

**LETHAL AND SUBLETHAL EFFECTS OF THE
WATER-SOLUBLE FRACTION OF
COOK INLET CRUDE OIL ON PACIFIC HERRING
(*CLUPEA HARENGUS PALLASI*) REPRODUCTION**

by

Stanley D. Rice, Malin M. Babcock, Christine C. Brodersen,
Mark G. Carls, Jessica A. Gharrett, Sid Kern, Adam Moles,
and Jeffrey W. Short

Auke Bay Laboratory
Northwest and Alaska Fisheries Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
P.O. Box 210155, Auke Bay, Alaska 99821

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EXECUTIVE SUMMARY

The proposed sale of continental shelf leases for petroleum development in Bristol Bay, Alaska, is generating concern about the possible effects on valuable fishery resources, such as Pacific herring (*Clupea harengus pallasii*), in the area. Herring from Bristol Bay are marketed primarily for roe (exported to Japan for human consumption) and are an important prey of several commercially valuable fishes. The inshore spawning strategy of herring **makes them** particularly vulnerable to the effects of an oil spill. Spawning adults congregate at sites where they and their maturing gonads could be exposed to spilled oil, which could harm their reproductive success. Spawned eggs, and larvae, would have to survive and grow in an oil-contaminated environment.

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 as well as on hatching success of eggs from exposed adults. We studied also the effects of feeding oil-contaminated prey to herring larvae. The results of our study are summarized:

- * **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.
- * Eggs of adults exposed 12 days to 1.6 ppm had normal hatching success.
- * Eggs exposed 2 days to 5.3 ppm had normal hatching success; eggs exposed 12 days had an LC_{50} of 1.5 ppm.

- * Yolk-sac larvae exposed ≤6 hours to 6.0 ppm survived; yolk-sac larvae exposed from 16 hours to 6 days had LC₅₀'s of 2.8 to 2.3 ppm.
- * Feeding larvae exposed 7 days had an LC₅₀ of 1.8 ppm, and 21 days, 0.36 ppm.
- * Tissue (muscle, liver, testes, and mature and immature ovaries) uptake of hydrocarbons in adult herring was rapid, but equilibrium was not reached in 10 days of exposure.
- * Muscle tissue generally accumulated the highest levels of hydrocarbons; immature ovarian tissue accumulated almost two times the levels found in mature ovarian tissue.
- * In adults, initial deputation was rapid but slowed after 24 hours, and 10% of the hydrocarbons were still present after 7 days of deputation in clean water. Hydrocarbon levels after 14 days were not significantly higher than control levels.
- * Uptake in larvae was more rapid than in adults and reached equilibrium within 4 hours. Retention was less in larvae than adults, and after 24 hours, only 2% of the ¹⁴C-labeled naphthalene remained in larval tissues.
- * Growth of larvae was significantly reduced after 7 days of exposure to 0.3 ppm, and reductions were greater after longer exposures and higher concentrations.
- * Growth of larvae was not significantly reduced by a diet of oil-contaminated prey.

We conclude that the life stage at which the reproductive success of Pacific herring is most likely to be impaired by oil is feeding larvae: Larvae are killed by shorter exposures and lower concentrations than are the eggs or the adult reproductive products or the **adults**

themselves. We conclude also that even if **oil** is present at levels too low to threaten the survival of herring, the fisheries could be impacted because the rapid **bioaccumulation** of oil hydrocarbons in the edible muscle and ovarian tissues could make the herring unmarketable.

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INTRODUCTION

The Pacific herring (*Clupea harengus pallasii*) is an important prey species and supports the valuable commercial fisheries in the coastal waters of Alaska. Effects of oil pollution are of particular concern because of the inshore spawning strategy of herring. Each herring stock spawns near shore within a restricted natal range in spring. Adults typically congregate for several weeks near prospective spawning sites while their reproductive organs mature, then they spawn en masse over a period of a few days. Herring spawn in 0-20 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 fishery was for a high-volume and low-priced product of 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, and 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 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 (Kühnhold 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 pollution effects 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 (ppb)) decreased survival of ova in the gonads and also of resulting spawn. Struhsaker (1977) concluded that spawning fish are a very sensitive life stage. 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 and, thus, affect 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 LC_{50} for prespawning adult Pacific herring exposed to WSF of Cook Inlet crude oil; 2) uptake and deputation of aromatic hydrocarbons in gonads, liver, and muscle tissue of mature herring; 3) survival and viability of eggs spawned from adult herring exposed to WSF; 4) survival and viability of herring eggs and newly hatched (yolk-sac) larvae exposed to WSF; and 5) survival, growth, and hydrocarbon accumulation of feeding larvae exposed to WSF or fed WSF-contaminated food.

METHODS

Test Animal Collections

Mature adult Pacific herring (*Clupea harengus pallasii*) for adult herring exposures and for artificial spawning in the egg tests were caught by a standard commercial fishery purse seine near Juneau, Alaska, in 1984 and 1985. Fish were held in 1,000 L fiberglass tanks containing low salinity (15‰) running water for the first 5 days. Salinity was gradually increased during the next 5 days to 30‰. Holding herring in low salinity water minimizes hemorrhaging due to scale loss, by reducing osmotic stress. Nets were never used to transfer fish because they cause massive scale loss; all fish transfers were 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. Substrate for collected eggs was *Fucus distichus*. Larvae hatched from these eggs were used in the yolk-sac and feeding larvae experiments.

Dosing and WSF and Tissue Analyses

The WSF of Cook Inlet crude oil was supplied by a flow-through, WSF generator (Moles et al. 1985) that dripped 10 L/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. 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.

Aromatic hydrocarbon levels in WSF's of crude oil were determined by gas chromatography of methylene chloride extracts of WSF samples. All glassware and containers used in processing the samples were cleaned with detergent and water, then rinsed twice with distilled hexane. Samples (750 ml) of WSF 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 1-1 separator 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-Packard¹ model 5880A gas chromatography equipped with a 12 m fused silica dimethyl silicone capillary column and a flame ionization detector. The

¹Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

N_2 carrier gas was held at **70** kPa inlet pressure. Temperature of the inlet, outlet, and detector was held at **250°C**. The injection volume was 1 μ l. The column temperature program was as follows: 20°C for 2 minutes, followed by a temperature ramp of **10°C/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 parts per million (**ppm**) aromatic hydrocarbons. Our **WSF's** were dominated by mononuclear aromatic hydrocarbons (about 95%); **dinuclear** aromatic hydrocarbons constituted about 5% (Table 1).

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, then 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 **70-80°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 30 ml collected. Two to 5 g of Na_2SO_4 was added and allowed to stand at

Table 1. --Distribution of individual aromatic hydrocarbons in water-soluble fraction (WSF) of Cook Inlet crude oil and in tissues of mature female Pacific herring (*Clupea harengus pallasii*) after 6 days of exposure to 1.2 ppm aromatic hydrocarbons (mean of three samples). Concentrations are in parts per million and 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	*		*		.550	45.8
Toluene	9.443	20.4	2.965	6.7	.377	31.4
Ethyl benzene	2.582	5.6	.746	1.7	.044	3.7
m- and p-xylene	6.399	13.8	1.584	3.6	.107	8.9
o-xylene	3.596	7.8	1.064	2.4	.065	5.4
Cumene	.879	1.9	.441	1.0	.008	.7
Mesitylene	.733	1.6	.449	1.0	.005	.4
n-propyl benzene	.581	1.3	.519	1.2	.006	.5
P-cymene	.256	.6	.161	.4	.004	.3
Naphthalene	3.722	8.1	4.069	9.1	.020	1.7
2-methyl naphthalene	2.787	6.0	6.264	14.1	.025	2.1
1-methyl naphthalene	2.540	5.5	4.969	11.2	.008	.7
2,6-dimethylnaphthalene	.308	.7	1.277	2.9	.009	.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	.061	5.1
Mono- and dinuclear aromatics	46.213	100.0	44.501	100.0	1.201	100.0

least 10 minutes. Samples were decanted into 50 ml pear-shaped flasks, **evaporated to 2 ml on a rotary** evaporator, then 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 **(4:1)**. The aromatic fraction was evaporated to 1 ml on a rotary evaporator, transferred to a 1.8 ml gas chromatography vial with a Teflon-lined septum, and frozen until gas chromatography analyses. Analyses were performed by a Hewlett Packard model 5880A gas chromatography equipped with a 12 m fused silica **dimethyl** silicone capillary column and a flame ionization detector. The **N₂** carrier gas was held at 70 kPa inlet pressure. Temperature of the inlet, outlet, and detector was **held at 250°C**. The injection volume was 1 µl. The column temperature program was as follows: **30°C** initial temperature for 1 minute, 5°C/minute temperature ramp for 2 minutes, **10°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 (0-4 hours) and depuration (0-24 hours) in larvae and prey were measured with liquid scintillation techniques using **¹⁴C-labeled naphthalene**. Labeled **naphthalene** dissolved in acetone was added to seawater and stirred 15-20 minutes before larvae or the prey, **Artemia nauplii, were** added. Initial concentration of **naphthalene** was 0.33 (0.10 to 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** (Sol uene). A 10 ml scintillation cocktail (**Dimilume**) was then added as **diluent**. A 1 ml **subsample** in additional 10 ml **Dimilume** was counted with a liquid **scintillator**.

Adult Herring Toxicity, Uptake, and Deputation

The **LC₅₀** 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 L tanks, and their survival was monitored. Exposure levels in all subsequent, sublethal tests (Appendix) were selected as percentages of the 12-day **LC₅₀** 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 (10-day) exposures. Groups of adult herring were exposed either 2 days to 0, 18, 43, 45, or 55% of the **LC₅₀** of **WSF** of Cook Inlet crude oil or 10 days to 0, 14, 18, or 30% of the **LC₅₀**.

In the deputation phase of this study, herring were exposed 4 days to 30% of the **LC₅₀** of **WSF**, then placed in clean, flowing seawater for 14 days. All tests were in 800 L fiberglass tanks with appropriate WSF and dilution water (flow rate, 8 L/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, before artificial spawning, to WSF's 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 60 days. Each treatment was replicated at least three times (Appendix).

