

EFFECTS OF OILED SEDIMENT ON JUVENILE KING CRAB

by

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ABSTRACT

This 1-year project to determine effects of oiled sediment on juvenile king crab began in April 1982. Most of the experiments and observations were completed by October 1982. Chemical and statistical analyses were completed in FY 83. Juvenile king crab (Paralithodes camtschatica) were exposed to the water-soluble fraction of Cook Inlet crude oil (flow-through, stable concentrations for 30 days) or to oiled sediments for 3 months. The higher exposure concentrations of the water-soluble fraction were toxic and affected survival, growth, feeding rate, and scope for growth. Adverse effects were visible in just a few days. In contrast, the oiled sediments did not cause any measurable adverse effects on survival, feeding rate, growth, molting success, or scope for growth during the 3-month exposure, including those crabs exposed to the highest concentration--2%. Aromatic hydrocarbons were detected in some tissues of the crabs, including the crabs exposed to oiled sediment. Most experimental evidence suggests that exposures to oiled sediment will have minimal impact directly on survival and growth of juvenile king crab. However, the fact that hydrocarbons were detected in the tissues means that there is some contact with the hydrocarbons, and adverse effects are possible, although exposures longer than 3 months would be required.

INTRODUCTION

Oil may enter the environment in a variety of ways and subsequently cause short-term or long-term effects on both the habitat and the resident animals. Short-term effects on animal survival or physiology are caused by water-soluble fractions (WSF'S) of oil, which contain toxic aromatic hydrocarbons. However, toxic effects of WSF'S in the environment are usually transient because of rapid dilution, evaporation, and biodegradation. In contrast, oiled sediment may persist for years. Because the hydrocarbons in sediment are not as readily available to animals as in the WSF, biological effects may not be evident until animals have been exposed for long periods.

There are few accounts in the literature on the long-term effects of oiled sediment on survival and physiology of marine animals, but there is substantial documentation that oil in sediment can persist for years. Krebs and Burns (1977) traced the effects of a spill of fuel oil at West Falmouth, Massachusetts, on the fiddler crab (*Uca pugnax*) that burrowed in the resultant contaminated sediment. After 7 yr, the oiled sediment still held enough hydrocarbons to have a serious impact on the crabs. Anderson (1982) set pans of artificially oiled sediment in the intertidal zone of Sequim Bay, Washington, and found that if the particle sizes of the sediment were very fine, the concentration of hydrocarbons dropped only about 20% in nearly 10 months. McCain et al. (1978) set up laboratory tanks of running seawater with oiled sediment and found that 75% of the initial concentration persisted after 4 months.

The outer continental shelves of Alaska are rich in commercial fishery resources, including king crab, and the same areas are expected to become important producers of petroleum. Although we can predict that spilled oil will persist in sediments for a long time, the long-term effects of this exposure on the survival and growth of king crabs are unknown. This is the final report of the study on the effects of oiled sediment on juvenile king crab that was contracted by OCSEAP to the Auke Bay Laboratory.

The specific objectives of the study were to (1) determine the effects of long-term exposures of oiled sediment on the survival, molting success,

growth, feeding rates, and energetic (scope for growth) of juvenile king crab; (2) determine the effects of long-term exposures of the WSF of the same oil to the same parameters of juvenile king crab; and (3) determine the uptake of hydrocarbons into the tissues of king crab exposed to oiled sediments and WSF'S.

The probability of WSF exposures in the environment is much less than the oiled sediment exposures, but there is more literature on WSF tests. We included the same measurement of juvenile king crab response to both types of exposures so that they could be compared and put into perspective.

METHODS

Experimental Design

Juvenile king crab were exposed either to oiled sediment for 3 months or to the WSF of crude oil for 1 month and compared with unexposed control crabs. Several exposure concentrations were used in each test: five in the oiled sediment exposures (0-2% oil added) and eight in the WSF exposures (0-3 ppm). Sample size was 15-16 crabs in each dose. The crabs were monitored daily for survival, molting success, and feeding rate. Growth, energetic (scope for growth), general behavior, and condition were measured weekly in the WSF tests and biweekly in the oiled-sediment tests. Each crab was tagged so that data could be collected on individuals. In addition, uptake of hydrocarbons in crab tissues was measured from crabs sampled periodically from the highest sublethal concentrations of both WSF and oiled sediment.

Oil concentrations in sediment were analyzed by infrared spectrophotometry and in the WSF by gas chromatography. Tissue concentrations were measured by gas chromatography.

Oiled Sediment

The sediment for experiments was collected by dredge from an area in Auke Bay known to be used by crabs. It was kept frozen in 5-gal. buckets until

use. Five concentrations of oiled sediment were prepared by mixing measured volumes of oil (2%, 0.8%, 0.2%, 0.05%, and 0%) with sediment in a fiberglass-lined cement mixer. Experiments were conducted in fiberglass tanks (three replicate tanks per dose) with the bottoms covered by 5-cm layers of sand, pea gravel, and oiled sediment to a total depth of 15 cm, with the oiled sediment on top. Clean running seawater from Auke Bay was supplied to each tank for 18 d before the crabs were added.

Samples of oiled sediment were taken periodically from the tanks and were analyzed for hydrocarbon content. The surface 1 cm and subsurface sediments (three replicated each) were analyzed from each of the three replicate exposure tanks (18 analyses per dose). An aqueous slurry of each sample was acidified, extracted into Freon, and its absorbance at 2,930 nm measured by infrared spectrophotometer for comparison with similar extractions of whole crude oil. The changes in concentration over time for each initial concentration were developed by combining analyses of samples from all experiments.

Water-Soluble Fractions

The WSF of Cook Inlet crude oil was generated using our standard method, which involves dripping seawater through a floating layer of the oil (Moles et al., in press). The WSF and diluent seawater were delivered to continuous-flow exposure tanks through glass tubing. All WSF concentrations were monitored in terms of ppm of aromatic hydrocarbons using fluorescence spectrophotometry and gas chromatography. For specifics of analysis methods see Gordon et al. (1973).

