Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms

Proceedings of a Symposium
November 10-12, 1976, Olympic Hotel, Seattle, Washington

Edited by
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Sponsored by
National Oceanographic and Atmospheric Administration
Environmental Protection Agency

PERGAMON PRESS
Oxford / New York / Toronto / Sydney / Paris / Frankfurt

1977

CHAPTER 13

EFFECT OF CRUDE OIL ON TROUT REPRODUCTION

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Abstract

Prudhoe Bay crude oil was incorporated into the diet (1 g oil/kg food) of adult rainbow trout during sexual maturation to assess the effects of long-term petroleum exposure on salmonid fish reproductive success. Parallel control fish received identical rations, except without added petroleum. When the fish reached maturity, six to seven months after initiation of petroleum exposure, a total of 31 test and 10 control crosses were made. Mean survival through hatching was 86% and 90% for test and control eggs, respectively; the difference was non-significant ($P = 0.10$). Test and control males were virtually identical in fertility. Mean survival from hatching to swim-up fry stage of development was 76% for test and 91% for control fish; again the difference was not significant ($P = 0.10$). In addition, no gross morphological or histological abnormalities were observed in offspring of petroleum-fed fish. The results of these studies were, therefore, that there was no significant impairment of reproductive success detected from this type of dietary exposure to petroleum.

Key words: Fertility, hatching success, histology, Prudhoe Bay Crude, rainbow trout

Introduction

Although in several studies there have been reports of the effects of crude oil and refined petroleum products on viability and of structural and functional abnormalities of eggs, sperm, and juveniles of aquatic species (Mironov 1969a, 1969b, Morrow 1974, Renzoni 1975, Rice et al 1975, Struhsaker 1974), we are aware of no information on the consequences of petroleum exposure on sexual maturation of fish. Interference by petroleum with sexual maturation processes could result in infertile gametes and teratogenic effects on progeny. These kinds of effects were demonstrated for trout exposed to DDT. In lake trout (*Salvelinus namaycush*), adults were exposed to DDT as a result of its use in insect control and, although they remained apparently unaffected, toxic quantities of DDT or its metabolites were deposited in eggs, resulting in high mortality of offspring (Burdick, *et al* 1964). In studies with brook trout (*Salvelinus fontinalis*), DDT fed to maturing adults also resulted in lower survival of offspring (Macek 1968).

Previous studies in our laboratory have elucidated endocrinological and biochemical mechanisms of maturation of salmonid fishes (Gronlund 1969, Gronlund and Hodgins 1970, Gronlund et al 1973). We have shown that initiation of sexual maturation takes place well in advance of outward signs of maturity and that the synthesis and storage of gonadal material is occurring at least six months prior to spawning. During this period of maturation, anadromous fishes are actively feeding at sea; as they approach estuarine waters and natal streams, the risk of exposure to pollutants increases.

The present study was initiated to test the effects of chronic dietary exposure to crude oil on salmonid fish reproduction. Rainbow trout (*Salmo gairdneri*) were used that were maturing for the first time and which were representative of steelhead trout, the anadromous form of the species. Trout were exposed to large quantities of crude petroleum incorporated into the diets, beginning in early summer of 1975 and continuing past spawning in the winter of 1976. These animals were compared with control animals maintained on a diet without added petroleum. The primary objective of the study was to evaluate effects of long-term dietary petroleum exposure on reproductive success, represented by successful hatching and by survival of normal-appearing alevins. If reproduction was impaired by the large amounts of dietary petroleum in these exploratory studies, a more in-depth examination of threshold doses and mechanisms involved was to be undertaken.

Materials and Methods

Experimental Animals

Fish used were 3-year-old rainbow trout of Cape Cod strain, obtained in June of 1975 from the Washington State Department of Game Hatchery in Spokane, Washington. At the beginning of the study the fish measured 41 to 53 cm in fork length and weighed 1.0 to 1.8 kg.

Upon arrival at the Northwest and Alaska Fisheries Center of the National Marine Fisheries Service (NMFS), Seattle, Washington, the fish were randomly placed in approximately equal numbers in one or the other of two adjacent circular fiberglass tanks (1.8 m diameter) continuously supplied with dechlorinated city water at 30 l/min and maintained at a depth of 0.8 m. Water temperature was controlled at 11 $\pm$ 1°C and artificial light was maintained to correspond to a natural light cycle.

Petroleum Exposure

Petroleum-containing food was routinely prepared in the following manner: Two kg of 1/4 inch diameter Oregon moist pellets were placed in a 4 l. glass beaker. Two g (2.6 ml) of Prudhoe Bay crude oil were mixed with 148 ml of FREON (R) TF[1/] solvent (trichlorotrifluoroethane) and poured over the food. The food and oil were thoroughly mixed and the food was spread over porcelain-covered metal trays for 90 min of air drying in a fume hood. The food was then weighed into daily aliquots, sealed in plastic bags, and frozen until used. Food for control experiments was prepared identically except the crude oil was omitted.

