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SUBLETHAL EFFECTS OF THE WATER-SOLUBLE FRACTION OF EKOFISK CRUDE OIL ON THE EARLY LARVAL STAGES OF COD (GADUS MORHUA L.)

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ABSTRACT

Continuos exposure of cod eggs and larvae to 50 and 250 ppb of the water-soluble fraction (WSF) of Ekofisk crude oil for 14 days, caused significant reduction in growth and change in neutral buoyancy. The larvae exposed to 250 ppb showed malformation in the foremost part of the head and jaw, which reduced their ability to capture prey organisms at first feeding.

Cod larvae lost coordination of their swimming movements after one hour exposure to 0.9 ppm of the WSF of oil hydrocarbons.

First feeding cod larvae did not recover from one hour exposure to 4 ppm of the WSF of oil hydrocarbons when tested in feeding experiments after 24 hours in clean sea water. The feeding incidence was reduced by 50% compared to unexposed larvae.

INTRODUCTION

During an oil spill situation in the open sea, concentrations of the total dissolved oil hydrocarbons in the water column, seldom exceeds 100 ppb (Grahl-Nielsen et. al., 1976, 1979, Law, 1978). In shallow water, however, it might as well reach concentrations of 300-400 ppb or even higher (Blackman and Law 1981).

Laboratory experiments on embryoes and larvae of various marine organisms, indicate that these low levels of dissolved oil hydrocarbons, or selected components involve sublethal effects such as morphological deformations, retarded growth and changes in hatching time (Mazmanidi and Bazhashvili, 1974., Struhsaker, 1976, Eldridge et. al., 1977, Kühnold, 1979, Kühnold et. al., 1979, Leung and Bulkley, 1979 and Linden et. al., 1980).

The present paper presents some results of the effect of the water soluble fraction (WSF) of Ekofisk crude oil at concentrations of 50 ppb to 4.5 ppm on cod larval (Gadus morhua L.) growth, early development, feeding activity and behaviour.

MATERIAL AND METHODS

Biological material

Cod eggs were artificially fertilized in the laboratory after being stripped from ripe ovaries of coastal cod (Gadus morhua L.). The eggs were gently washed in clean sea water 2 hours post fertilization. Dead eggs sank to the bottom and were removed. After 8 hours 10 ml eggs were transferred to each of several 10 liter black plastic aquaria with white bottoms. Antibiotics were administrated according to Shelbourne (1963), and 2500 IE Mycostatin/liter was also added. These doses were administrated only once. The aquaria were placed in termostat controlled waterbaths at 5^{o}C. During incubation, filtered air (0.2 µm Millipore bacteria filters), was gently bubbled through the aquaria.

Ten days post fertilization, about one week prior to hatching, 2 ml eggs were transferred to each of six aquaria in a biotest oil exposure system, and exposed to sea water contaminated with the WSF of Ekofisk crude oil. The larvae were not fed during the incubation in the biotest system. The exposure experiments were terminated two weeks post hatching, three days past the point of no return. Exposure experiments were run on two groups of cod eggs/larvae from two different female fish.

The biotest oil exposure system

The oil polluted sea water was made in a flow-through doseing system described by Johannessen (1978). This doseing system was connected to the biotest system (Fig. 1). The biotest system consists of three parallel subunits, (one presented in Fig. 1), one for each of the two oil concentrations selected in the present experiment and one control unit. The flow rate through each aquarium was about 100 ml/minute. To minimize bacterial growth an UV-sterilizing unit was connected to the sea water inlet of the doseing system. For the same reason, the crude oil-sea water mixer unit of the doseing system consisted of two parallel units. The sea water and oil flow were switched from one unit to the other once a week. The mixer not in use was then cleaned and sterilized.

Chemical analysis

The concentration and composition of the dissolved fraction of Ekofisk crude oil hydrocarbons were analysed and monitored by a gaschromatographic-masspectrophotometric system according to the methods described by Grahl-Nielsen et. al., (1979).

Effect studies

Two exposure experiment were run, one on each of two cod eggs/ larval groups. Both groups were continously exposed to about 250 ppb and 50 ppb of the WSF of Ekofisk crude oil. The exposure lasted from one week prior to hatching to fourteen days post

hatching. During this period, 10 larvae were sampled daily and preserved on 4% formalin in 10 °/oo sea water. Larval standard length (nearest 0.1 mm), dry weight (nearest 1 µg, Chan electrobalance) and the larval morphological development were studied daily.

The change in neutral bouyancy of cod eggs and larvae during the eposure experiments was observed according to the methods of Tilseth and Strømme (1976).

The ability of the oil exposed larvae to capture and digest micro zooplankton was tested in feeding experiments. Natural zooplankton was collected from 15 meters depth in the Byfjord, close to the Institute of Marine Research, Bergen, by a automatic plankton sampler system (Fig. 2). The concentrated plankton sample was allowed to stand for one hour in a 2 liter plastic beaker. Dead organisms were removed and two subsamples of 10 ml were analysed to identifie and count the plankton. The feeding experiments were run in 4 liter glassjars in clean or oil contaminated sea water, zooplankton were added to a density of 500 organisms per liter. Twenty larvae were transferred to each glassjar, and the feeding experiments were run for one hour at 5°C and about 100 lux light intensity. At the end of the test, the larvae were preserved on 4% formalin in 10 °/oo sea water.