Crabs

The crabs were all juvenile red king crab (Paralithodes camtschatica), between 25 and 50 mm in shell length and weighing 10-80 g. They were collected from Auke Bay by scuba divers. Each crab was marked by gluing a numbered 3x5-mm plastic tag to the carapace with Eastman 910 "super glue." Within a few days after any crab molted, its retrieved tag was replaced on its new carapace so each individual could be identified throughout the 3-month experiment.

Test crabs were fed on mussels (Mytilus edulis) throughout the tests. Excess mussels *were* offered so crab feeding was never limited by availability of food. We have held juvenile king crab for as long as 11 months, feeding them nothing but mussels. During this time, they molted 3-4 times, grew to 6-10 times their original weight, and always appeared normally active; therefore, we are confident that a diet of mussels provided adequate nutrition for the 3 months of oiled-sediment exposure.

Test crabs were exposed at ambient seawater temperatures of Auke Bay, which ranged from 6° to 9°C.

Observations and Measurements

Survival: Deaths of crabs were recorded and all dead crabs removed from test tanks daily. Mean lethal concentrations (LC50'S) were determined from mortality counts, using logit analysis (Silverstone 1957) when possible (when two or more concentrations contained both live and dead crabs). The Spearman-Kärber method (Finney 1971) was used when less than two concentrations contained both live and dead crabs.

Growth and Molting: Molts were noted and removed from tanks daily. Growth of WSF-exposed crabs was measured weekly and biweekly in oiled-sediment-exposed crabs. Carapace lengths were measured from the posterior edge of the eye socket to the center posterior and live weights measured after gently shaking off excess water. Missing legs, general condition, and incidence of disease were also recorded at the same time as growth data.

Feeding Rate: Crabs were fed daily on mussels that were split open but still attached to the shell. The shells, with any uneaten mussel tissue attached, were retrieved 23 h later. All mussels fed to crabs were weighed before and after feeding to determine feeding rate. Mussels that have been split open will, consequently, lose some weight just by soaking in water 24 h. We determine that weights of the remains of mussel tissue retrieved after feeding, should be corrected by a factor of 0.929 before subtracting from prefeeding weight. The resulting measure of wet mussel tissue eaten was converted to dry weight by multiplying by a factor of 0.238. The calculated

dry weights of tissue eaten by each group of crabs were then divided by the total live weight of those crabs to calculate a feeding rate comparable to rates for other groups of crabs; this rate was then multiplied by the live weights of individual crabs to determine approximate individual feeding rates for calculating scope for growth.

Energy Balance: Scope for Growth: Scope for growth was calculated for individual crabs by subtracting maintenance energy (respiration and excretion) from the calories consumed. Crabs in the oiled sediment tests were measured at the end of each month of exposure. Crabs in WSF tests were measured before exposure and after 1, 2, 3, and 4 weeks of exposure. Each crab was isolated for 6 h in a 350-ml plastic chamber through which waterflow could be precisely controlled and its oxygen consumption and nitrogen excretion rates measured. Absorption efficiencies were determined from samples of feces collected from the chambers after crabs were returned to their test tanks. Scope for growth is defined as $C - (F + R + U)$ where C = food energy consumed, F = energy lost as feces, R = energy respired, and U = energy excreted, all in calories. Detailed methods are given in Shirley and Stickle (1982a, b).

Tissue Levels of Hydrocarbons: Tissue samples from each of seven crabs were taken periodically from groups of 30 crabs held on 2% oiled sediment or in 0.5-ppm WSF. Crabs were sampled from the sediment group after 4, 10, 30, and 90 d of exposure and from the WSF group after 1, 4, 10, and 30 d of exposure. Unexposed crabs were also sampled. The crabs were killed and preserved by placing them in clean glass jars and freezing them. Hydrocarbon levels in leg muscle tissue and hepatopancreas (digestive gland) were determined later. Crabs were thawed and dissected and one pooled sample of muscle tissue and one of hepatopancreas tissue collected from each sample of seven crabs. Samples were weighed, heated, and digested in 10N NaOH, extracted in hexane, and had their aromatic and aliphatic contents separated using columns of silica gel. Individual hydrocarbons were separated and quantified by packed column gas chromatography.

RESULTS

Chemical

Exposure concentrations remained relatively constant in both the WSF and the oiled-sediment tests. As hydrocarbons in the WSF tests were lost from the test containers, they were continuously replenished by new solution during the month-long tests.

Hydrocarbons in the sediment exposures were added only once, at the beginning of the test, and persisted at relatively high levels during the 3-month test (Fig. 1). Surface sediments were the least stable, losing about 35% of the initial concentration. Subsurface sediments lost about 15% of the initial hydrocarbons. The highest concentrations lost more hydrocarbons than lower concentrations, and losses occurred primarily within the first month. The highest concentration approached the upper limit of hydrocarbon-carrying capacity of the sediment; 2% oil was the highest oil concentration that we could mix into the sediment without producing a surface oil slick when water was added. There was a large amount of variation in the hydrocarbon content of the sediments between replicate tanks and between replicate samples from one tank (as shown by large standard deviations in Figure 1). The variability in the initial samples indicates that it is difficult to mix oil into sediments in a uniform manner.

Response of Crabs

All tested concentrations of oil in sediment were sublethal. No crabs exposed to the highest concentration of 2% crude oil in sediment, died during the tests. None of our measurements of growth, feeding, or scope for growth showed any significant differences between the crabs exposed to 2% and the control crabs (Table 1). These negative results are clear and consistent even though the crabs remained in constant contact with the contaminated sediment. They continually stirred up the surface of the mud by walking on it and dug themselves into slight depressions in it. They also ate some sediment (perhaps inadvertently with mussels), as indicated by the presence of sediment in their feces.

