumulation and depuration rates and resultant alterations in physicochemical properties of metal-exposed mucus.

VIII. CONCLUSIONS

The proposed research of this task is on schedule.

The ultrastructural changes in fish liver indicate that ingestion of Prudhoe Bay crude oil mixed with food is deleterious to salmonids. The depletion of energy reserves (glycogen and lipid) may reduce the capability of the animals to survive stress conditions, such as predation or diseases. The increase in rough endoplasmic reticulum, a subcellular system that is implicated in protein synthesis, including enzyme synthesis, is perhaps indicative of an attempt to detoxify injurious materials.

Marine organisms, selected for chemosensory-behavior studies, represent a broad range of chemosensory responses and taxonomic groups: avoidance response in a benthic sea cucumber, reproductive response in a pelagic copepod, feeding response in shrimp, and olfactory discrimination in salmon.

In the invertebrate studies, animals have been secured and larvae of shrimp are now available for testing. Histological techniques have been perfected and work is currently in progress to ascertain the characteristics of normal tissue for comparative purposes.

Methods have been established for collection of mucus and skin samples. Analytical methods to examine mucus and skin from test animals were applied to control fish and the procedures have been standardized for maximum efficiency. The tanks and related equipment are now installed and exposure studies will begin in the next quarter.

IX. NEEDS FOR FURTHER STUDY

Because the studies are largely in early phases it is premature to delineate needs for further study in the present report. Nevertheless, some implications for future work are arising from present studies. These implications will be evaluated in the next quarter.

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ANNUAL REPORT

TITLE: Research Unit 74. Identification of major processes in biotransformations of petroleum hydrocarbons and trace metals

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ANNUAL REPORT FOR RESEARCH UNIT 74

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

The objectives of research unit 74 are the identification and evaluation of certain physiological and biological effects of petroleum hydrocarbons on salmon, flatfish, and spotted shrimp and their larvae. Also included are studies on the effects of trace metals on salmon and flatfish. The effects of petroleum hydrocarbons will be evaluated in terms of uptake and depuration studies in which hydrocarbons are administered by diet as well as by exposure to water-soluble fractions via immersion. Experiments will include the use of petroleum oil, fractions thereof, and selected radioactively-labeled aromatic hydrocarbons. Studies on the effects of trace metals will involve exposures, via immersion, to radioactively-labeled compounds. In both the hydrocarbon and trace metal studies, chemical and physiological parameters will be correlated with data obtained by microscopic techniques.

To date, emphasis of this research unit has been largely on the establishment and standardization of experimental techniques and protocols. In this regard, the installation of a continuous flow-through system for exposing marine organisms to chronic levels of water-soluble hydrocarbons at our Mukilteo field station is considered significant. As a consequence of this advance in experimental capabilities it is now possible to obtain data on the chronic effects of soluble water-borne hydrocarbons on marine organisms. In addition, a micro-modification of the above system was developed for exposing shrimp larval forms to minute (ppb) levels of radioactively-labeled water-soluble aromatic hydrocarbons. Moreover,

analytical procedures have been developed for detailed analyses of tissues in studies on the accumulation and depuration of radioactively-labeled hydrocarbons, total metabolites, and individual metabolic products. In studies involving non-radioactively-labeled hydrocarbons (e.g., crude oil fractions), spectroscopic and chromatographic methods are now established for use in the detailed identification of hydrocarbon constituents of biological samples.

Experimental regimes for exposing salmon and flatfish to isotopically-labeled Pb, Cd, and V are almost completed. Techniques for analyzing for radioactive metals in key tissues are perfected. Moreover, chromatographic methods are established for examining interactions of trace metals with biological systems, such as liver proteins. Autoradiographic techniques have been tested and found to be suitable for studying the distributions of radioactive metals in fish tissues by microscopy. The nearly completed installation of scanning and transmission electron microscopes at the NWFC will permit us to relate challenge conditions and chemical parameters to morphological changes in key tissues.

We anticipate that much of the work during the next quarter will involve the conduction of challenge experiments and the acquisition of data relevant to the objectives of this research unit.

II. INTRODUCTION

A. General nature and scope of study

The studies are intended to elucidate biological effects induced in marine organisms by exposure to petroleum hydrocarbons and trace metals under conditions consistent with arctic and subarctic environments.

B. Specific objectives

The work involves the measurement and evaluation of a number of biological parameters: uptake, accumulation and depuration of petroleum oil fractions and radioactively-labeled aromatic compounds using salmonids, flatfish, larval shrimp and molluscs as experimental animals. The study is oriented toward an understanding of relations existing between (1) levels of dietary and water-borne hydrocarbons, exposure times, degrees of hydrocarbon accumulations, and (2) metabolic conversions, depurations, and morphological alterations evidenced by microscopic techniques. Comparable studies are conducted to relate experimental conditions to the biological fate and effects of trace metals on salmonids and flatfish.

C. Relevance to problems of petroleum development

Very little is known about the impact of petroleum oil and trace metals on organisms indigenous to arctic and subarctic marine waters. A preliminary understanding of the effects on these biota of such contaminants requires additional data relating the type and degree of exposure to accumulation, metabolic conversions, and depuration of tissues. Moreover, these data should be evaluated in terms of concommittent alterations in cellular and subcellular structure. Well-designed experiments leading to acquisition of this information will allow balanced judgments to be made about the impact of oil operations on arctic and subarctic organisms and ecosystems.

It is particularly noteworthy that the larval forms are planktonic in their early stages and would be directly affected by oil spills in an area where they are present, both because of their lack of

ability to avoid the contaminant and because of their proximity to the oil at the water surface. Shrimp, in the early stages of their life cycle, appear to be extremely sensitive to oil pollution.

III. CURRENT STATE OF KNOWLEDGE

Few studies are documented which provide details on the fate and effects of petroleum hydrocarbons on marine organisms, with the following notable exceptions: Roubal, Collier, and Malins (1975) described the accumulation and metabolism of aromatic hydrocarbons in salmon; Corner (1973) delineated naphthalene metabolism in crabs; and Lee (1972) studied naphthalene and benzopyrene metabolism in some eastern fish species. In view of the paucity of knowledge mentioned, the studies presently underway are designed to answer, at least in part, the following questions in relation to the objectives of research unit 74: (1) What compounds or classes of compounds in crude oil are taken up by fish and what is the degree of accumulation? Are certain classes of compounds preferentially accumulated?, (2) Does mode of entry of hydrocarbons into fish (e.g., via the water or via the food) make a difference in uptake, metabolism, and accumulation of hydrocarbons and their metabolites?, and (3) Are ultrastructural changes produced in key organs in relation to specific hydrocarbon structure? If so, how are such changes related to the conditions of these experiments?

Very little work has been conducted on the effects of hydrocarbons on larval shrimp. Most work to date on larvae of invertebrates has involved static systems and the study of acute toxicity. There are indications that soluble fractions of oil cause molting delay in larvae, however, long-term implications of this data have not been pursued. In fact, the cellular morphology and biochemistry of normal larval shrimp is largely unknown.

Larval molluscs have been studied usually with the standard 96-hour LC_{50} bioassay. This is the time of development to the straight-hinge stage and is the point at which they begin feeding. Due to the complexity of their feeding, little work has been conducted on post straight-hinge stage molluscs.

Most studies reported thus far on water-borne lead, cadmium, and vanadium focus on establishing 96-hr LD_{50} levels of the metal. The likelihood of acutely toxic levels being reached in the marine environment is remote, except for certain isolated cases. There is very little information on the effects of these metals on marine organisms (in the 5 to 500 ppb range) for extended periods of time (up to one month). Questions such as rates of accumulation and depuration and effects on behavior, physiology, feeding, immune response, growth and mortality, clearly need to be answered.

IV. STUDY AREA

All experiments on arctic and subarctic species are conducted in laboratories that are part of the Northwest Fisheries Center.

V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

All data in R.U. 74 result from laboratory exposures of selected species to experimental conditions. The experimental designs are described in this section.

Three regimes for exposure to hydrocarbons are employed:

1. Fish are exposed to soluble petroleum hydrocarbons via the water (uptake primarily a function of gills and/or skin).

2. Fish are exposed to petroleum hydrocarbons (Prudhoe Bay crude oil) via the food (uptake primarily a function of transport across the gastrointestinal tract).

3. Selected radioactively-labeled aromatic compounds are employed in a feeding study designed to determine: (a) extent or degree of passage of aromatic compounds across the gastrointestinal tract, and (b) the degree and nature of the accumulations in key tissues (e.g., gall bladder, liver, kidney, light and dark muscle, brain, and blood). The experimental protocols will allow the assessment of variations in uptake and accumulation of petroleum hydrocarbons and allow for the determination of metabolites. Exposure conditions will be related to concentration of contaminants in food or water, and duration of exposure.

A. Flow-through studies with water-soluble fractions

Salmon smolt (30-40 g) acclimated to salt water will be exposed (initially at 10°C) to the water-soluble fraction of crude oil using a specially designed flow-through system. Tissues will be analyzed on a weekly basis by photofluorometry. Other samples will be examined via gas liquid chromatography and/or mass spectrometry as seems appropriate. In addition, morphological examination of liver, and possibly the gastrointestinal wall and other organs, will be carried out in order to delineate cellular and subcellular alterations. One to two month exposure and depuration periods are anticipated, depending on the nature of the early results.

Considerable time and effort was expended in the design and perfection of a flow-through system because of the importance of this mode of exposure and the necessity for carrying out such experiments under reproducible conditions. Although no data are available to date, the project is on schedule and it is expected that samples for analysis will be available shortly (starting in the next quarter).

B. Feeding studies using Prudhoe Bay crude oil in food

Coho salmon smolts (100-150 g), acclimatized in holding pens in Puget Sound (ca. 8°C), will be fed a diet comprising Oregon moist pellet (OMP) prepared in our laboratories; two batches of food will be prepared. One batch will contain 5 ppm added Prudhoe Bay crude oil; the other batch will contain 10 ppm. In separate feeding regimes of 5 and 10 ppm of treated food, weekly fluorescence analyses of tissues (together with selected GLC and/or MS examinations) for aromatics and certain metabolites, will be conducted. In each study, feeding will be for a 4-week period. Thereafter, fish will be maintained on nontreated food and the depuration of hydrocarbons will be investigated weekly over an additional 4-week period. These feeding studies started in February, 1976.

C. Feeding studies using selected aromatic compounds present in crude oils

Feedings of carbon-14 labeled compounds (e.g., naphthalene and anthracene), followed by an assay of carbon-14 labeled hydrocarbon structure and total labeled aromatic metabolites (including, in the case of naphthalene, certain determinations of total aromatic metabolites), is presently being carried out. Assays for radioactive aromatic structures are being conducted 0.25, 0.50, 1.0, 2.2, 4.0, 8.0, 16.0, 24.0, and 48.0 hrs after feeding of 5.8 μC (207 ng) per animal.

D. Exposure of invertebrate larval forms to petroleum hydrocarbons

Animals will be subjected to the soluble fraction of crude oil and to aromatic hydrocarbons, at a constant concentration using

continuous flow-through conditions. Various concentrations will be used. Larval shrimp will be tested at each stage separately and also continuously from stage I to stage VI to determine effects on molting, behavior, physiology, histology, and biochemistry (uptake, metabolism, depuration) from chronic exposure. The aromatic compounds will be presented in native form and/or complexed to proteins. Since aromatic compounds with molecular weights greater than naphthalene are only slightly soluble in water the complexing of these compounds with proteins renders them water-soluble. Protein and other macro structures are likely complexing agents in the natural marine environment. Animals subjected to the various petroleum compounds will be sampled periodically to determine: (1) uptake rates; (2) biochemical transformation of the natural hydrocarbons with delineation of the metabolites formed; and (3) induced non-communicable disease through examination of the tissue for altered morphology. These methods are consistent with the objectives of a research effort to provide data on the biotransformation of hydrocarbons in selected arctic and subarctic species.

E. Studies with water-borne trace metals

Exposure experiments on coho salmon are carried out in cooperation with R.U. 73. The fish will be exposed in a semi-closed recirculating saltwater system at Mukilteo (where salt water is available). These systems are temperature-controlled. The exposures will consist of one tank of control animals and a test tank containing from 5 to 500 ppb Pb as radioactive Pb $^{210}(NO_3)_2$. Each experiment will be done at 4° and 10°C to assess the effects of temperature. The first exposure will be with 5 ppb of Pb and will begin during the next

quarter. Test animals will be exposed for up to 30 days. At ten-day intervals fish will be taken for analysis. These fish will be dissected and analyzed for total Pb and amount of Pb in key organs (e.g., liver, kidney, brain, muscle tissue, spleen). Liver and kidney cytosol fractions will be passed through a gel chromatography column to identify any buildup of protein structures which preferentially bind heavy metals. Autoradiography will be carried out on tissue sections to identify cellular sites of Pb deposition. At the end of the 30-day metal exposure, fish will be placed in "metal-free" seawater for 14 days to follow the elimination of metals. During the exposure, fish will be observed with regard to gross changes in appetite, behavior, mortality, and other factors.

Since Dr. Varanasi (see R.U. 73) will remove mucus from these same fish, our results will be correlated with her data.

VI. RESULTS

A. Flow-through studies (exposures via the water column)

The development of a continuous flow-through system for exposure of marine organisms to water-soluble fractions of crude oil is virtually completed. This system is the result of considerable effort in development and will be described in detail. An all-glass system comprised of three separate modules will be employed for preparing water-soluble fractions of Prudhoe Bay crude oil. This module, in conjunction with a heating-cooling unit, forms the basis for controlled temperature exposures of fish to soluble petroleum hydrocarbons. An all-glass construction insures that no leaching of contaminants from construction materials will occur. This will be especially important

when working at low exposure levels of hydrocarbons. The total system operates in the following manner: crude oil, at the rate of 0.18 ml/min, is metered by a positive displacement pump into a mixing module with incoming sea water flowing at the rate of 1400 ml/min. The mixing module consists of three separate portions. In the first portion, the oil and incoming water are churned to a froth by a variable, high-speed stirrer of stainless steel construction. The froth, consisting of dissolved compounds together with large and small oil droplets and films of oil, then flows through a V-shaped channel (middle portion of the mixer) where much of the oil film and undissolved oil droplets rise to the surface and are swept away to an overflow chamber. A portion of the churned water, comprising soluble compounds together with many small oil droplets, is diverted from the bottom of the middle chamber (700 ml/min outflow) to a second module, the baffle module. Here, remaining droplets of undissolved crude oil float to the surface and are swept away via an overflow standpipe. Additional untreated sea water (2400 ml/min) is also added to the baffle chamber. Water from the bottom of the end portion of the baffle module is metered (1200 ml/min) to a headbox from which flowing streams of treated water are routed to individual aquaria (20 gal all-glass units) equipped with overflow standpipes.

Under the operating conditions presently employed, the level of total solubles in the headbox water, as determined by infrared analysis, is 3 ppm. Initial exposure studies will be performed at the level of 0.5 ppm; this is accomplished by adding water as a dilutant to the aquaria along with treated water in the correct

proportion. All water containing solubles or floating films of oil are purged of solubles and films by a process of settling, followed by filtration of the flowing streams through special glass-wool and charcoal filters contained in enameled 55 gallon drums.

Exposure studies will be initiated in the next quarter. Conditions are now standardized and animals are being acclimated prior to exposure to water-soluble fractions of Prudhoe Bay crude oil.

B. Feeding of hydrocarbons: Prudhoe Bay crude oil in feed

Two net pens containing 150 coho salmon each (average weight ca. 200 g) are established at Manchester and the fish are currently being acclimated. One group will be fed 5 ppm Prudhoe Bay crude oil in the diet and the other 10 ppm. The analyses (fluorometry; GLC-MS) described previously will be carried out on samples which will be collected weekly, starting in the next quarter.

C. Feeding studies using selected aromatic compounds

Studies were initiated on the incorporation of carbon-14 labeled naphthalene and anthracene into key tissues of coho salmon (ca. 140 g); the isotopically-labeled hydrocarbons are administered in glyceride oils and the accumulation of hydrocarbons and metabolites in intestine, liver, brain, kidney, gall bladder, and blood serum are determined. Results to date are too preliminary for cogent interpretation and discussion; however, the data being acquired will provide valuable insight into the degree and nature of hydrocarbon accumulation in salmonids exposed to low levels of petroleum hydrocarbons.

Studies of naphthalene and naphthalene bound to a protein (bovine serum albumin, BSA) in stage V spot shrimp has shown that 100% mortality is produced by both these compounds in a 36-hour exposure to concen-

trations of 10 ppb. The unfed shrimp take the material from the water very rapidly reaching maximum values in approximately 8 hours. The naphthalene is accumulated to much higher concentrations than the BSA-complexed naphthalene; however, the amount of metabolized aromatic is approximately the same. After being subjected to the naphthalene for 24 hours, the animals, when placed in clean sea water, retained one-third of the radioactive material after five days.

