NOAA Technical Memorandum NMFS F/NWC-111

Lethal and Sublethal Effects of the Water-Soluble Fraction of Cook Inlet Crude Oil on Pacific Herring (Clupea harengus pallasi) Reproduction

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February 1987

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

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ABSTRACT

Pacific herring (Clupea harengus pallasi) are a valuable commercial fishery in the northwestern Pacific Ocean, where they are harvested primarily just before spawning, when the edible ovaries are at maximum ripeness. Our studies examined the effects of oil on important stages of the herring reproductive process. Using the water-soluble fraction (WSF) of Cook Inlet crude oil, we studied effects of lethal and sublethal exposures on prespawn adult Pacific herring, eggs,. yolk-sac larvae, and feeding larvae. We studied also the effects of feeding oil-contaminated prey to herring larvae. The results of our study are summarized:

1. Prespawn adult herring exposed to WSF had a 2- and 12-day LC_{50} (the median concentration that killed 50% of the herring) of 2.3 parts per million (ppm) aromatic hydrocarbons.
2. Adults exposed 12 days to 1.6 ppm produced eggs that had normal hatching success.
3. Eggs exposed 2 days to 5.3 ppm had normal hatching success; eggs exposed 12 days had an LCs_0 of 1.5 ppm.
4. Yolk-sac larvae exposed <6 hours to 6.1 ppm survived; yolk-sac larvae exposed from 16 hours to 6 days had LCsO's of 2.8 to 2.3 ppm.
5. Feeding larvae exposed 7 days had an LCsa of 1.8 ppm; exposed 21 days, 0.36 ppm.
6. Uptake of hydrocarbons in adult herring was rapid in muscle, liver, testes, and mature and immature ovaries, but equilibrium was not reached in 10 days of exposure.

7. Muscle tissue generally accumulated the highest levels of hydrocarbons; immature ovarian tissue accumulated almost two times the levels found in mature ovarian tissue.
8. In adults, initial depuration was rapid but slowed after 24 hours, and 10% of the hydrocarbons were still present after 7 days in clean water. After 14 days, hydrocarbon levels were not significantly higher than control levels.
9. Uptake in larvae was more rapid than in adults and reached equilibrium within 4 hours. Retention was lower in larvae than adults, and after 24 hours, only 2% of the ^{14}C-labeled naphthalene remained in larval tissues.
10. Larval growth was significantly reduced after 7 days of exposure to 0.3 ppm, and growth reductions were greater after longer exposures and higher concentrations.
11. Growth of larvae was not significantly reduced by a diet of oil-contaminated prey.

Feeding larvae of Pacific herring are killed by shorter exposures and lower concentrations than are the eggs or the adults. This is therefore the life stage most likely to be impaired by oil. Even if oil is present at levels too low to threaten the survival of herring, fisheries could be impacted if the rapid bioaccumulation and persistent retention of oil hydrocarbons in the edible ovarian tissues made the herring unmarketable.

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INTRODUCTION

'The Pacific herring (Clupea harengus pallasi) is an important prey species and supports valuable commercial fisheries in the coastal waters of Alaska. Effects of oil pollution are of particular concern because each herring stock spawns in spring within a restricted natal range near shore. Adults typically congregate for several weeks near prospective spawning sites while their reproductive organs mature, then spawn en masse over a period of a few days. Herring spawn in O-20 m depths on almost any available substrate (Jones 1978; Carlson 1980). Larvae hatch after approximately 20 days at Alaska spring temperatures (5-8°C) and live off yolk reserves for about a week prior to initial feeding. Larvae are weak and drift passively in waters near the spawning areas for several months before they transform into juveniles (Jones 1978).

Oil pollution in' the spawning habitat could contaminate adult herring and their gonads or harm the spawn and larvae.;. Because herring spawn en masse, entire stocks and year classes are vulnerable to oil pollution at the time of spawning.

Herring have been fished in Alaska since 1882, but until 1970, the products were high-volume and low-priced--salted herring, fish meal, fish oil, or bait. Beginning about 1970, demand by Japan for herring roe caused a resurgence of the fishery. Gonads (roe) of mature females immediately prior to spawning, and the spawned eggs, are highly valued in foreign markets. The wholesale price of salted roe and eggs is more than $6.00 per pound, higher than the wholesale price for chinook salmon (Oncorhynchus tshawytscha) ($2.10 in 1983) or salmon eggs ($3.10 per

pound in 1983) (Alaska Department of Fish and Game 1984). The 1983 wholesale value of herring products from Alaska was $67 million.

Although studies on the effects of contaminants on reproductive processes in fish are rare, sensitivities of herring eggs and larvae to crude oil have been examined (Kihnhold 1974; Eldridge et al. 1977; Linden 1978; Cameron and Smith 1980). Most fish larvae are relatively sensitive to oil, but eggs are usually more resistant (Rice 1985). In contrast to eggs and larvae, little attention has been directed at the effects of pollution on gamete formation, mainly because of difficulties in capturing, transferring, and holding mature adults in quantities sufficient to ensure valid statistical results. In one such study on Pacific herring, Struhsaker (1977) demonstrated adverse effects of benzene, a toxic and highly soluble component of crude oil, on developing ova, embryos, and larvae. Short-term (48-hour) benzene exposures (800 parts per billion) decreased survival of ova in the gonads and also of resulting spawn. Struhsaker (1977) concluded that the spawning process is very sensitive in herring. Her study received considerable attention, partly because viability of ova in the gonads was reduced at parts per billion concentrations and also because no similar studies had been done. However, benzene effects may not be representative of effects, of exposure to the water-soluble fractions (WSF's) of crude oil, a more likely environmental scenario.

Even if not immediately toxic, hydrocarbons from an oil spill in the habitat of spawning herring may be accumulated by gonads and spawned eggs, affecting their marketability. Detection of hydrocarbons in the habitat may lead to displacement of mature adults to less suitable spawning locations.

The objectives of our study were to determine 1) the median concentration of Cook Inlet crude oil WSF that would kill 50% of prespawning adult Pacific herring exposed (LC_{50}); 2) the uptake and depuration of aromatic hydrocarbons in gonads, liver, and muscle of mature herring the survival and viability of eggs spawned from adult herring exposed WSF 4) the survival and viability of herring eggs and newly hatched (yolk-sac) larvae exposed to WSF; and 5) the survival, growth, and hydrocarbon accumulation of feeding larvae exposed to WSF or fed WSF-contaminated food.

METHODS

Test Animal Collections

Mature adult Pacific herring for exposures to WSF and for artificial spawning in egg tests were caught by a standard commercial purse seine near Juneau, Alaska, in 1984 and 1985. Fish were held in l,OOO-liter fiberglass tanks containing low salinity (15°/oo) running water for the first 5 days to reduce osmotic stress and minimize hemorrhaging due to scale loss. Salinity was gradually increased during the next 5 days to 30°/oo. Nets were never used to transfer fish because they cause massive scale loss; all fish were transferred in buckets with water. Mortality was less than 1% during holding periods.

Naturally spawned herring eggs were collected from the Kahshakes fishery near Ketchikan, Alaska, in March 1985, and from Auke Bay in early June 1984 and 1985. The substrate for collected eggs was the seaweed Fucus distichus. Larvae hatched from these eggs were used in the yolk-sac and feeding larvae experiments.

Production and Supply of WSF

The WSF of Cook Inlet crude oil was supplied by a flow-through WSF generator (Moles et al. 1985) that dripped 10 liter/minute of Auke Bay seawater through a continuously replenished 40-cm layer of Cook Inlet crude oil. The resulting WSF was collected from below the slick and pumped into head tanks after the dispersed oil floated out. The WSF and dilution water were delivered to test containers by appropriate manifold systems. Flow rates of WSF and of dilution water were held constant for each test group to maintain stable concentrations during the exposure periods. See Appendix for details of exposures of each life stage to WSF.

Chemical Analysis of WSF and Tissues

Aromatic hydrocarbon levels in WSF's of crude oil were determined by gas chromatography of methylene chloride extracts. All glassware and containers used in processing the samples were cleaned with detergent and water, then rinsed twice with distilled hexane. Samples of WSF (750 ml) were transferred from appropriate head tanks or exposure containers by small-bore siphon into a graduated cylinder. Methylene chloride used for the extractions was spiked with a known quantity of 1,3,5 triisopropyl benzene as an internal standard. Each sample was transferred to a l-liter separatory funnel and extracted by 25 ml methylene chloride (shaken by hand 1 minute and allowed to settle 5 minutes). This procedure was repeated for a total of 50 ml collected in glass vials and frozen. Analyses were performed by a Hewlett-Packard' model 5880A gas chromatograph equipped with a 12-m fused

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silica dimethyl silicone capillary column and a flame ionization detector. The N_2 carrier gas was held at 70 kPa inlet pressure. Temperatures of the inlet, outlet, and detector were held at 250°C. The injection volume was 1 u1. The column temperature program was as follows: 20°C for 2 minutes, followed by a temperature ramp of lO°/minute to a final value of 200°C. Concentrations of individual aromatic hydrocarbons "in the samples were determined by the internal standard method. Concentrations are reported in Table 1 in parts per million (ppm) aromatic hydrocarbons. Our WSF's were dominated by mononuclear aromatic hydrocarbons (about 95%); dinuclear aromatic hydrocarbons constituted about 5%.

Aromatic hydrocarbon levels in tissues were determined by gas chromatography of hexane extracts of tissue samples digested in sodium hydroxide. All implements and containers that came in contact with the samples were glass or Teflon and were cleaned with detergent and water, rinsed twice with distilled hexane, and baked at 440°C for 8 hours. Tissues were dissected from freshly killed herring and immediately frozen in glass jars with Teflon-lined lids. Samples were thawed just before processing and weighed in tared 500-ml screw-cap Teflon centrifuge tubes. To each tube, we added 10 ml 10N NaOH, 2 ml hexane, and 0.1 ml internal, standard (a known quantity of 1,3,5 triisopropyl benzene). The tubes were heated 3 hours at 70-8O°C, shaken well once each hour, then cooled to room temperature. Next, 5 ml 10% NaCl and 10 ml hexane were added to each tube, samples were centrifuged at 10,000 rpm for 10 minutes, and the supernatant hexane was aspirated into a 50-ml flask. This procedure was repeated three times for a total of

Table 1. --Distribution of individual aromatic hydrocarbons in the water-soluble fraction (WSF) of Cook Inlet crude oil and in tissues of mature female Pacific herring (Clupea harengus pallasi) after 6 days of exposure to 1.2 ppm aromatic hydrocarbons- (mean of three samples). Concentrations (in parts per million) were measured by capillary column gas chromatography. Asterisk indicates benzene was probably present in significant concentrations in tissues but eluted out with the solvent and could not be determined precisely.

Compound	Mature ovary		Muscle		WSF	
	Mean	%	Mean	%	Mean	%
Benzene	*		*		0.550	45.8
Toluene	9.443	20.4	2.965	6.7	0.377	31.4
Ethylbenzene	2.582	5.6	0.746	1.7	0.044	3.7
m- and p-xylene	6.399	13.8	1.584	3.6	0.107	8.9
o-xylene	3.596	7.8	1.064	2.4	0.065	5.4
Isopropylbenzene	0.879	1.9	0.441	1.0	0.008	0.7
1,3,5- trimethyl-benzene	0.733	1.6	0.449	1.0	0.005	0.4
n-propylbenzene	0.581	1.3	0.519	1.2	0.006	0.5
p-isopropyltoluene	0.256	0.6	0.161	0.4	0.004	0.3
Naphthalene	3.722	8.1	4.069	9.1	0.020	1.7
2-methylnaphthalene	2.787	6.0	6.264	14.1	0.025	2.1
1-methylnaphthalene	2.540	5.5	4.969	11.2	0.008	0.7
2,6-dimethylnaph-thalene	0.308	0.7	1.277	2.9	0.009	0.7
Total mononuclear aromatics	38.064	82.4	25.028	56.2	1.140	94.9
Total dinuclear aromatics	8.149	17.6	19.473	43.8	0.061	5.1
Mono- and dinuclear aromatics	46.213	100.0	44.501	100.0	1.201	100.0

30 ml collected. Two to five grams of Na_2SOL_4 were added and allowed to stand at least 10 minutes. Samples were decanted into 50-ml pear-shaped flasks, evaporated to 2 ml on a rotary evaporator, and layered onto a silica column (5 g silica in hexane in 0.8-mm column). The aliphatic fraction was washed out with 14 ml hexane, and the aromatic fraction with 18 ml hexane/methylene (4:l). The aromatic fraction was evaporated to 1 ml on a rotary evaporator, transferred to a 1.8-ml gas chromatograph vial with a Teflon-lined septum, and frozen until analyses. Analyses were performed by the same gas chromatograph used to analyze WSF's. The N_2 carrier gas was again held at 70 kPa inlet pressure, and inlet, outlet, and detector temperatures at 250°C; the injection volume was again 1 u1. The column temperature program was as follows: 30°C initial temperature for 1 minute, 5°C/minute temperature ramp for 2 minutes, lO°C/minute temperature ramp for 21 minutes, and final temperature at 250°C for 12 minutes. Concentrations of individual aromatic hydrocarbons were determined by the internal standard method.