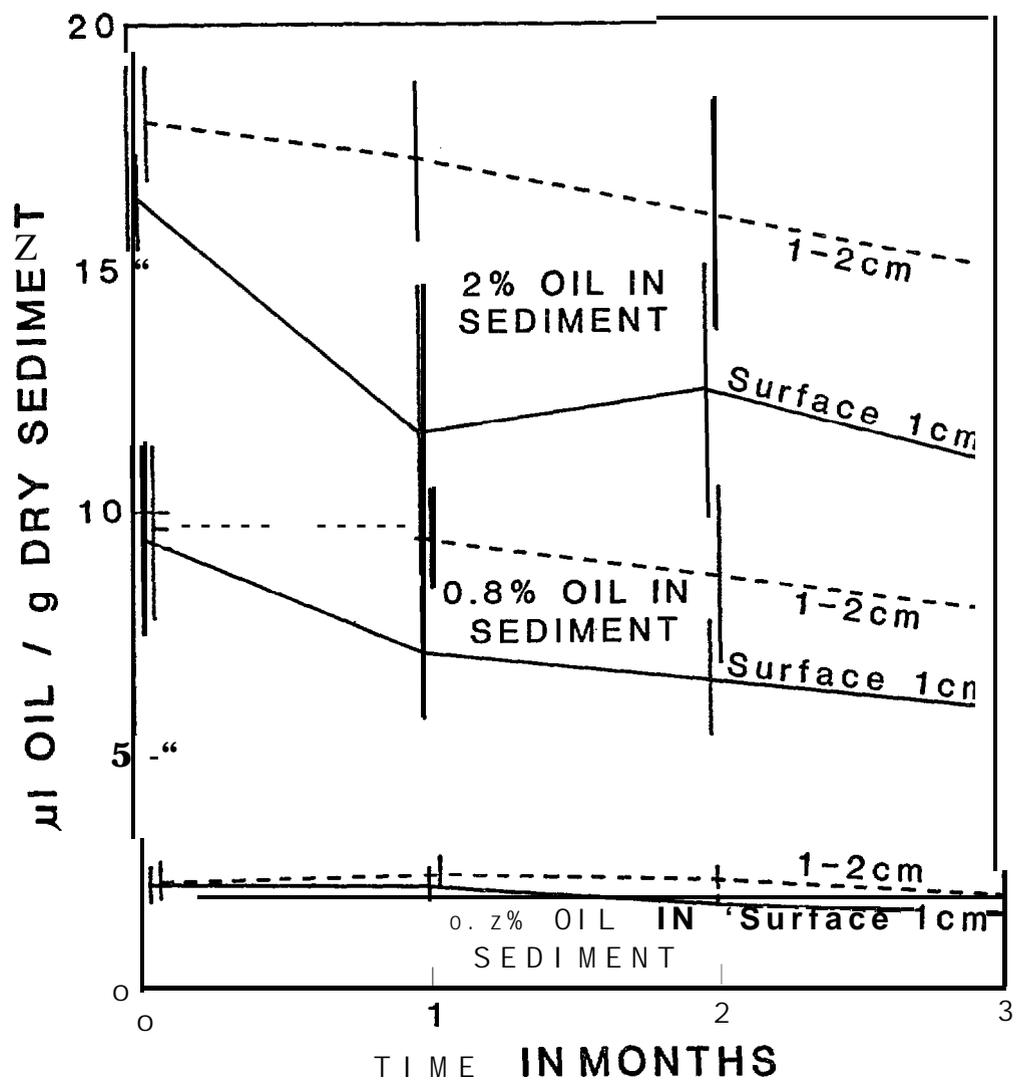


Figure 1. --Loss of oil hydrocarbons over time from three concentrations of Cook Inlet crude oil mixed into sediment. Samples were taken from the top centimeter of sediment and from the centimeter immediately below. All points are means of replicate samples within tanks and between tanks of several similar experiments. Bars are standard deviations of the means.

Table 1. --Comparison of juvenile king crab living for 11 weeks on sediment mixed with 2% Cook Inlet crude oil with crab living on uncontaminated sediments. The two groups do not differ significantly on any parameter.

	CONTROL	2% OIL IN SEDIMENT
DEATH RATE		
% dying of all causes, 24 May - 10 Aug.	13% (3/24)	0% (0/16)
MOLT RATE		
% molting at least once	100%	100%
% molting twice	13% (3/24)	6% (1/16)
GROWTH IN LENGTH		
Carapace length on 10 Aug. Carapace length on 24 May (excluding crabs molting twice)	$\bar{X} = 1.27$ $s = .036$	$\bar{X} = 1.27$ $s = .068$
GROWTH IN WEIGHT		
Live weight on 10 Aug. Live weight on 24 May (excluding crabs molting twice)	$\bar{X} = 2.14$ $s = .118$	$\bar{X} = 2.12$ $s = .169$
FEEDING RATE		
mg dry mussel tissue eaten / g live crab / day	$\bar{X} = 8.45$ $s = 1.41$	$\bar{X} = 7.87$ $s = 1.48$
SCOPE FOR GROWTH		
Calories / crab / day	$\bar{X} = 663$ $s = 155$	$\bar{X} = 782$ $s = 191$

The higher tested WSF concentrations were lethal. The crabs had an LC50 of 1.5 ppm of aromatic hydrocarbons for 4 d of exposure and an LC50 of 1.4 ppm for 1 week of exposure. Although the highest concentration used for measurement of sublethal effects --0.5 ppm--did not kill any crabs in the first 3 weeks of exposure, it did stop the crabs from feeding.

Feeding rate of crabs was inversely related to WSF concentration (Fig. 2). This relationship may be seen most clearly when inconsistencies in the WSF concentrations within a single dose are considered. Figure 2 compares the mean WSF concentration for each week of exposure for each group of crabs with the mean feeding rate of that group for that week. Feeding rates for the "0.1-ppm" group were similar to those for the control group. Feeding rates for the "0.3-ppm" group were low at first and then returned to control level at least partially because WSF concentrations fell slightly and perhaps also due to some acclimation or compensation by the crabs. Crabs in the "0.5-ppm" group did not eat at all except in the third week when their exposure levels dropped for a few days.

Failure to feed had a direct effect on other measured parameters. Scope for growth paralleled feeding rate, but there were no concentration-related effects on oxygen consumption, nitrogen excretion, or absorption efficiency. The WSF concentrations >0.3 ppm caused reduced scope for growth, and concentrations >0.5 ppm caused negative scope for growth or a net loss of calories to the crabs (Fig. 3). Failure to feed would obviously have a direct effect on any measure of growth rate; however, the length of the period of exposure was too short to clearly demonstrate differences in growth between feeding (controls) and nonfeeding crabs. Failure to feed would also eventually be lethal and, indeed, there were deaths in the 0.5-ppm group during the fourth week of exposure, bringing the 28-d LC50 down to 0.64 ppm of aromatic hydrocarbons.

