

EFFECTS OF THE WATER SOLUBLE FRACTION OF CRUDE OIL ON HERRING
EGGS AND PIKE FRY

by

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Abstract

In the lowest oil concentration tested (0.6 mg/l), the hatching percentage of herring larvae was 50 %, being greater than in the control (32 %). The percentage declined as the oil concentration increased, and in the highest oil concentration (36 mg/l) only 3.5 % of the eggs hatched. For pike fry, the 96-hour LC50 value was 43 mg/l and the corresponding LC90 value was 64 mg/l. The growth of pike fry was significantly poorer in the two higher oil concentrations than in the control. In the gills of pike fry exposed to oil, the secondary lamellae were bent and changes were observed in the lamella epithelium. The epithelium of the intestine was also damaged.

Résumé

Le pourcentage d'éclosion des larves de hareng a été le plus grand (50 %) dans la plus petite teneur en pétrole (0.6 mg/l) et plus grand que celui (32 %) dans l'essai de contrôle. Mais autrement, le pourcentage d'éclosion s'est diminué à mesure de l'augmentation de la teneur en pétrole, étant 3.5 % dans la plus grande teneur en pétrole (36 mg/l). Le taux LC50 de cinq jours pour les fretins de brochet a été noté à 35 mg/l et, également, le taux LC90 à 61 mg/l. La croissance des fretins de brochet a été dans les deux plus grandes teneurs en pétrole moindre (signification statistique) que dans le groupe de contrôle. Les lamelles secondaires dans les branchies des fretins de brochet, exposés au pétrole, ont été courbées, et il y a eu des changements dans l'épithélium des lamelles. Aussi, l'épithélium de l'intestin a été déformé.

Introduction

Economically, the herring is the most important species fished off the coast of Finland. Also of importance is the pike, which is a good sport-fishing species. In the event of an oil accident these species, and especially their eggs and young, are likely to be exposed to oil.

The herring spawns in shallow bays near the coast (EHNHOLM 1951) and the pelagic larvae are abundant close to the water surface at night in the early summer (SJÖBLOM & PARMANNE 1978). The eggs and fry of pike also occur in shallow water in the littoral zone (HÄKKILÄ & NIEMI 1973). According to KÜHNHOLD (1972), fish fry cannot avoid water contaminated by oil, because their chemoreceptors rather rapidly become blocked or destroyed. The embryo and yolk-sack stages are the developmental stages most sensitive to the effects of oil and oil components (KÜHNHOLD 1972, HÄKKILÄ & NIEMI 1973).

In this study we examined the effects of the water-soluble fraction of Russian crude oil on herring embryos and pike fry. Russian crude oil constitutes about 65 % of all the oil imported into Finland. The acute toxicity and effect on growth were determined for pike fry, and tissue damage was also studied.

Material and methods

All the experiments were done in brackish water (salinity 6 ‰). The crude oil used in the tests was imported from the Soviet Union. Ten millilitres of crude oil was shaken vigorously in one litre of brackish water for 5 minutes. After shaking the oil and water phases were allowed to separate for 1-2 hours. The water phase was then used as the stock solution and diluted to the desired concentrations. The oxygen concentration and pH were measured at the end of each test and the temperature was measured daily. At the beginning and end of each test a sample of the test solution was taken for measurement of the oil concentration. All the results of the water analysis are presented in table 1.

To determine the rate of evaporation of oil from water, the stock solution was diluted 1:2 and 1:20, and samples of these dilutions

were taken after 0, 6, 12, 24 and 48 hours. The test vessels were held in a water bath whose temperature was about 14 °C. The evaporation of the oil is presented in fig. 1.

The concentrations of oil in the water samples were determined by the Institute of Marine Research with a fluorescence spectrophotometer, by the method of AHNOFF et al. (1974) and AHNOFF & JOHNSON (1977).

Hatchability test. Spring-spawning herring (Clupea harengus) caught in a trap-net were used as the parent fish. Eggs and sperm were stripped straight on to glass plates (12 x 3 cm) and mixed with a little water. Untertilized eggs were removed before the beginning of the experiment. At the beginning of the test there were 49-94 eggs on the glass plates. Fourteen hours after fertilization the glass plates were transferred to glass jars containing 0.8 litres of test solution. The glass jars were kept in the dark, in a constant-temperature water bath. Dead eggs were removed daily. The hatched fry were counted and removed once a day, and checked for malformations.