Fish were fed the above diets at a rate of 1.5 to 2.5% of body weight (wet weight of food) each week day starting in July 1975 and continuing through August 1976.

Maturity Assessment and Spawning Procedures

In late November 1975, all fish were examined for sex and degree of maturity. Subsequent biweekly and then weekly examinations were made. As females ripened they were spawned within one day of examination and eggs were fertilized using standard trout-culture methods (Leitritz 1959). Ripe males were consistently available for the duration of the spawning period from January through February, 1976. All eggs from control fish were divided into equal aliquots and one aliquot was fertilized with sperm from one test male and the other with sperm from one control male. Ten of the test females were similarly treated; eggs from the remaining test females were fertilized with test sperm only. A total of 31 test and 10 control crosses were made, that is, crosses utilizing different fish. Eggs were incubated in Heath trays (Heath Tecna Plastics, Inc.) at 7 to 9°C. Mortality data were collected through the yolk-sac resorption developmental stage and statistically analyzed using the Mann-Whitney modification of Wilcoxon's sum of ranks test (Langley 1971).

Chemical Analyses

Samples of adult tissue, eggs, and alevins were collected and frozen for later analysis for petroleum. Some samples were collected at the time of spawning, others were obtained four to five months after spawning. The analytical procedures followed methods of Warner (1976) utilizing alkaline digestion, solvent extraction, and silica gel column chromatography. To reduce losses of volatile compounds of Prudhoe Bay crude oil, the alkaline digestion procedure was modified as follows: 6 ml of 4N NaOH were added to 10 g of sample, which was digested at 30°C for a minimum of 16 hours. Column chromatographic fractions were analyzed by spectrofluorometry.

Fraction III from the modified Warner method, containing the fluorescent aromatic compounds, was concentrated to 2.0 ml, which were analyzed using an Aminco-Bowman spectrofluorometer, with a Model 4-8912 radio mode accessory (American Instrument Company, Silver Springs, Maryland).

Dilute solutions of Prudhoe Bay crude oil (0.1 µg/ml to 10.0 µg/ml) in methylene chloride: petroleum ether (20:80 v/v) were used as standards for the spectrofluorometric quantitation of the samples. The maximum excitation wavelength and maximum emission wavelength for Prudhoe Bay crude oil were found to be 262 nm and 364 nm, respectively.

All solvents used were either Burdick and Jackson "Distilled-in-glass" grade or Mallinckrodt "Nanograde." All glassware used in the preparation of samples for spectrofluorometric analyses was given special cleaning by immersion in boiling concentrated HNO_3 overnight, then rinsing in distilled water and drying at 120°C.

1/Trade names referred to in this publication do not imply endorsement of commercial products by the National Marine Fisheries Service.

Histology

Samples were collected for histology and prepared in the following manner: Fry were fixed in 0.75% glutaraldehyde, 3% formaldehyde, 1% acrolein in 0.1 M sodium cacodylate buffer with 0.02% $CaCl_2 \cdot H_2O$, 0.02 M S-collindine and 5.5% sucrose (Hawkes 1974). After a buffer wash the samples were post-fixed in 2% osmium tetroxide dehydrated in an ethanol series and embedded in Spurr plastic (Spurr 1969).

Sections were cut with glass knives at 0.75 to 1.0 μ thickness and stained with Richardson's stain.

Results

Mortality

Totals of 12 control (not fed petroleum) and 48 test (fed petroleum) fish were available for reproduction studies. Due to holding facility failure an additional 33 control fish died two months prior to spawning which resulted in fewer control crosses than anticipated. There was a substantial post-spawning mortality in the petroleum-fed group in which 15 fish died one to three months after spawning; all of these animals were heavily infected with fungus. None of the controls were similarly affected.

Maturation

The first males were in spawning condition by mid-December 1975, and the first females were ripe two to three weeks later (Fig. 1). Although the first ripe fish were from the test group, there appeared to be no pronounced acceleration or retardation of maturity related to petroleum exposure. Eggs were collected from ripe females starting on January 6, 1976, and collections continued weekly through February 17, 1976.