Uptake of Hydrocarbons by Crabs

Aromatic and aliphatic hydrocarbons were found in the tissues of crabs exposed to either 0.5-ppm WSF or 2% oil in sediment. However, the patterns of

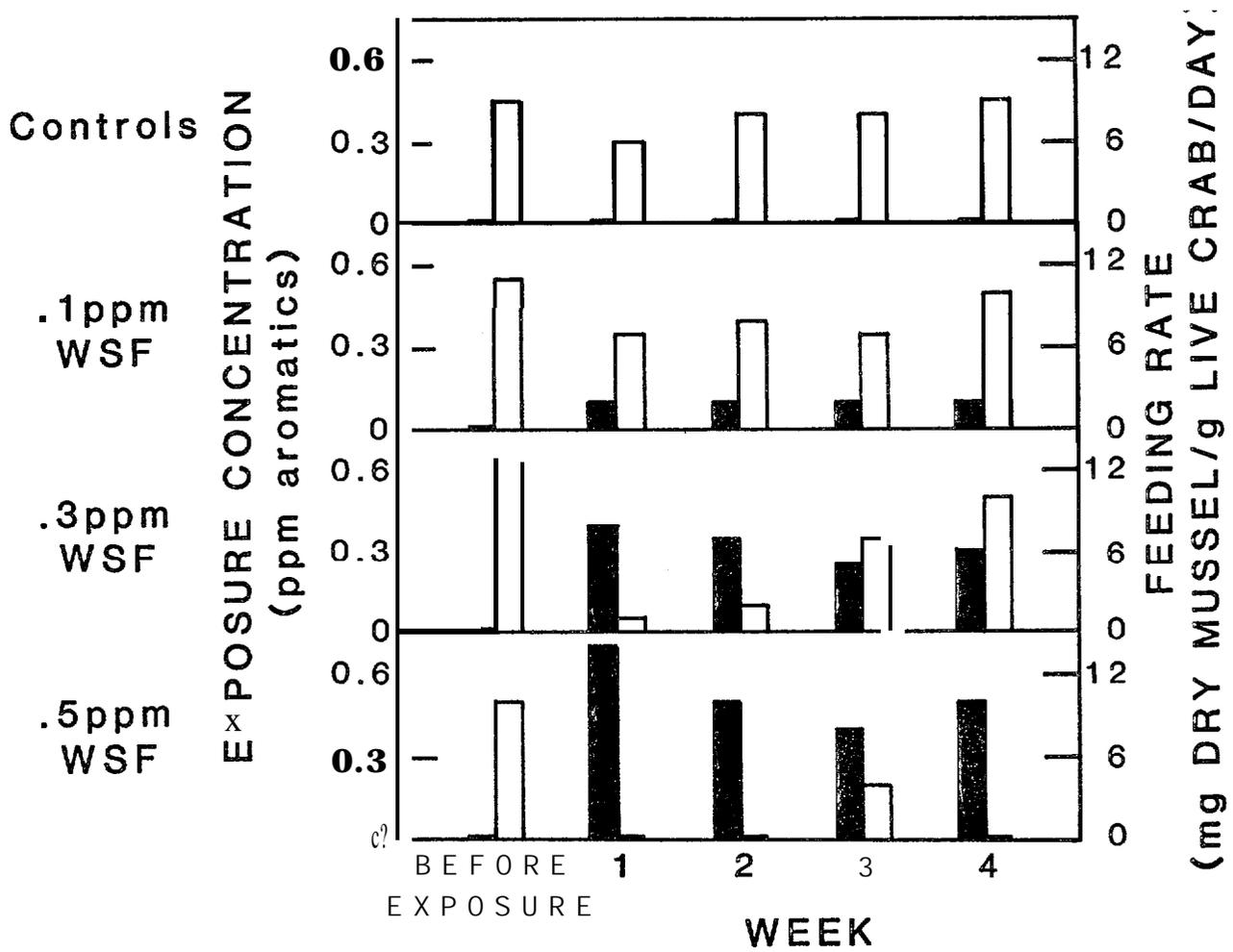


Figure 2. --Effect of weekly mean exposure level (solid bars) on weekly mean feeding rate (white bars) for juvenile king crab fed on blue mussels during exposure to the water-soluble fraction of Cook Inlet crude oil."