Short-term uptake (O-4 hours) and depuration (O-24 hours) in larvae and prey were measured with liquid scintillation techniques using ^{14}C-labeled naphthalene. Labeled naphthalene dissolved in acetone was added to seawater and stirred 15-20 minutes before larvae or prey, Artemia nauplii, were added, The initial concentration of naphthalene averaged 0.33 (0.10-0.66) ppm. Herring larvae (20-100) were placed in 2 cm x 15 cm glass tubes with fine mesh-net bottoms. Tubes were drained and transferred quickly from clean water to labeled solutions. Artemia nauplii were washed onto plankton netting, then resuspended in the labeled solution. Labeled naphthalene was recovered by filtering

samples through paper or glass fiber filters. Filters were then cut into small pieces and digested 24-48 hours in 5 ml tissue solubilizer (Soluene). A l0-ml scintillation cocktail (Dimilume) was then added as diluent. A l-ml subsample in additional 10 ml Dimilume was counted with a liquid scintillator.

Adult Herring Toxicity, Uptake, and Depuration

The LCs_0 of adult herring was determined by an acute bioassay in a flow-through exposure system. Six groups of 12 fish were exposed to different WSF concentrations in six 600-liter tanks, and their survival was monitored. Exposure levels in all subsequent, sublethal tests (Appendix) were selected as percentages of the 12-day LC_{50} but are reported in parts per million.

Uptake of hydrocarbons by adult herring was measured in two series of flow-through WSF exposures representing short-term (2-day) and long-term (lo-day) exposures. Groups of adult herring were exposed either for 2 days to 0, 18, 43, 45, or 55% of the LC_{50} of WSF of Cook Inlet crude oil or for 10 days to 0, 14, 18, or 30% of the LCs_0.

In the depuration phase of this study, herring were exposed 4 days to 30% of the LCs_0 of WSF, then placed in -clean, flowing seawater for 14 days. All tests were in 800-liter fiberglass tanks with appropriate WSF and dilution water (flow rate, 8 liter/minute). Tissues (muscle, liver, testes, and mature and immature ovaries) were sampled during and after exposure to WSF and were analyzed for aromatic hydrocarbon content. Values reported are means of tissue samples from three fish.

At 2 and 12 days, ovaries of six fish from the 1.6 ppm exposure group and ovaries of six control fish were examined to determine whether ovarian tissues and egg development were affected by WSF exposure.

Gonads, Eggs, and Yolk-Sac Larvae

Adult fish with mature gonads were exposed to WSF's, before artificial spawning, for 2 or 12 days; eggs were exposed within 5 minutes after spawning and fertilization to WSF's for 2 or 12 days; and yolk-sac larvae were exposed within 24 hours after hatching to WSF's for various periods from 20 minutes to 6 days. Each treatment was replicated at least three times (Appendix).

All eggs were held in identical incubators that were 28 cm long by 15 cm diameter PVC pipe fitted with clear Plexiglas bottoms. Seawater (with WSF for exposures) was delivered at 50 ml/minute into these incubators by peristaltic pumps, and exited through four screened ports near the bottom of each incubator. Temperature fluctuations were minimized, and water levels maintained, by standing each incubator in a bucket that was bathed in circulating seawater. Slides of fertilized eggs were suspended within the incubators in plastic and stainless steel slide holders. The holders hung by monofilament line from an apparatus that raised and lowered the slides (six times per minute).

Eggs were observed daily. The day after hatching was first observed, we began counting eggs at 2-day intervals. Numbers of dead eggs, live unhatched eggs, and hatched eggs (empty egg cases) were recorded.

Gonad (Adult) Exposures

Mature adult herring were exposed either 2 or 12 days to WSF concentrations up to 1.6 ppm, 75% of the LC_{50}. From each WSF exposure, six females were used for artificial spawning and three males for artificial fertilization. Eggs from each female were squeezed (in a

single layer of two rows) onto two glass microscope slides (45-130 eggs/slide). Eggs were fertilized by dipping them into a suspension of fresh milt in seawater. These eggs were transferred to incubators and held until hatching.

Egg Exposures

Herring eggs obtained from unexposed adults by artificial spawning and fertilizing (as described above) were transferred on slides to incubators where they were exposed for 2 or 12 days to WSF concentrations of 0.8 to 5.3 ppm. After exposure, eggs were moved to incubators supplied with clean seawater and held until hatching.

Yolk-Sac Larvae Exposures

Naturally spawned eggs were held in clean, flowing seawater until hatching. Within 24 hours after hatching, yolk-sac larvae were exposed for 20 minutes to 6 days to WSF. After 6 days, unfed larvae absorb their yolk and begin to die of starvation.

The 6-day WSF exposures of yolk-sac larvae used the same type of incubator as did egg exposures. About 30 larvae were in each incubator, and three incubators were used for each concentration. Larvae receiving shorter exposures were held in 2-cm ID glass tubes that had nylon screen bottoms and were suspended in the incubators. Triplicate tubes, each holding eight larvae, were used in each concentration. Larvae exposed <6 days were placed in clean, flowing seawater for the remainder of the 6-day period. All larvae were checked after 24 hours of exposure or at the end of the exposure period or both and were checked again when they were 6 days old. They were counted and classified as swimming, not swimming, or dead.

Feeding Larvae and Oil-Contaminated Prey Exposures

Larvae hatched from Kahshakes and Auke Bay herring eggs were used in the feeding larvae exposures. Kahshakes eggs were incubated at 6-7.5°C in test chambers or in 600-liter fiberglass tanks supplied with flowing seawater until the majority hatched. Auke Bay eggs began hatching upon arrival in the laboratory. These larvae were maintained in 600-liter fiberglass tanks until they were transferred to test chambers, where all larvae were held 17-18 days before tests began.

Larvae were fed rotifers (Brachionus spp.), then were gradually shifted to the larger Artemia nauplii. These prey items have been used successfully to rear larvae in other studies (Struhsaker et al. 1974; Eldridge et al. 1977). Rotifers were reared on cultured Stephanoptera spp. in 20-liter plastic buckets. Rotifers were harvested every 2-3 days, washed onto a net, and resuspended in clean seawater or on algae in seawater. Artemia cysts were incubated for 3 days in 20-liter plastic buckets in seawater with vigorous aeration.

The test chambers were identical for exposures to WSF or oil-contaminated prey. Larvae were reared and tested in 40-liter black circular fiberglass chambers with concave bottoms. Seawater (28°/oo salinity) and food were introduced at 0.7 liter/minute through a diffuser chamber (height, 5 cm; diameter, 8 cm) designed to keep the bottom clean and create a gentle upwelling in the tanks. Overflow water exited through a 363-micron sleeve stretched over an 8-cm diameter standpipe protector at the surface center. In WSF tests, seawater was delivered to controls and seawater and WSF were delivered to exposure tanks from head tanks to Teflon mixing funnels at the head of each manifold. Overflow water filled an external water jacket that minimized

temperature fluctuations. A simulated die1 environment of a 16-hour photoperiod and 15-minute "sunrise" and "sunset" periods was maintained with fluorescent lights. Tanks were treated as needed with erythromycin to control bacterial growth.

In contaminated prey tests, some of the Artemia nauplii, which were harvested daily, were poured into conical nylon nets and exposed for 16 hours to WSF (O-6.3 ppm). Nauplii exposed to the lower WSF concentrations were fed directly to herring larvae, whereas nauplii in the highest WSF concentration were filtered and resuspended in clean seawater immediately before they were fed to the larvae.

Methods of observing larvae were identical in exposures to WSF and oil-contaminated prey. Mortality of feeding larvae was calculated indirectly; direct observations of mortality were impossible because of large tank volumes, high larval abundance, waste food, and rapid decomposition after death. Mortality was estimated from the total number of larvae per liter per tank that remained at the end of the experiment and was adjusted relative to controls. Alternatively, mortality also was estimated from bottom subsamples in tanks when most or all larvae stopped swimming. These two estimates agreed closely.

Feeding status was observed daily (without capture or disturbance) through the transparent integuments of the larvae (z = 96 per dose) at 24 predetermined points in each tank. The first larva sighted at or nearest each point was scored for presence or absence of food in its gut. Feeding status was also noted during collection for length and weight measurements. In WSF tests, recovery potential of larval feeding was measured daily by observing feeding frequencies after exposing larvae to 0.9 ppm WSF for 2, 4, or 8 days. For comparison, controls

were starved for 8 days and then their feeding recovery potential was measured.

Hydrocarbon uptake by predator and prey was measured by gas chromatography after 0, 1, 2, 4, and 16 days of exposure. Larvae were collected in early morning before the first feeding to reduce possible effects of contaminated prey on the analysis. Larvae were captured, filtered onto a fine mesh net, rinsed with seawater, weighed, and frozen until analysis. Artemia nauplii were collected for tissue analysis immediately after removal from the WSF. Nauplii were also sampled to determine the rates of hydrocarbon loss before and after they entered the herring test tanks. Artemia nauplii were filtered through a 100-micron plankton net, rinsed, and stored until analysis.

Short-term uptake (0-4 hours) and depuration (0-24 hours) in larvae and prey were measured using ^{14}C-labeled naphthalene as described earlier.

Measurement of larval growth required several steps. Twenty to 25 living larvae were collected weekly from each tank, placed in 20-ml glass scintillation vials, and preserved in Dietrich's solution. Samples were allowed to equilibrate at least 1 month before measurement. Preserved specimens were arranged on a petri dish and photographed with a reference scale. Negatives were enlarged 9 X, and each specimen was traced onto a sheet of paper. To determine notochord length, tracings were analyzed with a digital pad (calibrated to the reference scale). To correct length measurements for fixative effects, random samples of living larvae were measured directly with an ocular micrometer, photographed, and preserved for subsequent measurement.

Mathematical and Statistical Analyses

Median concentrations causing death (LC_{50}) and swimming or feeding inhibition (EC,,) were determined by logit analysis (Finney 1952; Berkson 1957) or by Spearman-Karber techniques (Hamilton et al. 1977). Correction for control response (Abbott 1925) was applied as necessary. Tukey's a posteriori multiple comparison tested differences between responses of dosed groups and controls, or Scheffe's test (Scheffe 1953) compared several groups simultaneously. Unless stated otherwise, all results are defined as statistically significant at $P < 0.05$. To control type I error in time series data, values were tested at 0.05/n, where n was the number of sets of comparisons in a series.

RESULTS

Adult Herring Exposures

The acute toxicity (LC_{50}) of the WSF of Cook Inlet crude oil to adult herring was 2.3 ppm after 2 days and throughout 12 days of exposure (Table 2). All mortalities occurred within the first 36 hours.

Uptake of aromatic hydrocarbons into tissues was rapid, and by 24 hours, concentrations were 20-35 ppm in herring exposed to 0.6 ppm (Fig. 1). Uptake was affected by exposure times and concentrations and by tissue types. Tissue uptake continued throughout the l0-day period (Fig. 11, indicating tissue equilibrium with the WSF concentrations takes longer than 10 days. Tissue concentrations were influenced directly by exposure concentrations (Fig. 2). Muscle tissue usually accumulated the highest concentrations (Figs. 1, 2); testes and liver tissues generally had the lowest concentrations. Immature ovarian

Table 2.--Sensitivity of several life stages of Pacific herring' (Clupea harengus pallasi) exposed to water-soluble fractions (WSF) C k Inlet crude oil for 2, 6, 12, or 21 days. Mortality of gonads and of eggs was defined as failure to hatch.