D. Exposure to metals

In the metal studies to date, the fish exposure facilities have been designed and the equipment ordered and installed. Each fish-holding unit is self-contained and has its own refrigeration system. In addition to designing, assembling and installing the exposure facilities, autoradiographic techniques and chemical analytical methods have been established. Methods for counting tissue samples via liquid scintillation are proven and gel chromatography procedures for separating protein fractions have been standardized. Animals will be exposed to Pb in initial experiments and samples will be taken at 10-day intervals. This aspect of the study will start next quarter and chemical analyses will be carried out on samples as obtained.

VII/VIII DISCUSSION AND CONCLUSIONS

In virtually all phases of the work suitable experimental protocols are established and it is now possible to initiate challenge experiments and conduct necessary examination of sacrificed animals. Organisms exposed to hydrocarbons under varied conditions will be evaluated with regard to hydrocarbon accumulations in key tissues and for the capability to discharge hydrocarbons and related metabolic products. Studies of this type are greatly facilitated by the availability of a new continuous flow-through system for exposing organisms to water-soluble fractions of crude oil.

Initial studies with stage V larvae indicate that the petroleum component naphthalene is acutely toxic at the level of a few ppb. Further data on the biological effects of aromatic hydrocarbons on shrimp larval forms will accrue from data acquired in the next quarter.

Studies on the biological effects of metals will yield data relevant to the objectives of this work unit next quarter.

The studies with radioactive hydrocarbons and certain other aspects of the work are supported, in part, by NMFS base funds to the Northwest Fisheries Center.

All phases of the project are essentially on schedule.

IX. NEEDS FOR FURTHER STUDY

Because the studies are largely in early phases, it is premature to delineate needs for further work on this occasion. Nevertheless, some implications for future work are arising from present studies. These implications will be evaluated in the next quarter.

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ANNUAL REPORT

TITLE: Research Unit 75. Assessment of available literature on effects of oil pollution on biota in arctic and subarctic waters

PRINCIPAL INVESTIGATORS:

Dr. Donald C. Malins and Mr. Maurice E. Stansby, National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard East, Seattle, Washington

April 1976

ANNUAL REPORT FOR RESEARCH UNIT 75

April 1, 1976

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

The objective of this project is to summarize knowledge already available regarding effects of petroleum and especially petroleum industrial activities in the outer continental shelf areas upon marine biota with special reference to the Arctic and subarctic environment. This information is being developed both in the form of extensive bibliographic reference lists, tabulated under 20 subject headings, and by preparation of a series of critical reviews prepared by scientific specialists from the information gleaned from available reports compiled in the bibliography. In general, considerable is know already concerning effects on a world-wide basis of petroleum upon the marine biota. Much less research, however, has been carried out in an Arctic environment.

II. INTRODUCTION.

A. General nature and scope of study.

This project consists of carrying out a literature search and then using the results to compile (1) an extensive bibliography and (2) a series of critical review reports. Literature is being reviewed backwards from the present time to as far back as anything can be found. While a few references are found dating back as much as 50 years, a preponderance of the material has been published during the last 10 years, much of it during the past three or four years.

B. Specific objectives.

Specifically what is most needed is information concerning effects of petroleum in an Arctic or perhaps to some extent subarctic environment upon marine biota. Since almost all of the research conducted in the past along such lines has been not on effects in the Arctic but on effects in more temperate zones, it has been necessary to broaden the scope to include sufficient results in nonarctic environmental areas to be able to extrapolate and hypothesize what might happen in Arctic areas for such aspects for which no published information dealing with Arctic conditions is available.

C. Relevance to problems of petroleum development.

A knowledge of what is already known is necessary for most phases of all the OCSEAP program in order to avoid needless duplication in research being carried out. The bibliography and critical reviews being prepared are general enough that they will be of interest to most investigations in the OCSEAP work.

III. CURRENT STATE OF KNOWLEDGE.

The entire object of RU-75 is to find out what the current state of knowledge is and then to prepare material revealing the state of knowledge to those in the OCSEAP activities needing such information.

IV. STUDY AREA

Petroleum effects are being examined on a wide area of marine biota. Major consideration is given to effects on such organisms as fish, shellfish, marine mammals, and plankton because scientists in National Marine Fisheries Service have greatest expertise in these areas. In the area of effects on birds a scientist outside NOAA is preparing a special report.

As indicated above primary importance is placed upon knowledge of effects in an Arctic and subarctic environment. Owing, however, to paucity of such information considerable attention is having to be paid to work conducted in more temperate areas.

V. SOURCES, METHODS, AND RATIONALE OF DATA COLLECTION.

Whenever current extensive bibliographies are already available, we are making use of them. For example, we are using as a prime source of literature references the extensive (over 2,000 references) bibliography on effects of petroleum published by Marine Biological Association of the United Kingdom which is current to June 1975. Use of this and other similar existing bibliographies are supplemented by direct literature searches of abstract journals. We use computerized searches for much of such activity and are using the NOAA OASIS computerized search facility, based upon key word supplied, to gain maximum pertinent retrieval. For very current information not yet reported in abstract journals, sometimes not yet even published, direct inquiry to research agencies is uncovering pre-publications and progress reports such as the Interim Reports of the Beaufort Sea Project of Environment Canada.

VI. RESULTS.

We are submitting with this report our Subject Classified Literature Reference report current to date. This listing of over 150 pages, although still in rough draft form, gives a good idea of the kind of bibliography which will be processed in complete and revised form before the end of the contract period.

VII. DISCUSSION.

As has been indicated, the output of this Research Unit will consist not only of the list of references appended to this report but also a

series of critical reviews. In these reviews will be a discussion of the knowledge reported in the bibliography references. The critical reviews are still at any early stage of preparation. Attached to this report are a set of titles and in most cases outlines for these critical reviews together with a listing of the scientists who are preparing them.

VIII. CONCLUSIONS.

The carrying out of Research Unit 75 is well on schedule. The first end result of the project, a detailed subject classified bibliography, is 85% complete. Work is well underway on preparation of critical reviews describing the status of knowledge on important aspects. Both the bibliography and critical reviews will be submitted with the final report at the end of the contract period. It is also planned to use the critical reports as the basis of a scientific report to be published, possibly in book form.

IX. NEEDS FOR FURTHER STUDY.

It is desirable to continue tabulation of references and preparation of annual summaries of research results currently entering the scientific literature for the duration of the OCSEAP program. Such work can be accomplished by present staff working on RU-75 if the contract is extended beyond October 1, 1976. Availability of updated digests of material on research papers pertinent to OCSEAP programs would be of considerable assistance to many portions of the OCSEAP operations.

PROVISIONAL TITLE: EFFECTS OF PETROLEUM OIL ON ARCTIC AND SUBARCTIC MARINE ENVIRONMENTS AND ORGANISMS

EDITOR: Donald C. Malins
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Northwest Fisheries Center
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MARCH 1976

Chapter I. Petroleum: Properties and Analyses in Biotic and Abiotic Systems

by

Robert Clark
Environmental Conservation Division

and

Donald Brown
NOAA National Analytical Facility

I. PHYSICAL PROPERTIES OF PETROLEUM
- A. General description
- B. Measurable characteristics
  1. Specific gravity
  2. Viscosity
  3. Pour point
  4. Distillation temperatures

II. CHEMICAL PROPERTIES OF PETROLEUM
- A. Hydrocarbons
  1. Paraffins
  2. Naphthenes and cycloparaffins
  3. Olefins
  4. Aromatics
- B. Non-hydrocarbon components
  1. Nitrogen compounds
  2. Sulfur compounds
  3. Oxygen compounds
  4. Metals
- C. Chemical properties compared with refining properties
- D. Composition of unrefined petroleum
  1. Prudhoe Bay crude oil
  2. South Louisiana crude oil
  3. Kuwait crude oil
- E. Composition of refined petroleum products
  1. No. 2 fuel oil
  2. Bunker C

III. ANALYSES FOR PETROLEUM HYDROCARBONS IN MARINE SYSTEMS
- A. Sampling and preservation
- B. Extraction
- C. Separation
- D. Identification and quantification

IV. COMPARISON OF HYDROCARBONS IN PETROLEUM WITH THOSE IN MARINE ORGANISMS
- A. Volatile paraffins
  1. Normal paraffins
     a. Petroleum
     b. Organisms

2. Branched paraffins
    a. Petroleum
    b. Organisms
B. Non-volatile paraffins
    1. Petroleum
    2. Organisms
C. Naphthenes and cycloparaffins
D. Olefins
    1. Petroleum
    2. Organisms
        a. Volatile
        b. Non-volatile
F. Non-hydrocarbon components
G. Basic differences between biotic and abiotic systems

Chapter II. Inputs, Transport Mechanisms and Observed Concentrations of Petroleum in the Marine Environment

by

Robert Clark
Environmental Conservation Division

and

William MacLeod
NOAA National Analytical Facility

I. MAGNITUDES AND SOURCES OF POTENTIAL PETROLEUM INPUTS
    A. Land-based discharges
        1. Refineries and petrochemical
        2. Waste oils, runoff and sewage
    B. Marine operation losses
        1. Tankers
        2. Bilge discharges
    C. Accidental losses
    D. Offshore production
    E. Oil seeps

II. TRANSPORT MECHANISMS
    A. Evaporation
    B. Dissolution
    C. Emulsification
    D. Sinking
    E. Bacterial degradation
    F. Photochemical modification
    G. Ingestion and compaction
    H. Tar ball formation
    I. Ice dispersal and albedo change

III. OBSERVED CONCENTRATIONS
- A. Marine organisms
  - 1. Unexposed baseline samples
  - 2. Exposed samples
  - 3. Laboratory exposed samples
- B. Sediment
- C. Water
- D. Tar balls

Chapter III. Physico-Chemical Associations of Petroleum Oil with the Physical Forms of Water and Other Substrates

by

William MacLeod
NOAA National Analytical Facility

Chapter IV. Alterations in Petroleum Oil Resulting From Physico-Chemical and Microbiological Factors

by

Neva Karrick
Environmental Conservation Division

I. PARTITIONING OF PETROLEUM IN THE MARINE ENVIRONMENT
   A. Description of partitioning processes
   B. Factors that affect types and rates of partition
      1. Properties of the petroleum
      2. Environmental conditions
   C. Physical and chemical changes in petroleum after partitioning
      1. Altered characteristics of petroleum
      2. Deposition of petroleum

II. DEGRADATION OF PETROLEUM
   A. Chemical: Photo-oxidation and other processes
   B. Microbiological
      1. Bacteria
         a. Bacteria that consume different classes of petroleum compounds
         b. Geographic distribution of hydrocarbon-utilizing bacteria in Arctic and Subarctic areas
         c. Effects of environmental conditions on bacterial degradative processes
         d. Role in removal of petroleum from the marine environment
      2. Other organisms
   C. Products formed from transformations and degradation of petroleum

III. SUMMARY
   A. Potential environmental impact from degraded petroleum in Arctic and Subarctic waters

Chapter V. Biological Effects of Petroleum Oil: Alterations in Life Processes and in Community Structures

Acute Toxic Effects

by

Donovan Craddock
Environmental Conservation Division

I. INTRODUCTION
   A. Acute toxicity studies: importance, scope and limitations
   B. Acute bioassay techniques

II. DATA FROM ACUTE BIOASSAY STUDIES
   A. Tabulation: species, contaminants, test parameters, remarks and references
   B. Interpretation of results in relation to phylogenetic differences and other factors

III. SUMMARY AND CONCLUSIONS

IV. REFERENCES

Marine Birds

by

W.N. Holmes
Department of Biological Science
University of California
Santa Barbara, California

Marine Mammals

by

Mark Keyes
Marine Mammal Division
Northwest Fisheries Center

Pathology

by

Harold Hodgins, Bruce McCain and Joyce Hawkes
Environmental Conservation Division

Behavioral and Physiological Effects Following Sublethal Exposure

by

Douglas Weber and Fred Johnson
Environmental Conservation Division

I. INTRODUCTORY REMARKS
   - A. Phylogenetic considerations
   - B. Physiological changes
   - C. Behavioral changes
   - D. Growth, development and reproduction
   - E. Trace metals

II. PROTISTA

III. PLANTS

IV. ANIMALS
   - A. Mesozoa
   - B. Porifera
   - C. Others through phylum Chordata as appropriate

Within each of the above first and second order headings, the following categories will be considered though not necessarily delineated as third and fourth order headings.

1. Physiological changes
   - a. Metabolism (whole organ, tissue homogenate, respiration)
   - b. Osmoregulation
   - c. Feeding and nutrition (changes in relative concentrations of proteins, amino acids, carbohydrates, lipids and intermediates)
   - d. Photosynthesis
   - e. Alteration in blood proteins
2. Behavioral changes
   - a. Loss of equilibrium
   - b. Change in respiration and feeding rates
   - c. Modification in locomotor and activity patterns
   - d. Chemoreception (modification in avoidance, feeding and reproductive behavior)
3. Growth, development and reproduction
   - a. Rates of growth (changes in shell production, molting frequency, cell proliferation)
   - b. Developmental changes (larval stages, allometric relationships, ontogenetic survival)
   - c. Success of fertilization (gamete production and survival, breeding behavior)
   - d. Success of second generation reproduction

Bioaccumulations, Biochemical Transformations and Excretions of Petroleum Oil and its Constituents By Marine Organisms

by

Usha Varanasi and Donald Malins
Environmental Conservation Division

I. INTRODUCTION

II. UPTAKE AND DISTRIBUTION OF PETROLEUM OIL AND CONSTITUENTS
- A. Fractions of crude oil
  - 1. Plankton
  - 2. Invertebrates
  - 3. Fish
- B. Aliphatic hydrocarbons
  - 1. Plankton
  - 2. Invertebrates
  - 3. Fish
- C. Aromatic hydrocarbons
  - 1. Plankton
  - 2. Invertebrates
  - 3. Fish
- D. Trace metals
  - 1. Plankton
  - 2. Invertebrates
  - 3. Fish

III. BIOCHEMICAL TRANSFORMATIONS OF HYDROCARBONS AND TRACE METALS
- A. Metabolic conversions and transport mechanisms
  - 1. Hydrocarbons
    - a. Plankton
    - b. Invertebrates
    - c. Fish
  - 2. Trace metals
    - a. Plankton
    - b. Invertebrates
    - c. Fish
- B. Excretion and depuration
  - 1. Hydrocarbons
    - a. Plankton
    - b. Invertebrates
    - c. Fish
  - 2. Trace metals
    - a. Plankton
    - b. Invertebrates
    - c. Fish

IV. BIOCHEMICAL CONSEQUENCES OF PETROLEUM OIL
- A. Alterations in enzyme activities
  - 1. Hydrocarbons
  - 2. Trace metals
- B. Interaction with macromolecules
  - 1. Hydrocarbons
  - 2. Trace metals
- C. Biomagnifications
  - 1. Hydrocarbons
  - 2. Trace metals

V. SUMMARY

VI. REFERENCES

Habitats, Populations, Communities and Ecosystems

by

Herbert Sanborn
Environmental Conservation Division

I. BACKGROUND

II. HABITAT: PETROLEUM HYDROCARBONS AND HEAVY METALS
- A. Intertidal
  - 1. Rocky
  - 2. Sandy
  - 3. Mud flats
- B. Salt marsh
- C. Subtidal

III. POPULATIONS AND COMMUNITIES: PETROLEUM HYDROCARBONS AND HEAVY METALS
- A. Plankton
- B. Benthos
- C. Nekton
  - 1. Invertebrates
  - 2. Vertebrates
- D. Seabirds and waterfowl
- E. Marine mammals

IV. BIOLOGICAL SUCCESSION: CASE STUDIES FROM PETROLEUM SPILLS
- A. Tampico
- B. Arrow
- C. Torrey Canyon
- D. General M.C. Meigs
- E. West Falmouth

V. ECOSYSTEMS: PETROLEUM HYDROCARBONS AND HEAVY METALS
- A. Estuaries
- B. Coastal areas
- C. Open ocean

Chapter VI. Observed Effects of Oil Spills

by

Robert Clark and John Finley
Environmental Conservation Division

I. GENERAL EFFECTS BASED ON TYPE OF OIL AND TYPE OF ORGANISMS
   A. Petroleum
      1. Crude oil
      2. Refined products
      3. Oil plus dispersants
   B. Organisms
      1. Bacteria and plankton
      2. Finfish
      3. Shellfish
      4. Marine plants
      5. Waterfowl and mammals
   C. Habitat - sediments

II. MAJOR OIL SPILLS
   A. Summary of biological impacts
   B. Long-term studies
      1. Tampico
      2. Torrey Canyon
      3. General M.C. Meigs
      4. West Falmouth

III. ARCTIC AND SUBARCTIC SPILLS
   A. Terrestrial
   B. Marine
      1. Arrow
      2. Deception Bay
      3. Alert Bay
      4. Coastal Maine
      5. Resolute Bay

Chapter VII. Taint as a Flavor in Fish from Petroleum Pickup

by

Maurice E. Stansby
Northwest Fisheries Center

I. INTRODUCTION
   A. Scope of problem
   B. Difficulties in studying problem

II. GENERAL NATURE OF FLAVORS AND ODORS IN FISH
   A. Subjective nature
   B. Natural flavors in fish

C. Unnatural flavors in fresh fish
   1. Flavors from feed of fish
   2. Flavors from contact with foregin substances
D. Spoilage or decomposition flavors in fish

III. RESEARCH ON FLAVORS PICKED UP FROM PETROLEUM
A. Source of petroleum
   1. Contact of fish with petroleum
   2. Petroleum in feed of fish
B. Identification of nature of petroleum component in fish
C. Confusion between petroleum-like flavors and flavors derived from petroleum

IV. DISCUSSION AND CONCLUSIONS

SUBJECT CLASSIFIED LITERATURE REFERENCES
ACCUMULATED BY JANUARY 14, 1976 FOR USE

by

OSCEAP RESEARCH UNIT 75

"ASSESSMENT OF AVAILABLE LITERATURE ON EFFECTS OF
OIL POLLUTION ON BIOTA IN ARCTIC AND SUBARCTIC WATERS"

References selected and subject classified by Maurice Stansby, NWFC, Seattle
Typed and assembled by Isabell Diamant, NWFC, Seattle

Partial List of Sources of References

1. OASIS Computerized Search.
2. Marine Biological Association of the United Kingdom. Bibliography on Marine and Estuarine Oil Pollution, June 1975.
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Codes for the Above References

1. "N" preceding number.
2. Numbers only.
3. "A" preceding number.
4 &
5. "O" preceding number indicates miscellaneous items.
* Indicates complete abstract.