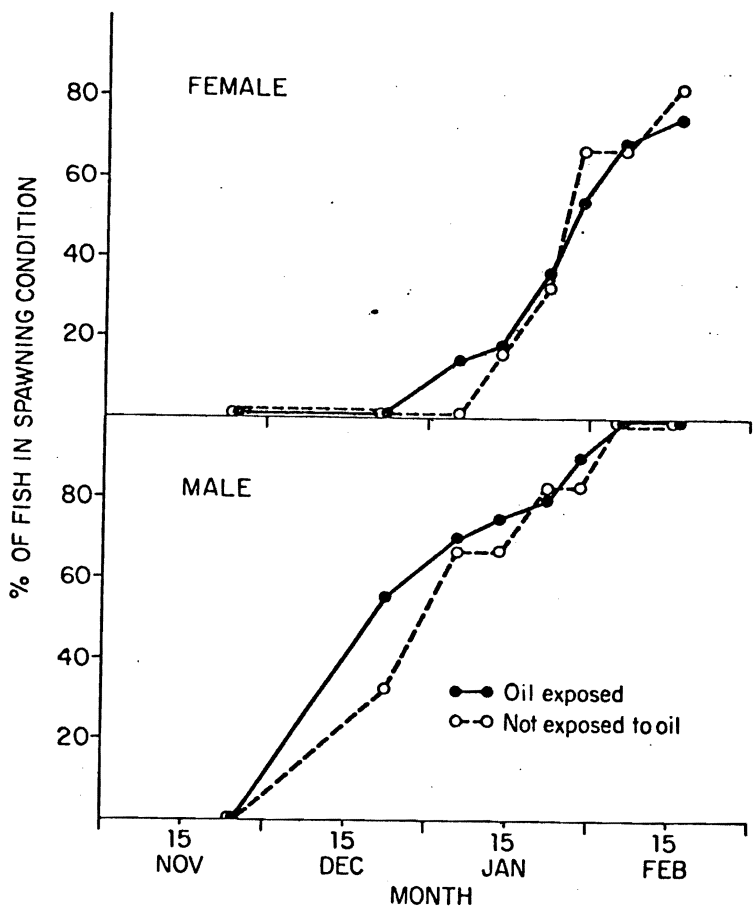

Fig. 13.1 Timing of maturation for petroleum-exposed and non-petroleum exposed rainbow trout.

Reproductive Success

No significant difference (n_A=14, R=197, P=0.10) was observed in hatching success (percent survival) among crosses in which sperm was used from petroleum-fed and non-petroleum-fed males (Table 1). One particular cross did, however, result in very low survival (5.1%) and slightly lowered the average percent hatching success of eggs fertilized with sperm from a non-petroleum-fed male.

TABLE 1. Survival of Eggs Fertilized with Sperm from Petroleum-Exposed and Non-Petroleum-Exposed Male Rainbow Trout through Hatching

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

[a]Pool of eggs from two females.
[b]Pool of eggs from three females.

Hatching success ranged from 32.4% to 99.5% for eggs from petroleum-exposed females and from 79.2% to 96.8% for non-petroleum-exposed eggs (Table 2), but the respective means of 86.4% and 90.3% were not significantly different (n_A=5, n_B=15, R=41.5, P=0.10). Eggs from two test females with 32% and 37% survival lowered the average survival of the test group.

Average survival of alevins was higher, although not significantly (n_A=4, n_B=10, R=19, P=0.10), for control than for test fish (Table 3). Again, low survival occurred in one petroleum-exposed group.

Chemical Analyses

Interference from fluorescing compounds prevented precise quantitation of Prudhoe Bay crude oil from adult trout muscle and eggs; only qualitative and semiquantitative results were possible. An emission maximum (364 nm) superimposed on the background of fluorescing compounds was observed for all samples from fish fed Prudhoe Bay crude oil; this maximum was not observed for any of the samples from fish fed the control diet (Fig. 2). The ratios of the average relative intensities at an excitation wavelength of 262 nm and an emission wavelength of 364 nm of petroleum-fed fish to control fish for muscle tissue and eggs were 2.8:1 and 3.8:1, respectively. A total of 14 analyses of muscle and 7 analyses of eggs from petroleum-fed fish and four analyses of muscle and two of eggs from control fish were performed. The background of fluorescing compounds was sufficiently high for the control and petroleum-impregnated food so that no definitive results could be obtained via spectrofluorometry.

Histology

No alevins with gross deformities were observed as a result of oil exposure. There was also no indication of abnormality in low magnification (X70) cross sections of anterior and posterior body areas in three fry from a petroleum-exposed female/petroleum-exposed male cross. At higher magnifications (X690), blood cells, muscle, mucous glands, and kidney tubules appeared normal. There were, however, instances of potentially deleterious histo-

logical abnormalities of eye lenses and livers of adult trout exposed to petroleum in these studies. These anomalies will be discussed in another paper (Hawkes 1976).

TABLE 2. Survival of Eggs from Petroleum-Exposed and Non-Petroleum-Exposed Female Rainbow Trout through Hatching

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

[a]Pool of eggs from 2 females.
[b]Pool of eggs from 3 females.
[c]Pool of eggs from 4 females.

TABLE 3. Survival of Alevins from Petroleum-Exposed and Non-Petroleum-Exposed Female Rainbow Trout

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

[a]Pool of offspring from 2 females.
[b]Pool of offspring from 3 females.
[c]Pool of offspring from 4 females.