Stage	LC_{50} (ppm) ± 95% CI			
	2 days	6 days	12 days	21 days
Ripe adults	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	--
Gonads	>1.6	>1.6	>1.6	--
Eggs	>5.3	--	1.5 ± 0.1	--
Yolk-sac larvae	3.2 ± 1.0	2.3 ± 0.2	--	--
Feeding larvae	--	2.0 ± 0.2	0.9 ± 0.1	0.36 ± 0.1

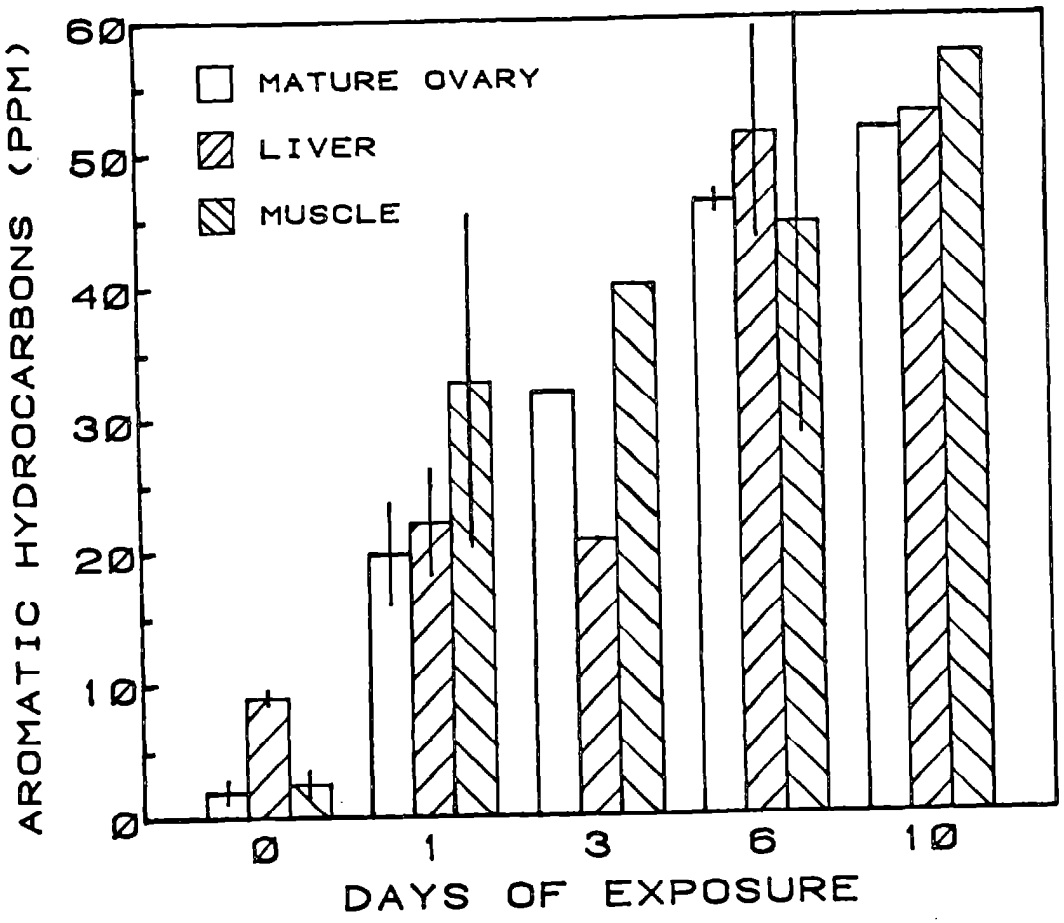

Figure 1.--Accumulation of aromatic hydrocarbons in tissues of adult Pacific herring (Clupea harengus pallasi) during 10 days of exposure 0.6-ppm water-solublefraction NSF) of Cook Inlet crude oil. Vertical bars = standard error.

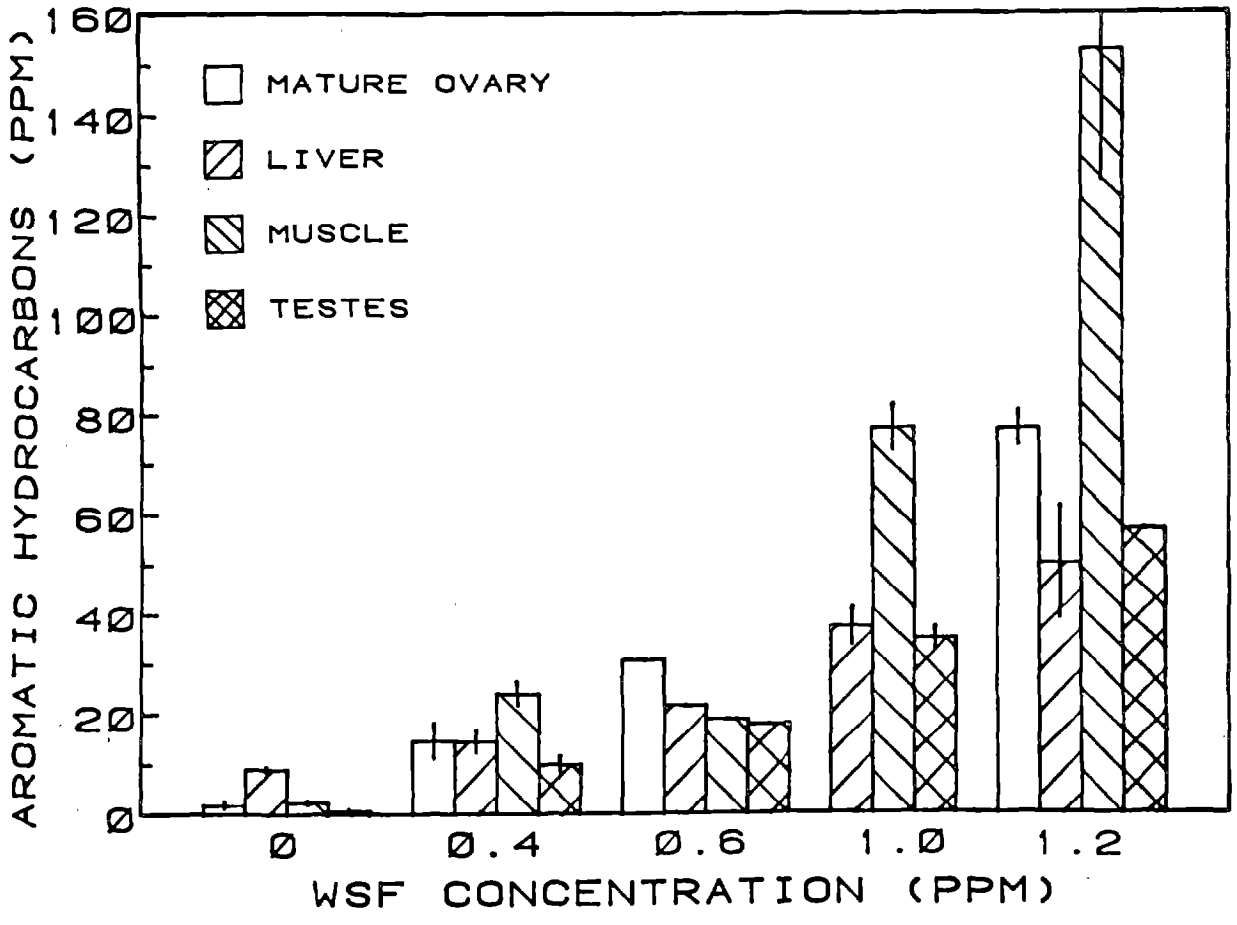

Figure 2.--Concentration of aromatic hydrocarbons in adult Pacific herring (Clupea harengus pallasi) tissues after 48-hour exposure to different of water-soluble fraction (WSF) of Cook Inlet crude oil. Vertical bars = standard error.

tissue consistently accumulated more than did mature ovarian tissue (Fig. 3). After 48 hours, immature ovarian tissue from fish exposed to 1.2 ppm WSF accumulated 120 ppm compared with 75 ppm for mature ovaries.

The distribution of aromatic hydrocarbons in the WSF was dominated by monoaromatic hydrocarbons (95%), and concentrations of larger compounds declined in approximate proportion to their solubilities (Table 1). The distribution of aromatic hydrocarbons in tissues was not as simple. Monoaromatics dominated in the ovarian tissue, but in muscle tissue, the concentrations of diaromatic compounds were nearly equal to monoaromatics. Concentration differences between tissues and WSF demonstrated that lipophilic tissues can bioconcentrate larger aromatic hydrocarbons, even though their proportions in the WSF are relatively low. Polyaromatic hydrocarbon concentrations were highly variable in tissues of exposed and control fish; therefore, quantification of uptake in the experimental animals, was impossible. However, because polyaromatics have low water solubility, virtually none occurred in the WSF. All the polyaromatic hydrocarbons in herring tissues almost certainly were of biogenic origin. Differences in distribution of aromatic compounds between ovarian and muscle tissues were probably caused by differences in the lipid content of the tissues, in the access to the tissues by the blood, and in the ability of the tissues to metabolize and remove hydrocarbons.

Depuration was rapid in muscle and ovarian tissues during the first 24 hours in clean water; about 50% of the hydrocarbons were lost (Fig. 4). Depuration was much slower after the initial 24-hour period. Two more days were required to lose 50% of the remaining hydrocarbons, and another 50% was lost between Day 4 and 7. By Day 14, hydrocarbon

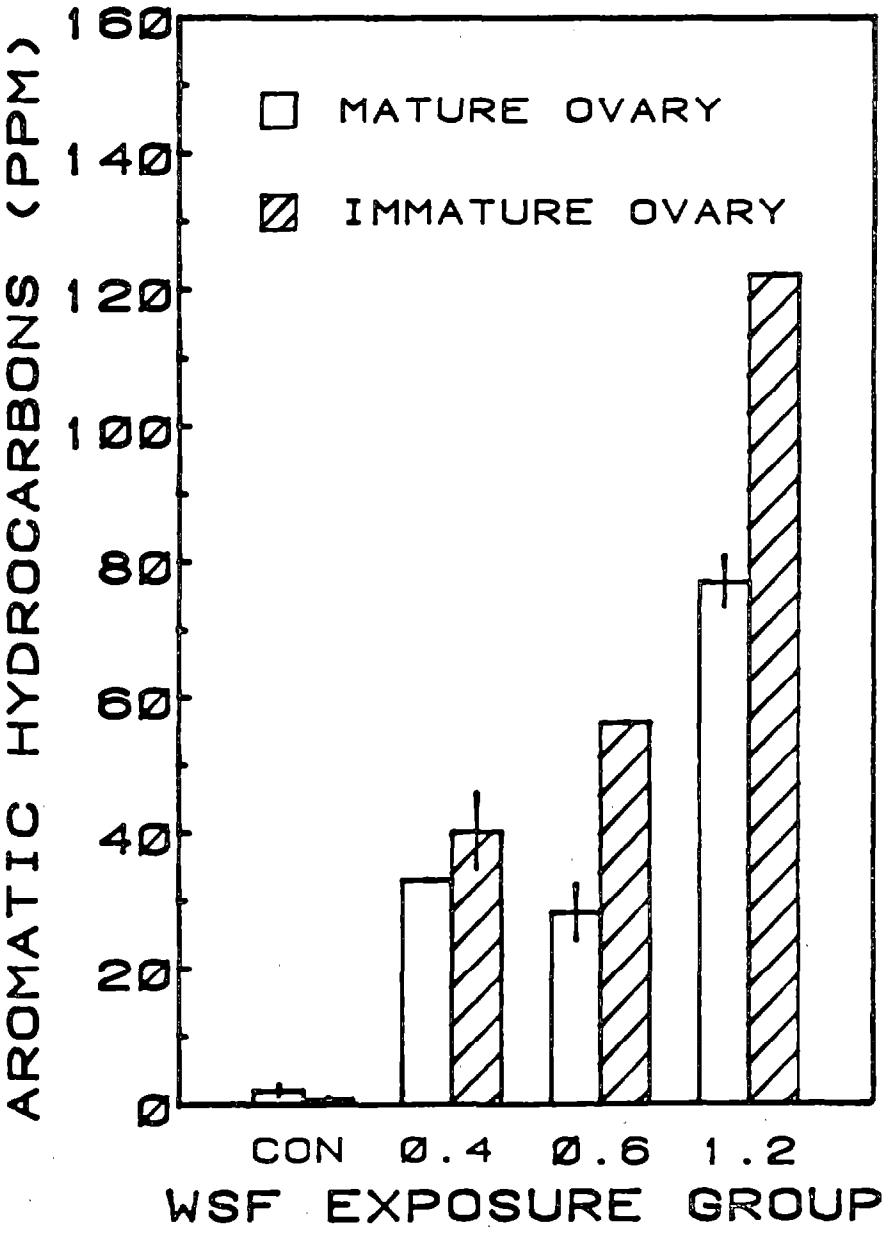

Figure 3. --Accumulation of aromatic hydrocarbons in mature and immature ovaries of prespawning Pacific herring (Clupea harengu; pailasi): CON = control group; vertical bars= standard error.