PETROLEUM LITERATURE SUBJECT CATEGORY LIST OF NOV. 5, 1975 - M. E. STANSBY (amended as of Nov. 13, 1975).

1. Ice and snow (oil under or on ice and snow, its physical movements, etc., and effects on biota).

2. General effects of cold (background information on effects of lowered temperature on significant parameters).

3. Mortality effects (largely studies where the significant measurement was whether the organism was living or dead; this category also includes general effects and toxicity not specified in the title which may go beyond mortality).

4. Effects on reproduction and growth.

5. Effects on behavior (behavior includes wide range of phenomenon mostly those determinable by mere observation).

6. Effects on physiological processes (includes also unspecified sublethal effects).

7. Effects on biochemical processes (enzymes, metabolism, et al., also photosynthesis).

8. Effects on micro-organisms and their activity (overlap in part - No. 11, Weathering).

9. Pathological effects (disease, carcinogens).

10. Composition of oil (chemical composition of oil and emulsions; also spreading action).

11. Weathering of oil (includes fate of oil studies).

12. Metabolites in oil.

13. Levels of oil components and degradation products (in biota and water; also buildup and release).

14. Sediments, mud, silt (including oil and heavy metals therein, adsorption of oil on silt, and geological aspects).

15. Taint as a flavor in fish from oil pickup.

16. General effects of trace metals (includes levels of trace metals, etc., like 13 for oil).

17. Description of spills and subsequent biota recovery.

18. Effects of oil drilling operations (also from refineries and from natural oil seeps).

19. Analytical methods (methods highly specific to pertinent problems; includes bioassays and model systems for testing toxicity).

20. Miscellaneous (includes very general articles; e.g., ecological effects).

[illegible] applicable mainly [illegible] category. The first [illegible] categories [illegible] apply to both oil and trace metals.

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ANNUAL REPORT

Contract # 03-5-022-56
Research Unit # 123
Reporting Period 7/1/75 - 3/31/76
Number of Pages 13

ACUTE EFFECTS - PACIFIC HERRING ROE IN THE GULF OF ALASKA

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Institute of Marine Science
University of Alaska

April 1, 1976

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

Orderly planning of resource utilization and management requires knowledge of the basic biology and ecological relationships of the organisms comprising the resource, knowledge of current methods relating to both utilization and management, knowledge of the impact (both economic and emotional) of the resource on the community and knowledge of the impact or potential impact of the community on the resource. None of these aspects can be ignored if meaningful plans and meaningful choosing of alternatives is to take place.

One important resource in Prince William Sound is herring, *Clupea pallasii*. Herring caught in Alaska has been used primarily for reduction (processing into meal or oil) with some use for bait and food. Recently a herring roe fishery has developed in which the roe, attached to algae or marine grass, is harvested and sold in the Japanese gourmet trade.

A useful evaluation of herring roe in Prince William Sound should eventually include all the areas mentioned above. Relevant to Outer Continental Shelf energy development, we must ask how will this fishery (and organism) be affected by exploration and development? How susceptible are adults, larvae and eggs to toxic effects from oil spills and seepage? What critical times of the year exist with respect to herring and oil? Some aspects of these questions will be answered by this project as well as those of Rice & Karinen (NMFS, Auke Bay) and Malins (NMFS/NWFC). Specifically our objective is to delineate the toxicity of soluble components of

crude oil under simulated natural conditions. Toxicity will be measured in terms of hatching success and gross morphological abnormalities.

II. INTRODUCTION

A. General Nature and Scope of Study

This is an experimental study of limited scope. We plan to examine the effect of exposure to oil-equilibrated seawater on hatching success and prevalence of morphological abnormalities in herring larvae.

B. Specific Objectives

1. We will measure hatching success of herring eggs maintained for 24 hours, 48 hours, 1 week and continuously in crude oil-equilibrated seawater. These success rates will be compared to those for control groups (no exposure).

2. Evaluate the frequency of occurrence of morphological abnormalities in experimental vs control larvae.

3. measure uptake of hydrocarbon components in larvae and unhatched eggs.

C. Relevance to Problems of Petroleum Development

As discussed below, herring spawn in a habitat which is particularly susceptible to the influence of crude oil. Many of the roe are deposited in the intertidal, the larger usually being deposited highest on the beach. Since the larger eggs normally produce the larvae with the greatest chance

of reaching adulthood, the presence of oil on the water and on the beach will select against the highest quality of eggs in particular and will cause an increased mortality in general. Spills or seepage during the three to four week reproductive period could have significant impact on egg and larval mortality. These mortality rates are already high in nature. Therefore, development activities could have a major impact on the herring fishery in Alaska.

III. CURRENT STATE OF KNOWLEDGE

Pacific herring spawn during a relatively short period in spring in water shallower than twenty meters. Eggs are demersal and stick to the substratum. Usually the eggs become attached to marine grass, kelp or other algae although spawning over gravel apparently also takes place. Significant amounts of the spawn are deposited above mean low water and much of the total spawn is deposited intertidally. Thus, the developing embryos may be subject to fluctuations in exposure, desiccation, salinity, temperature and predation in the natural environment. The spawn is thus precariously positioned in areas of actual or potential oil spillage. Coating of the embryos with oil would be disasterous to development. Hatching time varies with temperature, being on the order of 15 days from spawning to hatching. Spawning adults and immobile roe will be particularly susceptible to surface pollution for a period of about one month. Larvae, once hatched, are still very delicate and very subject to environmental influences. While prehatching mortality varies perhaps from 60-90%,

larval mortality is thought to be as much as 99%. Herring are undoubtedly a significant link in the food webs of Prince William Sound. Adults are preyed upon by larger fish such as salmon. Pelagic larvae are preyed upon by the other fish species as well as by some invertebrates such as chaetognaths. Roe may be subject to predation by fish, intertidal invertebrates (such as crabs), shore birds and even some mammals. Depletion or destruction of roe might have significant implications on migrating waterfowl. Clearly, more information about predation on herring roe would be very useful in predicting the possible impact of ecological upsets such as oil spills.

Although much has been published on many aspects of the life history of Pacific herring, *Clupea pallasii* (see Taylor, 1964; Stevenson and Outram, 1953; McMynn and Hoar, 1953; Taylor, 1971; Jones, 1972; Tester, 1955; Svetividov, 1949), very little information exists on Alaskan stocks, especially those of Prince William Sound. Most papers relevant to Alaskan stocks are rather old (Kahlgren and Kolloen, 1943; Kolloen and Smith, 1953; Rounsefell, 1930a; Rounsefell, 1930b; Rounsefell, 1931; Rounsefell and Dahlgren, 1932; Rounsefell and Dahlgren, 1935) although the two recent papers, one on catch statistics (Skud *et al.*, 1960), the other on age and growth of adults (Reid, 1971) have appeared.

A number of pertinent references exist which deal with pollution effects on herring. These studies deal with the effects of vinylchloride production (Braaten *et al.*, 1974), sulfuric water pollutants (Kinne and Rosenthal, 1967), benzene (Struhsaker *et al.*, 1974), crude oil-emulsifier mixtures (Rosenthal and Gunkel, 1967), water soluble components from crude

oil (Kühnhold, 1969), and oil disperants (Wilson, 1972). An additional body of knowledge exists on pollution effects on other fish taxa. In addition to the above topics, thermal effects are being widely studied.

IV. STUDY AREA

The study area for this project is the Gulf of Alaska.

VI. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

Since no data have been collected this section will deal with the experimental design as we envision it.

A. Sources of Experimental Material (Herring Eggs)

We have a committment from Stan Rice, NMFS, Juneau, to supply us with herring eggs if they are accessable in the Juneau - Auke Bay area. These eggs will be air shipped to Seward via Anchorage. Since this source is somewhat uncertain we are making arrangements with two men in Seward for getting eggs from Resurrection Bay or other locations in Prince William Sound. One of these, Keith Knighten, runs Harbor Air Service. He may be able to get us to spawning beaches via float plane. The other man, Martin Gorsin, participates in the herring roe commercial fishery and will be picking up eggs this spring. We feel confident that one of these sources will provide us with the eggs we need.

B. Experimental Design

These experiments will be conducted at the IMS marine station in Seward. There will be 15 aquaria, three each for controls (no oil), 24 hour exposure, 48 hour, 1 week and continuous exposure. Experimental eggs will be exposed to seawater equilibrated with Prudhoe Bay crude oil. We feel it is much simpler to use equilibrated seawater than actually introducing oil into the aquaria. Although we do not know what concentrations of hydrocarbons will exist in the equilibrated seawater we will analyze it at various points during the course of the experiments. Preparation of the mixture will be carried out by vigorous mixing of oil and seawater in 50 liter glass carboys. Equilibrated seawater will be drawn off the bottom of the carboy. Since a delivery system capable of matching flow rates to twelve different aquaria would be extremely complicated, we have elected to use a static system. Aquaria will be initially filled with seawater or equilibrated seawater and will be changed at least every 48 hours. All aquaria will be placed in a large wooden tank which will serve as a constant temperature bath. We anticipate temperatures between 6°C and 10°C. Blaxter, in his work on rearing herring eggs, found that a static tank which was changed every other day resulted in hatching success of 70-85%.

C. Analytical Procedures

Procedures to be used for analyzing hydrocarbon uptake into eggs and larvae were outlined in our original work statement. We do not envision any changes in these procedures.

VI. RESULTS

There have been no results since no experiments have been conducted. We have, however, performed a bibliographic search. A list of pertinent references dealing with herring life history and the effects of oil pollution on fishes is included.

VII. DISCUSSION

A discussion of results is not possible at this time since the field and laboratory work has not begun. These experiments will begin in late April.

VIII. CONCLUSIONS

See discussion.

IX. NEEDS FOR FURTHER STUDY

It is difficult to assess what additional studies are required. This project is only one of a number of OCS studies relating oil toxicity to marine fishes. We are not aware of the results of any other "effects studies". In looking over the original work statements, however, it appears that impacts on the alimentary canal have not been approached. If time permits, we hope to approach this subject area during the laboratory work in Seward.

X. SUMMARY OF 3rd QUARTER OPERATIONS

A. Laboratory Activities

1. Field schedule

   a. A trip to Seward, where the actual experiments will be performed, was made on March 5, 1976.

   b. Our best estimate of the actual startup date of experiments in Seward is April 20, 1976.

2. Scientific party

Ronald L. Smith	IMS	Principal Investigator
John Pearson	IMS	Associate Investigator
Jane Anne Cameron	IMS	Research Assistant

3. Methods

   To date the only methods utilized are TLC techniques for separation of hydrocarbons and associated densitometric techniques.

4. Sample localities

   Not applicable as no samples have been obtained.

5. Data collected or analyzed

   None.

B. Problems Encountered

Our associate investigator, John Pearson, was involved in an automobile accident which incapacitated him for over a month. Thus, we are not as far along in setting up and calibrating some of our analytic procedures as we had intended.

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OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 18 R.U. NUMBER: 123

PRINCIPAL INVESTIGATOR: Dr. R. L. Smith

No environmental data are to be taken by this task order as indicated in the Data Management Plan. A schedule of submission is therefore not applicable[1].

NOTE: [1] Data Management Plan has been approved and made contractual.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 18

PRINCIPAL INVESTIGATOR: Dr. Ronald L. Smith

Period July 1, 1975 -- March 31, 1976* (9 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	31,863.00	4,581.14	27,281.86
Staff Benefits	5,417.00	778.79	4,638.21
Equipment	2,000.00	1,284.36	715.64
Travel	1,400.00	-0-	1,400.00
Other	3,100.00	639.15	2,460.85
Total Direct	43,780.00	7,283.44	36,496.56
Indirect	18,225.00	2,620.41	15,604.59
Task Order Total	62,005.00	9,903.85	52,101.15

* Preliminary cost data, not yet fully processed.

Following is part 2 of the quarterly report R.U.# 123 for the period ending December 31, 1975. This was received after the printing of the Quarterly Reports, July - September 1975, therefore is included here.

OCS COORDINATION OFFICE

University of Alaska

Quarterly Report for Quarter Ending December 31, 1975

Project Title: Effects of Crude Oil on Herring Roe

Contract Number: 03-5-022-56

Task Order Number: 18

Principal Investigator: Dr. Ronald L. Smith

I. Task Objectives

Objectives for this quarter included completion of literature search, pursuing calibration of analytical techniques and planning the laboratory and experimental design.

II. Field and Laboratory Activities

No field activities were conducted. Laboratory activities have centered around working out the chromatographic techniques for the hydrocarbon analyses which will follow the pollution experiments.

III. Results

Thin layer chromatographic techniques seem to be well in hand at this time. We are still setting up the gas chromatographic techniques.

Experimental design is still in a state of flux. We have found that during the commercial season on herring roe, the packing companies working in Prince William Sound fly the roe from the outer Sound, where they are gathered, to Cordova for packing. Flights come into Cordova daily. This shipping system might prove an alternative to supply from Auke Bay, should that source be unavailable for any reason. We are exploring the possibilities of obtaining eggs in Cordova and flying them to Seward for our studies.

In talking with the Facilities Manager at our Seward Station, it is evident that the running water system will not be operating at that time. Therefore, instead of a flowthrough experimental design, we are now considering a static experiment. Thermal equilibration would be feasible, if our tanks were immersed in a larger tank, which would be drained and refilled fairly often (and by hand) to maintain some sort of stability.

The only other option would be to use a walk-in cold room but the one at Seward is at present inoperable, requiring $500 - $1,000 for repairs. This cold room has electrical outlets for aerators.

IV. Problems Encountered

None to date.

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 18 R.U. NUMBER: 194

PRINCIPAL INVESTIGATOR: Dr. R. L. Smith

No environmental data are to be taken by this task order as indicated in the Data Management Plan. A schedule of submission is therefore not applicable(1).

NOTE: (1) Data management plan was submitted to NOAA in draft form on October 9, 1975 and University of Alaska approval given on November 20, 1975. We await formal approval from NOAA.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 18

PRINCIPAL INVESTIGATOR: Dr. Ronald L. Smith

Period July 1 -- December 31, 1975 * (6 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	31,863.00	552.84	31,310.16
Staff Benefits	5,417.00	93.98	5,323.02
Equipment	2,000.00	1,204.00	796.00
Travel	1,400.00	-0-	1,400.00
Other	3,100.00	41.59	3,058.41
Total Direct	43,780.00	1,892.41	41,887.59
Indirect	18,225.00	316.22	17,908.78
Task Order Total	62,005.00	2,208.63	59,796.37

* Preliminary cost data, not yet fully processed.