All eggs exposed as gonads or as fertilized eggs, and unexposed eggs (controls), were held in identical incubators. The incubators were 28 cm long X 15 cm diameter PVC pipe fitted with clear Plexiglas bottoms. Seawater (plus WSF for the 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 slowly (6 times/minute).

Eggs were observed daily. The day after hatching was first observed, we began counting eggs at 2-day intervals. Numbers of dead eggs, unhatched live 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 from 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 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 L 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 L 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 L plastic buckets. **Rotifers** were harvested every 2-3 days, washed onto a net, then resuspended in clean seawater or on algae in seawater. **Artemia** cysts were incubated for 3 days in 20 L 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 L black circular fiberglass chambers with concave bottoms. Seawater (**28‰**) and food were introduced at 0.7 L/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 **diel** 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 (0-5.5 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.

In exposures to WSF and oil-contaminated prey, larval observation methods were identical. 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 also from the number of larvae **per liter remaining at** the end of the experiment and was adjusted relative to controls, **Mortality was** estimated also from tank bottom **subsamples when most or all larvae stopped swimming. These two estimates agreed closely.**

Feeding status was easily determined: Food could be viewed through the transparent, **larval** integument without **larvae** being disturbed. The

presence or absence of food in the gut was observed during collection for other measurements, or visually from above, Larvae were observed at the same 24 predetermined nodes in each chamber. In WSF tests, recovery potential of larval feeding was measured by observing feeding frequencies after exposing larvae to 0.9 ppm WSF for 2, 4, or 8 days. We also starved controls for 8 days, then measured their feeding recovery potential for comparison.

Hydrocarbon uptake by predator and prey was measured by gas chromatography after 0, 1, 2, 4, and 16 days of exposure. Larvae were collected early in the 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 100 micron plankton net, rinsed, and stored until analysis.

Short-term uptake (0-4 hours) and deputation (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 with Dietrich's solution. Samples were allowed to equilibrate at least 1 month before measurement. Preserved specimens were arranged on a petri dish and photographed against a black background. Negatives were enlarged 8-10 X, and each specimen was traced onto a sheet of paper. Tracings were analyzed with

a digital pad to determine notochord lengths. To correct length measurements for fixative effects, random samples of living larvae were measured directly with an eyepiece micrometer, photographed, and then preserved for subsequent measurement.

Mathematical and Statistical Analyses

Median concentrations causing death (LC_{50}) and swimming or feeding inhibition (EC_{50}) were determined by logit analysis (Finney 1952; Berkson 1957) or by Spearman-Kärber 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 Scheffé's test (Scheffé 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 WSF of 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 10-day period (Fig. 1), indicating tissue equilibrium with the WSF concentrations

Table 2. --Sensitivity of several life stages of Pacific herring (*Clupea harengus pallasii*) exposed to water-soluble fractions (WSF) of Cook 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	(*)9 ± 0.1	0.36 ± 0.1

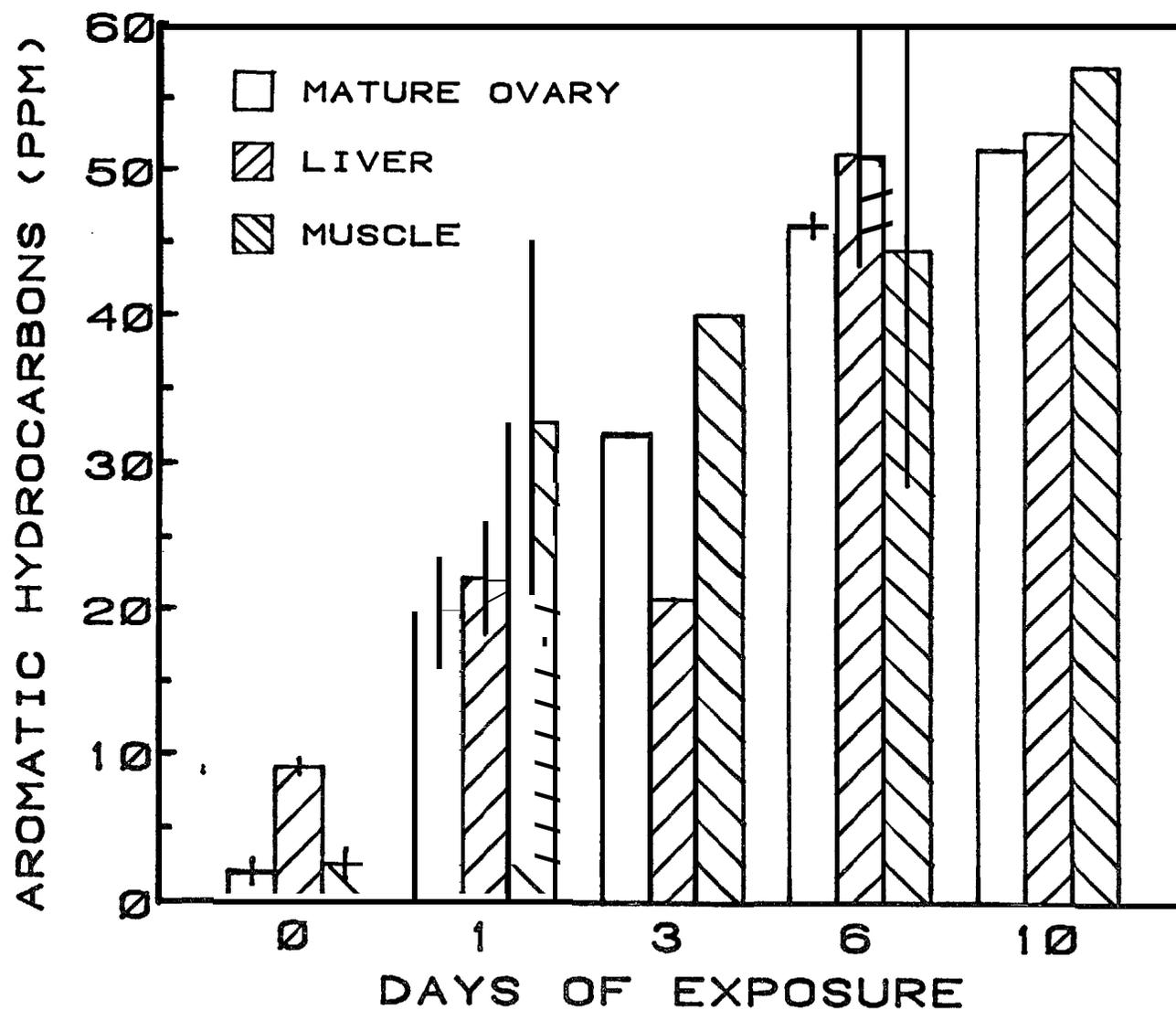


Figure 1.--Accumulation of aromatic hydrocarbons in tissues of adult Pacific herring (*Clupea harengus pallasii*) during 10 days of exposure to 0.6 ppm water-soluble fraction (WSF) of Cook Inlet crude oil. Vertical bars = standard error.

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 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). In contrast, the distribution of aromatic hydrocarbons in tissues was not as simple. **Monoaromatics** dominate in the ovarian tissue, but in muscle tissue, the concentrations of **diaromatic** compounds were nearly **equal** to monoaromatics. Concentration differences between tissues and WSF demonstrate that **lipophilic** tissues can bioconcentrate larger aromatic hydrocarbons, even though their concentrations 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 occur in the WSF. All the **polyaromatic hydrocarbons** in herring tissues are almost certainly of **biogenic** origin. Differences in distribution of aromatic compounds between ovarian and muscle tissues are probably caused by differences in the lipid content of the tissues, in the access to the tissues by the blood, and in the ability to metabolize and **remove hydrocarbons from tissues.**