Acute toxicity test. The length of the pike (Esox lucius) fry was about 20 mm. They were reared in lake water and before the experiment they were acclimated for 2-3 weeks in brackish water. The fry were fed with zooplankton. The fish were kept in glass aquaria (35 x 22 x 18 cm, 2.5 l test solution), placed in a water bath. There were 14 fry in each aquarium. Dead fry were counted and removed after 1/2, 1, 3, 6, 12 and 24 hours, and after that once a day for 7 days.

Present age mortality in each concentration was calculated by the method of BLISS (1935), which takes into consideration mortality in the control aquarium. LC values were then calculated according to LITCHFIELD & WILCOXON (1949).

Growth test. Ten pike fry were put into three different oil concentrations and a control aquarium without oil. The aquaria, each containing 5 litres of test solution or water, were kept in a water bath. The fry were fed with zooplankton three times a day. Growth was determined by measuring the length of the fry at the beginning of the experiment and 15 days later.

Histology. For the histological examinations 20 pike fry were kept in three different oil concentrations and a control aquarium without oil. Five fry were removed and fixed from each concentration after 6, 12, 24 and 48 hours. The fixative used was 4 % neutralized formaldehyde.

Results and discussion

Hatchability test. The herring eggs began to hatch 7 days after fertilization and hatching continued for 7 days. Hatching was better in low oil concentrations (fig. 2). The higher the oil concentration, the greater were the numbers of dead larvae. The swimming movements of a few larvae were abnormal. Such larvae had a bent backbone or distended yolk sack. Fewer live larvae with abnormalities were observed than in earlier studies (STRUHSAKER et al. 1974, LINDÉN 1976). It is remarkable that the hatching percentage was higher in the oil concentration of 0.6 mg/l than in the control. There were also fewest dead hatched larvae in this concentration. According to ELDRIDGE et al. (1977) a small concentration of benzene or xylene may give better hatching than in the control.

Acute toxicity test. After half an hour in the strongest oil concentration 50 % of the fry had spasms, and in the concentration of 50 mg/l the corresponding proportion was 36 %. After 3 hours in 25 mg/l, 86 % of the fry had lost their balance, and in the other concentrations the proportion was even bigger. The fry lay with their bellies or sides upwards at the water surface. After 48 hours most of the fry that were still living had recovered. After 72 hours some of the fry were swimming normally, but some still had difficulties with their equilibrium. This condition continued in the aquaria until all the fry had died. The control fry behaved normally all the time. The recovery from disturbances in equilibrium was evidently connected with evaporation of oil from the water (fig. 1), because oil was not added during the test. HÄKKILÄ & NIEMI (1973) also noted that the narcotic effect of oil was reversible. The behaviour of the pike fry during the two first days corresponds with the results obtained by ROSENTHAL & GUNKEL (1967) and STRUHSAKER et al. (1974). They observed decreased swimming and feeding movements and disturbances in equilibrium.

The 96-hour LC50 value calculated on the basis of the mortality percentages (table 2) was 43 mg/l. The 96-hour LC90 value was 64 mg/l. According to CRADDOCK (1977), the acute toxicity of oil (expressed as LC50 values) ranges from 0.81 to 80 000 ppm, depending on - among other things - the quality of the oil, arrangement of the tests and species of fish.

Growth test. The length of the pike fry at the beginning of the test was 19.0-24.0 mm and after 15 days it was 21.0-29.0 mm. The growth of the fry was significantly poorer in the test solutions than in the control (table 3). The poor growth may have been due to a decrease in the oxygen supply caused by damage to the gills of the fry, or by decreased food utilization, because changes were observed in the epithelium of the intestine. During the first two days the fry also took less food in the higher oil concentrations than in the lowest concentration and the control. The growth of herring in water containing oil was also poor (STRUHSAKER et al. 1974).

Histology. In the oil concentration of 2.2 mg/l, changes were observed in the gills of the pike fry after 24 hours, and in the greater concentrations after as little as 12 hours. The secondary lamellae were bent parallel to the filaments, with which they normally form an angle of 60°. In some gills the epithelium of the lamellae was also detached from the pillar cell system or the plasma membrane of the epithelial cells was folded. The damage was worst in the greatest oil concentration.

In pike fry exposed to the lowest oil concentration for one day, the intestine had swollen epithelium. In fry kept in the oil concentration of 2.2 mg/l the cells of the epithelium were detached at their bases after 12 hours. After longer exposure to this concentration the damage was similar, but in the greater oil concentrations the epithelium also contained necrotic cells. Comparable changes in histology have been observed in rainbow trout (Salmo gairdneri) exposed to oil (McKEOWN & MARCH 1978).

These results suggest that an oil accident can cause great harm to fish eggs and fry, if it happens during the spawning time and near spawning places.