ANNUAL REPORT

Contract # NOAA 03-5-022-68
Task Order # 3
Research Unit # 183
Reporting Period: 7/1/75-5/15/76
Number of Pages: 31

Acute and Chronic Toxicity of Seawater Extracts Of Alaskan Crude Oil to Zoeae of the Dungeness Crab, Cancer magister Dana

Richard S. Caldwell

Oregon State University
Marine Science Center
Newport, Oregon 97365

May 27, 1976

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

The objective of the study is to attempt to determine the incipient toxic concentration of a seawater soluble fraction of Alaskan crude oil to larval crabs under conditions of long-term exposure in laboratory culture. The study is emphasizing the commercially important Dungeness crab, Cancer magister. The incipient toxicities of benzene and naphthalene, two major aromatic compounds in crude oil, are also being determined under comparable experimental conditions.

The full strength seawater soluble fraction of Cook Inlet crude oil is acutely toxic to first instar C. magister larvae but we have found no lethal or sublethal effects of an approximately 1/10 dilution of this fraction during a 28-day continuous exposure period. In similar long-term exposures, 0.16 ppm naphthalene, the highest concentration tested, is also without effect on the larvae but 7.2 ppm benzene and possibly also 1.4 ppm benzene result in reduced larval survival. The effects of benzene appear to be manifested at the time of the first zoeal molt in these long-term exposures. A comparison of the lethal concentration of benzene with the estimated concentration of this aromatic compound in the full strength seawater soluble fraction of crude oil suggests that benzene may account for a major portion of the toxicity of this fraction.

Since the concentrations of many of the seawater extractable components of crude oil may be expected to decline rapidly under natural environmental conditions as a result of dilution, evaporation, and metabo-

lism by micoorganisms, these studies suggest that crude oil contaminations of seawater may not seriously affect decapod larvae as long as the larvae do not contact the oil/water interface. The study does not provide any information, however, on the effects of an oil water dispersion on crab larvae, a condition that could be encountered under certain circumstances in the natural environment. We suggest, therefore, that additional studies both to determine the vertical distribution of decapod larval stages in the water column and the toxicity of oil-in-water dispersions would be helpful to further evaluate the effects of oil spillage on crab larvae.

INTRODUCTION

General Nature and Scope of Study

The overall objective of the study is to attempt to determine the concentrations of seawater extractable components of Alaskan crude oil which have an incipient detrimental effect on larval crabs. A particular emphasis is being placed on the commercially important Dungeness Crab, Cancer magister. The primary criterion used for evaluation is the success of development of larvae throughout the zoeal stages during continuous exposure to seawater extracts of crude oil and to dissolved naphthalene and benzene, two constituents of oil.

Specific Objectives

The specific objectives as originally stated are:

1. To determine the concentrations of the total seawater soluble extract of Alaskan crude oil which causes mortalities in the first zoeal stage of the crab in

96 hr. acute exposures.

2. To determine the concentration of the total seawater soluble extract of Alaskan crude oil which affects hatching success of eggs and prezoeal development.
3. To determine in continuous exposure tests, the concentration of the total seawater soluble extract of Alaskan crude oil and possibly 1 or 2 specific components (e.g. naphthalene, methylnaphthalene, benzene) which impairs the normal development of crab larvae from the 1st zoeal stage to at least the 5th (last) zoeal stage and, if possible, through the megalopae to 1st postlarval crab. Some chronic experiments may employ toxicant exposures during only limited segments of the developmental sequence in order to identify the most sensitive stages.
4. To attempt to characterize in such variously exposed larval crabs such sublethal effects as impaired tolerance of unnatural salinities or temperatures or impaired behavior (e.g. swimming abnormalities, abnormalities of photo- or geotrophic responses, etc.).

Relevance to Problems of Petroleum Development

The Dungeness crab fishery represents an important resource to the State of Alaska, accounting in recent years for from 10 to 40% of the total harvest of this species from the Pacific coast of North America (Pacific Marine Fisheries Commission, 1974). Taking 1970 and 1971 as representative years, 9.7 and 3.7 million pounds were the respective annual landings of this crab at Alaskan ports (National Marine Fisheries

Service, 1970 and 1971). These catches were valued at 1.4 and 0.6 million dollars, respectively. Since the value of the fishery is roughly the same for each of British Columbia, Washington, Oregon and California, areas where Alaskan crude oil may be shipped to refineries, the importance of studying the potential effects of Alaskan oil on this species is evident.

CURRENT STATE OF KNOWLEDGE

Studies of the biological effect of oil pollution in marine waters has intensified in recent years as a result of the publicity created by large oil spills such as that associated with the Torrey Canyon shipwreck. Recent reviews have summarized much of the pertinent data (Nelson-Smith, 1970; 1973). The seawater soluble components of oils have generally been considered to be the most toxic to marine organisms (Dunning and Major, 1974; Struhsaker et al., 1974). Although the developmental stages of organisms are usually more sensitive to toxicants than adults, relatively little work has yet been done on these forms. Kuhnhold (1970), Struhsaker et al. (1974) and others have studied the effects of oil and water soluble components of oil, on developmental stages of fish, but studies on larval stages of invertebrate species are few. Wells (1972) reported that emulsions of crude oil were lethal to lobster larvae. Larval stages of crabs and shrimp are affected by 1-100 ppb of various oil products (Mironov, 1969). Katz (1973) reported that larvae of the crab Neopanope texana showed reduced survival when exposed to seawater that had been polluted with 10ml/liter of Venezuelan crude oil. Only larvae exposed during the earliest developmental stages were affected,

however. We are unaware of any studies that have attempted to document sublethal effects of oil on crustacean larvae even though such sublethal effects have been reported for other toxicants (DeCoursey and Vernberg, 1972; Vernberg et al., 1973).

STUDY AREA

Gulf of Alaska

METHODS AND MATERIALS

Rearing Method

Long term exposure of crab larvae under laboratory rearing conditions to seawater soluble fractions of Cook Inlet crude oil and to benzene and naphthalene dissolved in seawater are being conducted in flowing water systems. Ovigerous Female crabs, Cancer magister and Hemigrapsus nudus, collected in the vicinity of Newport, are held in the laboratory until hatching begins. First stage zoeae are collected within a few hours of hatching and cultured under conditions similar to those previously described (Buchanan et al., 1975). The procedure involves rearing of larvae, 20 to a beaker, in 250 ml beakers having a small screened opening near the bottom. The beakers are held in glass aquaria measuring 28 cm x 28 cm on a side and 10 cm deep. By means of an automatic siphon, the water level in the aquaria is made to fluctuate causing seawater to enter and exit the beakers through the screened holes.

Larvae are fed three times per week with nauplii of San Francisco brine shrimp, Artemia salina. During the culture period larvae are

also transferred to clean sterilized culture beakers three times per week to minimize the incidence of deaths due to buildup of pathogenic organisms in the culture beakers. In addition, the entire culture system, including the culture aquaria and the diluter system are occasionally cleaned and sterilized. Mortality and molt data are recorded daily.

Preparation and Serial Dilution of Seawater Extracts

Seawater extracts of crude oil and solutions of benzene and naphthalene are prepared daily using a procedure similar to that of Anderson et al., (1974). The materials to be tested are added to the surface of the seawater in 5 gal pyrex bottles, and the contents of the containers are then stirred non-turbulently with magnetic stirrers for 18-20 hours at 13°C. The stirrers are turned off 2-3 hours before transfer, and the contents are allowed to remain undisturbed to insure phase separation. Daily transfer of the seawater/toxicant solutions to the Mariotte bottles is accomplished by pressurizing the mixing bottles with nitrogen which forces the solutions through a glass piping system (figure 1). Following transfer, the mixing bottles are cleaned, sterilized, and the preparation of fresh toxicant solutions is initiated.

The amount of crude oil and pure chemicals used in the preparation of the stock solutions are as follows: Crude oil, 180 ml; Benzene, 3.0 ml; and naphthalene, 360 mg, each to 18 liters of sea water. The resulting concentrations of stock solutions immediately following transfer to the Mariotte bottles and approximately 20 hrs. later are listed in Table 1. The data are for the period from April 16, 1976 to May 13, 1976, coinciding with the long term tests with C. magister. A daily decline in concentration

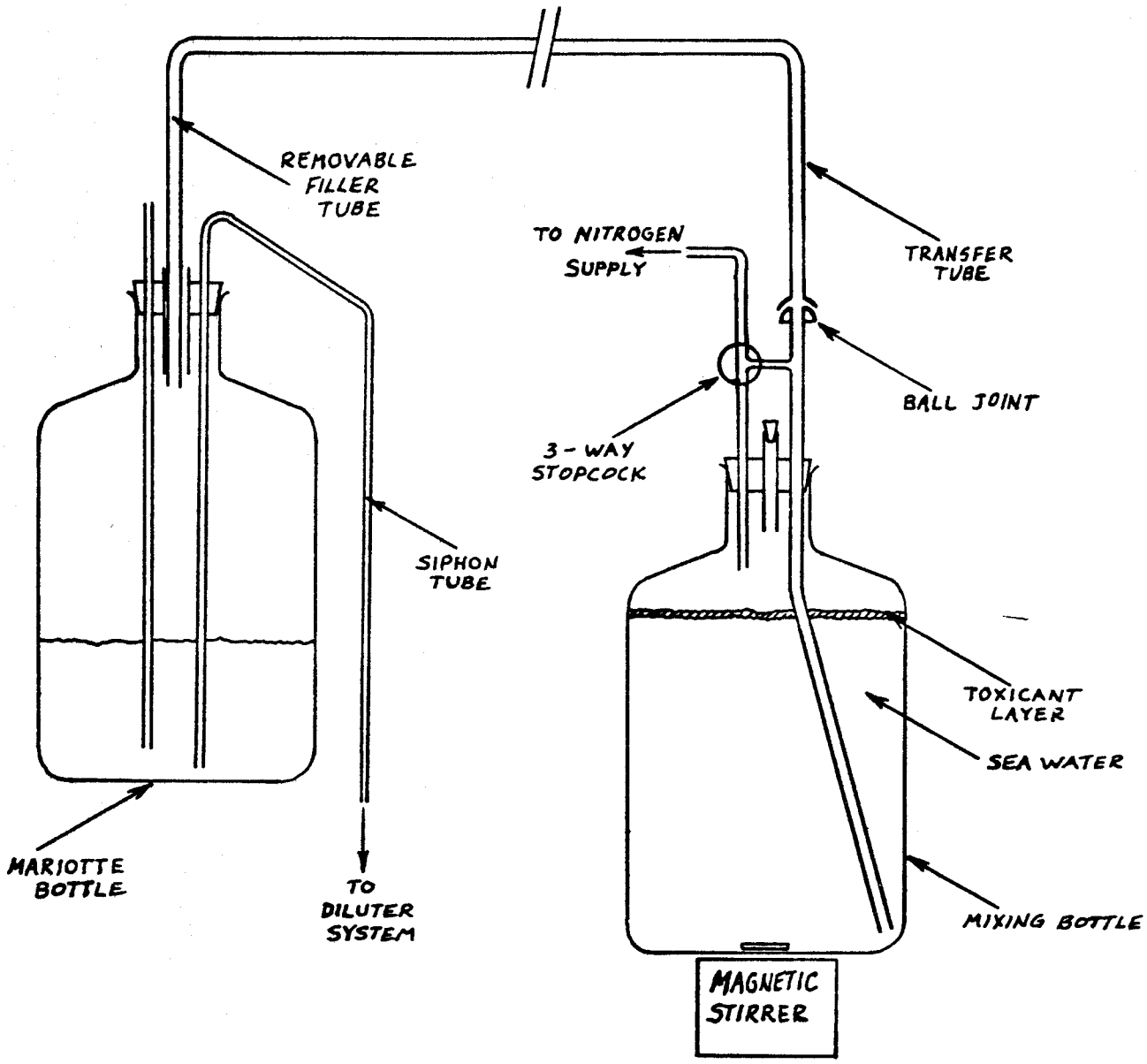

Figure 1. Toxicant mixing and delivery system

Table 1. Concentration of toxicants in stock solutions immediately following transfer and approximately 20 hr. later. Data are for the period from April 16, 1976 to May 13, 1976.

Toxicant	Concentration[1] (ppm)	Number of Analyses
Crude Oil		
immediately after transfer	0.109 ± 0.006[2]	28
20 hr. after transfer	0.100 ± 0.014	27
Benzene		
immediately after transfer	138 ± 4	28
20 hr. after transfer	117 ± 6	26
Naphthalene		
immediately after transfer	2.39 ± 0.08	28
20 hr. after transfer	2.30 ± 0.11	27

[1] For crude oil the concentration is expressed as naphthalene by measurement of the 221 nm peak.

[2] mean ± standard deviation.

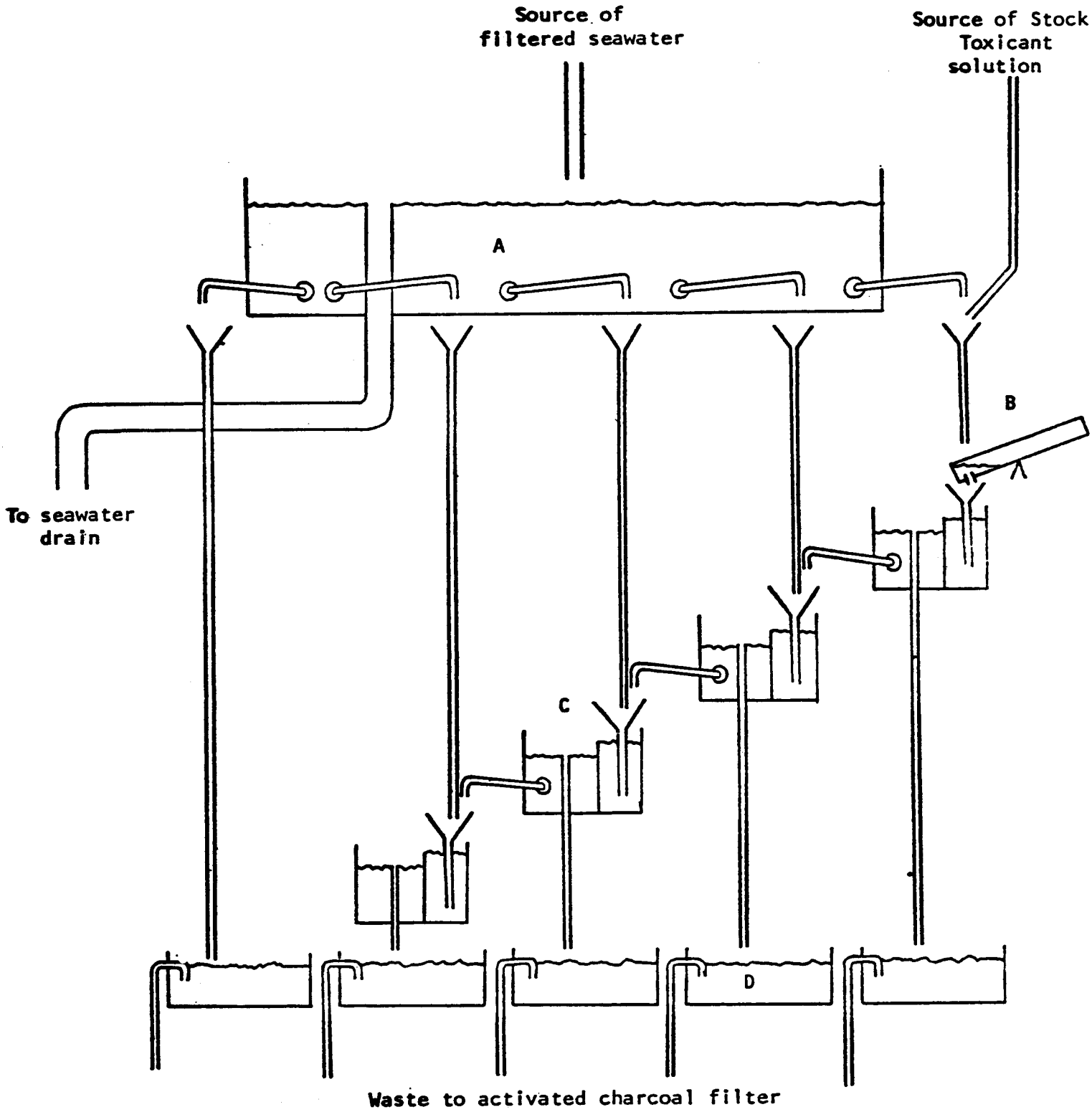

Figure 2. Schematic of serial dilution system. A, constant level seawater head tank; B, safty device for cutoff of toxicant flow in the event of a seawater supply failure; C, mixing-diluting boxes; D, exposure aquaria into which larval culture beakers are immersed.

was noted for all three toxicants averaging 8.3%, 15.2%, and 3.8% for crude oil, benzene and naphthalene, respectively. The large decline for benzene is presumed, in part, to be due to evaporation into the increasing air space in the Mariotte bottle. The smaller declines noted for naphthalene and crude oil probably are due to metabolism by microorganisms. The extent of decline in concentrations during the 20 hour period, especially in the naphthalene bottle, tends to increase over a period of several days and requires sterilization of the Mariotte bottles and glass transfer systems at regular intervals.