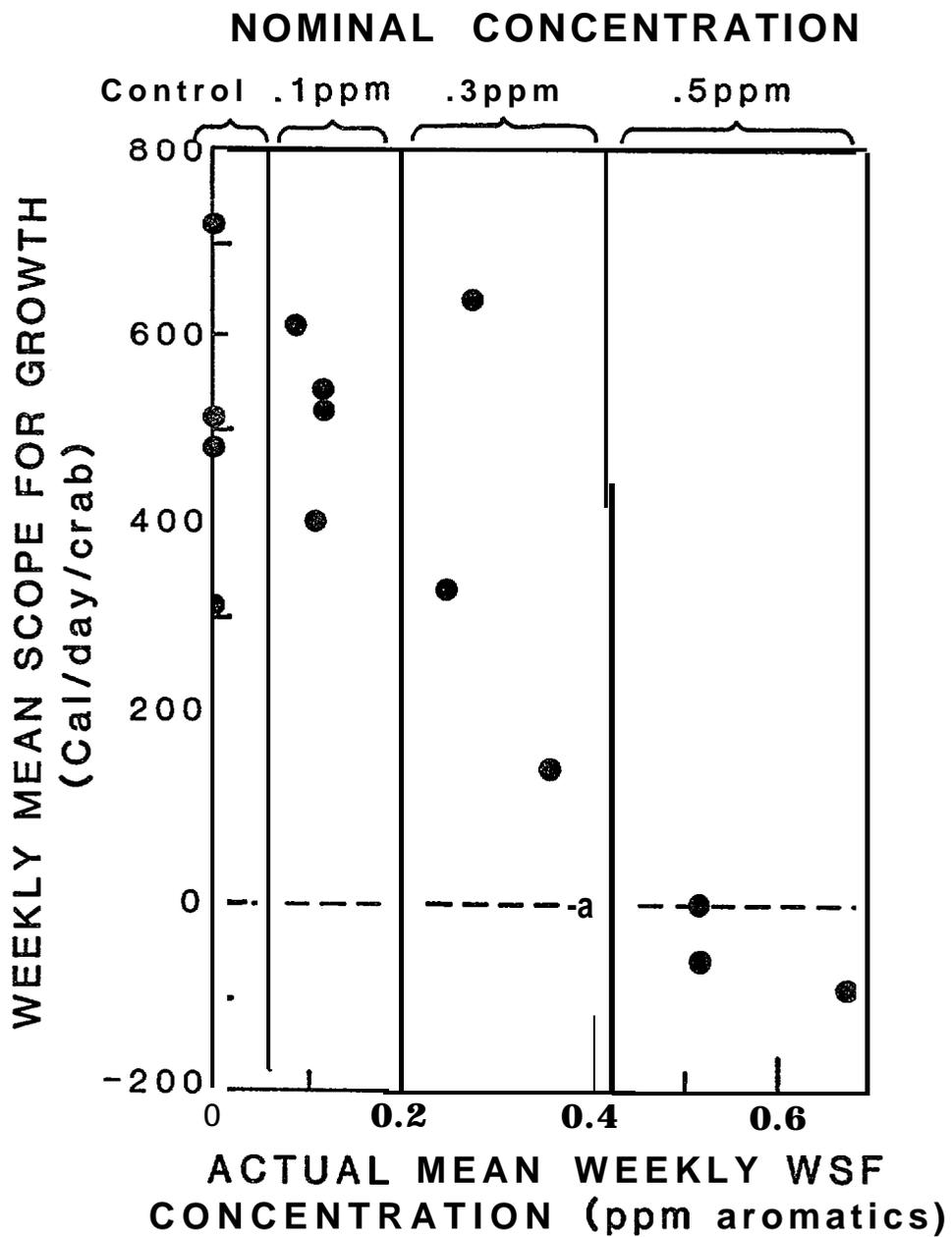


Figure 3. --Weekly scope for growth plotted against the mean exposure concentration for the same week.

uptake were quite different for crabs in the two different exposure methods (Table 2).

The hydrocarbon accumulation in muscle tissues of crabs exposed to either WSF or oiled sediment was low (Fig. 4). Muscles of **WSF-exposed** crabs had slightly elevated levels of lighter aromatic hydrocarbons but control level concentrations of **aliphatics**. In contrast, muscles of sediment-exposed crabs had elevated **aliphatic** levels compared with control crabs. The aromatic hydrocarbon levels in control and oiled-sediment-exposed crabs were low.

The accumulation of aromatic and **aliphatic** hydrocarbons was much greater in the hepatopancreas than in muscle. Hepatopancreas tissues of **WSF-exposed** crabs were consistently high in hydrocarbons (Fig. 5). They contained lower boiling aromatic hydrocarbons, the lowest boiling **aliphatics**, and a low envelope of unresolved higher boiling **aliphatics**.

In sediment-exposed crabs, the hydrocarbon concentrations in **hepatopan-**creas tissue increased over time (Fig. 6). These samples contained unresolved envelopes (groups of peaks that overlap each other so much that they cannot be differentiated) of aromatic hydrocarbons in a higher boiling range than the aromatic peaks of WSF crabs that increased from 210 ppm at 4 d to 270 ppm at 10 d and to 370 ppm at 90 d. Hepatopancreas tissue also contained **aliphatic** peaks throughout the sampled range with unresolved envelopes in the midrange. **Aliphatics** increased from 160 ppm at 4 d to 380 ppm at 10 d and to 670 ppm at 90 d. Accumulation on hydrocarbons in **the** sediment-exposed crabs was expected because some sediment was consumed and passed through the digestive system. However, the surface sediments were decreasing in concentration over time while the concentrations in **hepatopancreas** tissue were increasing.