References

- AHNOFF, M. et al. 1974: Analysis of sea water and sediments with emphasis on environmental contamination. Gothenburg to Uddevalla. - Dept. of Analytical Chemistry, University of Göteborg. Report on the chemistry of sea water 14: 1-24 (mimeogr.).
- AHNOFF, M. & JOHNSON, L. 1977: Quantitation and characterization of petroleum hydrocarbons in Baltic Sea water. - Report to the Soviet-Swedish Expert Meeting on Evaluation of Results from the Joint MUSSON-Expedition 1976 and Planning of Future Cooperation, Göteborg, April 24-30, 1977. 33 pp. (mimeogr.).
- BLISS, C.I. 1935: The calculation of the dosage - mortality curve. - Ann. appl. Biol. 22: 134-167.
- CRADDOCK, D.R. 1977: Acute toxic effects of petroleum on arctic and subarctic marine organisms. - In: MALINS, D.C. (ed.), Effects of petroleum on arctic and subarctic marine environments and organisms II. Biological effects: 1-93. New York/San Francisco/London.
- EHNHOLM, G. 1951: Studier över strömmingen i östra Kvarken. - Helsingfors, 94 pp.
- ELDRIDGE, M.B., ECHEVERRIA, T. & WHIPPLE, J.A. 1977: Energetics of Pacific herring (*Clupea harengus pallasii*) embryos and larvae exposed to low concentrations of benzene, a monoaromatic component of crude oil. - Trans. Amer. Fish. Soc. 106:452-461.
- HÄKKILÄ, K. & NIEMI, A. 1973: Effects of oil and emulsifiers on eggs and larvae of northern pike (*Esox lucius*) in brackish water. - Aqua Fennica 1973: 44-59.
- KÜHNHOLD, W.W. 1972: The influence of crude oils on fish fry. - In: RUIVO, M. (ed.), Marine pollution and sea life: 315-318. FAO Fishing News (Books), Ltd., London.
- LINDÉN, O. 1974: Effects of oil spill dispersants on the early development of Baltic herring. - Ann. Zool. Fennici 11: 141-148.
- 1976: The influence of crude oil and mixtures of crude oil/dispersants on the ontogenic development of the Baltic herring, *Clupea harengus membras* L. - Ambio 5: 136-140.

- LITCHFIELD, J. T. Jr. & WILCOXON, F. 1949: A simplified method of evaluating dose-effect experiments. - J. Pharmacol. Exp. Ther. 96: 99-113.
- McKEOWN, B. A. & MARCH, G. L. 1978: The acute effect of bunker C oil and an oil dispersant on: 1 serum glucose, serum sodium and gill morphology in both freshwater and seawater acclimated rainbow trout (*Salmo gairneri*). - Water Research 12: 157-163.
- ROSENTHAL, H. & GUNKEL, W. 1967: Wirkungen von Rohöl-Emulgatorgemischen auf marine Fischbrut und deren Nährtiere. - Helgoländer Wiss. Meeresunters. 16(4): 315-320.
- SJÖBLOM, V. & PARMANNE, R. 1978: The vertical distribution of Baltic herring larvae (*Clupea harengus* L.) in the Gulf of Finland. - Finnish Fish. Res. 2: 5-18.
- STRUHSAKER, J. W., ELDRIDGE, M. B. & ECHEVERRIA, T. 1974: Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. - In: VERNBERG, F. J. & VERNBERG, W. B. (ed.), Pollution and physiology of marine organisms: 253-284, Academic press, New York/San Francisco/London.

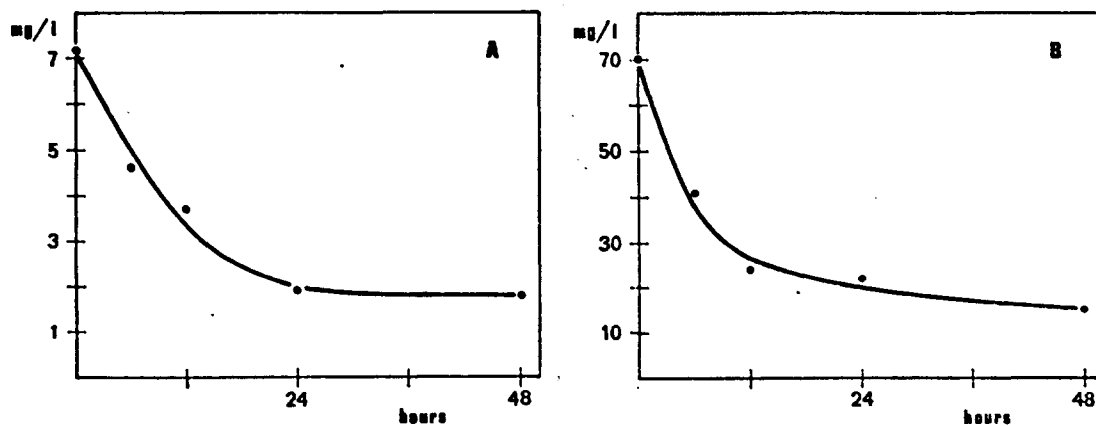


Fig. 1. Evaporation of oil from test media.