Dilution of the stock solutions is accomplished using the serial diluter system shown in figure 2. Careful checks of all flow rates are made daily to ensure uniform dilutions within the system and with time. In the experiments with C. magister, the concentrations of toxicants in the highest level exposure aquaria have been determined by analysis and the concentrations in the lower level aquaria estimated assuming a dilution factor of 0.2. Since this probably results in overestimation of concentrations in the lower aquaria, we will attempt in the future to directly analyze these lower concentrations to the detection limits of our analytical procedures. In the experiment with H. nudus all exposure concentrations were estimated assuming a 0.1 dilution factor from the stock solutions in the Mariotte bottle to the first dilution unit, and dilution factors of 0.2 thereafter.

Analytical Methods

Routine analyses of toxicant concentrations in seawater are performed by UV absorption methods, using a Bausch and Lomb Spectronic 505 Spectrophotometer. Extraction of toxicants from seawater is made

into Burdick and Jackson glass distilled UV-quality n-hexane such that extraction efficiencies are greater than 95% and absorbances at the measuring wavelengths are greater than 0.3. Typical absorption spectra are shown in figures 3, 4, and 5. Benzene is determined at the 255 nm maximum; naphthalene at 221 nm. In the case of crude oil, concentration is determined by measurement of the 221 nm peak and expressed as naphthalene.

RESULTS

Preliminary 96-hr acute toxicity tests indicated that the seawater extract of crude oil exhibited a low toxicity; effects being apparant only in the undiluted extract. Accordingly, we used the highest possible concentrations of the seawater extract in the long-term rearing experiments; beginning with a one tenth dilution. This was determined by the requirement for a minimal flushing rate for the culture beakers and also by the maximum quantity of stock extract available for a 24 hr period.

To date, two experiments with the crude oil extracts have been conducted: one, which is still in progress, with the larvae of Cancer magister; the other with larvae of the shore crabs, Hemigrapsus nudus. The crude oil extract has had no apparent affect on survival of either of these species. In the experiments with H. nudus, the survival of larvae in all cultures was poor after the third or fourth day (figure 6). We believe that the small first instar zoeae of this species were unable to consume newly hatched Artemia larvae and that deaths after four days were due primarily to starvation. Nevertheless, within a period of eight days the highest concentration of crude oil extract, 8.4 ppb as

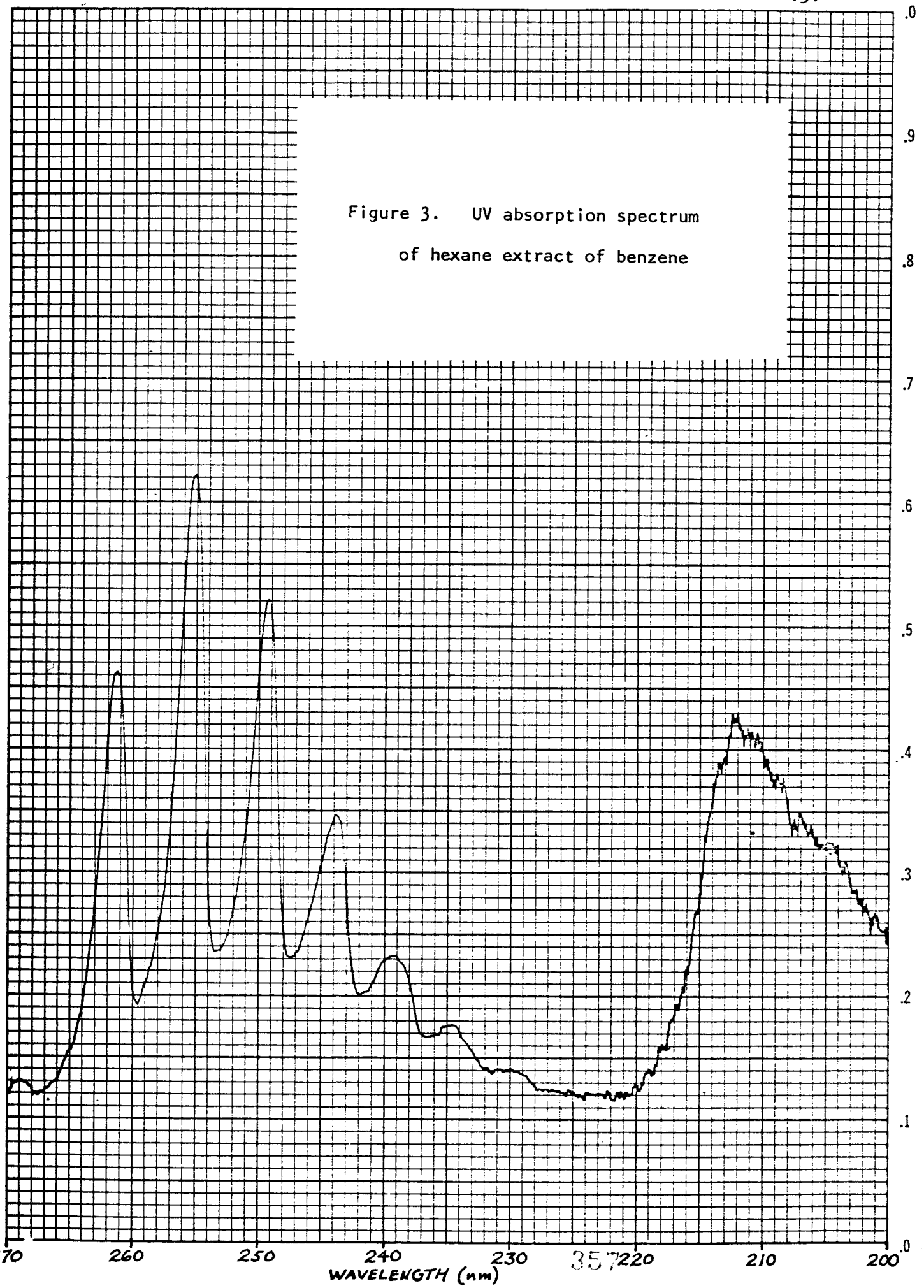

Figure 3. UV absorption spectrum of hexane extract of benzene

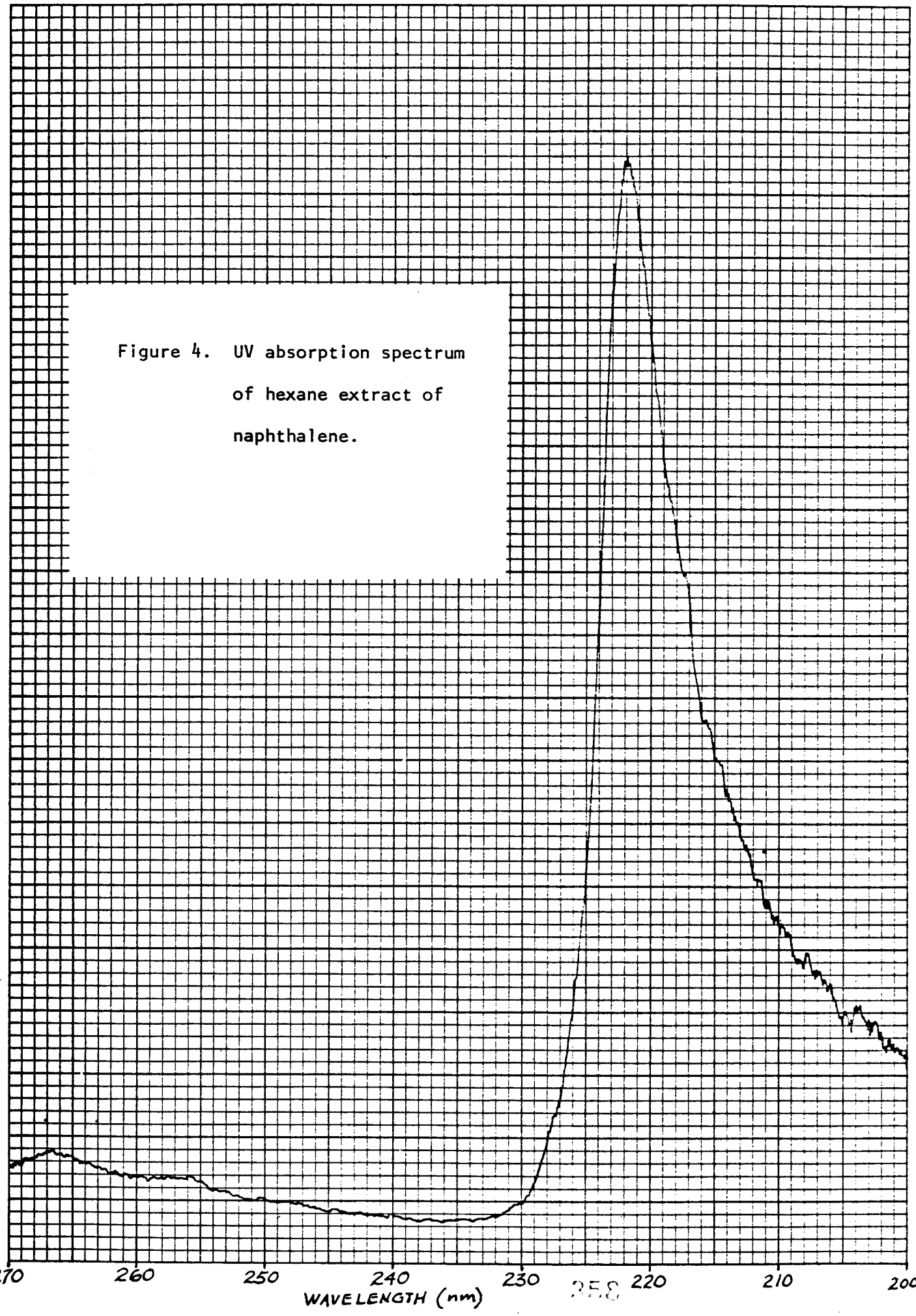

Figure 4. UV absorption spectrum of hexane extract of naphthalene.

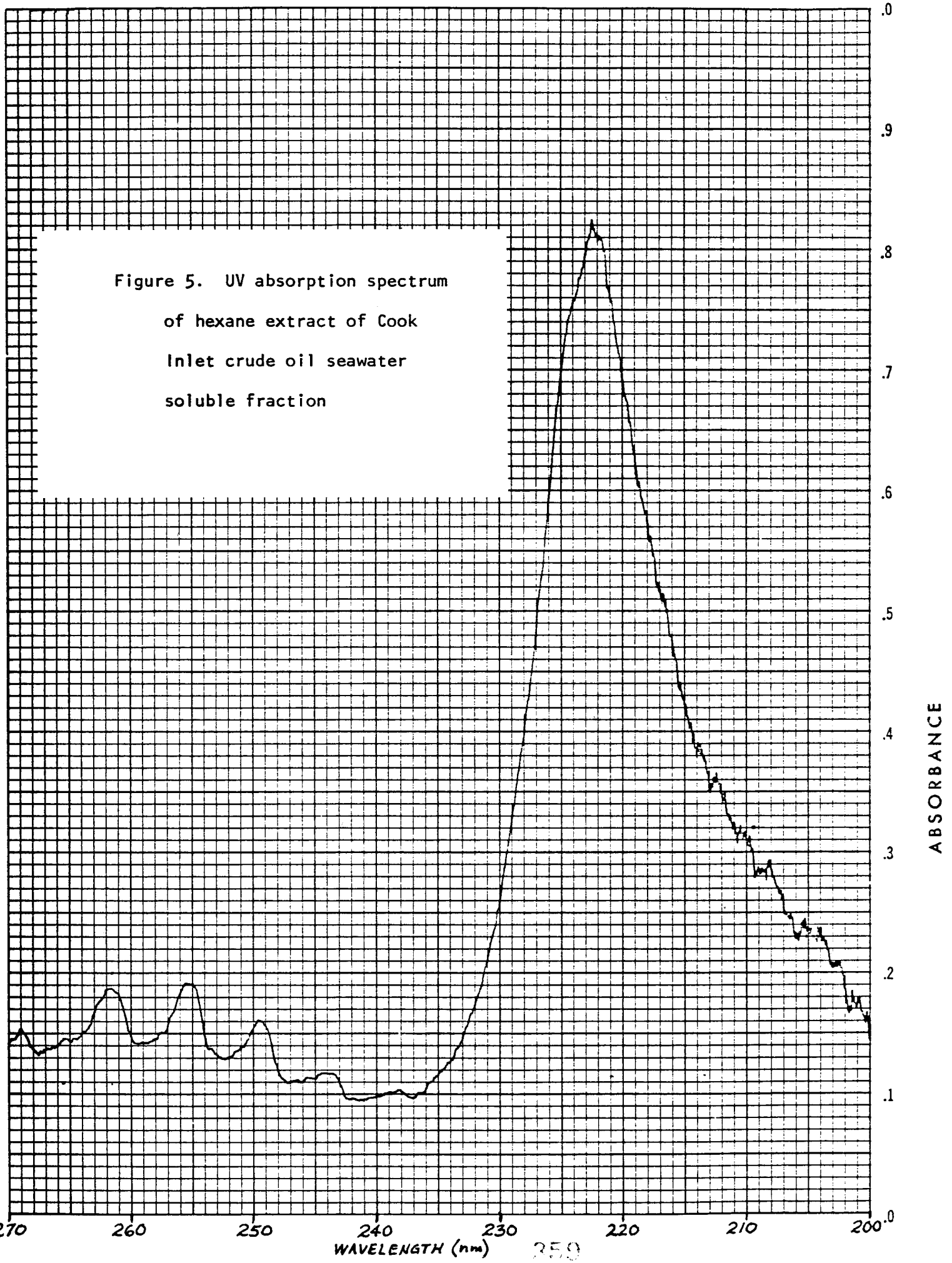

Figure 5. UV absorption spectrum of hexane extract of Cook Inlet crude oil seawater soluble fraction

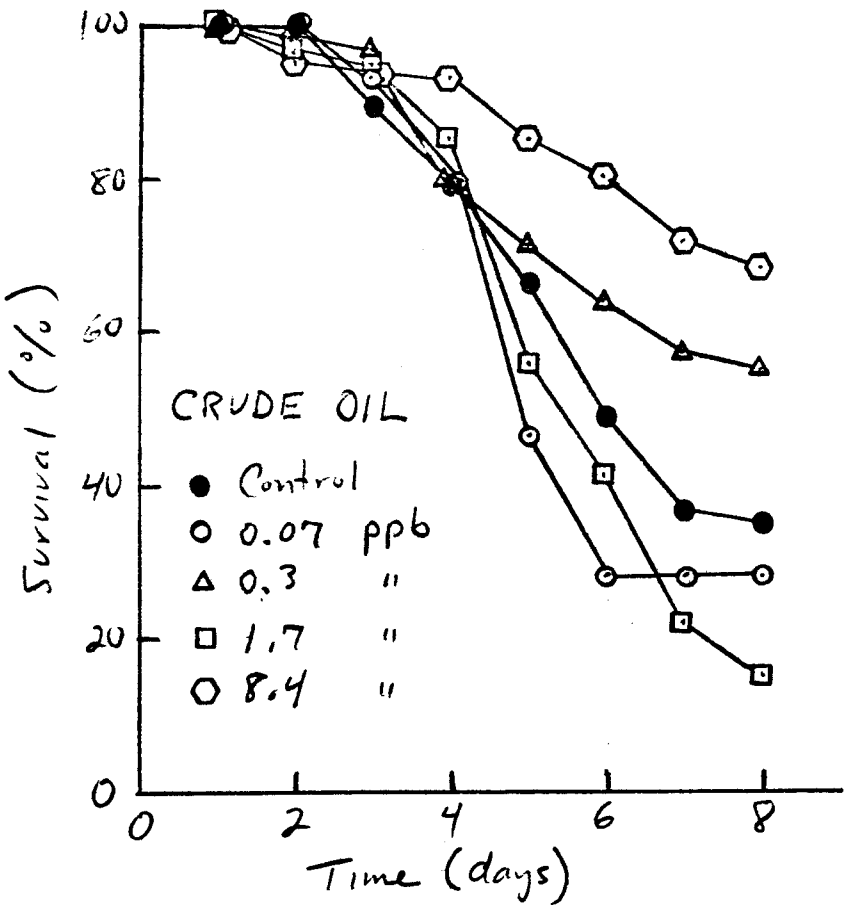

Figure 6. Effect of continuous exposure to the seawater soluble fraction of Cook Inlet crude oil on the survival of Hemigrapsus nudus zoeae. The concentrations of oil are expressed as naphthalene (absorption at 221 nm). and are estimates based on fixed dilutions of stock solutions of known concentration (84.3 ± 28.2 ppb; N = 14).

naphthalene, did not adversely affect survival; survival was 68% compared to only 35% in the controls. We attach no significance to this difference inasmuch as the survival after eight days was highly variable in the several treatments and showed no obvious pattern of treatment effect. No larvae had molted to the second zoeal stage by the time the experiment was terminated on day eight.