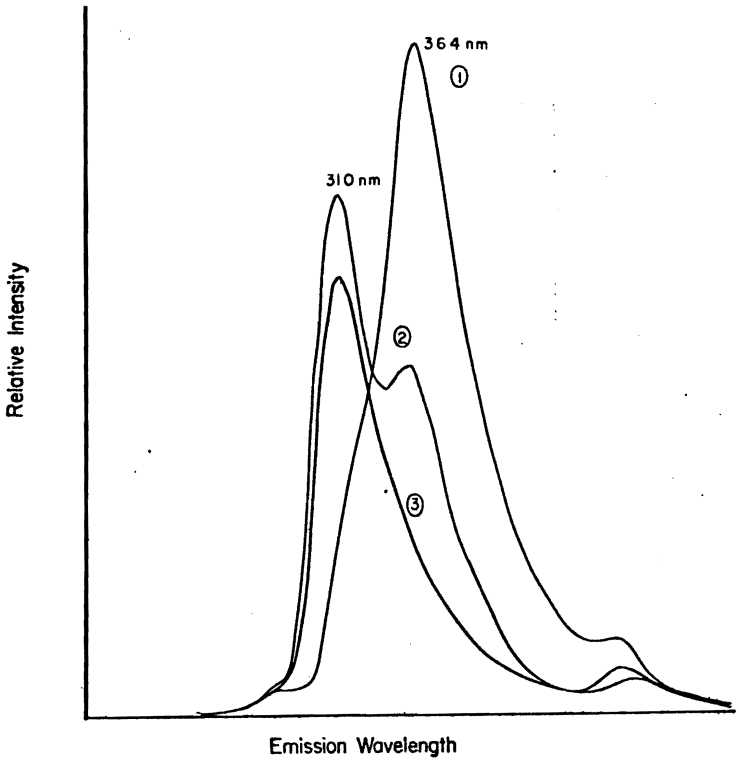

Fig. 13.2 Typical spectrophotofluorometric curves from the analyses of standard Prudhoe Bay crude oil and extracts of fish eggs.
Excitation at 262 nm
Solvent: methylene chloride in petroleum ether, 20:80 (v/v)
Curve No. 1 - Prudhoe Bay crude oil (10 µg/ml) standard
Curve No. 2 - Oil-fed fish egg extract
Curve No. 3 - Control fish egg extract

Discussion

The quantities of petroleum components consumed by test fish almost surely exceeded that which would be encountered in natural food supplies; however, it was our intention to examine an extreme case of exposure. The fish readily consumed the petroleum-impregnated food and continued to grow and develop. Although there were no mortalities of petroleum-fed fish prior to spawning, the post-spawning mortality of petroleum-exposed trout with fungus infections suggests some possible interaction between petroleum exposure and recovery from spawning. It is also possible, however, that the differential mortality between the test and control groups may have been related to a greater density of fish in the test tank compared to that in the control tank.

There was no significant impairment of hatching success related to the petroleum exposure. Survival percentages of 86 to 90% compare well with survivals of 90 to 95% for the hatchery program from which the fish were obtained (M. Albert, personal communication, 1976), as well as with published values for other studies using rainbow trout (Anon. 1973). However, eggs from two of the test females had low survivals, and it may be that certain individual fish were adversely affected by the petroleum exposure.

There is no indication that the dietary petroleum exposure had any effect on male fertility. In only one case, the hatching survival of eggs was greatly different for eggs fertilized with sperm from both best and control fish; in fact, the lowest survival was associated with a control male (Table 1). Of course, many other behavioral and physiological aspects of natural reproduction were not examined in these studies. Clearly, activities such as homing, mate selection, redd-building behavior, and territoriality could be disrupted by petroleum consumption and contribute to poor reproductive success in the natural environment.

The fluorescence spectra associated with the trout muscle indicated that certain fluorescing compounds were mobilized from the food through the circulatory system in the fish, and localized in the tissues. Similarly, as shown in Fig. 2 , evidence suggests that trout are capable of transporting certain fluorescent hydrocarbons into eggs when the fish are exposed to petroleum in food.

There is no evidence from these studies to suggest that a chronic dietary exposure to concentrations of the less volatile components of Prudhoe Bay crude oil that are likely to occur in the environment would result in reproductive failure of rainbow or steelhead trout. The histological abnormalities of eye lenses and livers observed in adult fish exposed to petroleum are potentially deleterious, however. New studies are in progress to determine if the eye and liver changes develop in young fish of the same stock fed either the same large quantity of Prudhoe Bay crude oil or 1% of that amount.

Acknowledgement

We are indebted to Linda Rhodes and Teresa Scherman for expert technical assistance.

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