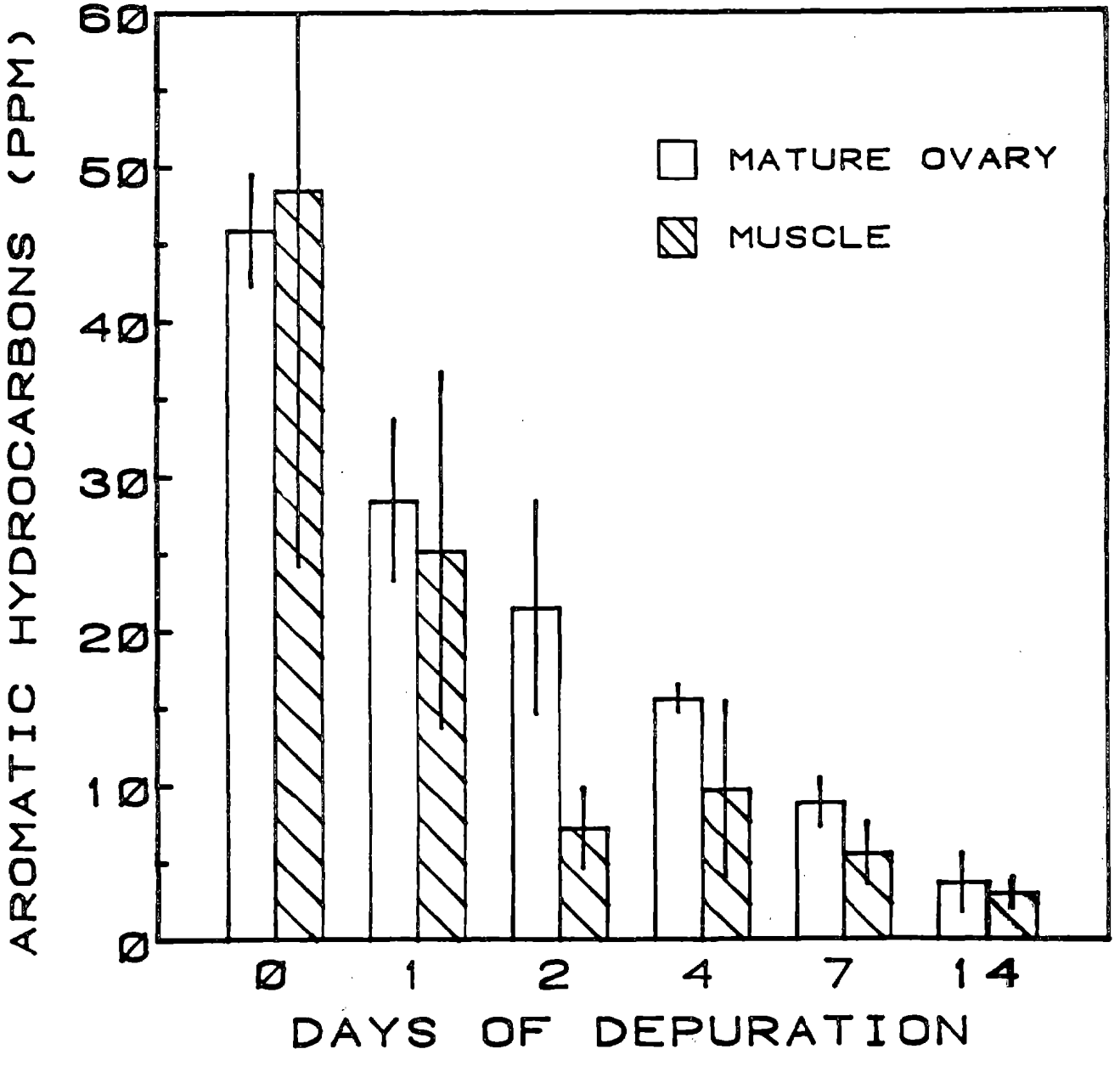

Figure 4.--Depuration of aromatic hydrocarbons from adult Pacific herring (Clupea harengus pallasi) previously exposed 96 hours to 0.6 ppm water-soluble fraction (WSF) of Cook Inlet crude oil. Vertical bars = standard error.

levels in muscle and ovarian tissues were not significantly higher than levels in tissues of unexposed fish.

WSF exposures to prespawn adult herring did not damage ovaries; all exposure groups appeared normal. There was no evidence of increased number of dead eggs, of atretic follicles, or effect on egg sizes.

Gonad (Adult), Egg, and Yolk-Sac Larvae Exposures

There was no effect on survival of spawn from oil-exposed, mature adults (Fig. 5). Hatching success of eggs spawned from adult herring that were exposed to WSF for 12 days varied between 78 and 85% and did not differ significantly from controls. The highest concentration tested was 1.6 ppm, 75% of the LCs_O for adult herring (85%' of the herring survived at this exposure). If the adults survived oil exposure, hatching rates were normal.

Relatively long-term (12-day) WSF exposures of artificially spawned eggs affected hatching rates (Fig. 5), whereas short-term (2-day) exposures did not. Eggs exposed 2 days to WSF concentrations as high as 5.3 ppm hatched at rates between 78 and 81%, and rates were not significantly different between exposed or control eggs. In contrast, eggs exposed 12 days hatched at rates between 0 and 84% and had an LCsa of 1.5 ppm aromatic hydrocarbons (Table 2).

Yolk-sac larvae exposed to WSF ≤6 hours were less affected than the larvae exposed >16 hours. Larvae exposed <6 hours to 6.1 ppm aromatic hydrocarbons (the highest concentration tested) survived. Six hours of exposure to 4.8 ppm caused swimming failure in 57% of larvae and 6.1 ppm caused 71% swimming failure, but the effect was transitory; larvae regained swimming ability within 24 hours after exposure. Larvae

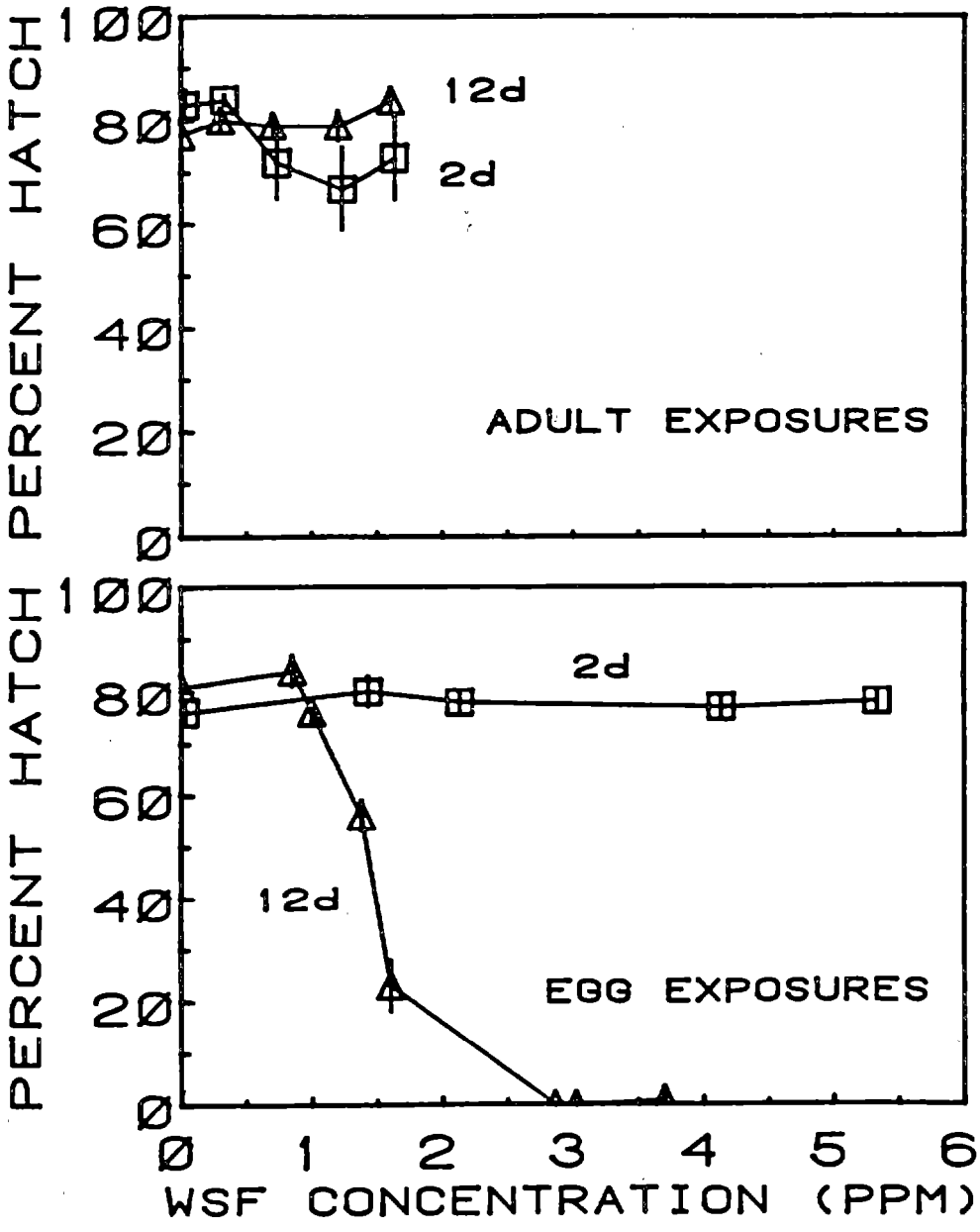

Figure 5.--Percent hatch of Pacific herring (Clupea harengus pallasi) eggs from adults exposed 2 or 12 days to water-soluble fraction (WSF) (1.5 ppm, 75% of the LC_{50} 3 was the highest WSF concentration used in the adult exposures), and percent hatch of artificially spawned and fertilized eggs exposed 2 cr 12 days to WSF. Vertical bars = standard error.

exposed 16 hours had an LC_{50} of 2.8 ppm (95% CI = t0.6), and larvae exposed 6 days had an LC_{50} of 2.3 ppm (95% CI = +O.2) (Fig. 6).

Feeding Larvae and Contaminated Prey

Survival, feeding, and growth of herring larvae were affected by direct exposure to WSF. Feeding the herring oil-contaminated prey had less influence than did direct exposure to aromatic hydrocarbons; larval survival was eventually reduced by the most contaminated prey, but feeding rates and growth were little affected.

Direct exposure to WSF caused high larval mortality. The LC_{so} dropped from 1.85 ppm WSF on Day 7 to 0.36 ppm on Day 21 and remained unchanged through Day 28 (Fig. 7). WSF concentrations >0.8 ppm were fatal within 3 weeks. Herring fed contaminated prey had higher survival rates than did larvae exposed directly to WSF. The most contaminated prey (59 ppm average tissue concentration) caused a significant ($p = 0.94$) fraction, 51%, of the larvae to die relative to controls (Fig. 81, and after 3 weeks, their effects were equivalent to effects seen in the 0.3 ppm WSF exposure. Larvae that survived the indirect exposures appeared robust. Survival in control groups was above 90% throughout the study '(Fig. 8).

Larval swimming was -inhibited before death occurred by exposure to WSF (Fig. 7). Swimming was inhibited more rapidly as doses increased. For example, larvae stopped swimming and settling occurred in about 3.5 days at 1.7 ppm WSF, whereas settling occurred in about 6 days at 0.9 ppm. Mortality and swimming inhibition were inseparable after larvae had been exposed >14 days (Fig. 7). Eating oil-contaminated prey did not cause noticeable swimming inhibition in larvae. The

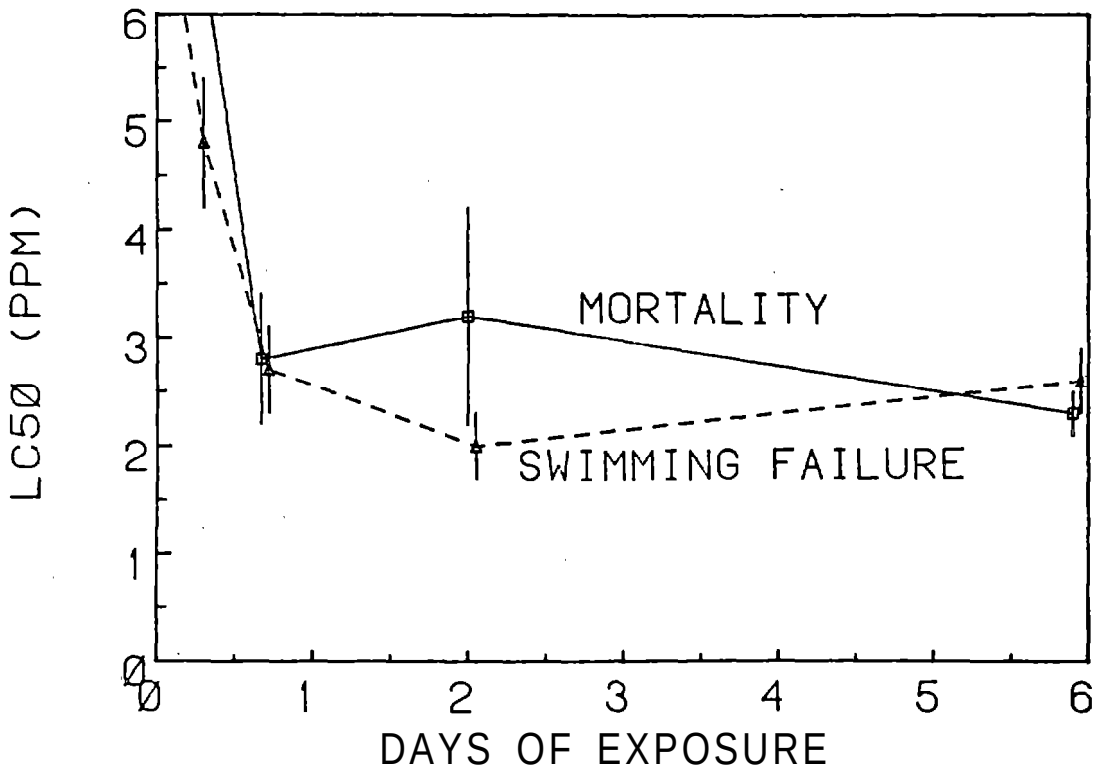

Figure 6.--Mortality (LC,,) and swimming ability (EC,,) of yolk-sac Pacific herring (Clupea harengus pallasi) larvae exposed to water-solubmactmF) of Cook Inlet crude oil. Vertical bars = 95% confidence intervals