Similarly, we have observed no effects of the crude oil extracts on survival of Cancer magister zoeae after 28 days of culture (figure 7). Survival of all larvae in this experiment has been substantially poorer than we have previously reported (Buchanan et al., 1975). The larvae used in this experiment were the progeny of a female crab which, at the time of collection late in the season, was nearly spawned out and the embryos may have been in poorer than usual condition. In addition, we believe that the high rate of mortality during the initial five days of culture in all treatments may be attributed in part to starvation effects since the larvae were not fed until they were five days old (day 2 of toxicant exposure).

Neither have we observed any sublethal effects of the crude oil extract in C. magister at the highest exposure concentration. By day 28 all surviving larvae were in the 3rd zoeal stage and there were no apparent affects of the extract on the timing of either the first or second molt (figure 8). Surviving larvae at all oil extract concentrations appear behaviorally and morphologically similar to the controls.

In the experiment involving exposure of benzene to H. nudus larvae the results appear somewhat ambiguous (figure 9). Survival of larvae was generally poorer in the highest benzene concentration (12.5 ppm) than in the controls, but in 2.5 ppm benzene survival was better than

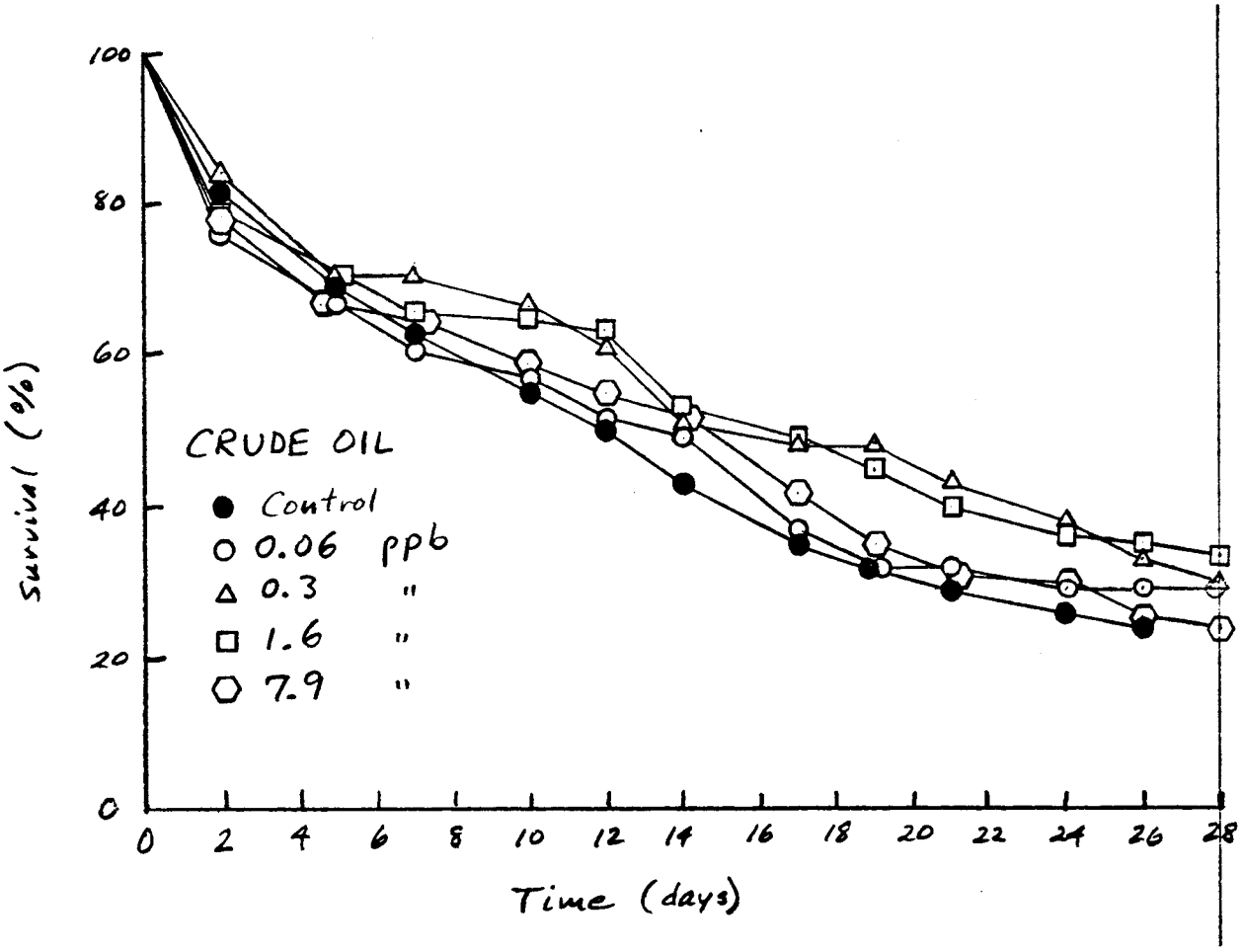

Figure 7. Effect of continuous exposure to the seawater soluble fraction of Cook Inlet crude oil on the survival of Cancer magister zoeae. The concentrations of oil are expressed as naphthalene (absorption at 221 nm) The highest concentration represents a measured value with a standard deviation of ± 1.9 ppb (N = 10). Lower concentrations are estimated values based on a serial dilution scheme with a dilution factor of 0.2 at each step.

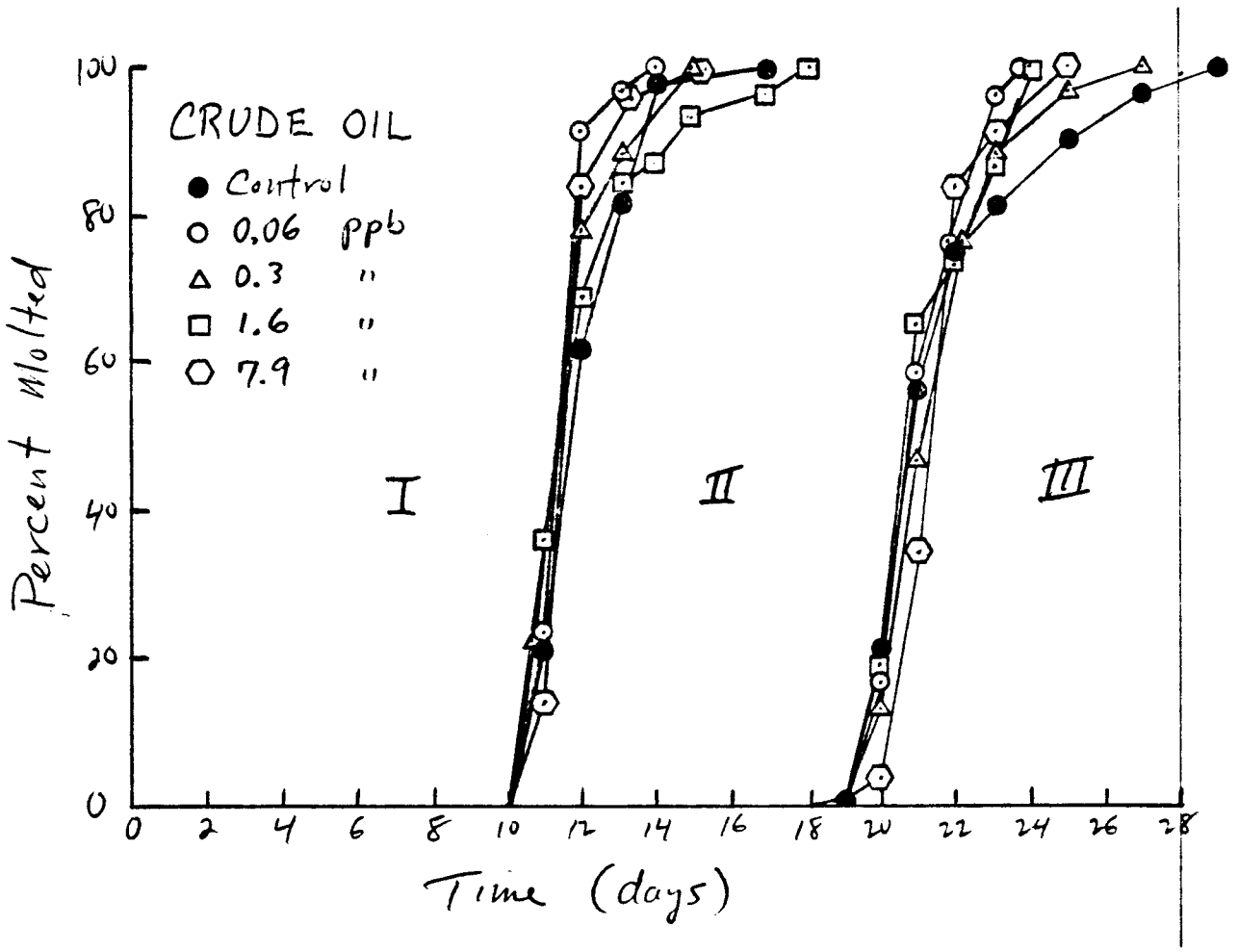

Figure 8. Effect of continuous exposure to the seawater soluble fraction of Cook Inlet crude oil on molting of Cancer magister zoeae beginning with the first zoeal molt. At each molt the percent molted is based on the total number of zoeae completing that molt. Roman numerals indicate the zoeal instar at each stage in the developmental sequence. The concentrations were determined as in Figure 7.

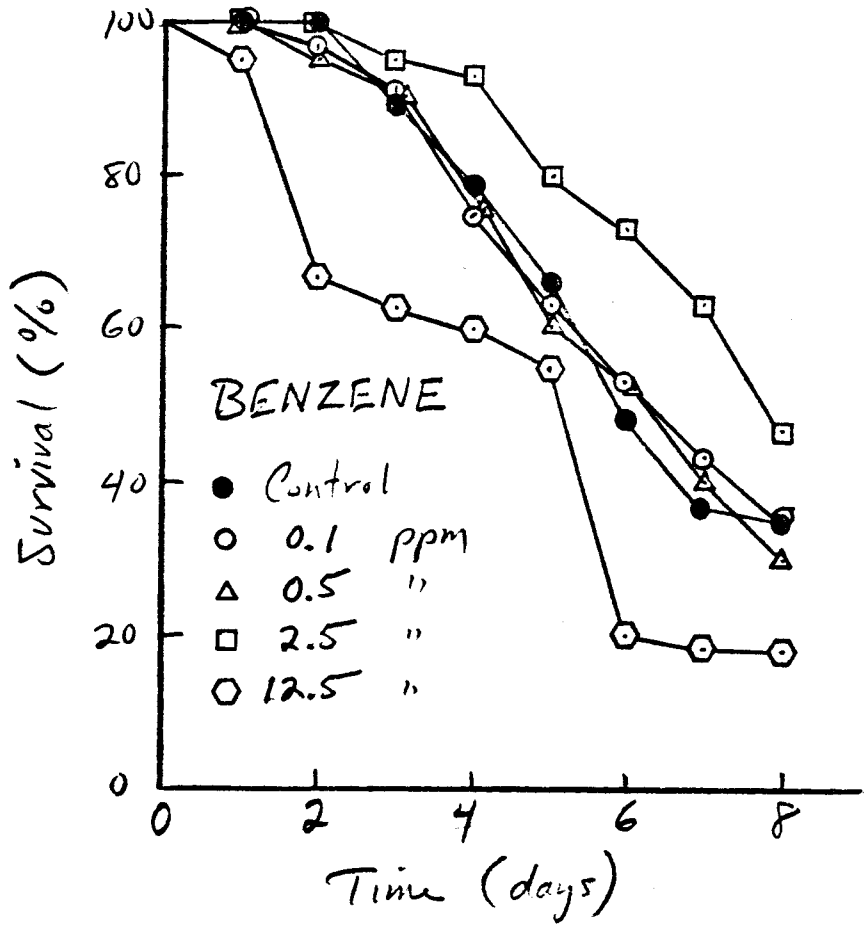

Figure 9. Effect of continuous exposure to benzene dissolved in seawater on the survival of Hemigrapsus nudus zoeae. The concentrations are estimates based on fixed dilutions of stock solutions of benzene of known concentrations (125 ± 9 ppm; N = 20).

in the controls.

High exposure concentrations of benzene do appear to have an adverse effect on C. magister larvae but the effect did not become apparant until after 10 days (figure 10). Nearly half of the original number of larvae exposed to 7.2 ppm benzene died between days 10 and 14, and all were dead by day 19. The highest rate of dying in this group and the group exposed to 1.4 ppm benzene occurred coincident with the time of molting to second stage zoeae in control crabs (figure 11). Only one of the zoeae exposed to 7.2 ppm benzene actually molted to the second zoeal stage. Zoeae exposed to 0.3 and 0.06 ppm benzene appear to be surviving as well as the controls by day 28.

Although death of larvae exposed to high concentrations of benzene appears to be related to molting, no delay in molting was observed for those crabs which survived this period (figure 11). The situation during the second molt, however, appears to be more ambiguous since larvae exposed to 0.06 and 1.4 ppm benzene experienced about a three day delay of molting compared to controls, but larvae exposed to the intermediate concentration of 0.3 ppm were not delayed in molting.

As was true of crude oil extracts, naphthalene did not appear to affect survival of either H. nudus (figure 12) or C. magister (figure 13) at the highest concentrations employed, 0.2 and 0.16 ppm respectively. In addition there were no apparant sublethal effects, either in the timing of molting in C. magister (figure 14) or in gross behavioral or morphological observations with either species.

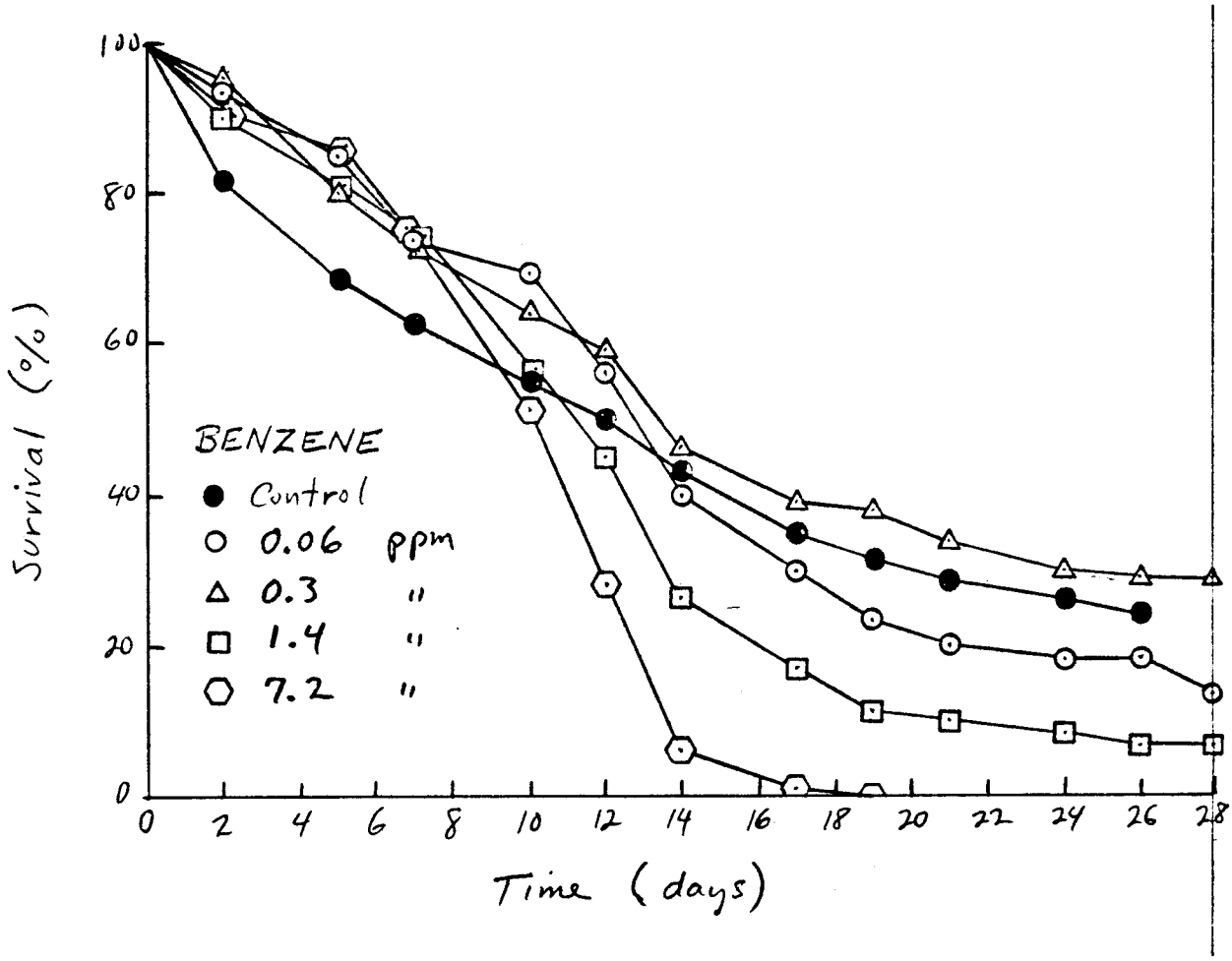

Figure 10. Effect of continuous exposure to benzene dissolved in seawater on the survival of Cancer magister zoeae. The highest concentration represents a measured value with a standard deviation of ± 1.5 ppm (N = 8). Lower concentrations are estimated values based on a serial dilution scheme with a dilution factor of 0.2 at each step.