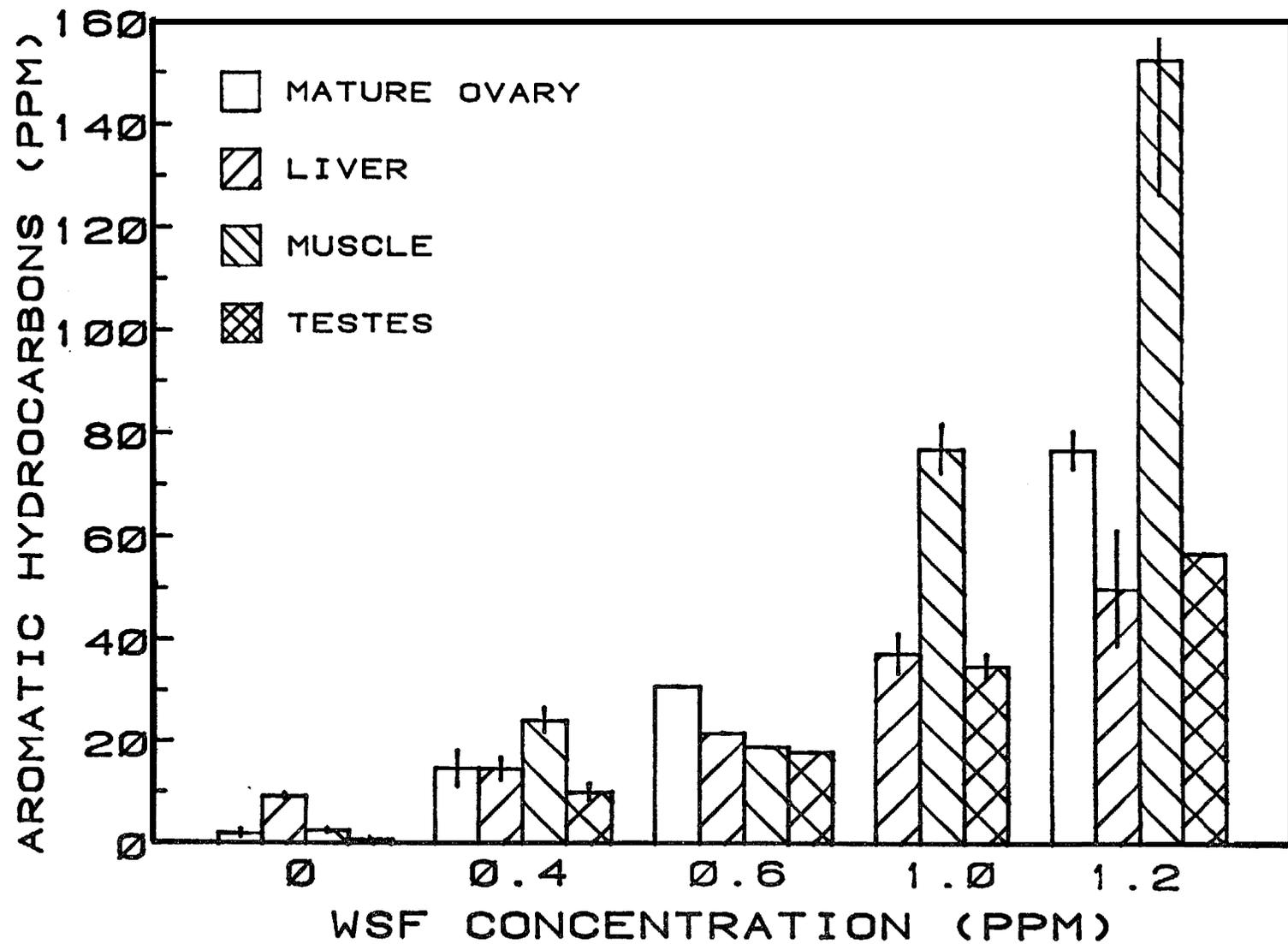


Figure 2.—Concentration of aromatic hydrocarbons in adult Pacific herring (*Clupea harengus pallasii*) tissues after 48-hour exposure to different levels of water-soluble fraction (WSF) of Cook Inlet crude oil. Vertical bars = standard error.

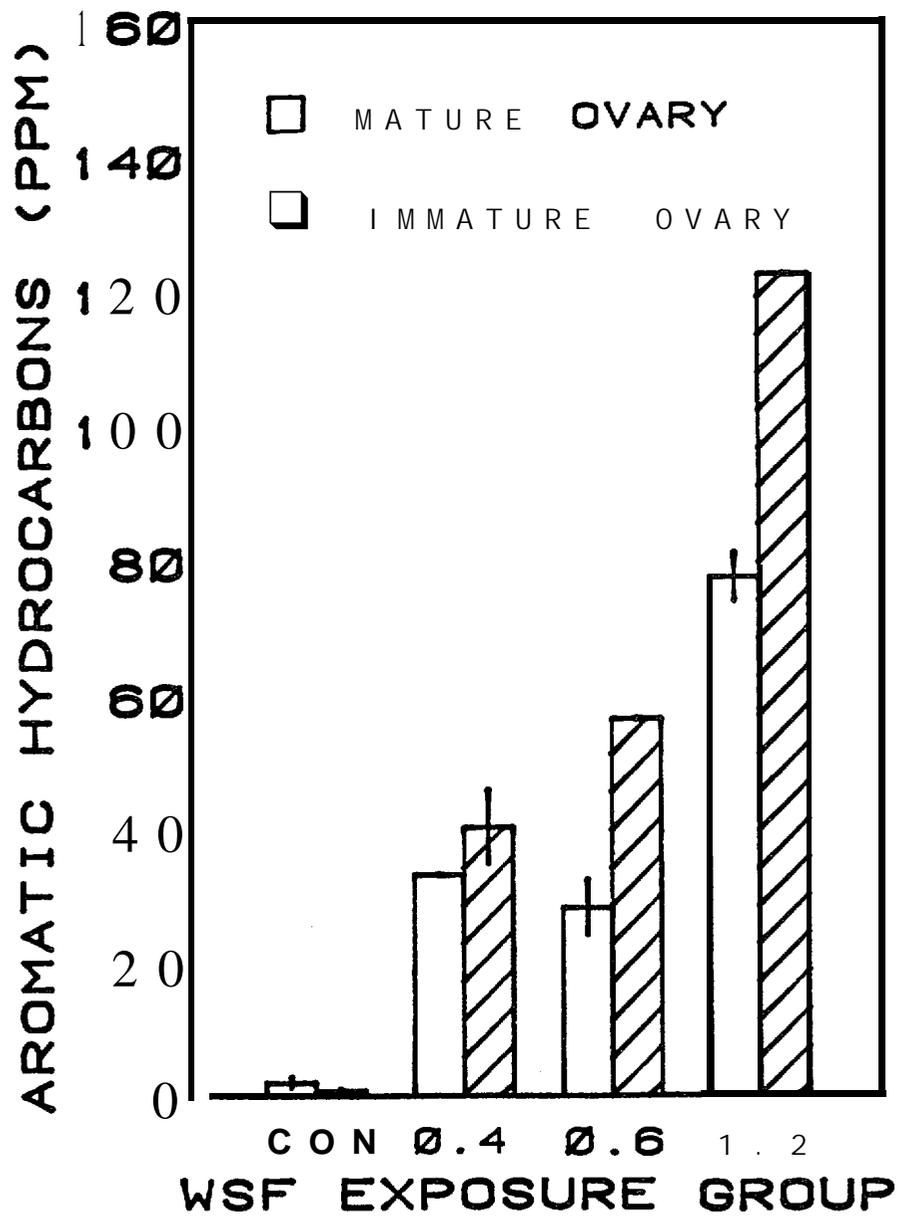


Figure 3. --Accumulation of aromatic hydrocarbons in mature and immature ovaries of prespawning Pacific herring (*Clupea harengus pallasii*). CON = control group; vertical bars = standard error.

Deputation was rapid in muscle and ovarian tissues during the first 24 hours in clean water; about 50% of the hydrocarbons were lost (Fig. 4). Deputation 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 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 LC_{50} 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 affect hatching rates. 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 LC_{50} 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

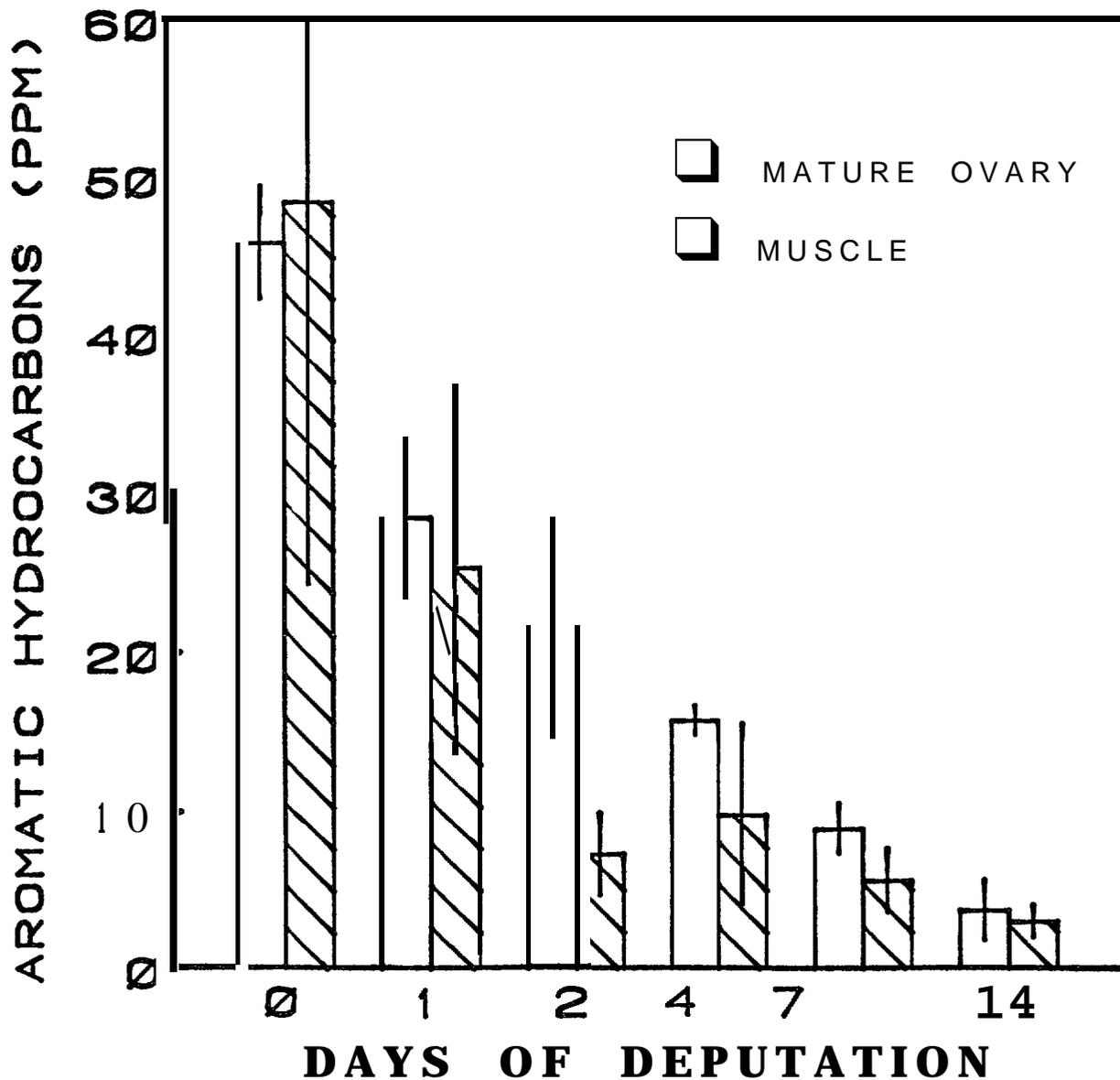


Figure 4. --Deputation of aromatic hydrocarbons from adult Pacific herring (*Clupea harengus palasi*) previously exposed 96 hours to 0.6 ppm water-soluble fraction (WSF) of Cook Inlet crude oil. Vertical bars = standard error.