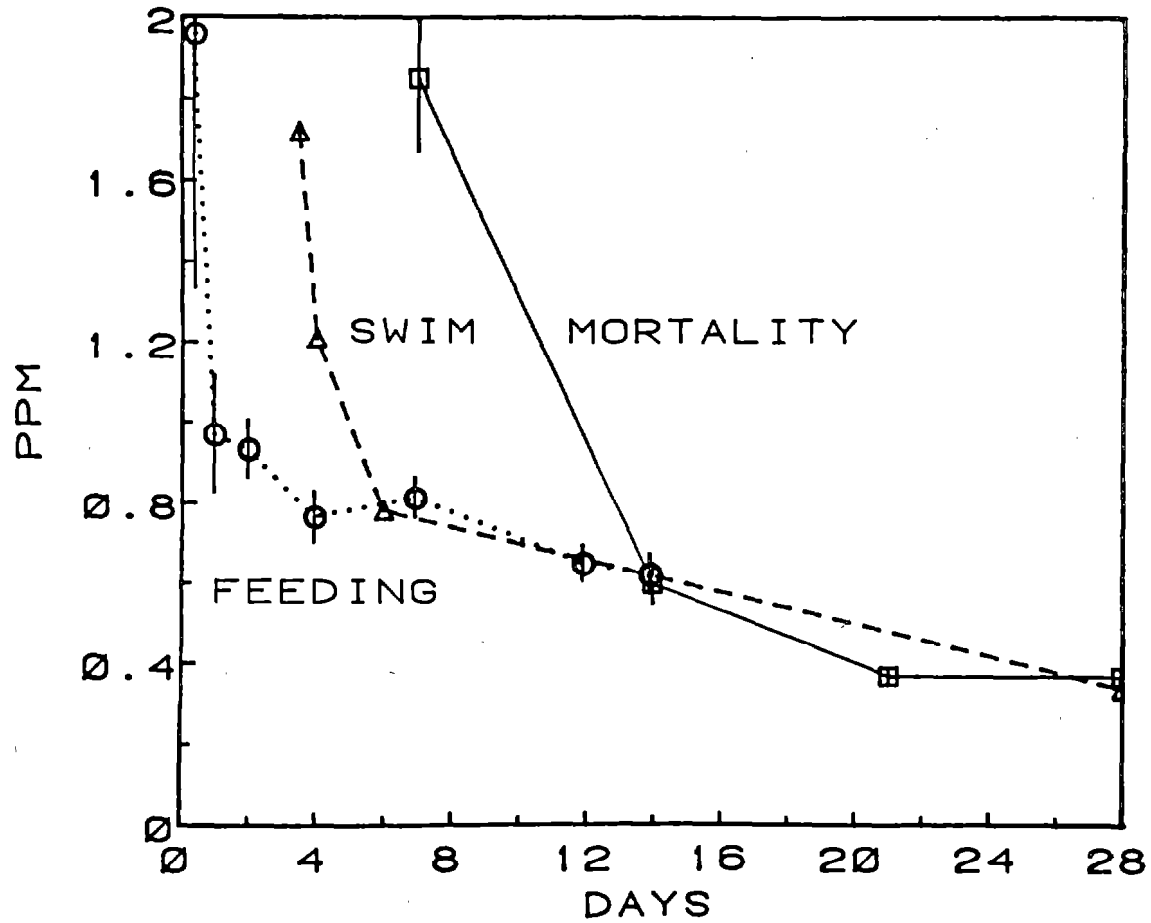

Figure 7.--Mortality (LC_{50}) and swimming ability and feeding frequency (EC& of feeding Pacific herring (Clupea harengus pallasi) larvae exposed to water-soluble fraction (WSF) Cook Inlet crude oil. Vertical bars = 95% confidence intervals.

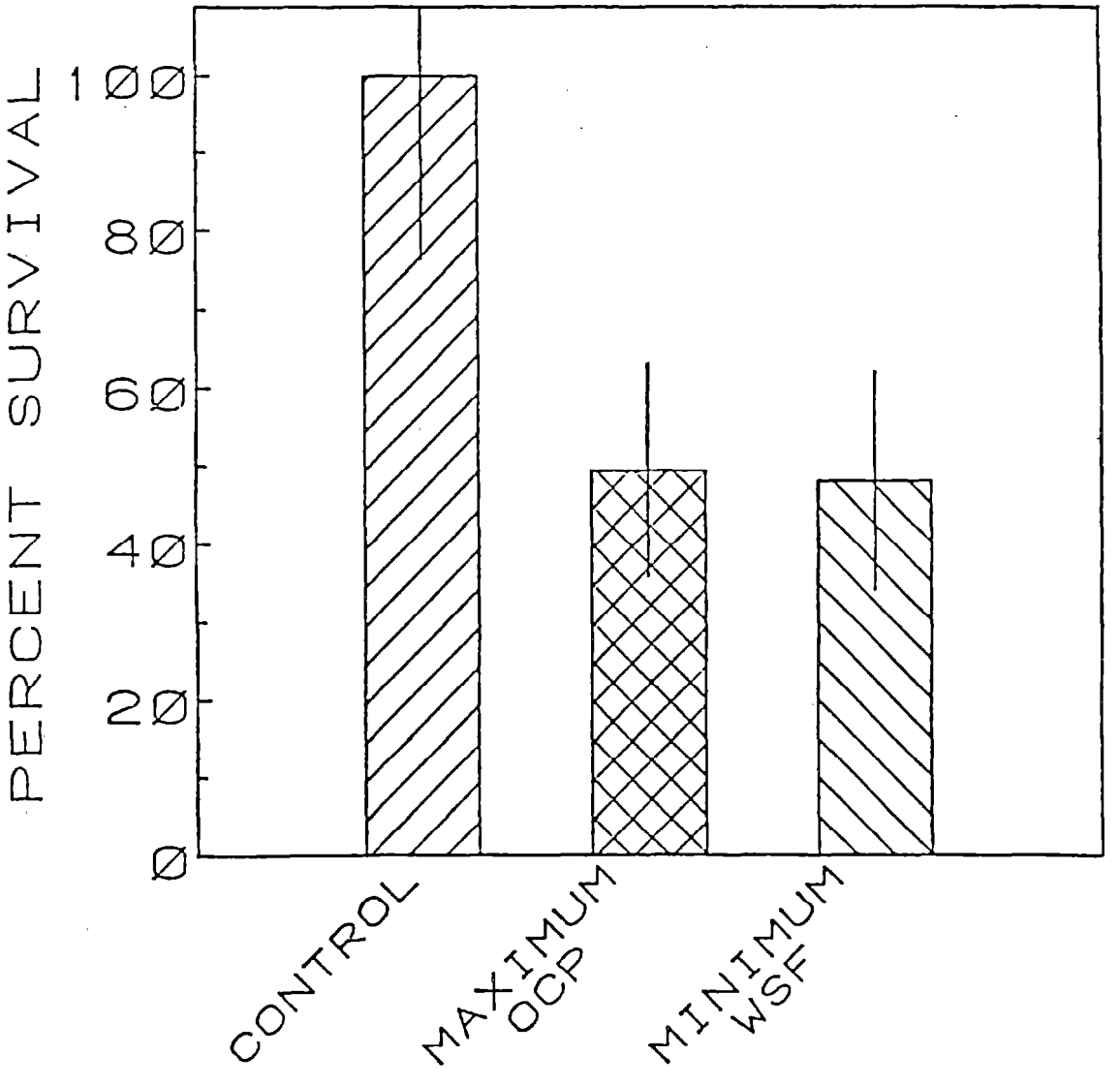

Figure 8.--Survival of Pacific herring (Clupea harengus pallasi) control larvae and of larvae exposed 16 days to maximum oil-contaminated prey (OCP) doses (59 ppm average tissue concentration) or to minimum WSF doses (0.3 ppm). Vertical bars = kl standard error.

swimming ability of the prey, Artemia nauplii, was reduced by exposure to highest WSF concentrations.

Feeding frequencies (EC_{50} s) were significantly reduced by exposure to WSF before swimming ability was reduced or significant mortality occurred (Fig. 7). Concentrations causing half the larvae to cease feeding decreased to 2.0 ppm WSF after 9 hours, 1.0 ppm on Day 1, 0.8 ppm on Day 4, and 0.7 ppm on Day 12. Feeding frequencies at 0.9 ppm decreased significantly within half a day, continued to decline rapidly (Fig. 9, and ceased within 10 to 14 days of exposure. Feeding frequencies decreased at concentrations as low as 0.5 ppm. Contaminated food had no effect on feeding frequencies; daily feeding rates never differed significantly from controls (Fig. 9).

Larvae that survived exposure to 0.9 ppm WSF resumed feeding when transferred to clean water, but increased exposure times sharply reduced survival (Fig. 10). Feeding frequencies, measured after 2, 4, and 8 days of exposure, increased rapidly once WSF dosing stopped (Fig. 10). The longer the larvae were exposed to WSF, the more rapidly survivors' tended to resume feeding. However, increases in the exposure times sharply reduced larval survival ($Y^{\frac{1}{2}} = 10.01 - 0.71x$; $r^2 = 0.89$; Fig. 10). After 8 days of exposure, only 16% of the larvae survived; of these, 89% resumed feeding. Reductions in feeding frequencies after 12 days of exposure correlated with larval mortality measured at 3 weeks ($r^2 = 0.94$, $F = 222$, 1,15 d.f., $P < 0.001$; Fig. 11).

Growth (notochord length) in feeding herring larvae was strongly inhibited by WSF exposure, but not by indirect exposure (Fig. 12). After 1 week of exposure, concentrations >0.7 ppm reduced growth rate.

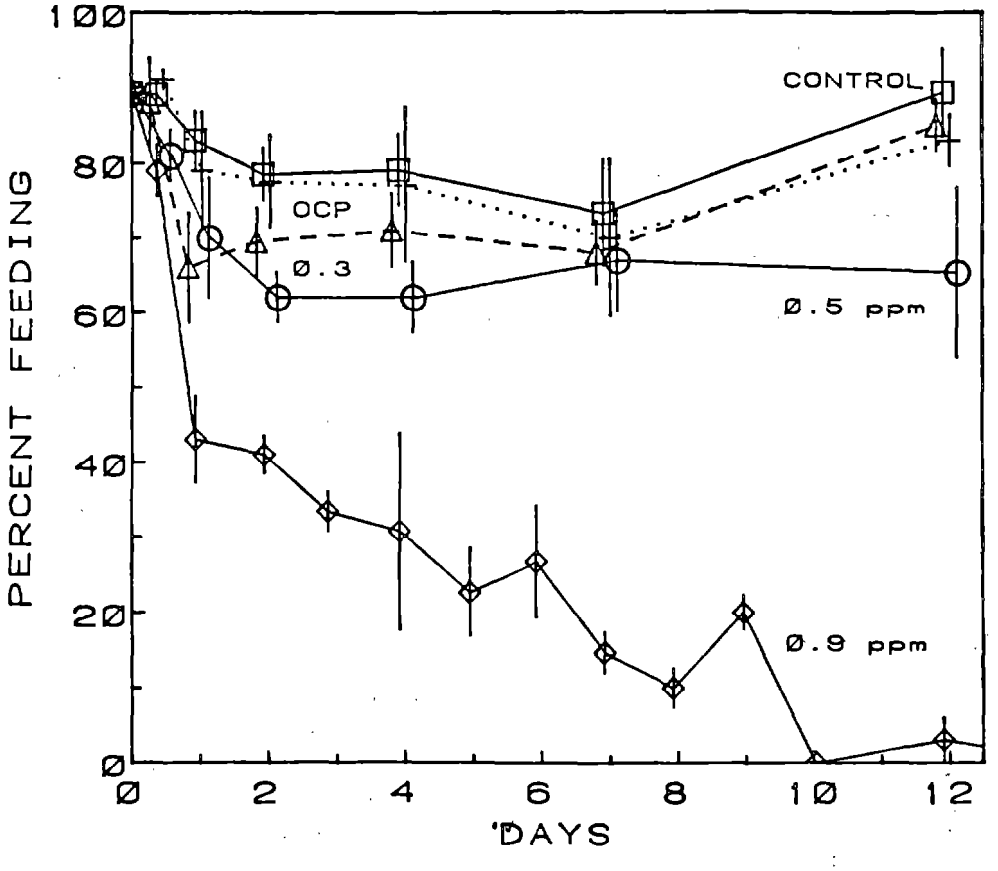

Figure 9. --Feeding frequency of Pacific herring (Clupea harengus pallasi) larvae exposed to water-s-fraction (WSF) of Cook Inlet crude oil and larvae fed oil-contaminated prey (OCP). Vertical bars = standard error.