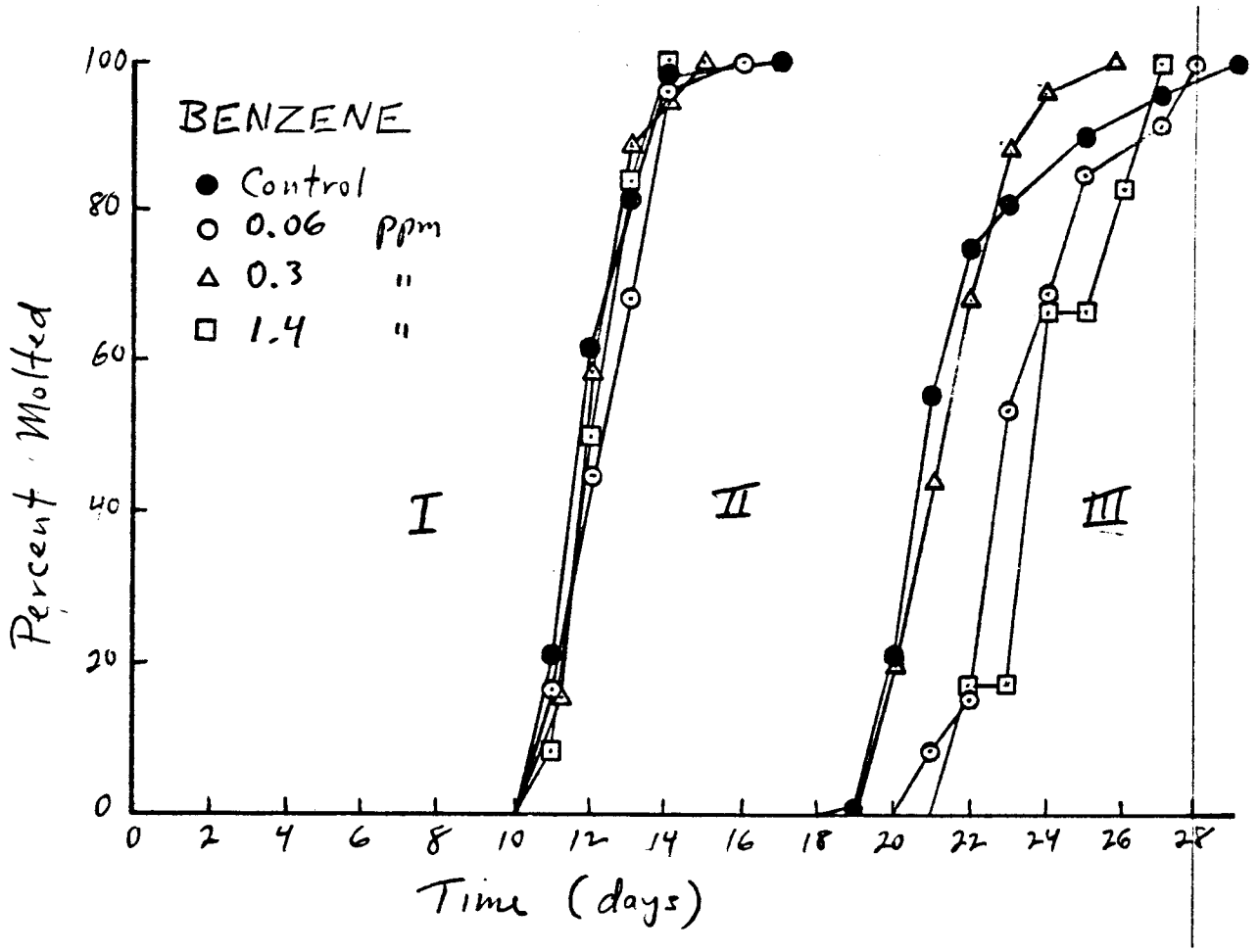

Figure 11. Effect of continuous exposure to benzene dissolved in seawater on molting of Cancer magister zoeae beginning with the first zoeal molt. At each molt the percent molted is based on the total number of zoeae completing that molt. Roman numerals indicate the zoeal instar at each stage in the developmental sequence. The concentrations were determined as in Figure 10.

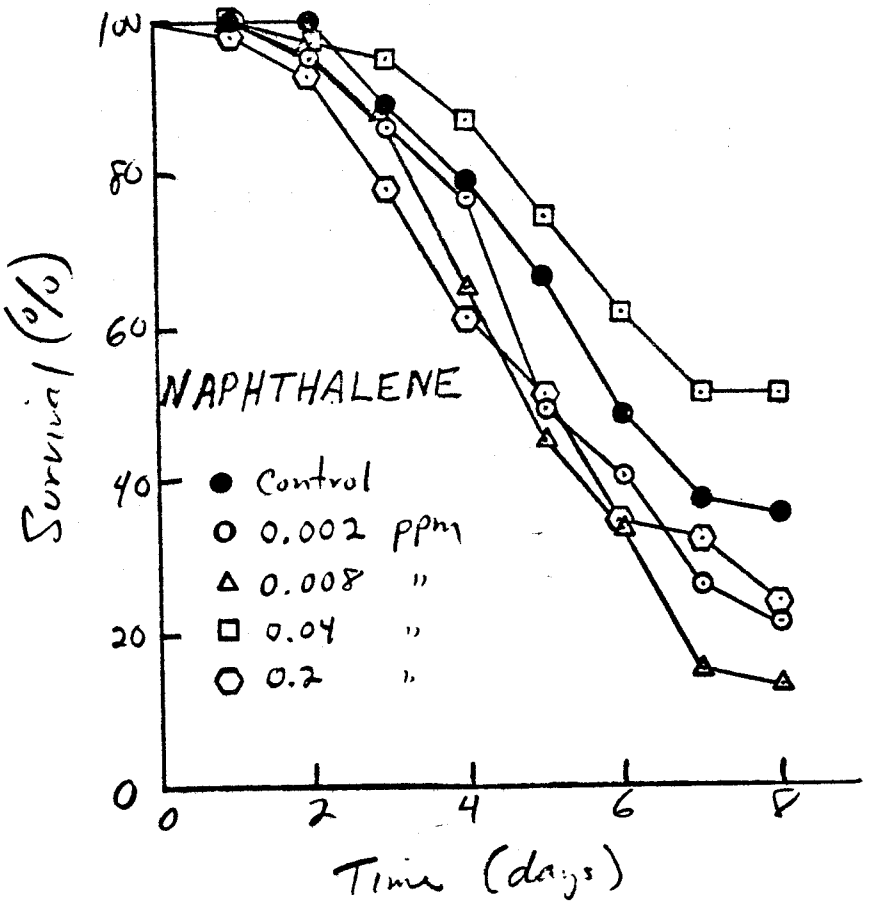

Figure 12. Effect of continuous exposure to naphthalene dissolved in seawater on the survival of Hemigrapsus nudus zoeae. The concentrations are estimates based on fixed dilutions of stock solutions of naphthalene on known concentrations (2.03 ± 0.28 ppm; N = 14).

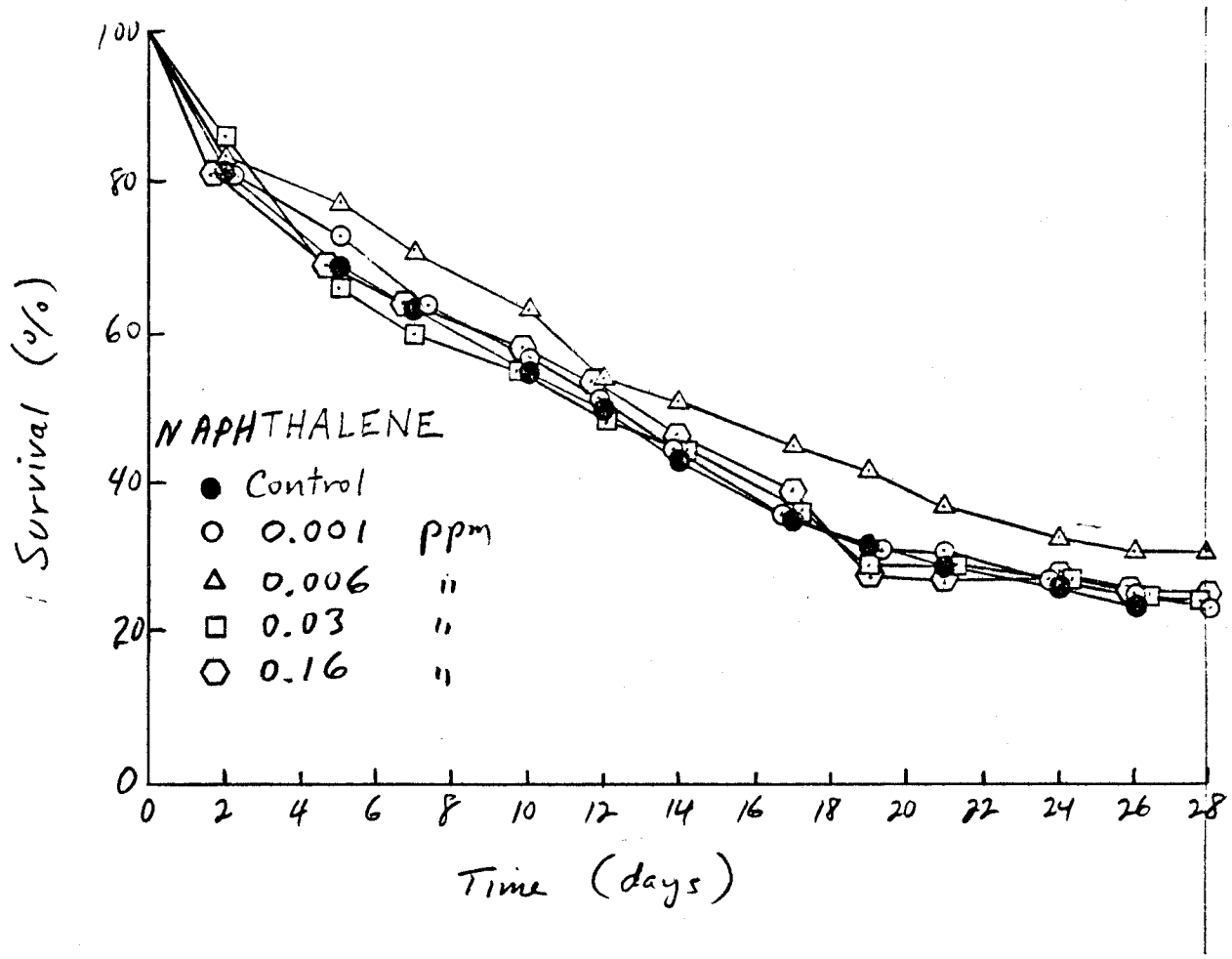

Figure 13. Effect of continuous exposure to naphthalene dissolved in seawater on the survival of Cancer magister zoeae. The highest concentration represents a measured value with a standard deviation of ± 0.06 (N = 11). Lower concentrations are estimated values based on a serial dilution scheme with a dilution factor of 0.2 at each step.

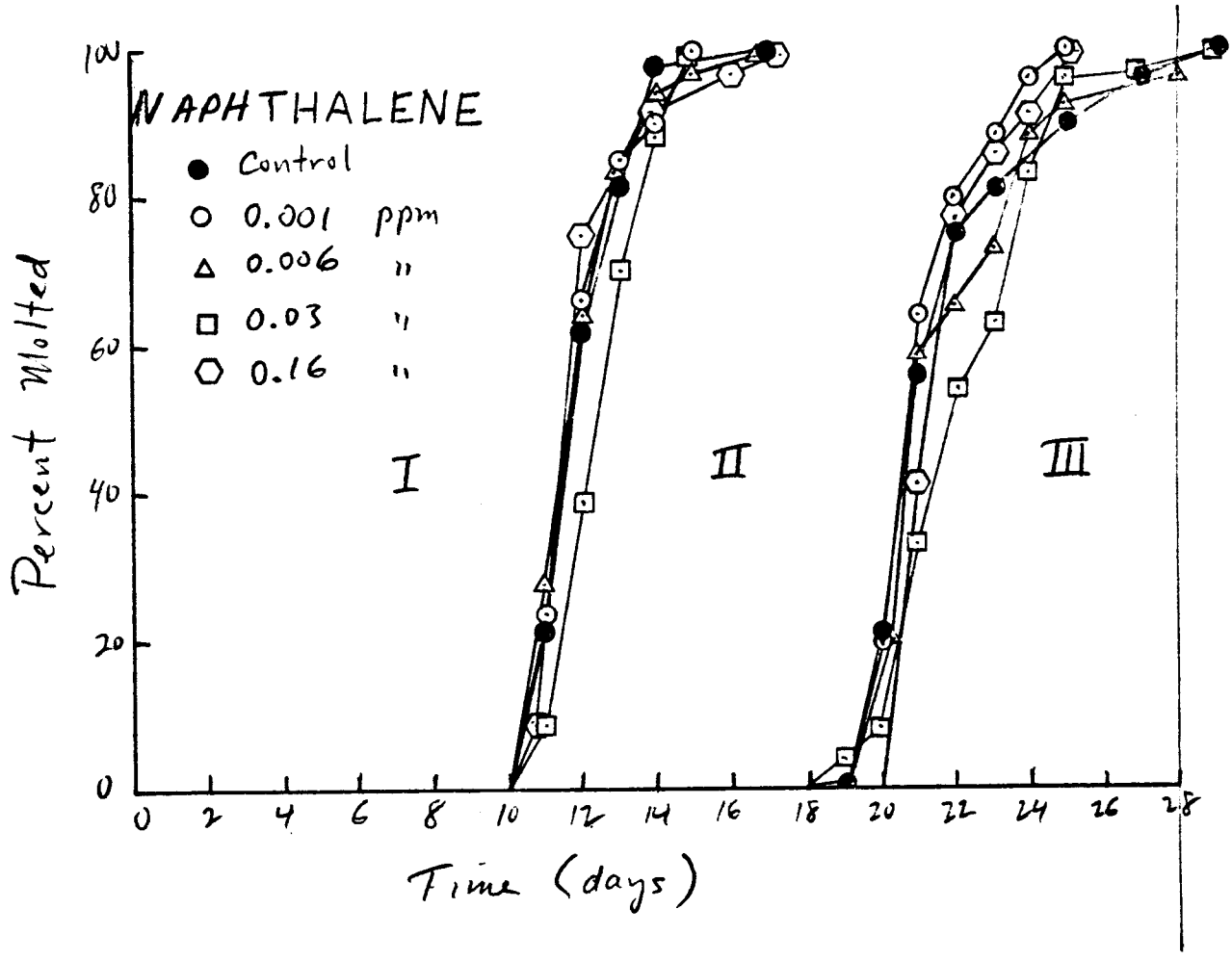

Figure 14. Effect of continuous exposure to naphthalene dissolved in seawater on molting of Cancer magister zoeae beginning with the first zoeal molt. At each molt the percent molted is based on the total number of zoeae completing that molt. Roman numerals indicate the zoeal instar at each stage in the developmental sequence. The concentrations were determined as in Figure 13.