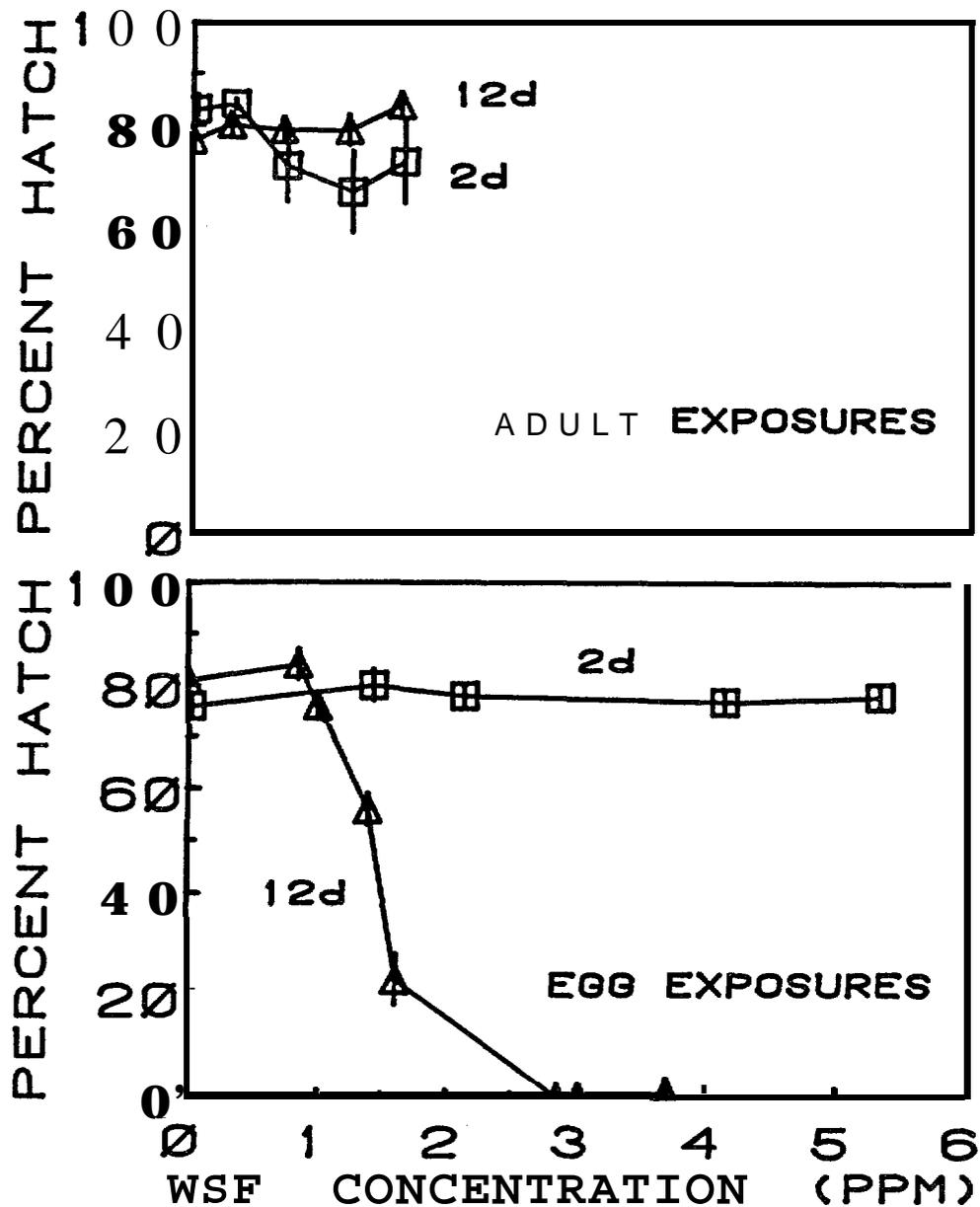


Figure 5.--Percent hatch of Pacific herring (*Clupea harengus pallasii*) eggs from adults exposed 2 or 12 days to water-soluble fraction (WSF) (1.6 ppm, 75% of the LC_{50} , was the highest WSF concentration used in the adult exposures), and percent hatch of artificially spawned and fertilized eggs exposed 2 or 12 days to WSF. Vertical bars = standard error.

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 exposed ≥16 hours had an **LC₅₀** of 2.8 ppm (95% **CI** = **±0.6**), and larvae exposed ≤6 days had an **LC₅₀** of 2.3 ppm (95% **CI** = **±0.2**) (Fig. 6).

Feeding Larvae and Contaminated Prey

Survival, feeding, and growth of herring larvae were affected by direct exposure to **WSF**. Oil-contaminated prey had a much smaller 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 **LC₅₀** 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 initial tissue concentration) caused a significant (**P** = 0.94) fraction, 51%, of the larvae to die relative to controls (Fig. 8), and after 3 weeks, effects of these contaminated prey were equivalent to effects seen in 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 by exposure to **WSF** before death occurred (Fig. 7). Swimming was inhibited more rapidly as doses increased. For example, larvae stopped swimming and settling occurred

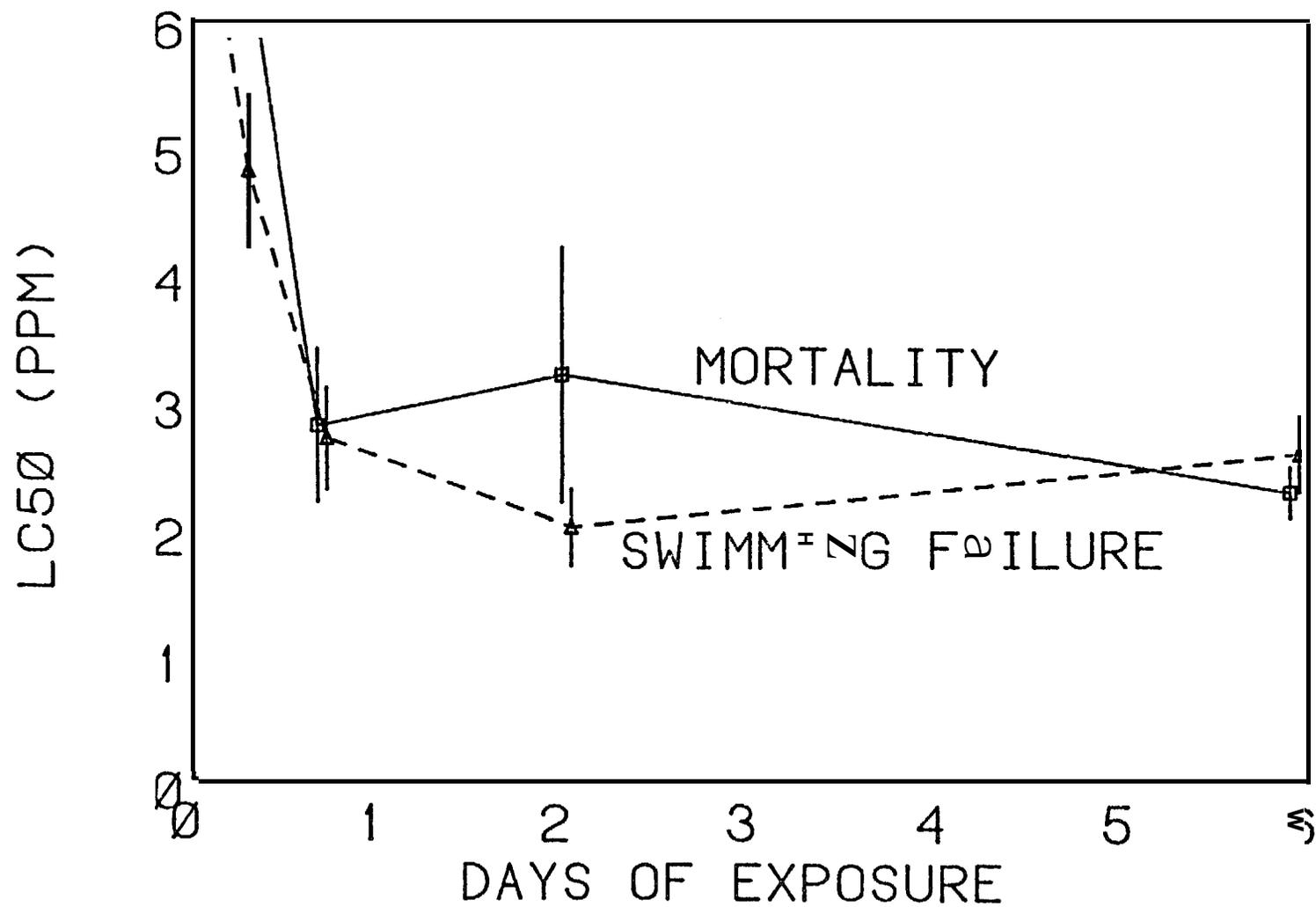


figure 6.--Effect of exposure to water-soluble fraction (WSF) Cook Inlet crude oil on Pacific herring (*Clupea harengus pallasii*) yolk-sac larval mortality (LC₅₀) and swimming ability (EC₅₀). Vertical bars = 95% confidence intervals