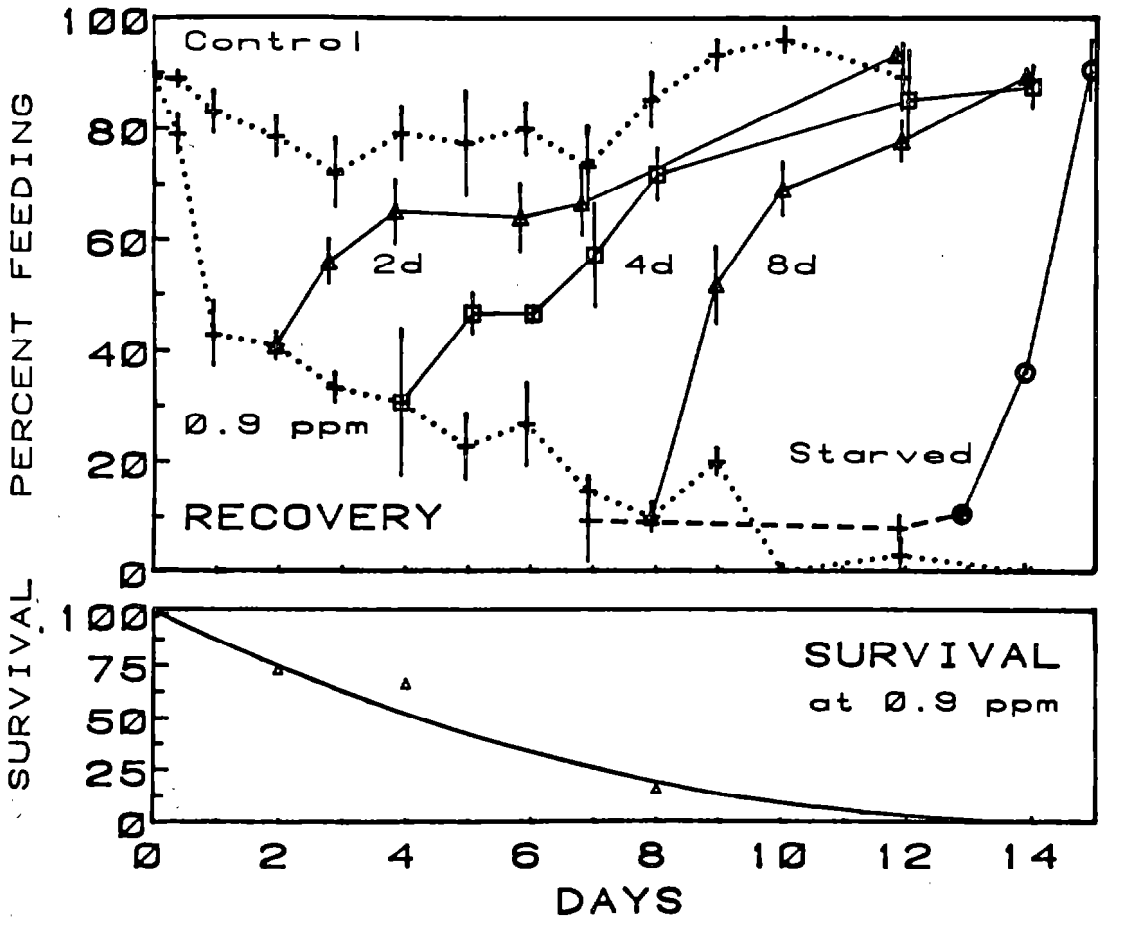

Figure 10. --Recovery of feeding response and survival of Pacific herring (Clupea harengus pallasi) larvae after exposure to 0.9 ppm water-solubl fraction for various time periods. Vertical bars = standard error.

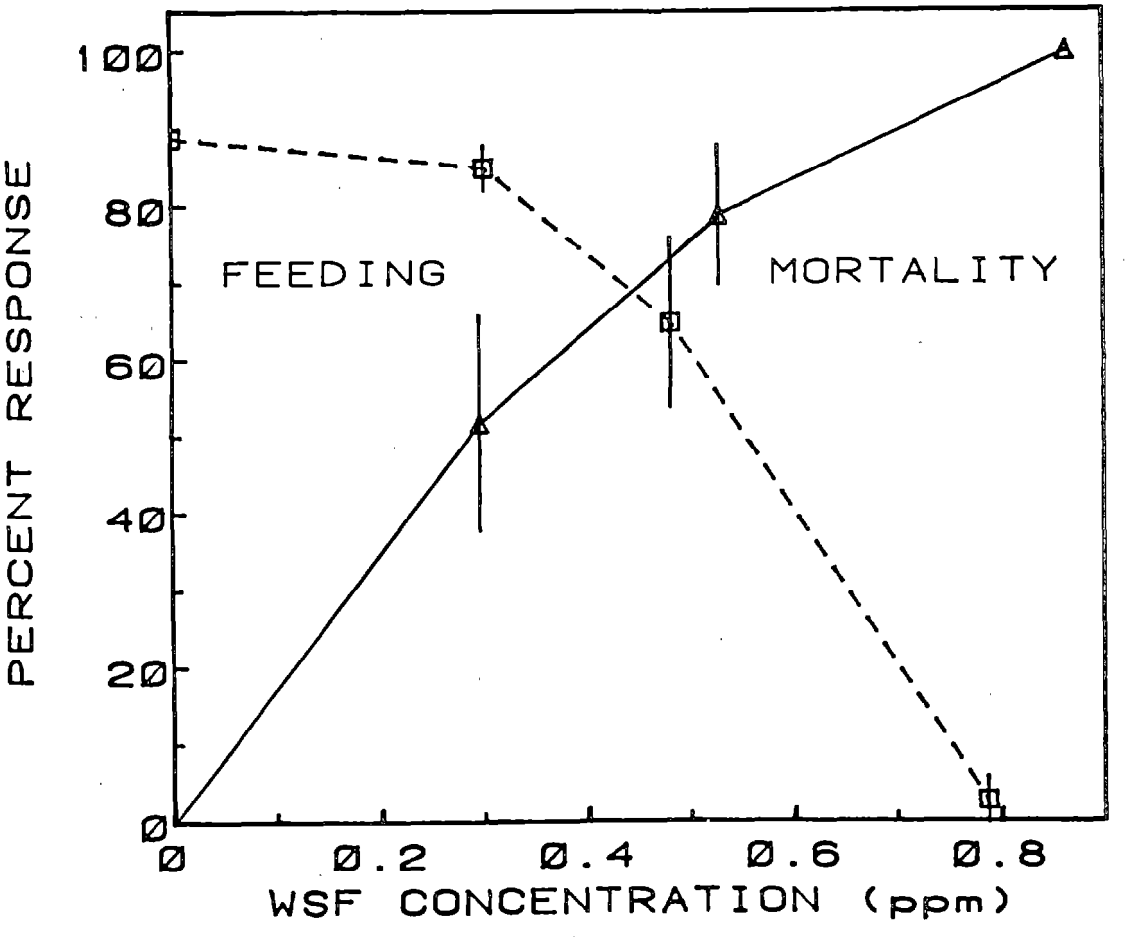

Figure 11. --Relationship of feeding frequency and mortality in Pacific herring (Clupea harengus pallasi) larvae. Feeding frequencies were determined after 12-day exposure to water-soluble fraction (WSF), and mortality, at 21-day exposure. Vertical bars = standard error.

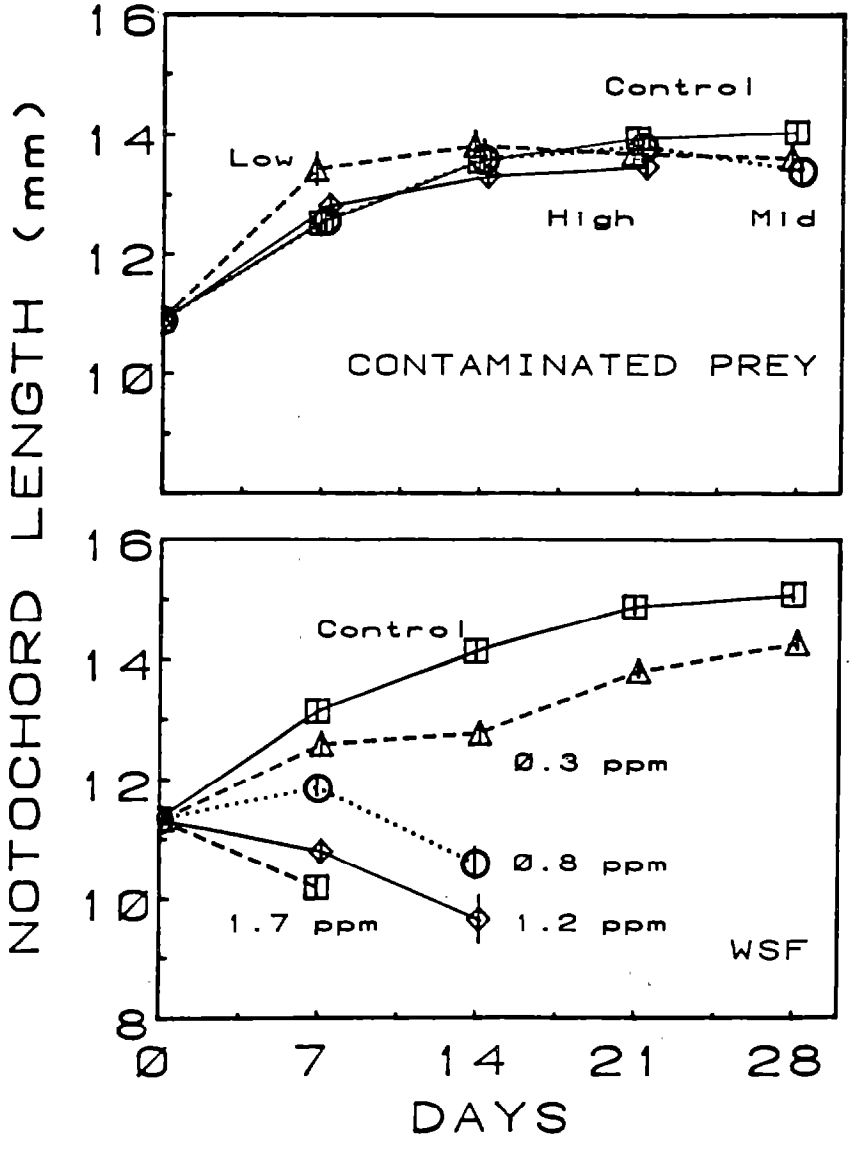

Figure 12.--Influence of water-soluble fraction (WSF) exposure and oil-contaminated prey (OCP) on growth of Pacific herring (Clupea harengus pallasi) larvae. Vertical bars = standardor.

After 2 weeks, the lowest (0.3 ppm) WSF dose also caused significant reductions. Growth became negative at concentrations >1.2 ppm during the first week and at 0.8 ppm during the second week. At these concentrations, mortality was 100% within 3 weeks.

Herring larvae and their prey exchanged hydrocarbons rapidly with surrounding water. Herring larvae accumulated radio-labeled naphthalene faster than did Artemia nauplii, but the nauplii retained a greater proportion of the ^{14}C-naphthalene (Fig. 13). Tissue concentrations of larvae reached equilibrium after 1 hour, and of Artemia nauplii, 10 hours. Depuration of ^{14}C-naphthalene was also rapid (Fig. 13). However, a substantial fraction (18%; standard error = 0.6) of the carbon-14 did remain in Artemia nauplii but not in herring larvae (2%; standard error = 0.3) after 24 hours. This result indicates naphthalene was either trapped in lipid-rich tissues or metabolized and permanently incorporated in nauplii tissues. Virtually no carbon-14 was permanently incorporated into larval fish tissues.

Herring larvae accumulated hydrocarbons when exposed to WSF, but larvae fed contaminated prey did not accumulate hydrocarbons to detectable levels. Bioaccumulation of WSF was low (0.9-2.1). For example, larvae exposed to 0.3 ppm WSF accumulated 0.3 ppm aromatic hydrocarbons.

Although larvae feeding on contaminated prey had hydrocarbon concentrations that were not detectable, the concentrations in prey were measured easily. In the most contaminated food group, aromatic hydrocarbon concentrations in Artemia nauplii tissues averaged 59 ppm at

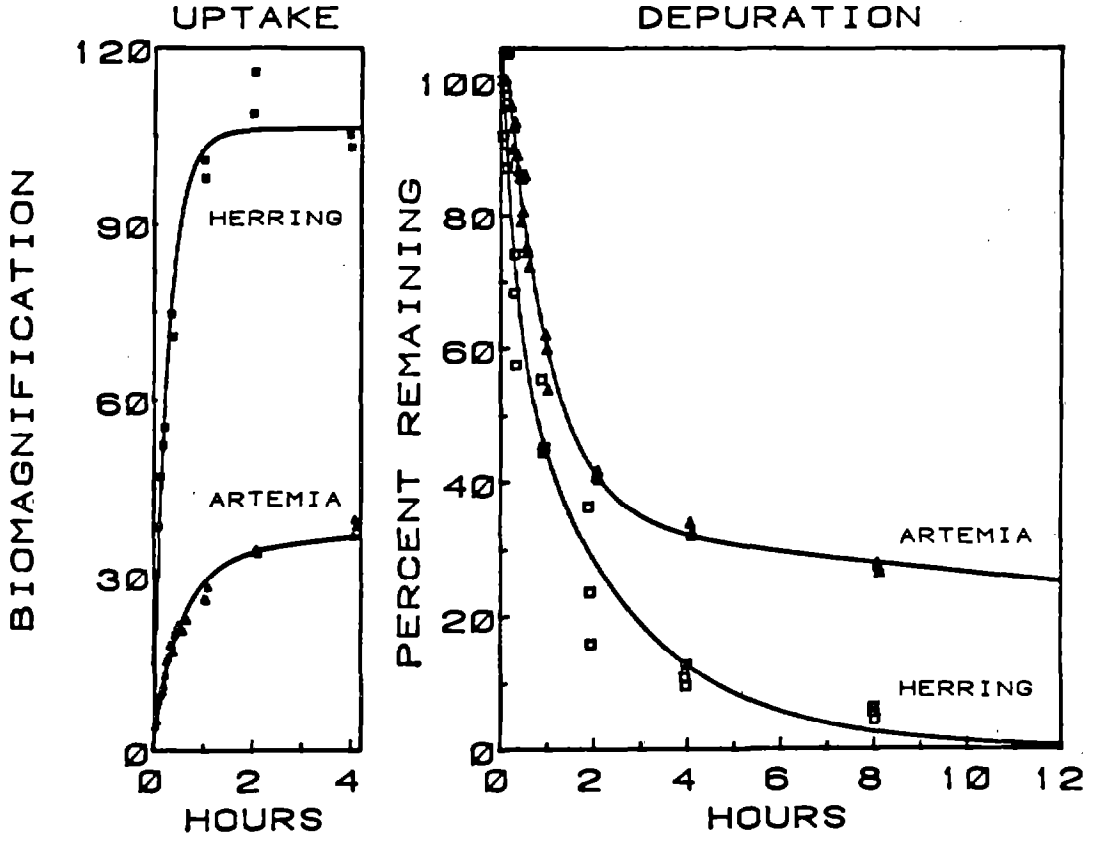

Figure 13. --Uptake and depuration of radio-labeled naphthalene by Pacific herring (Clupea harengus pallasi) larvae and their prey, Artemia nauplii. Vertical bars = standard error.

the end of exposure (Table 3) but dropped rapidly during holding and circulation in experimental tanks (Fig. 14). Average tissue concentrations at the time of consumption could not be determined precisely because of multiple feeding times (six per day), variable larval hunger, and imprecise knowledge of nauplii residence time (<8 hour). At the time of consumption, we estimated that 2-100% of the aromatic hydrocarbons remained in nauplii tissues and averaged about 14% (8.6 w).