DISCUSSION

Our results to date with larvae of both H. nudus and C. magister suggest that decapod larvae are affected by the seawater soluble fraction of crude oil only when it is undiluted. In our experiments we employed a water soluble fraction obtained from a 1:100 ratio of oil to seawater. In a preliminary acute experiment with C. magister we observed a significant rate of dying after one day of exposure of the larvae to the full strength seawater soluble fraction but zoeae exposed to a 1/5 dilution of this fraction survived for four days as well as the controls. In agreement with this preliminary experiment we have found that larvae of C. magister exposed in the flowing water system to a 1/10 dilution of the seawater soluble fraction did not experience greater mortality than did the controls over a period of 28 days.

These results appear to be in substantial agreement with the study of Katz (1973). Katz, prepared a seawater soluble fraction of Venezuelan crude oil using a 1:100 ratio of oil to seawater, and found that the larvae of Neopanope texana exposed to the full strength fraction from the day of hatching experienced about 60% mortality between days 2 and 5 of culture but, thereafter, experienced only a low rate of dying until the termination of the experiment on day 14. Larvae exposed to the seawater soluble fraction beginning with day 4 until the end of the experiment, however, did not die at a significantly greater rate than the control larvae. Although Katz did not employ dilutions of his seawater soluble fraction, his results with delayed exposures suggest that the full strength fraction was close

to the incipient lethal level. Our results with the seawater soluble fraction of the Alaskan oil suggest the same, since death occurred in the full strength fraction but not in a 1/5 dilution in acute tests or in a 1/10 dilution in chronic tests.

A difficulty encountered in making such interstudies comparisions resides in the uncertainty as to whether or not the water soluble fraction of one crude oil is qualitatively and quantitatively similar to that of another crude oil. Based on total organic carbon analysis, Katz estimated that his water soluble fraction had an approximate concentration of 4.0 ppm of crude oil hydrocarbons. We have not analyzed our seawater soluble fraction in the same way so that it is difficult to make direct quantitative comparisons. We believe, however, that the total level of crude oil hydrocarbons in our water soluble fraction is considerably less than an order of magnitude different than that of Katz since other investigators have found comparable levels of hydrocarbons in seawater soluble fractions prepared from different crude oils. Anderson et al., (1974) estimated that the total dissolved hydrocarbons in the seawater soluble fractions of South Louisiana crude oil and Kuwait crude oil were 24 and 22 ppm, respectively. In their analysis of Kuwait crude oil seawater soluble fraction, Boylan and Tripp (1971) reported a concentration of 1.5 ppm of total oil in water. The higher levels of crude oil hydrocarbons reported by Anderson et al. than those reported by Katz or Boylan and Tripp may be due to the higher ratio of oil to water used in their studies; 1 part oil to 10 parts water, compared with 1 to 100 and 1 to 60, respectively.

In another study of the effect of crude oil on a decapod larva

similar levels of toxicity were reported (Wells, 1972). Wells reported that a 100 ppm oil-in-water emulsion was lethal to lobster (Homarus americana) larvae and that sublethal effects were apparant at concentrations down to 1 ppm. Mironov (1969) reported that larval stages of crabs and shrimp are affected by only 1-100 ppb of various oil products.

Our experience with benzene suggests that this compound may account for a significant proportion of the toxicity of the seawater soluble fraction of crude oil. As previously mentioned, our preliminary acute toxicity experiment showed that the undiluted seawater soluble fraction of crude oil was toxic to C. magister larvae. Measurements of the 255 nm peak in the seawater soluble fraction indicate a benzene concentration of about 2.5 ppm. In our chronic tests with C. magister we have found that 7.2 ppm and possibly 1.4 ppm benzene is toxic to the larvae (figure 10).

It appears that naphthalene plays a lesser role in the overall toxicity of crude oil water soluble fraction. Continuous exposure of larvae to 0.16 ppm naphthalene for 28 days has not resulted in an observable effect on survival (figure 13). The concentration of naphthalene in the full strength seawater soluble fraction, as evidenced by absorption at 221 nm, is only 0.109 ppm (Table 1).

CONCLUSIONS

Because of the preliminary nature of the data to date only tentative conclusions are justified at this time. These are: 1) that crab larvae are affected only by an undiluted seawater soluble fraction of Cook Inlet crude oil, and 2) that benzene may account for a significant portion of the toxicity of this fraction. A second long-term experiment to assess

the toxicity of the seawater soluble fraction of oil, of benzene, and of naphthalene to C. magister zoeae is now underway and should provide confirmatory evidence for these conclusions.

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SUBLETHAL EFFECTS - EFFECTS ON SEA GRASS

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Institute of Marine Science
University of Alaska

March 31, 1976

I. SUMMARY

The focus of this study is an evaluation of selected volatile petroleum hydrocarbons on photosynthesis in the marine plant *Zostera marina*. The import of this study arises from the fact that in many Alaskan marine communities, *Z. marina* is the major contributor to primary productivity.

II. INTRODUCTION

A. General Nature of Study

This study is directed at evaluating the effects of selected petroleum hydrocarbons (dodecane, toluene, and napthalene) on the rates of photosynthesis in *Zostera marina*.

B. Specific Objectives

Specific aspects of this study include; a) an extension of the head space sampling technique to the hydrocarbons in the C_6 to C_{14} range; b) evaluation of baseline levels of volatile hydrocarbons in *Z. marina*; c) characterization of contaminant uptake rates; identification of primary absorption sites of selected contaminants (dodecane, toluene, and hapthalene); e) determining the effects of contaminants on photosynthesis by *Z. marina*.

C. Relevance

Since *Z. marina* is a major contributor to primary production in many ecological niches along the Alaskan coastline, it is imperative to understand what the possible effects of plant exposure to low-levels of the

more water soluble and toxic components of petroleum. Deleterious effects on sea-grass could disrupt whole food chains.

III. CURRENT STATE OF KNOWLEDGE

The current state of knowledge in this particular domain suffers from the fact that no studies have been carried out with alkyl aromatics and napthalenes on higher marine plants.

It is known that alkyl aromatics and napthalene are toxic to many marine biota. However, one can conjecture that the heavy cuticle wax of *Z. marina* serves as a protective barrier/sink. Adsorption of contaminants on article wax without incorporation might protect the plant.

IV. STUDY AREA

The initial proposed study site, Izembek Lagoon, was abandoned due to extensive ice cover. Gravena Pt. (near Cordova) was selected as an alternate and now Katchemak Bay is being considered.

V. SOURCES

Plants were collected with kelp rakes and anchors. No data was acquired because the shipper (Alaska Airlines) managed to lose key samples.

VI. RESULTS

The only results to date: the head space sampling technique has been extended to include volatile hydrocarbons with C<14.

VII. CONCLUSIONS

None

VIII. FURTHER STUDY

Needs for further study: time extension - see X-B, and possibly some additional funding for sample collection to make up for previous aborted attempt.

IX. SUMMARY

A. Ship or Laboratory Activities

1. A sampling trip to Pt. Gravena was carried out in early December on a chartered fishing vessel - the Aunt Martha.
2. The scientific party ~~included~~ a technician (L. M. Cheek) and the P.I. (John G. Pearson).
3. Plant samples were gained by uprooting plants with kelp rakes and a Danforth anchor.
4. Sample location: Pt. Gravena, Prince William Sound.
5. Data: None

B. Problems

This project appears to be suffering from snake bite. Initially, acquisition of equipment and materials was a major problem. Secondly, key samples were lost in shipment by a commercial air carrier thirdly, a key technicial was lost (discharged). And finally the principle investigator was involved in an automobile accident which incapitated him for six weeks.

The project is now on track, but will require a time extension.

The principle investigator apologizes for the poor quality of this report. He uses as an excuse that he was released from the hospital just prior to the due date for this report.

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 17 R.U. NUMBER: 305

PRINCIPAL INVESTIGATOR: Dr. J. G. Pearson

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to date as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]		
	From	To	Batch 1	2	
Cordova Area	12/9/75	12/12/75	None[2]		

Notes: [1] Data Management Plan has been approved and made contractual.

[2] As stated in quarterly report, all samples were apparently lost in transit by Alaska Airlines.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 17

PRINCIPAL INVESTIGATOR: Dr. John Pearson

Period July 1, 1975 - March 31, 1976* (9 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	28,460.00	7,211.70	21,248.30
Staff Benefits	4,838.00	1,225.98	3,612.02
Equipment	19,000.00	22,321.40	(3,321.40)
Travel	3,000.00	593.64	2,406.36
Other	6,100.00	2,651.53	3,448.47
Total Direct	61,398.00	34,004.25	27,393.75
Indirect	16,279.00	4,125.09	12,153.91
Task Order Total	77,677.00	38,129.34	39.547.66

* Preliminary cost data, not yet fully processed.

Following is part 2 of the quarterly report R.U.# 305 for the period ending December 31, 1975. This was received after the printing of the Quarterly Reports, July - September 1975, therefore is included here.

OCS COORDINATION OFFICE

University of Alaska

Quarterly Report for Quarter Ending December 31, 1975

Project Title: Sublethal Effects - Effects on Seagrass

Contract Number: 03-5-022-56

Task Order Number: 17

Principal Investigator: Dr. John G. Pearson

I. Task Objectives

Procurement of equipment and initial calibrations. Procure seagrass samples and initiate short-day studies. Determine baseline levels of napthalene, toluene and dodecane.

II. Field Activities

Early freeze up at Izembek Lagoon prevented sampling in the areas specified in our proposal. An alternate site for sampling was selected near Cordova and was approved by the Juneau office.

Samples were obtained at Harris Creek near Gravena Point on 9 December, 1975 and in Simpson Bay on 11 December, 1975. (See attached charts). Seagrass samples were obtained from the fishing vessel Aunt Martha (see comments) by grapple hooks. Thirty-two samples (eight whole plants per each sample) were frozen in foil lined glass jars and shipped to Fairbanks for baseline analysis. In addition 40 pounds of live seagrass were collected and shipped by air to Fairbanks for uptake studies.

This initial sampling venture was thwarted by the apparent inability of Alaska Airlines to effect proper delivery. One styrofoam container with frozen samples arrived at Fairbanks partially thawed and the remaining set of frozen samples have not arrived in Fairbanks (see comments - Section VI).

Of the three groups of live samples sent to Fairbanks, two were received in time for transfer to aquaria. The live samples are being maintained at 10°C with a 6 hour light period.

III. Results

Due to the problems cited above and in Section IV below, no data has been generated, however, by modifying the head-space extraction technique of the NBS group (Hertz, et al) we are

fully prepared to carry out baseline analysis. A modification of our approach to our field work which includes on-site extraction, will insure data production by 1 February, 1976.

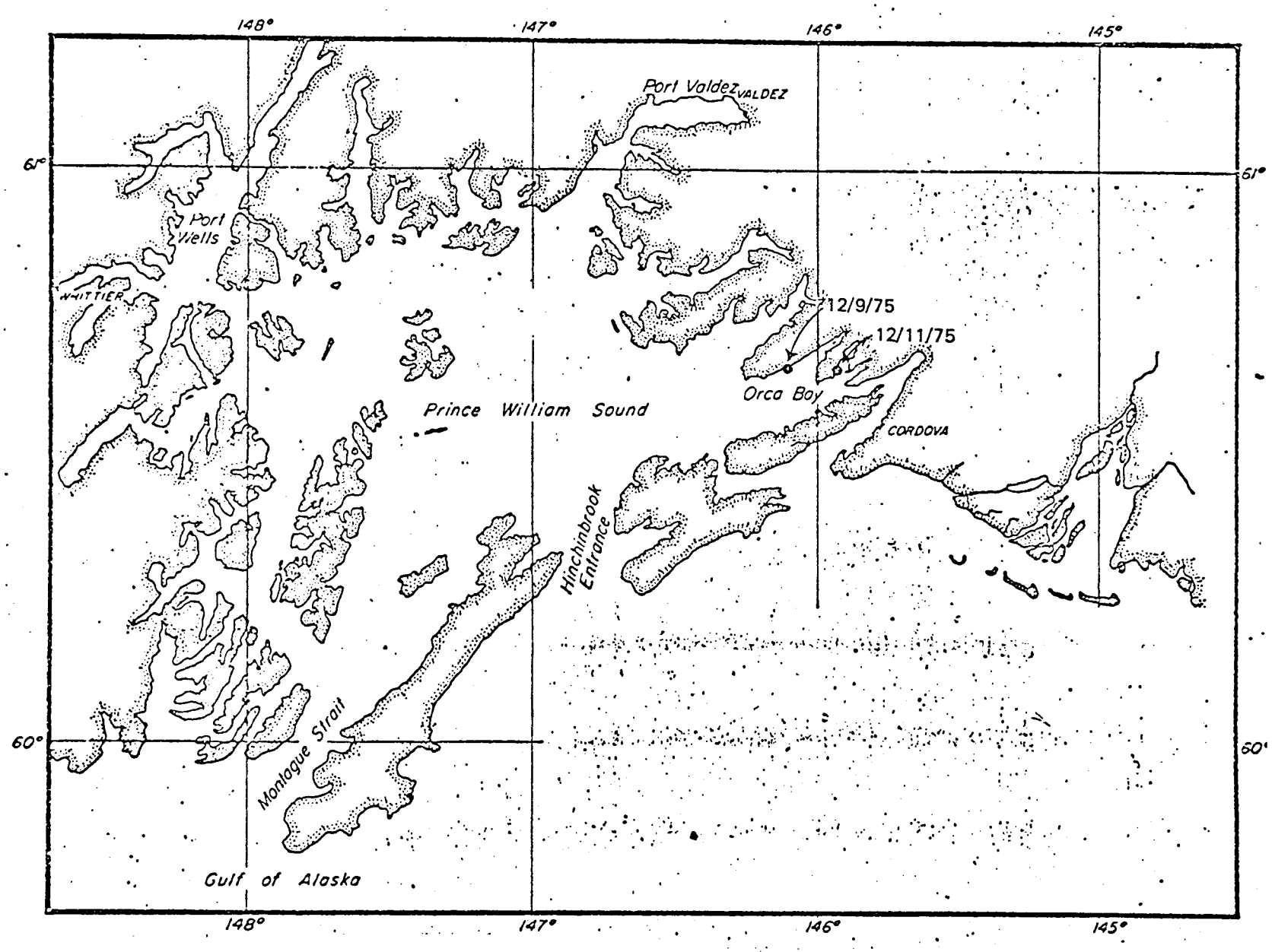
IV. Problems Encountered

Equipment: All equipment for baseline analysis has been received, modified and calibrated. The delay in approval of funding for this project has voided a package "deal" from Beckman Instruments. The package included a bio-oxidizer which Beckman has discontinued. (An alternate supplier has been found - at a higher cost). Apparently the loss of the bio-oxidizer from the package caused confusion in the offices of Beckman Instruments and a subsequent delay in delivery of the liquid scintillation counter. Mr. Sandford, of Beckman, has assured delivery of the counter near the first of January, 1976. This insturment is necessary for all uptake studies.

V. Comments

A. The Cordova area has several lush beds of seagrass that are accessable by boat. Mr. R. Rosenthal has information on beds other than those sampled. Boat charters are available from several local fishermen, including Mr. Bob Maxwell and Mr. Eric Johnson. Mr. Johnson was helpful and easy to work with and we chartered his boat for two days.

B. Rather than attempt a repeat sampling venture by air, we will return to Cordova by surface transportation (truck and ferry). Extraction of samples for baseline analysis can be carried out in Cordova. (Several cannery facilities are available for temporary small scale work.) This procedure will allow investigators to maintain contact with samples at all times.


148°
147°
146°
145°
61°
60°
Port Valdez
VALDEZ
Port Wells
WHITTIER
12/9/75
12/11/75
Orca Boy
Prince William Sound
CORDOVA
Hinchinbrook Entrance
Montague Strait
Gulf of Alaska

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 17 R.U. NUMBER: 305

PRINCIPAL INVESTIGATOR: Dr. J. G. Pearson

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to date as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]		
	From	To	Batch 1	2	
Cordova Area	12/9/75	12/12/75	None[2]		

Notes:

(1) Estimated submission dates are contingent upon final approval of NOAA of data management plan submitted in draft Oct. 9, 1975, approved by University of Alaska Nov. 20, 1975.

(2) As stated in quarterly report, all samples were apparently lost in transit by Alaska Airlines.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 17

PRINCIPAL INVESTIGATOR: Dr. John Pearson

Period July 1 -- December 31, 1975 * (6 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	28,460.00	6,778.06	21,681.94
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Equipment	19,000.00	22,205.00	(3,205.00)
Travel	3,000.00	95.75	2,904.25
Other	6,100.00	1,224.84	4,875.16
Total Direct	61,398.00	31,455.92	29,942.08
Indirect	16,279.00	3,877.05	12,401.95
Task Order Total	77,677.00	35,332.97	42,344.03

* Preliminary cost data, not yet fully processed.

* U.S. Government Printing Office: 1976-679-560/458 Region 8