A. Dilution 1:20 of stock solution.

B. Dilution 1:2 of stock solution.

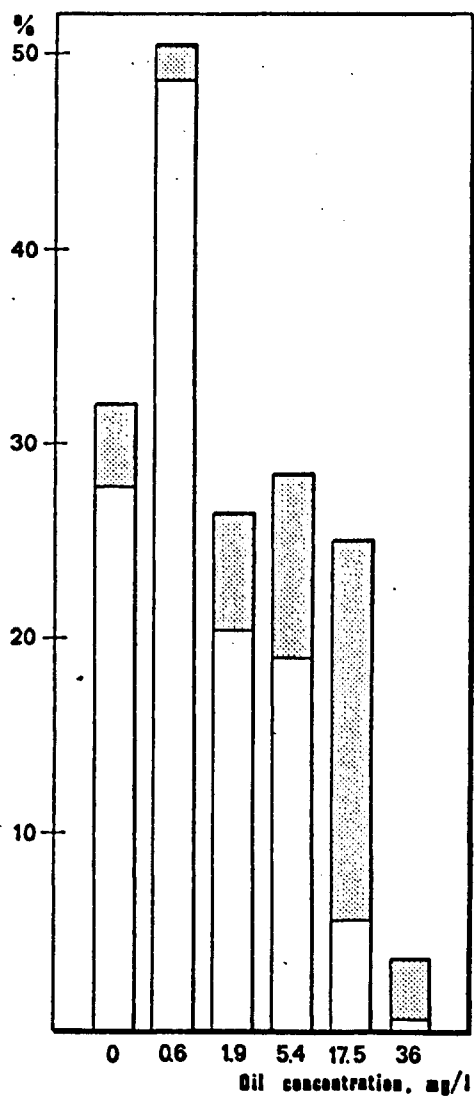


Fig. 2. Percentage hatching of herring larvae in different oil concentrations. The white part of the column represents living larvae and the shaded part dead hatched larvae.

Table 1. Results of the water analysis.

	Herring						Pike														
	Hatchability test						LC-test				Growth test				Histology						
Length of test, days	13						7				15				2						
Oil concentration, mg/l	start	0	0.6	1.9	5.4	17.5	36	0	20	25	38	50	69	0	0.1	2.2	5.2	0	0.4	2.2	-
	end	0	0.0	0.1	0.1	0.3	0.5	0	0.5	0.7	1.0	1.1	1.2	0	0.0	0.3	1.4	0	0.6	1.5	16
T °C	14.0 ± 0.9						16.3 - 18.0				14.3 - 18.0				16.2 - 16.9						
pH	7.6 - 7.7						7.8 - 8.0				7.6 - 7.7				7.9						
O ₂ , mg/l	7.3 - 8.0						5.4 - 7.9				3.8 - 4.6				6.9 - 8.8						

Table 2. Percentage mortality of pike fry in different oil concentrations.

h/d	Oil concentration, mg/l				
	20	25	38	50	69
3				28.6	100
6			9.1	42.9	100
12			14.3	71.4	100
24			14.3	71.4	100
2			14.3	71.4	100
3			14.3	71.4	100
4	9.1	9.1	14.3	72.7	100
5	9.1	30.0	50.0	80.0	100
6	60.0	83.3	100	100	100
7	100	100	100	100	100

Table 3. The growth of pike fry during 15 days in different oil concentrations. The numbers of dead larvae are also presented.

		Oil concentration, mg/l			
		0	0.1	2.2	5.2
Mean length, mm	start	22.2 ± 0.33	22.2 ± 0.36	21.1 ± 0.34	21.2 ± 0.37
	end	26.0 ± 0.42	25.6 ± 0.36	23.7 ± 0.53	23.2 ± 0.23
Growth, %		17.1	15.3	12.3	9.4
Significance			p < 0.1	p < 0.01	p < 0.001
Days		Dead larvae			
6				1	
10					1
12			1		
13		1		1	1
15			3		2