Table 3.--Concentrations of aromatic hydrocarbons (mono- and diaromatic) in Artemia nauplii that were fed to Pacific herring (Clupemngus pallasi) larvae. WSF = water-solumactlon of Cook Inlet crude oil; S.E. = standard error.

Dose group	n	WSF exposure (ppm)	Tissue concentration (mean ppm ± S.E.)	Biomagnification
Control	6	0.00	0.0 ± 0.10	-
Low	5	0.28	1.4 ± 0.53	5.0
Medium	5	0.82	6.8 ± 0.63	8.3
High	14	6.29	58.8 ± 9.54	9.4

DISCUSSION

Adult Herring

All tested herring tissues (muscle, liver, testes, and immature and mature ovaries) accumulated aromatic hydrocarbons, but continued accumulation, without reaching equilibrium, through Day 10 suggests that higher concentrations would be reached if exposure times were longer. All tissues, including ovaries, depurated hydrocarbons, but depuration after 24 hours was slow.

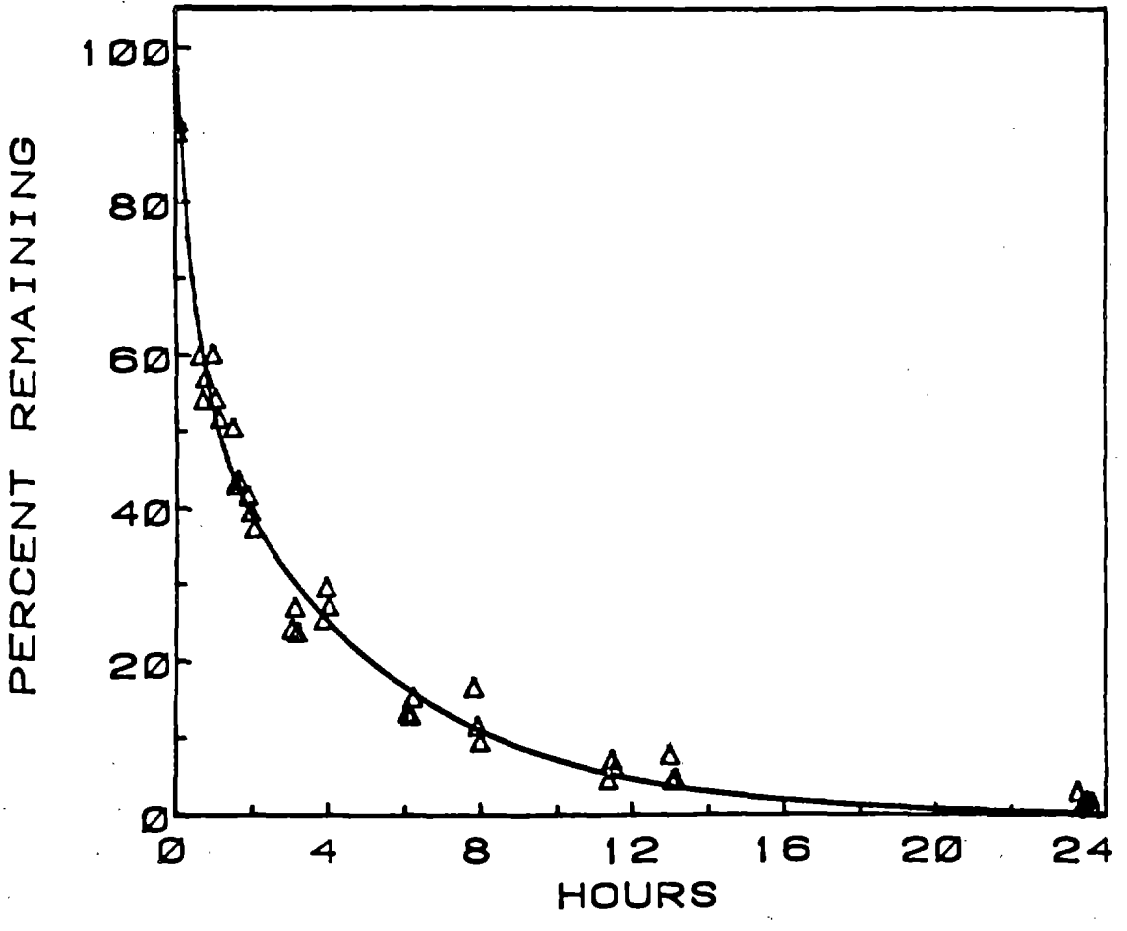

Figure 14.--Artemia nauplii depuration of radio-labeled naphthalene before they were fed to Pacific herring (Clupea harengus pallasi) larvae. Vertical bars = standard error.

This pattern of continuous accumulation of hydrocarbons through Day 10 is different from that in pink salmon (Oncorhynchus gorbuscha) fry exposed to WSF of Cook Inlet crude oil (Rice et al. 1977). Pink salmon reach equilibrium rapidly (24-48 hours), and their tissue concentrations return to background levels by 96 hours, even while the fish remain in the WSF. Pink salmon actively metabolize and excrete hydrocarbons at rates faster than incoming rates after 24 hours. In adult herring, prolonged accumulation of hydrocarbons is probably the result of two primary factors: the high lipid concentrations in their tissues and the suppression of aromatic hydrocarbon metabolism during the reproductive process. The length of time without tissues reaching equilibrium concentrations. suggests that hepatic aryl hydrocarbon hydroxylase (AHH) activity was low. Findings of other investigators (Walton et al. 1978; Spies et al. 1985) suggest that suppression of basal AHH activity in prespawn marine fish is a common occurrence, and that AHH is not inducible during late stages of gonad maturation (Walton et al. 1983). Adult herring in the prespawning condition have not been actively feeding for weeks, and their ability to metabolize and excrete aromatic hydrocarbons may be different during the summer when they are actively feeding and not in a reproductive mode.

Although aromatic hydrocarbons accumulated in herring tissues, particularly developing ovarian tissue, survival of ova and hatching success of spawn were not adversely affected. Our results contrast with those of Struhsaker (1977) who found that ova survival and hatching success decreased after 48-hour exposures to benzene. The two studies had two major differences: types of toxicants and stocks of fish.

The most obvious difference between the two studies--toxicants used--probably did not cause the differences in survival of ova and hatching success of spawn. Struhsaker (1977) exposed adult herring to 0.8 ppm benzene, and our WSF dose of 1.6 ppm aromatic hydrocarbons contained about 0.7 ppm benzene. In our study, 12 days of exposure should have been long enough for adverse effects on ova and on hatching success to become evident.

The factor most responsible for the differences in results between the two studies is probably the source of the two stocks of herring. Struhsaker (1977) captured herring in San Francisco Bay, where pollutant levels from a wide variety of sources are considered to be threatening to some fish and shellfish populations (Whipple et al. 1981). Hatching success of herring eggs from adults collected in San Francisco Bay was about 25% less than that of eggs from stocks collected outside the bay (Struhsaker et al. 1974). This difference was attributed to the greater amounts of accumulated pollutants in the bay (Struhsaker 1977). Herring in our study were collected in pristine waters in remote areas of southeastern Alaska, and the viability of the spawn was consistently high. The poor viability of herring eggs in the study by Struhsaker (1977) probably resulted from the combined effects of benzene exposure and the pollutant load accumulated from San Francisco Bay.

Gonads, Eggs, and Yolk-Sac Larvae

If mature adult herring survive WSF exposure, their eggs survive and develop. For 12 days, at least, the body of the adult protects its gametes from injury. Spawned eggs resist damage by WSF for at least 2 days but are killed by exposure to concentrations >1.5 ppm for over

12 days. In contrast, yolk-sac larvae can be killed in relatively short exposures, only 16 hours at 12.8 ppm.

Other studies on herring eggs exposed to WSF of crude oil or to selected monoaromatics have shown increased mortality and increased frequency of abnormalities in the embryos and larvae (Linden 1978; Smith and Cameron 1979; Vuorinen and Axell 1980). These crude oil studies were static, and fouling complications caused by bacteria and diatoms were reported by Smith and Cameron (1979). We did not observe any abnormalities, but did no extensive examination. Abnormalities may have become evident if the tests had ended after yolk absorption occurred rather than at hatching.

Feeding Larvae and Contaminated Prey

Larval survival was reduced by contaminated food and by direct WSF exposure, which was much more toxic. Highly contaminated food (about 59 ppm average tissue concentration) caused about half the herring larvae to die in 21 days, but the surviving larvae appeared robust. Contaminated food did not affect larval feeding and swimming. The vigor of larvae fed contaminated prey may have been due to a prophylactic effect of the oil, i.e., low concentrations of oil may have improved tank hygiene by killing bacteria and ciliates.

Although herring larvae were killed by low WSF concentrations (21-day LC_5 0.36 ppm); they reacted more slowly to the toxicant than did another pelagic species, walleye pollock (Theragra chalcogramma), in a similar study by Mark G. Carls at the Auke Bay Laboratory (unpubl. data). Herring larvae exposed to 1.7 ppm did not cease swimming for 3-4 days. In contrast, the swimming ability of T. chalcogramma larvae exposed to 1.2 ppm WSF of oil was reduced within 4 hours.

Food contamination probably contributed to WSF effects during direct exposure, but direct toxicity was more significant. Larvae exposed to WSF in our study were also exposed to contaminated prey because uncontaminated prey accumulated hydrocarbons rapidly in tissues after entering dosing chambers as food. Prey remaining several hours in the lowest (0.3 ppm) WSF dose would probably accumulate hydrocarbon concentrations equivalent to the lowest contaminated prey dose. No WSF exposure concentrations were great enough for prey to accumulate concentrations as high as the maximum (6.3 ppm) contaminated prey doses. Maximum contaminated prey doses caused mortality equivalent to minimum WSF doses.

Direct exposure to WSF reduced larval feeding, but the mechanism is obscure. Larvae exposed to 0.9 ppm WSF often continued to exhibit strike behavior. Strike speed and agility appeared uninhibited, Reductions in feeding were found in herring larvae (Struhsaker et al,. 1974) and in striped bass (Morone saxatilis) larvae exposed to benzene (Eldridge et al. 1981), in pink salmon juveniles exposed to naphthalene and WSF (Moles and Rice 1983), and in adult coho salmon (Oncorhynchus kisutch) exposed to WSF (Folmar et al. 1981). In our study, feeding reductions were not permanent; surviving larvae rapidly resumed feeding in clean water. Feeding frequencies were sensitive, easily measured indicators of toxicant effect. Changes in feeding predicted mortality and growth.

Starvation alone does not explain WSF-induced mortality. Although feeding rates declined rapidly in WSF treatments, larval survival (40%) after 8 days of starvation was much better than after direct exposure to

0.9 ppm WSF (16%) for the same time period. Furthermore, starved larvae resumed feeding more rapidly than did WSF-exposed larvae.

Growth of control larvae was not a linear function, but leveled off at about 1.4 cm. At the higher temperature (7.9°C vs. 6.4°C), feeding frequencies declined at 12 days when larvae were 1.25 cm; these declines correlated with asymptotic growth. We postulate that we reached our limit of ability to provide suitable nutrition in spite of an excess of food being available to the larvae. Other researchers found similar reductions in growth rate. Some marine larvae (e.g., plaice) are able to grow through metamorphosis on rather small prey (Brachionus spp.) but have depressed growth, whereas growth in other species, e.g., Pacific mackerel (Scomber japonicus), slows and few larvae survive to metamorphosis (Hunter 1981).