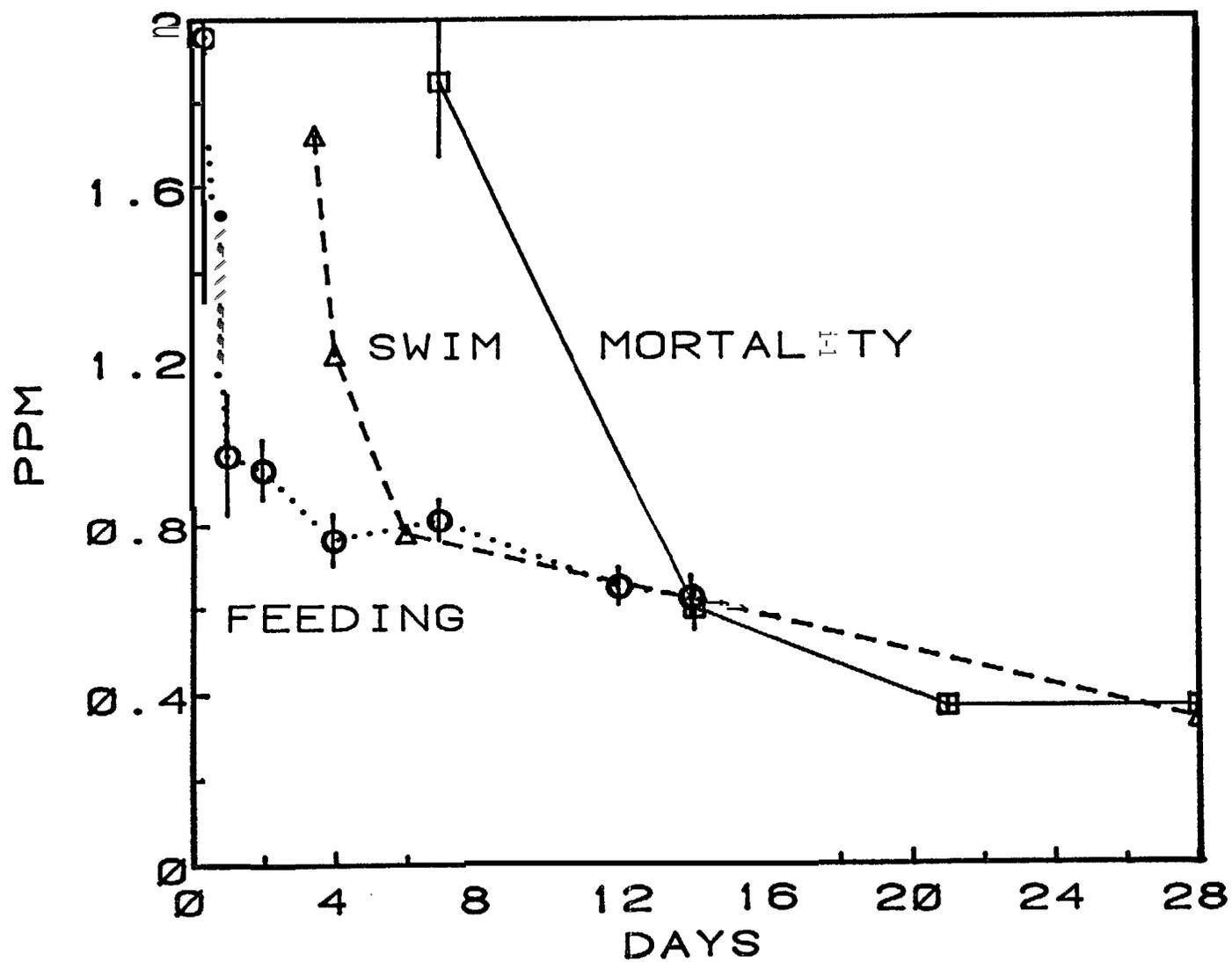


Figure 7.--Effect of exposure to water-soluble fraction (WSF) of Cook Inlet crude oil on feeding Pacific herring (*Clupea harengus pallasii*) larval mortality (LC₅₀) and swimming ability and feeding (EC₅₀). Vertical bars = 95% confidence intervals.

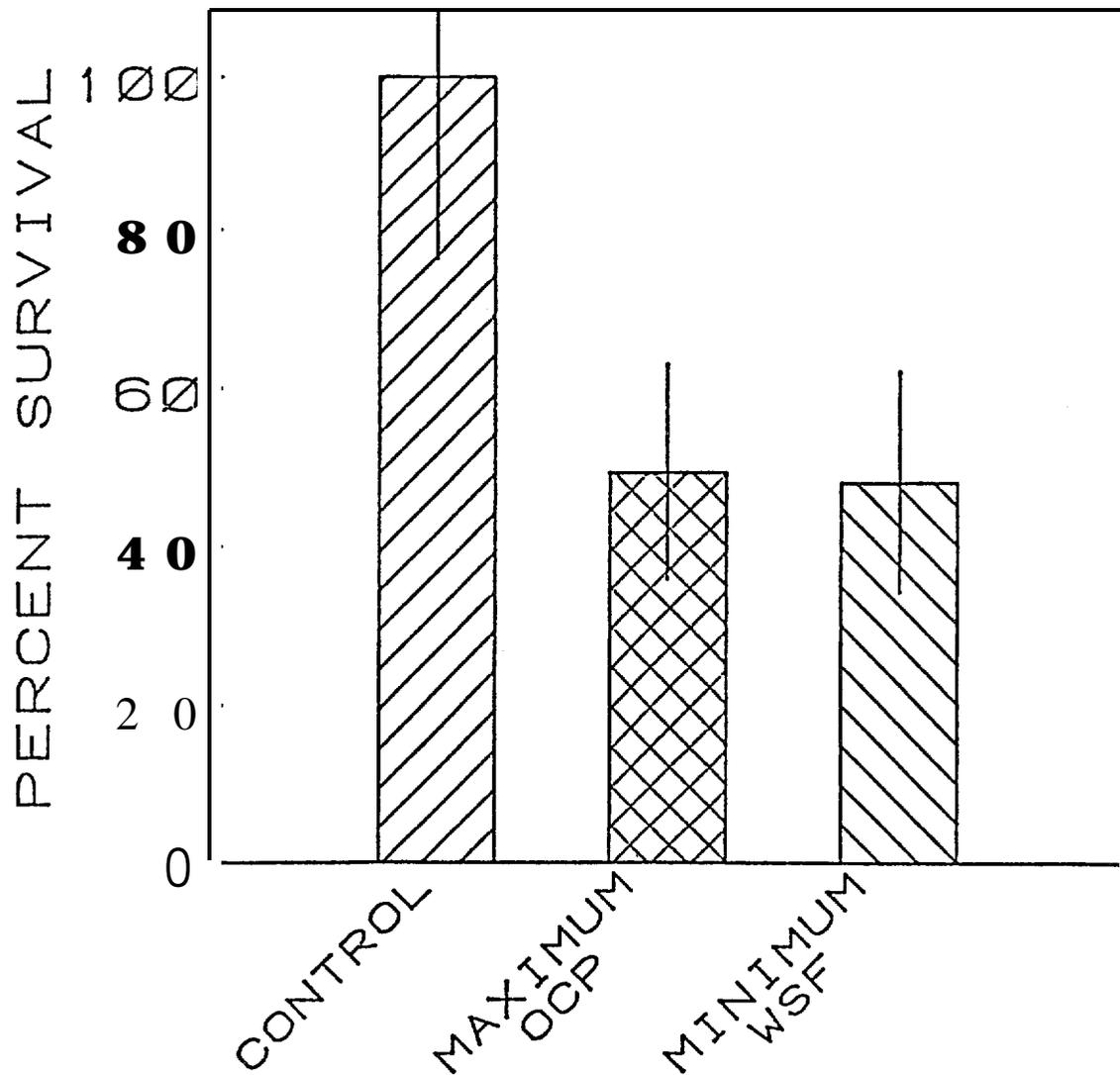


Figure 8.--Survival of Pacific herring (*Clupea harengus pallasii*) control larvae and of larvae exposed 16 days to maximum oil-contaminated prey (OCP) doses (59 ppm initial tissue concentration) or to minimum WSF doses (0.3 ppm). Vertical bars = ± 1 standard error.

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 foods did not cause noticeable swimming inhibition in larvae. Swimming ability of the prey, Artemia nauplii, was reduced by exposure to highest WSF concentrations.

Feeding frequencies 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 ($\underline{Y}^{\frac{1}{2}} = 10.01 - 0.71x$; $r^2 = 0.89$; 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).