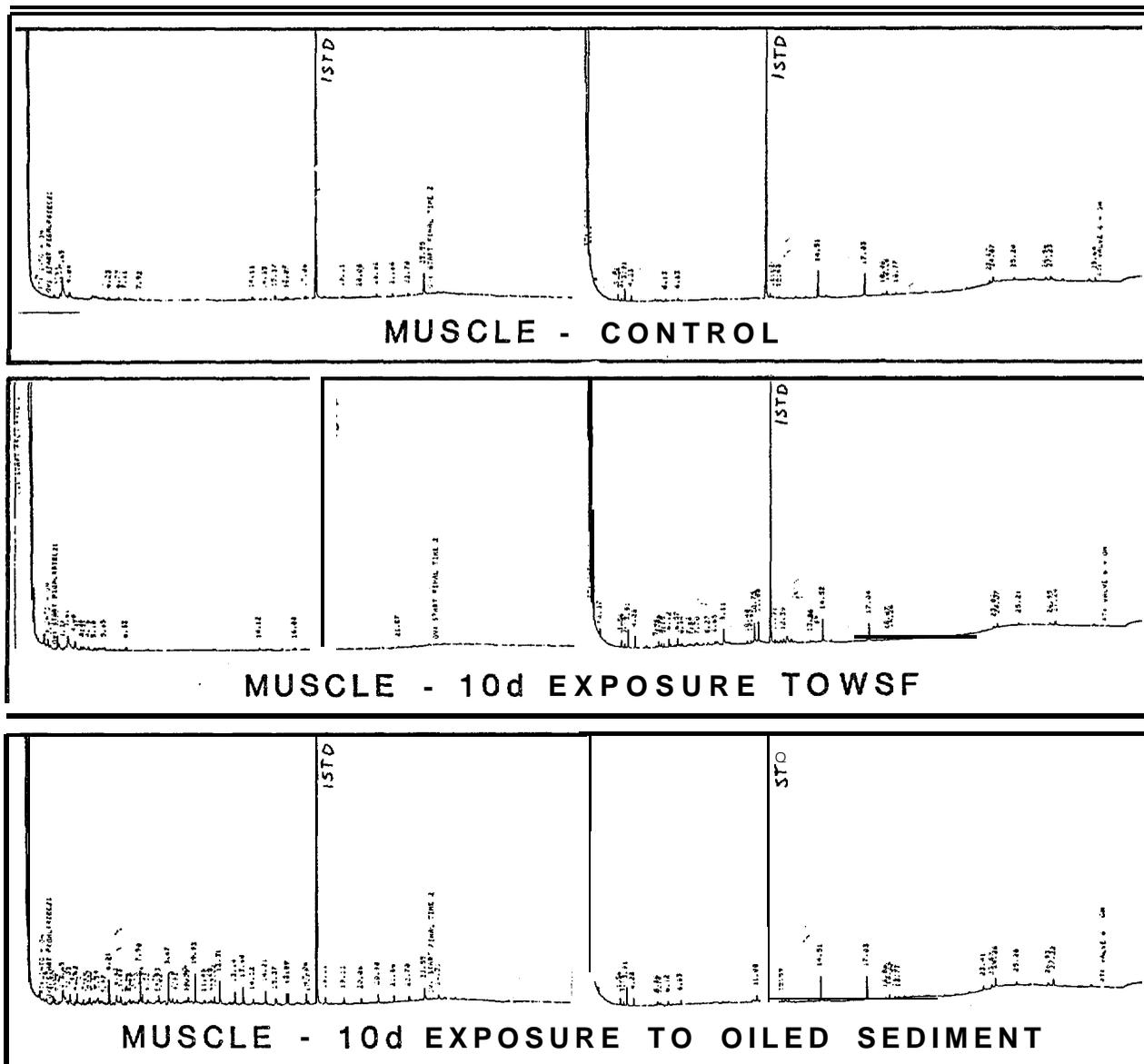
DISCUSSION

Juvenile red king crab can be affected by oil exposure, particularly if the exposure is to a **WSF** of oil. The WSF of Cook Inlet crude oil was toxic at the higher concentrations tested, and sublethal exposures stopped the feeding of juvenile crabs within the first few days of exposure. If feeding is inter-

Table 2.--Hydrocarbon content of tissues from oil-exposed juvenile king crab.

		HYDROCARBONS IN PPM (g HC/10 ⁶ g TISSUE)								
		DAYS OF EXPOSURE	TOLUENE	XYLENE (O,M&P)	NAPHTHALENE	2-METHYL-NAPHTHALENE	1-METHYL-NAPHTHALENE	TOTAL AROMATICS	TOTAL ALIPHATICS	
CONTROL		0	0	0	0	0	0	0	0	
MUSCLE TISSUES	EXPOSED TO WSF (.5ppm AROMATICS)	1	1	1	0	0	0	7	0	
		4	2	1	0	0	0	6	0	
		10	1	1	0	0	0	4	1	
		28	1	1	0	0	0	7	1	
	EXPOSED TO 2% OILED SEDIMENT	4	0	1	0	0	0	7	5	
		10	1	1	0	0	0	7	5	
		90	4	1	0	0	0	2	2	
	CONTROL		0	1	1	0	0	0	6	7
	HEPATOPANCREAS	EXPOSED TO WSF (.5ppm AROMATICS)	1	56	58	21	11	10	260	110
			4	39	46	17	19	20	300	82
10			1	17	12	16	16	180	110	
28			3	8	5	7	8	79	110	
EXPOSED TO 2% OILED SEDIMENT		4	0	1	0	1	0	210	160	
		10	1	2	0	1	0	270	380	
		90	1	1	0	2	1	370	670	

ALIPHATIC HYDROCARBONS AROMATIC HYDROCARBONS



ALIPHATIC HYDROCARBONS AROMATIC HYDROCARBONS

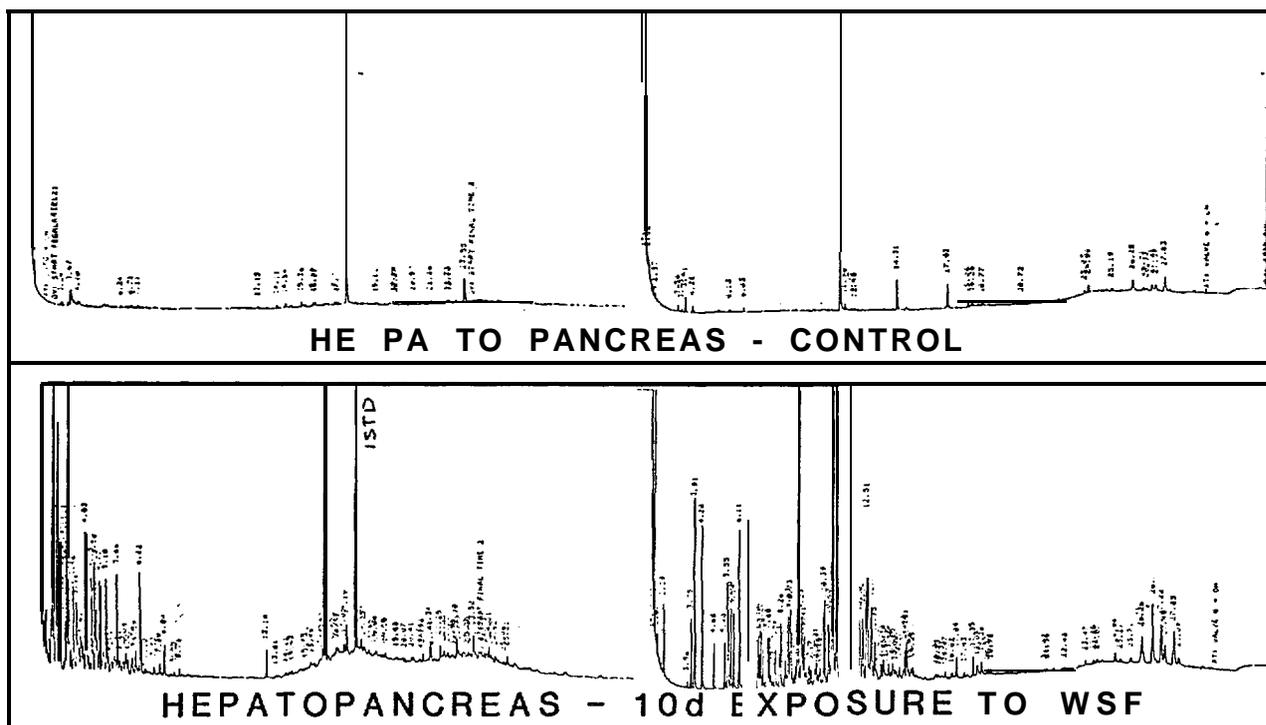


Figure 5. --GC scans of hydrocarbon content in hepatopancreas (digestive gland) of juvenile red king crab exposed to Cook Inlet crude oil WSF of 0.5 ppm aromatics. Samples taken 1, 4, 10, and 28 days after the start of exposure showed extremely similar scans.