Exposure to hydrocarbons decreased growth. Exposure of larvae to dietary sources of WSF resulted in slow, small growth reductions. Direct exposures caused rapid, dramatic effects at sublethal concentrations (0.3 ppm), primarily due to reduced feeding. Struhsaker et al. (1974) found that dissolved benzene decreased growth in correspondence with reduced feeding. Growth of pink salmon alevins (Rice et al. 1974) and juveniles (Moles and Rice 1983) has been shown to be partially due to decreased feeding. However, reductions in growth at sublethal exposures may also be due to changes in metabolic rate. For example, oxygen consumption and breathing rates of pink salmon fry exposed to toluene and naphthalene increased immediately upon exposure (Thomas and Rice 1979). Respiration of Fundulus heteroclitus embryos exposed to WSF increased briefly but was depressed after prolonged exposure (Sharp et al. 1979). Embryonic activity, a measure of

respiration, was initially increased by exposure to dissolved light oils and later depressed (Linden 1978).

Contaminated food inhibited growth only at very high concentrations and after long exposures. Other studies have also demonstrated that oil-contaminated food affects growth but requires time for the effects to become measurable. Growth of pink salmon fry was reduced by a diet of contaminated Artemia nauplii at hydrocarbon levels that would be lethal as WSF (Schwartz 1985). Chinook salmon (Oncorhynchus tshawytscha) gut tissue was damaged when exposed-to dietary hydrocarbons (Hawkes et al. 1980), and energy reserves were depleted and growth reduced in trout (Salmo spp.) (Hawkes 1977). In our study, hydrocarbons present in prey were partially depurated before consumption; nauplii in the pin.k salmon study were eaten rapidly before significant depuration could occur (Schwartz 1985).

The difference in rates of effects between WSF and contaminated food is due to the large difference in hydrocarbon accumulation rates.' Hydrocarbons accumulate rapidly in larval tissues if the WSF exposure is direct, reaching maximum levels in about 1 hour. Accumulation of hydrocarbons through contaminated food takes a long time, if it occurs at all, because larvae eat only a fraction of their body weight per day. Hydrocarbons taken up from contaminated food in 1 day are lost the next. Net accumulation from contaminated food can occur only if the absorbed hydrocarbons are trapped in the lipid-rich tissues and are not metabolized or excreted. The high surface area of larvae permits depuration into the clean surrounding water because most hydrocarbons in the WSF, particularly the monoaromatic hydrocarbons, are not tightly bound in the lipids. Eventually, contaminated food can affect larvae,

but at the low rate of hydrocarbon accumulation, it takes a long time before effects are evident.

The depuration of hydrocarbons by prey tends to obscure exact dose significance but emphasizes the importance of hydrocarbon loss by prey in the environment. Hydrocarbons present in prey were partially depurated before consumption. Artemia nauplii depurated WSF hydrocarbons rapidly (2% retention after 24 hours). Brachionus spp. depurated naphthalene rapidly during the first 2 hours, but depuration was much slower thereafter (33% retention after 24 hours). Other investigators have also observed rapid depuration of hydrocarbons from zooplankton: Coonstripe shrimp (Pandalus hypsinotus) zoeae depurated 97% in 1 day (Brodersen unpubl. data), Calanus helgolandicus depurated 35-85% in 1 day (Corner et al. 1976), and Euchaeta japonica depurated 60% in 1 day and about 80% in 2 days (Lee 1975). Generally, crustaceans depurate hydrocarbons rapidly (Anderson et al. 1974). Small amounts of hydrocarbons are often retained by zooplankters (e.g., Euchaeta japonica and Calanus helgolandicus) for long periods, but after 1 day, much of these hydrocarbons may remain as metabolites (Lee 1975; Corner et al. 1976).

In natural environments, contaminated food is not the major avenue of toxicant' exposure for herring larvae. Because prey and predator inhabit the same planktonic environment, both would be simultaneously exposed to the same levels of WSF contamination. Concentrations required to significantly contaminate prey (about 6 pp-m) are directly lethal to herring larvae. ', Further, because prey rapidly exchange hydrocarbons with their environment, they would not accumulate significant quantities of hydrocarbons days or weeks in advance and then

adversely affect larvae that begin feeding after the contaminant is gone.

Probable Effects of Oil on Fisheries

Catastrophic oil spills such as from the Amoco Cadiz on the north coast of Brittany in France (March 1978) and the grounding of the Metula in the Strait of Magellan (August 1974)--in regions similar to Alaskan waters--can result in the complete destruction of adjacent intertidal and subtidal floral and fauna1 communities. Crude oil accumulated in intertidal areas will persist and continue affecting these' ecosystems for many years (Glemarec and Hussenot 1982; Gundlach et al. 1982). Incubating herring eggs would be particularly vulnerable in this type of a situation.

Compared to oil in the intertidal area, oil in the water column may be more transitory. For example, concentrations dropped from greater than 1,000 pg/liter oil-in-water 9 days after the grounding of the Amoco Cadiz in Aber Wrac'h estuary to 60 pg/liter 48 days later (Calder and Boehm 1981). However, initial concentrations may well be within the range to impact herring, particularly the sensitive larval stages.

Potential impacts of WSF from an oil spill in an area where herring spawn can be predicted by our study. Concentrations of 1.6 ppm aromatic hydrocarbons will kill some adult fish; however, if the oil dissipates before the surviving fish spawn, their eggs probably will not be affected. If oil remains in the water column for no more than 2 days after spawning, eggs should develop normally a,nd hatch successfully. If the eggs are subjected to 1.5 ppm or more WSF for 12 or more days, at least half of them will fail to hatch. However, unless there is a large

oil spill or a chronic discharge of oil, WSF concentrations probably will not be high enough or persist long enough to cause such damage. Although herring eggs are more tolerant of WSF than are larvae, their lack of mobility makes them more vulnerable to oil stranded in the intertidal zone where herring spawn. Direct contact with beached oil could lead to massive mortalities from suffocation or toxicity,

Considering the aromatic hydrocarbon load in ovarian tissue and the slow rate of depuration, it is surprising we saw no increase in dead ova or in poor hatching of spawn from exposed adults. No effects on viability of spawn from exposed mature adults were detected: If the adults survived, the spawn hatched. Reproduction is usually considered a life process sensitive to pollutants; there are several possible reasons why that sensitivity was not evident in our study. One is that developing ova and embryos may be resistant to or tolerant of the accumulated aromatic hydrocarbons, which are sequestered primarily in the lipid portion of the eggs rather than in actively growing embryonic tissues. Thus, the developing embryos, as they slowly consume yolk for energy and tissue growth, may experience only low levels of hydrocarbons. Passive depuration may rid the yolk of the hydrocarbons before any significant damage occurs. A second possibility is that damage occurs, but remains hidden because yolk-sac larvae do not require full use of all their structures and tissues until after yolk absorption. Subtle deformities, particularly cellular damage, that are not evident at hatching may limit survival after yolk absorption.[a/]

In contrast to adults and eggs, herring larvae are affected rapidly by exposure to low concentrations of WSF. Yolk-sac larvae can be killed by WSF in a matter of hours. If oil is present at a concentration of

greater than 2.8 ppm for 16 hours, significant mortalities will occur. Feeding larvae cease to eat after 4 days at exposure concentrations as low as 0.8 ppm, causing increased mortality several days later. Herring larvae are fragile and, like most marine fish larvae, are poor swimmers and have low survival rates ,even in unpolluted environments. Larval survival has been considered one of the determinants of year-class strength in Pacific herring (Lasker 1985; Smith 1985); impact on the more sensitive larval stage could affect population levels.

The accumulation of aromatic hydrocarbons in herring tissues could affect the herring fishery, even without a direct effect on the reproductive process. An oil spill at a spawning site could contaminate the roe, making it unmarketable for human consumption. Because mature roe is harvested immediately before spawning, waiting for depuration to occur may not be practical; these fish may spawn before depuration has been completed.

CONCLUSION

Several life stages of Pacific herring were exposed to WSF of Cook Inlet crude oil. Direct effects on the reproductive process were not observed; if adult herring survived oil exposure, the resulting spawn hatched. Eggs were more resistant than adults to Z-day exposure but were more sensitive than adults to 12-day exposure. However, eggs spawned in the intertidal zone could be heavily impacted by stranded oil at low tides. Larvae were the most sensitive life stage observed.

The presence of oil for longer than 16 hours in larval rearing habitats could devastate an entire year class, for that, spawning population. Growth of larvae was decreased at WSF concentrations as low as 0.3 ppm aromatic hydrocarbons.

Herring fisheries could also be impacted by an oil spill through uptake of hydrocarbons into muscle and developing ovarian tissues. Pacific herring are a very valuable fishery because of the high value of the ovaries just prior to spawning. Accumulated hydrocarbons in ovaries depurate slowly and could result in unmarketable fishery products and fishery closures.

ACKNOWLEDGMENTS

This study was partially funded under a contract with the Alaska Outer Continental Shelf Environmental Assessment Program (OCSEAP), which is managed by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce through an interagency agreement with the Mineral Management Service, U.S. Department of the Interior. Additional funding was supplied by U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Auke Bay Laboratory Habitat Investigations.

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APPENDIX

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Table A.--Experimental conditions for exposures of Pacific herring (Clupea harengus pallasi) to the water-soluble fraction of Cook Inlet crude oil. This study was conducted by the Habitat Investigation Laboratory Unit, Auke Bay Fisheries Laboratory in 1984 and 1985.

Stage exposed	Exposure time	Parameters measured	Exposure concentrations (mean ppm aromatic hydrocarbons)	Individuals per concentration (No.)
Adult	12 days	Mortality (LC_{50})	0, 0.33, 0.68, 1.2, 2.7, 3.3	12 adults
Adult	48 hours (samples @: 0, 6, 12, 24, 48 hours)	Uptake in liver, muscle, testes, immature & mature ovary	0, 0.33, 0.63, 1.0, 1.2	Three adults per sample[a] (nine per sample time to supply all tissues)
Adult	10 days (samples @: 0, 24, 72, 144, 240 hours)	Uptake in liver, muscle, testes, immature & mature ovary	0, 0.28, 0.39, 0.61	Three adults per sample[a] (nine per sample time)
Adult	4 days (samples @: 0, 24, 48, 96, 168, 336 hours)	Depuration from muscle, mature ovary	0, 0.69	Three adults per sample[a] (six per sample time)
Gonad (mature adult)	2 days	Hatching success of eggs	0, 0.24, 0.54, 0.93, 1.47	575-750 eggs; eggs from six fish on 12 slides in three incubators[b]
Gonad (mature adult)	12 days	Hatching success of eggs	0, 0.38, 0.70, 1.18, 1.55	575-750 eggs; eggs from six fish on 12 slides in three incubators[b]
Eggs	2 days	Hatching success	0, 1.36, 2.07, 4.10, 5.30	575-750 eggs; eggs from three fish on 12 slides in three incubators[b]

Table A.--Continued.

Stage exposed	Exposure time	Parameters measured	Exposure concentrations (mean ppm aromatic hydrocarbons)	Individuals per concentration (No.)
Eggs	12 days	Hatching success	0, 1.00, 1.38, 2.86, 3.70	575-750 eggs; eggs from four fish on eight slides in two incubators[b/]
Eggs	12 days	Hatching success	0, 0.85, 1.60, 3.02	575-750 eggs; eggs from four fish on twelve slides in three incubators[b/]
Yolk-sac larvae	20 minutes 2 hours 6 hours[c/]	Mortality Swimming	0, 0.24, 0.49, 1.16, 2.21, 3.04, 4.81, 6.11	18-20 larvae in three tubes in one incubator[b/]
Yolk-sac larvae	16 hours[c/]	Mortality Swimming	0, 0.22, 0.42, 1.04, 2.21, 2.60, 3.90	13-19 larvae in three tubes in one incubator[b/]
Yolk-sac larvae	2 days	Mortality Swimming	0, 0.24, 0.42, 1.05, 2.08, 2.67, 4.09	17-20 larvae in three tubes in one incubator[b/]
Yolk-sac larvae	6 days[c/]	Mortality Swimming	0, 0.25, 0.44, 1.15, 2.12, 2.91, 4.23	76-108 larvae in three incubators[b/]
Feeding larvae	28 days	Mortality Swimming Feeding Growth	0, 0.3, 0.78, 1.21, 1.72	>2,000 larvae in four tanks
Feeding larvae	21 days	Mortality Swimming Feeding Growth	0, 0.3, 0.53, 0.86	>2,000 larvae in four tanks

Table A. --Continued.

Stage exposed	Exposure time	Parameters measured	concentrations (mean ppm aromatic hydrocarbons)	Individuals per concentration (No.)
Feeding larvae	28 days OCP[d]/	Mortality Swimming Feeding	Prey exposed to 0, 0.28, 0.82, 6.29 OCP[d]/ tissue levels = 0, 1.4, 6.8, 59 ppm	>2,000 larvae in four tanks

[a]/ Many adult herring were exposed to ensure finding three males, three immature females, and three mature females (externally alike) at each sampling period.

[b]/ Additional individuals and replicates were used for control groups.

[c]/ All yolk-sac larvae tests were monitored 6 days, regardless of exposure time to WSF. Larvae were placed in clean, flowing seawater following exposure.

[d]/ OCP = Oil-contaminated prey (Artemia nauplii).