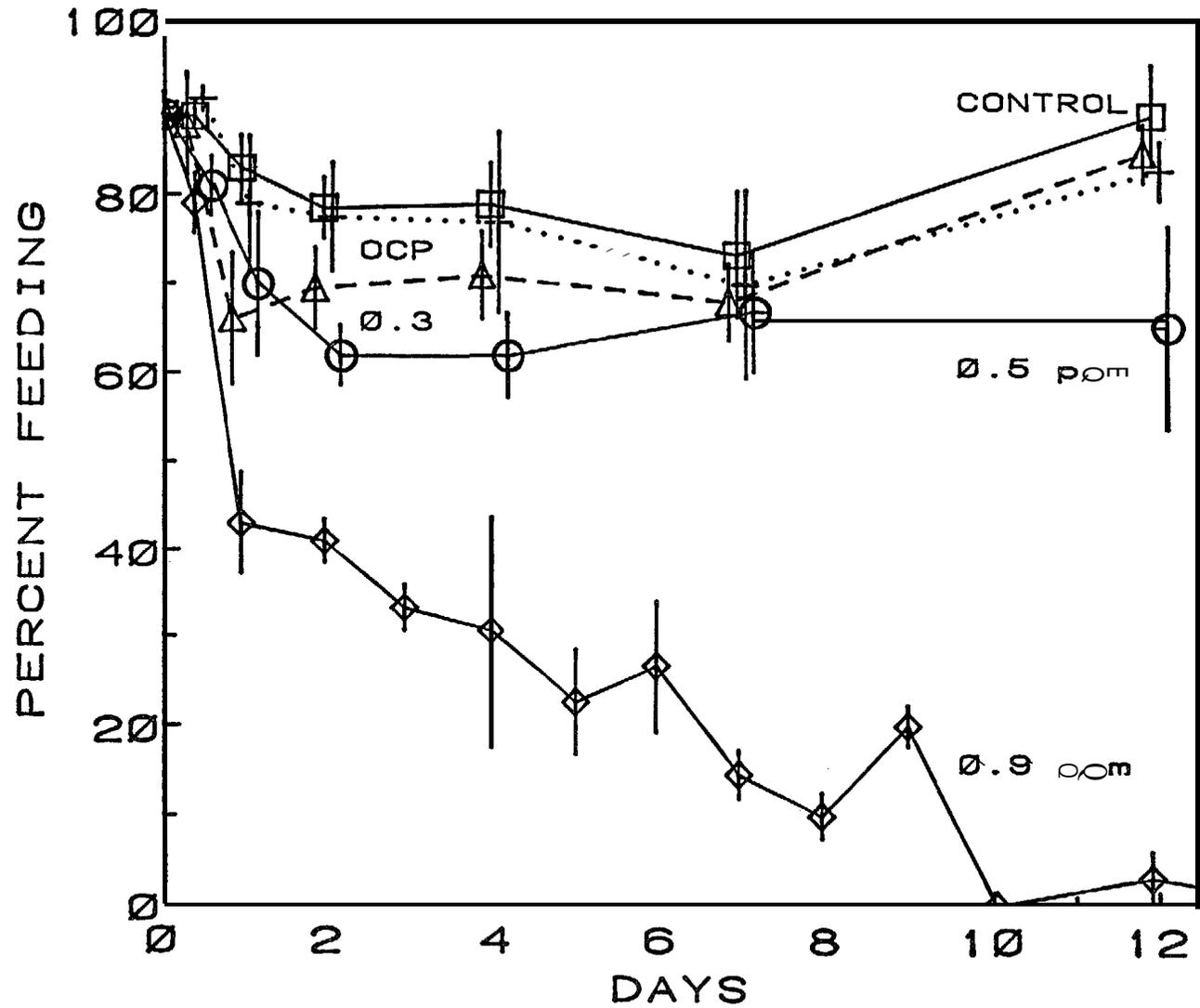


Figure 9.--Feeding frequency of Pacific herring (*Clupea harengus pallasii*) larvae exposed to water-soluble fraction (WSF) of Cook Inlet crude oil and larvae fed oil-contaminated prey (OCP). Vertical bars = standard error.

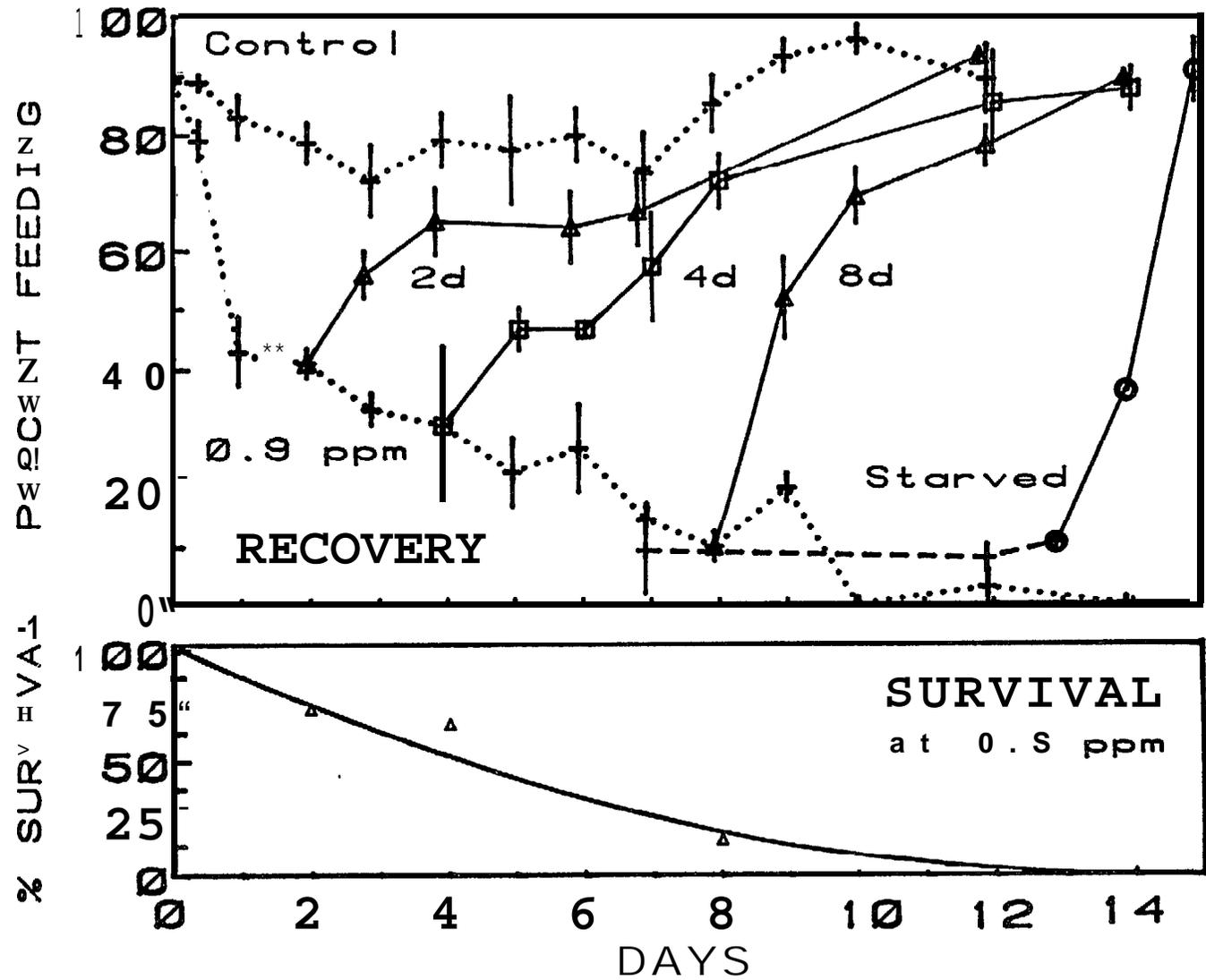


Figure 10. --Recovery of feeding response and survival of Pacific herring (*Clupea harengus pallasi*) larvae after exposure to 0.9 ppm water-soluble fraction (WSF) for various time periods. Vertical bars = standard error.

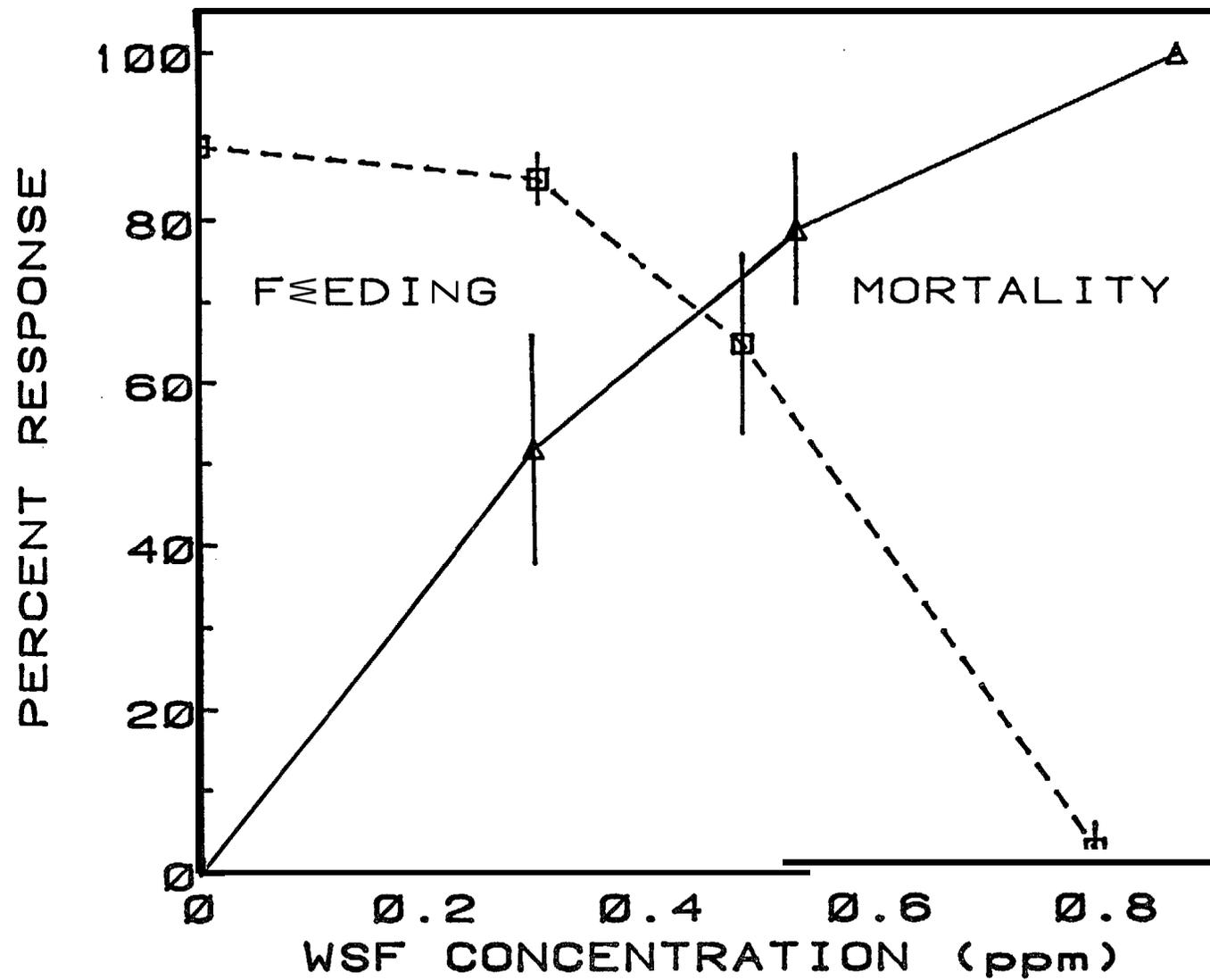


Figure 11.--Relationship of feeding frequency and mortality in Pacific herring (*Clupea harengus pallasii*) larvae. Feeding frequencies were determined after 12-day exposure to water-soluble fraction (WSF), and mortality, at 21-day exposure. Vertical bars = standard error.