ISTD = internal standard. Other peaks visible in control scans are contaminants and artifacts common to all scans shown.

ALIPHATIC HYDROCARBONS AROMATIC HYDROCARBONS

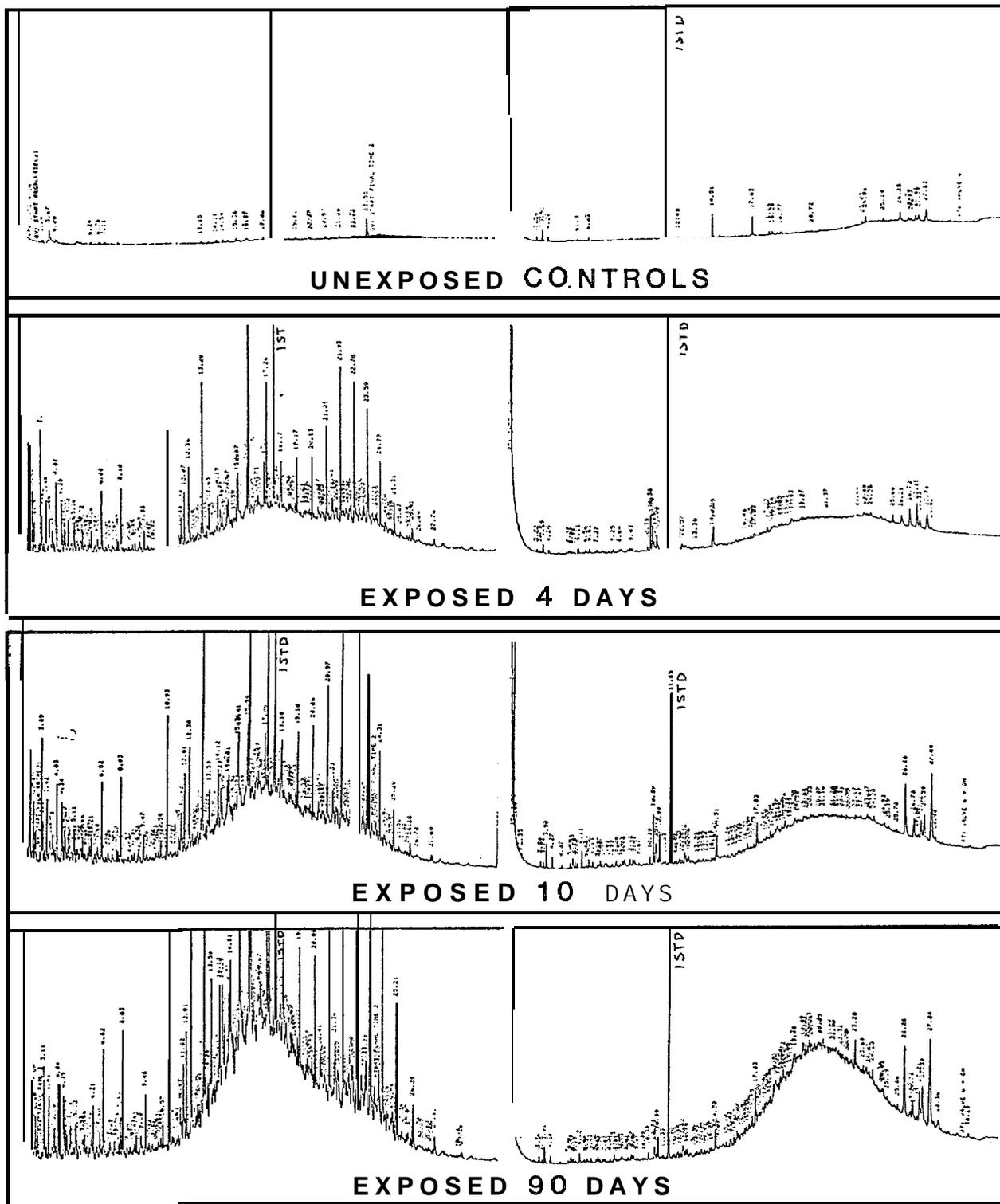


Figure 6. --GC scans of hydrocarbon content of hepatopancreas (digestive gland) of juvenile red king crab exposed to Cook Inlet crude oil mixed with sediment at 2% concentration for up to 3 months. ISTD = internal standard. Other peaks visible in control scans are contaminants and artifacts common to all scans shown.

rupted, it is not surprising that long-term exposures to the WSF of oil would have an effect on energetic (scope for growth) and eventually on growth and survival. The WSF of oil contains the lighter toxic aromatic hydrocarbons which, when present in the water column, are rapidly taken up by marine organisms.

In contrast, oiled sediments have a hydrocarbon composition that is similar to unweathered parent oil which is less available to marine organisms than WSF's. Our tests with juvenile king crab exposed to oiled sediment suggest that the oil did not adversely affect the crabs during the 3-month exposures. Survival, molting success, growth, and scope for growth were the same for control crabs as for those exposed to the highest concentration of oil (2%). Although this is a high concentration of oil, concentrations measured from actual spills have been reported to be of similar magnitude (Gilfillan et al. 1976; Krebs and Burns 1977).

King crabs live on the surface of the sediment, so direct contact with hydrocarbons in the sediment matrix is minimal. We measured hydrocarbon accumulation in muscle and in the hepatopancreas. We found some sediment in the guts of juvenile crab and, therefore, were not surprised that some hydrocarbons accumulated in tissues. But these accumulations had no apparent adverse effects on survival and growth. In other studies, oiled sediments have caused adverse effects on several species, but unlike king crab, the animals lived and burrowed in the sediment (Krebs and Burns 1977; Gilfillan and Vandermeulen 1978; McCain et al. 1978; Augenfeld et al. 1980; Fletcher et al. 1981).