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 the growth rate. After 2 weeks, the lowest WSF dose (0.3 ppm) 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-labelled naphthalene** faster than did Artemia nauplii, but the nauplii retained a greater proportion of the ¹⁴C-naphthalene (Fig. 13). Tissue concentrations of larvae reached equilibrium after 1 hour, and of Artemia nauplii, 10 hours. Deputation of ¹⁴C-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 **did** not remain 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 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

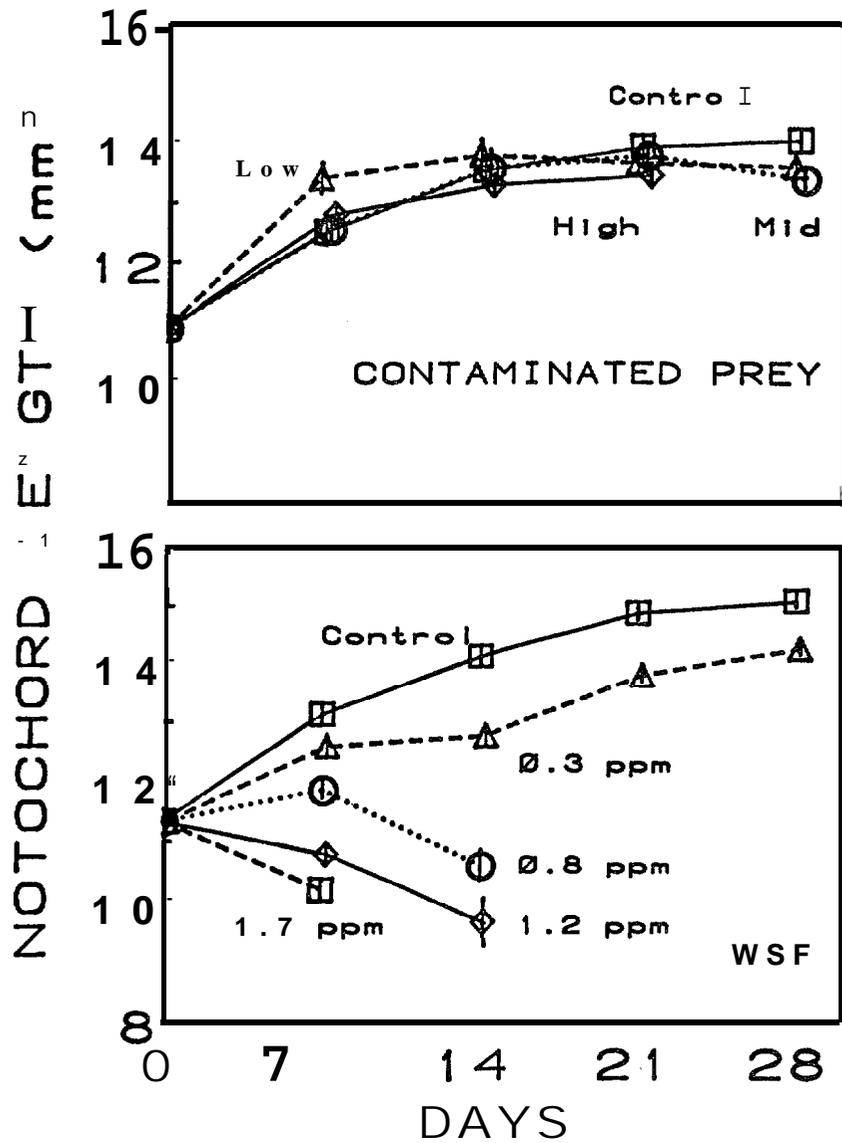


Figure 12. --Influence of water-soluble fraction (WSF) exposure and oil-contaminated prey (OCP) on growth of Pacific herring (*Clupea harengus*) larvae. Vertical bars = standard error.

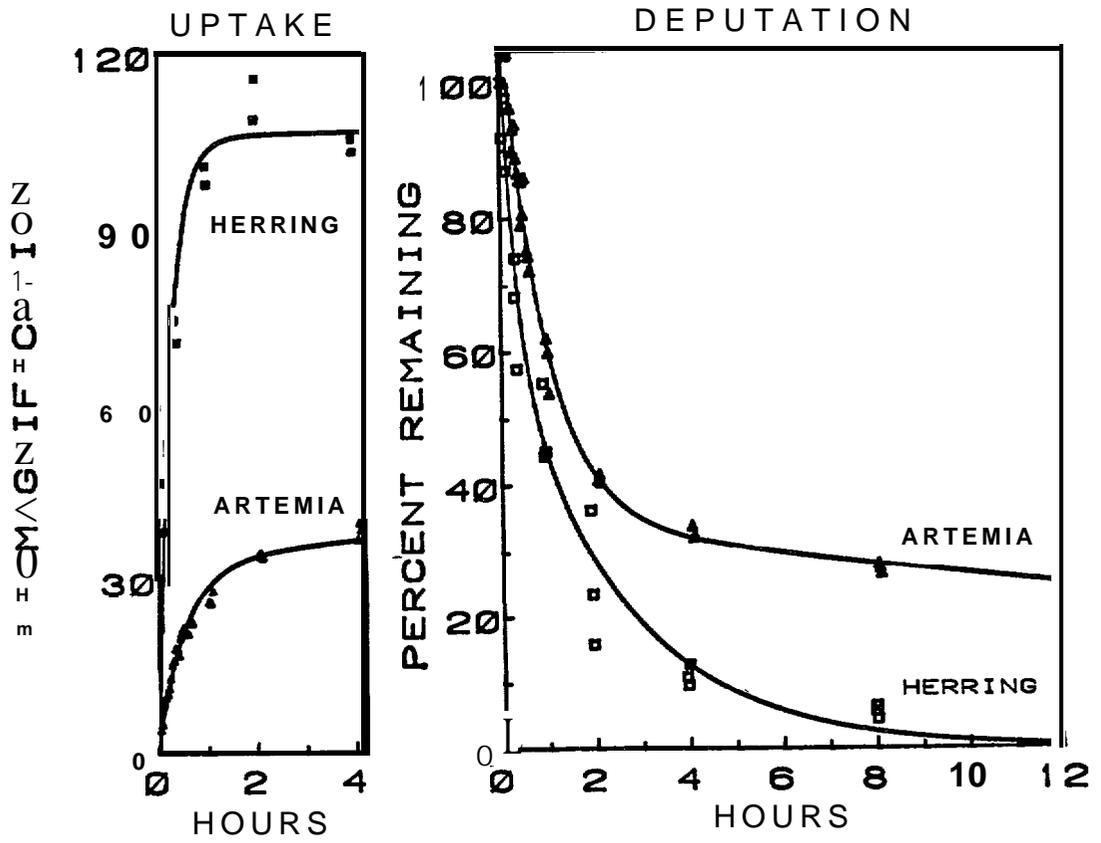


Figure 13. --Uptake and deputation of radio-labelled naphthalene by Pacific herring (*Clupea harengus pallasii*) larvae and their prey, *Artemia nauplii*. Vertical bars = standard error.

easily measured. In the most contaminated food group, aromatic hydrocarbon concentrations in Artemia nauplii tissues averaged 59 ppm at 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 ppm).

Table 3. --Concentrations of aromatic hydrocarbons (mono- and di-aromatic) in Artemia nauplii that were fed to Pacific herring (Clupea harengus pallasii) larvae. WSF = water-soluble fraction 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	0.23	1.4 ± 0.53	6.1
Medium	5	0.72	6.8 ± 0.63	9.5
High	14	5.52	58.8 ± 9.54	10.7

DISCUSSION

Adult Herring

All tested herring tissues (muscle, **liver**, testes, and immature and mature ovaries) accumulated aromatic hydrocarbons. Continued accumulation, without reaching equilibrium, through Day 10 suggests that