Although WSF exposures can be detrimental to crab survival and sublethal exposures can be damaging physiologically, the potential for harmful effects is minimal because exposures would be transient. A large spill could be violently mixed by wind and waves to produce an oil-water dispersion, but the toxic water would usually be rapidly diluted and the compounds rapidly degraded. In contrast, oiled sediment could persist for years. Our results indicate that exposures to oiled sediment for 3 months would not be harmful to survival and molting success of juvenile king crab. However, the tissue accumulation of hydrocarbons from the oiled sediments suggests that long-term

effects from prolonged exposure are possible, even **though lengthy** exposures may be required before damage is evident. For example, **McCain et al. (1978)** reported increased incidence of disease (tumors) in English sole (**Parophrys vetulus**) exposed to oiled sediments. Some of the hydrocarbons in oiled sediments are known carcinogens. **Fletcher et al. (1981)** have suggested that animals exposed to oiled sediments may be much more vulnerable to additional stress than unexposed animals. The flounders they studied survived **oiled-sediment** exposure at cold temperatures but succumbed at temperatures near their maximum tolerance level.

The hydrocarbon accumulation in the tissues of sediment-exposed crabs could cause tainting, and there could be some risk to consumers of **the** crabs because some hydrocarbons are carcinogens.

Additional studies of the potential for damage to king crab stocks exposed to oil should focus on very long-term (1 **yr** or more) exposures to oiled sediments. The uptake of hydrocarbons into several crab tissues should be measured, and the incidence of disease and tissue abnormality should be determined at the end of exposure. The ability of exposed crabs to withstand further stress, such as extremes of temperature, overcrowding, or limited food, should also be tested.

The risk to king crab populations from **oiled** sediment may be negligible in exposures of less than 3 months, but there could be adverse effects from very long exposures. Such effects, if any, are likely to be subtle and very difficult to assess.

CONCLUSIONS

(1) WATER-SOLUBLE FRACTION EXPOSURES: Survival, feeding, and energetic of juvenile king crab were effected by WSF exposures, 4-d **LC50 = 1.5** ppm; 28-d **LC50 = 0.64** ppm. Feeding stopped at 0.5 ppm.

(2) OILED-SEDIMENT EXPOSURES: The highest oiled-sediment concentration (2%, which was the maximum amount of oil that we could mix into the sediment) did not affect survival, molting success, feeding rates, growth, or energetic of juvenile king crab exposed to oiled sediment for 3 months. This concentration remained relatively constant during the 3-month study.

(3) TISSUE ACCUMULATION OF HYDROCARBONS: Hydrocarbons were accumulated into muscle and into the hepatopancreas of crabs exposed to WSF and oiled sediment. The patterns of uptake were different for the two exposure methods. The accumulation of hydrocarbons in tissues of oiled-sediment-exposed crabs proves that these crabs were in contact with oil possibly through ingestion of some sediment. However, no adverse effects were observed during the 3-month exposure.

(4) Although no adverse effects were observed in the 3-month oiled-sediment exposure of juvenile king crab, there is potential for long-term effects from the accumulated hydrocarbons in the tissues. Greater incidence of disease and other effects which require long time periods before they are visible, could be caused by the accumulated hydrocarbons.

LITERATURE CITED

- Anderson, J. W. 1982. The transport of petroleum hydrocarbons from sediments to benthos and the potential effects. Pp. 165-179 in G. F. Mayer (cd.), Ecological Stress and the New York Bight: Science and Management.
- Augenfeld, J. M., J. W. Anderson, D. L. Woodruff, and J. L. Webster. 1980. Effects of Prudhoe Bay crude oil-contaminated sediments on Protothaca staminea (Mollusca: Pelecypoda): hydrocarbon content, condition index, free amino acid level. Mar. Environ. Res. 4: 135-143.
- Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge Univ. Press, United Kingdom.

- Fletcher, G. L., J. W. Kiceniuk, and U. P. Williams. 1981. Effects of oiled sediments on mortality, feeding and growth of winter flounder Pseudopleuronectes americanus. *Mar. Ecol.* 4: 91-96.
- Gilfillan, E. S., and J. H. Vandermeulen. 1978. Alterations in growth and physiology of soft-shell clams, Mya arenaria, chronically oiled with bunker C from Chedabucto Bay, Nova Scotia, 1970-76. *J. Fish. Res. Board Can.* 35: 630-636.
- Gilfillan, E. S., D. Mayo, S. Hanson, D. Donovan, and L. C. Jiang. 1976. Reduction in carbon flux in Mya arenaria caused by a spill of no. 6 fuel oil. *Mar. Biol.* 37: 115-123.
- Gordon, D. C., Jr., P. D. Keizer, and N. J. Prouse. 1973. Laboratory studies of the accommodation of some crude and residual fuel oils in sea water. *J. Fish. Res. Board Can.* 30: 1611-1618.
- Krebs, C. T., and K. A. Burns. 1977. Long-term effects of an oil spill on populations of the salt-marsh crab Uca pugnax. *Science* 197: 484-487.
- McCain, B. B., H. O. Hodgins, W. D. Gronlund, J. W. Hawkes, D. W. Brown, M. S. Myers, and J. H. Vandermeulen. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (Parophrys vetulus), and pathological consequences. *J. Fish. Board Can.* 35: 657-664.
- Moles, A., S. A. Andrews, and S. D. Rice. In Press. Continuous-flow devices for the exposure of marine organisms to crude oil and components. The Tenth Annual Aquatic Toxicology Workshop, Halifax, Nova Scotia, Canada, 1983.
- Shirley, T. C., and W. B. Stickle. 1982a. Responses of Leptasterias hexactis (Echinodermata: Asteroidea) to low salinity: I. Survival, activity, feeding, growth, and absorption efficiency-. *Mar. Biol.* 69: 147-154.
- Shirley, T. C., and W. B. Stickle. 1982b. Responses of Leptasterias hexactis (Echinodermata: Asteroidea) to low salinity: II. Nitrogen metabolism, respiration and energy budget. *Mar. Biol.* 69: 155-163.
- Silverstone, H. 1957. Estimating the logistic curve. *J. Am. Stat. Assoc.* 52: 567-577.