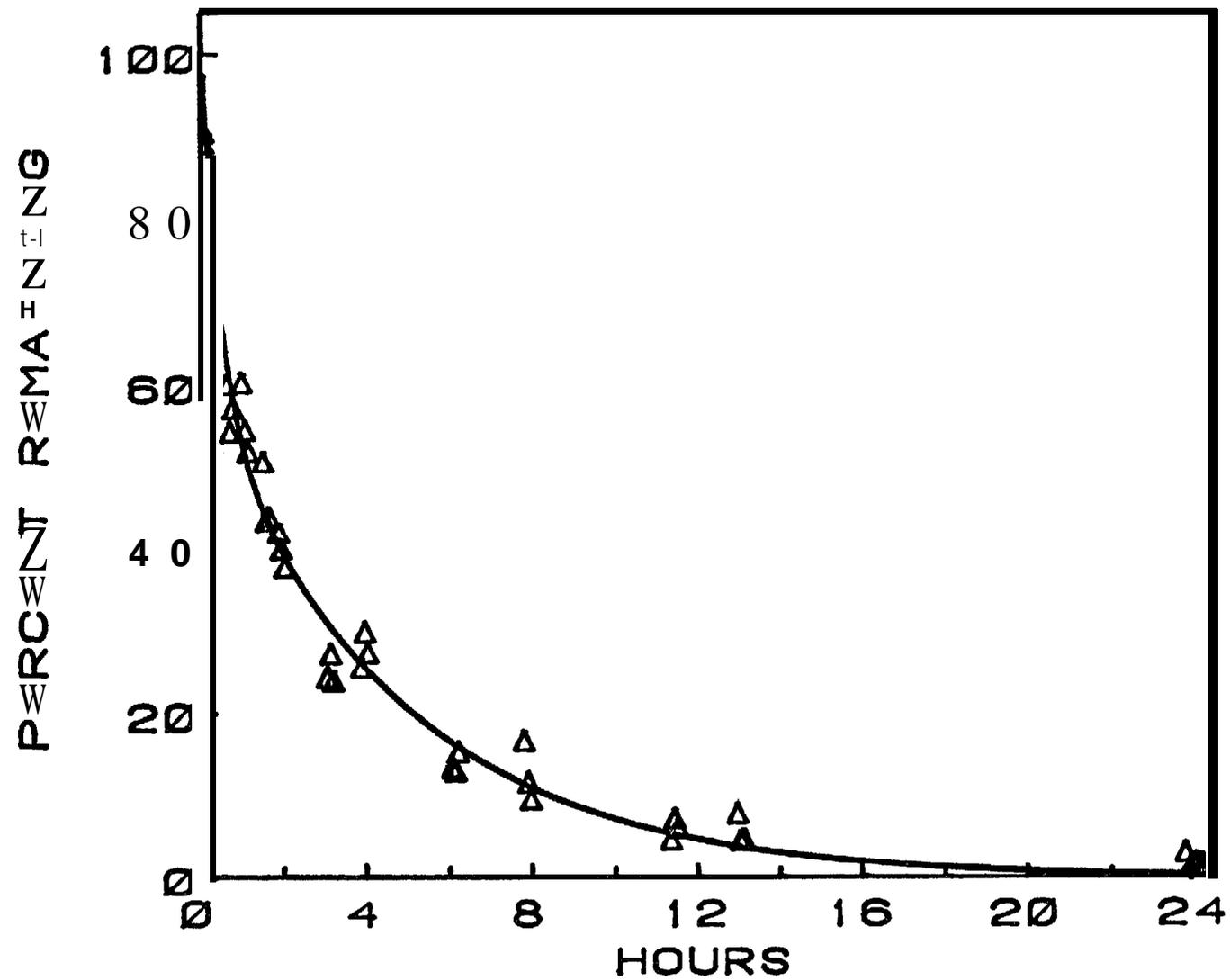


Figure 14.--*Artemia nauplii* deputation of radio-labelled naphthalene before they were fed to Pacific herring (*Clupea harengus pallasii*) larvae. Vertical bars = standard error.

higher concentrations would be reached if exposure times were longer. All tissues, including ovary, depurated hydrocarbons, but depuration after 24 hours was slow.

The pattern of continuous accumulation of hydrocarbons through Day 10 is different from that in pink salmon (*O. gorbuscha*) fry exposed to Cook Inlet WSF (Rice et al. 1977). Pink salmon reach equilibrium rapidly (24-48 hours), and 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 net result of two primary factors: high lipid concentrations in their tissues and the lack of aromatic hydrocarbon metabolism because of reproductive condition. The lengthy time without reaching equilibrium concentrations in the tissues 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 prespawning 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 summers 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: toxicants and stocks of fish.

The most obvious difference between the two **studies--toxicants--** 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 observed differences 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 reduction was attributed to differences in accumulated pollutants from the more polluted 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. Thus, poor viability of herring eggs in the study by Struhsaker (1977) probably resulted from the combined effects of benzene exposure along with 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 ≥ 2.4 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 extensive examination** was not done. 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 prey and by direct **WSF** exposure, which was much more toxic. Highly contaminated prey (about 59 ppm initial tissue concentration) caused about half the herring larvae to die in 21 days, but the surviving larvae appeared robust. Contaminated prey 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 improved tank hygiene by killing bacteria and **ciliates.**

Although herring larvae were killed by low WSF concentrations (21-day **LC₅₀**, 0.36 ppm), they reacted more slowly to the toxicant than did another pelagic species (**Theragra chalcogramma**) in a similar study (**Carl's unpubl.** data). Herring larvae exposed to 1.7 ppm did not **cease**

swimming for 3-4 days. **In** contrast, the swimming ability of **I. chalcogramma** larvae exposed to 1.2 ppm WSF of oil was reduced within 4 hours (**Carls unpubl.** data).

Prey 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 maximum (5.5 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, including those individuals with empty guts. 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 because 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' 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 1984).

Exposure to hydrocarbons reduced growth. Direct exposure caused rapid, dramatic reductions, whereas dietary sources resulted in slow, small reductions. Growth was sharply reduced by WSF exposures and was significantly affected at sublethal concentrations (0.3 ppm). Reductions in growth, particularly at the high concentrations, were primarily due to reductions in feeding. In other studies, growth of larval herring was reduced by dissolved benzene, and corresponded with reduced feeding (Struhsaker et al. 1974). Growth of pink salmon alevins (Rice et al. 1975) and juveniles was reduced at least partially by reduced feeding (Moles and Rice 1983). 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 then later depressed (Linden 1978).

Contaminated prey inhibited growth only at very high concentrations and after long exposures. Other studies have also demonstrated that oil-contaminated prey affect growth and require time for the effects to become visible. 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), energy reserves in trout (**Salmo spp.**) were depleted, and growth was reduced (Hawkes 1977). In our study, hydrocarbons present in prey were partially **depurated** before consumption; **nauplii** in the pink salmon study were eaten rapidly before significant depuration could occur (Schwartz 1985).

The difference in rate of effects between **WSF** and contaminated food is caused by the large difference in hydrocarbon accumulation rates. Hydrocarbon accumulation in larval tissues is rapid 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 prey in 1 day are lost the next. Net accumulation from prey 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 deputation into the clean surrounding water because most hydrocarbons in the WSF, particularly the **monoaromatic** hydrocarbons, are not tightly bound in the lipids. Eventually, contaminated prey can affect larvae, but the low rate of hydrocarbon accumulation takes a long time before effects are evident.

Deputation of hydrocarbons by prey tends to obscure exact dose significance but emphasizes the importance of hydrocarbon loss by prey in the real world. Hydrocarbons present in prey were partially deputed before consumption. Artemia nauplii deputed WSF hydrocarbons rapidly (2% retention after 24 hours). Brachionus spp. deputed **naphthalene** rapidly during the first 2 hours, but deputation was much slower thereafter (33% retention after 24 hours). Other investigators have also observed rapid deputation of hydrocarbons from zooplankton: Coonstripe shrimp (Pandalus hypsinotus) zoeae deputed 97% in 1 day, (Brodersen unpubl. data), Calanus helgolandicus deputed 35-85% in 1 day (Corner et al. 1976), and Euchaeta japonica deputed 60% in 1 day and about 80% in 2 days (Lee 1975). Generally, crustaceans deplete 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 the remaining hydrocarbons may be metabolized (Lee 1975; Corner et al. 1976).

Contaminated prey in natural environments 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 ppm) are directly and more rapidly lethal to herring larvae. Since prey (and predator) rapidly exchange hydrocarbons with their environment, prey cannot accumulate significant quantities of hydrocarbons days or weeks in **advance and then adversely affect larvae that hatch after the** contaminant is gone.

Probable Effects of Oil on Fisheries

Catastrophic oil spills such as from the Amoco Cadiz (March 1978) on the north coast of Brittany in France and from the grounding of the Metula in the Strait of Magellan (August 1974)--both in temperate, subarctic regions--can result in the complete destruction of adjacent intertidal and subtidal floral and **faunal** communities. Accumulated crude oil in intertidal areas will persist and its effects seen for many years (Glemarec and Hussenot 1982; Gundlach et al. 1982). Incubating herring eggs would be vulnerable in this type of a situation.

Although oil in the water column in the vicinity of a spill may be more transitory, Calder and Boehm (1981) show a drop from >1,000 µg/l oil-in-water (9 days after the grounding of the Amoco Cadiz in Aber Wrac'h estuary) to 60 µg/l 48 days later. 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 from our study. Concentrations of 1.6 ppm aromatic hydrocarbons or more will kill some adult fish; however, if the oil dissipates before the surviving fish spawn, the resultant 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 and successfully hatch. If the eggs are subjected to 1.5 ppm or more for 12 or more days, at least half of them will fail to hatch. Unless there was a large oil spill or a chronic discharge of oil, it is unlikely that WSF concentrations would be high enough or would persist long enough to damage herring eggs. Although herring eggs are more tolerant of WSF than are larvae, they are 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 effects.

In contrast to herring 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 >2.8 ppm for <16 hours there will be significant mortalities. Feeding larvae cease to eat after 7 days at exposure concentrations as low as 0.4 to 0.7 ppm, which leads to mortalities 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 determinant of year-class strength in Pacific herring (Lasker 1985; Smith 1985). Herring population levels could be affected through impact on the more sensitive larval stage.

Considering the aromatic hydrocarbon load in ovarian tissue and the slow rate of deputation, 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 stage sensitive to pollutants, but there are several possible reasons why that sensitivity was not evident in our study. One is 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 have to tolerate **only** low levels of hydrocarbons. Passive deputation eventually rids the yolk of the hydrocarbons, possibly before any significant amount of damage occurs. A second possibility is damage does occur, but the 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.

Accumulation of aromatic hydrocarbons in herring tissues indicates an oil spill could have an effect on the herring fishery, even though direct evidence of an effect on reproduction is lacking. An oil spill at a spawning site could contaminate the roe in adults, making it unmarketable for human consumption. Because mature roe is harvested immediately before spawning, waiting for deputation to occur may not be practical: These fish may spawn before deputation 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 2-day exposure but **were** more sensitive than adults to 12-day exposures. 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 longer than 16 hours in larval rearing habitats could devastate **an** entire year class. 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. Accumulated hydrocarbons and slow depuration rates may **result** in unmarketable fishery products and fishery closures.

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APPENDIX

Table A.--Experimental conditions for exposures of Pacific herring (*Clupea harengus pallasii*) 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 (LC50)	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)	Deputation 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 ^{b/} three incubators ⁻
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 ^{b/} three incubators ⁻
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 ^{b/} three incubators ⁻

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 eight slides in three incubators ^{b/}
Yolk-sac larvae	40 minutes 2 hours ^{c/} 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	12 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	>4000 larvae in four tanks
Feeding larvae	21 days	Mortality Swimming Feeding Growth	0, 0.3, 0.53, 0.66	>8,000 larvae in four tanks

Table A. --Continued.

Stage exposed	Exposure time	Parameters measured	Exposure 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 (Artemianauplii).