The swimming activity of oil exposed cod larvae was recorded by a low-light TV-camera on videotape. The larvae were transferred to a 50 cm in diameter, 5 cm deep plastic aquarium with a 1 cm^2 grid on the bottom. The aquarium was placed in a black plastic tent in a waterbath at 5°C. The light intensity was regulated to 100 lux. The larval swimming activity was recorded for 10 minutes after a adaption period of 30 minutes. The larval swimming frequency and speed were studied by tracking the larvae on the monitor screen, by playback of the videotape.

A few feeding and "recovery" experiments were run on larvae reared in clean sea water, after exposure to 0.9-4.5 ppm oil contaminated sea water for 1 to 2 hours. The recovery experiments were run as larval feeding experiments after transfer to clean sea water for 24 hours.

RESULTS

Analysis of the oil hydrocarbon concentrations in the exposure aquaria showed that cod eggs and larvae were exposed to 1/15 and 1/60 of the stock solution. The results of the GC, GC-MS analysis on the concentration of the WSF of oilhydrocarbons in the stock solution and in the water of the exposure aquaria is presented in Table 1.

Table 1. Concentration of the WSF of oil hydrocarbons and the consentration of benzene, toluene and xylene (BTX) in the stock solution and the exposure aquaria during two exposure experiments on cod eggs and larvae.

	Stock solu.				Exp. aquaria				
	GC				GC-MS	High		Low	
Exp.	Anal.	WSF	BTX	SD±	Anal.	WSF	BTX	WSF	BTX
1	13	1.5 ppm	3.0 ppm	0.17	3	100 ppb	145 ppb	19 ppb	28 ppb
2	7	1.7 ppm	2.6 ppm	0.24	2	136 ppb	129 ppb	29 ppb	37 ppb

The more volatile components, benzene, toluene and xylene constituted 60-70% of the total WSF of oil hydrocarbons in the stock solution and only 50-60% of the total WSF in the sea water of the exposure aquaria, clearly indicating evaporation of these more volatile components through the doseing biotest-exposure system.

Cod eggs and larvae were consequently, according to these results, exposed to the average concentrations of 245 ppb and 265 ppb at the highest exposure, and 47 ppb and 66 ppb at the lowest exposure to the total WSF of the Ekofisk crude oil hydrocarbons, during the two exposure experiments.

Effect studies

The concentrations of the total WSF of oil hydrocarbons were kept at a sublethal level during the two exposure experiment. At the end of the experiments the cod larval mortality was 10%

and 9% in larvae exposed to the highest concentrations (245 and 265 ppb), 8% and 7% in larvae exposed to the lowest concentrations of the total WSF of oil hydrocarbons, and 8% in both control groups.

Figs 3A,B and 4 shows that larvae exposed to oil polluted sea water obtained reduced growth, both in standard length and dry weight, compared to unexposed larvae. The reduction in standard length was smallest in larvae exposed to the lowest concentration of the WSF of oil hydrocarbons, However, the difference was significant in both experiments, $p < 0.001$ and $p < 0.01$ respectively (paired t-test). The larval dry weight was only measured during the last exposure experiment, and the difference was smallest in larvae exposed to the lowest oil consentration compared to unexposed larvae ($p < 0.01$ paired t-test).

Changes in the larval morphology during the early post hatching stages was observed in larvae exposed to the highest concentrations of oil contaminated sea water. As shown in Fig. 5 the front part-upper jaw of the larvae exposed to 245 ppb of the WSF of oil hydrocarbons for 14 days became malformed.

The oil exposed larvae, gradually developed a lower specific weight than larvae reared in clean sea water. This was recorded as changes in neutral bouyancy (Fig. 6) which became obvious from day 5 post hatching and most evident in larvae exposed to the highest concentrations of oil contaminated sea water.

The most dominant species of zooplankton in the feeding experiments were copepod eggs and nauplii, which constituted in numbers 80-90% of the zooplankton sample, while the remaining 10-20% was copepodites, rotiferes, trocophor larvae and bivalve veliger larvae, within the size range 100-500 microns. More than 90% of the cod larval stomach content was copepod nauplii, which was prefered to copepod eggs or other food particles. There was not observed any difference in preferance of different food particles between cod larvae exposed to oil and unexposed larvae. The larval ability to digest nauplii was studied by

catagorizing the ingested nauplii according to Ellertsen et. al. (1977) in three categories: 1) undissolved, 2) partly dissolved and 3) dissolved (only excuviae left). There was not observed any difference in the ability of digesting nauplii between larvae exposed to oil contaminated sea water and un-exposed larvae.

However, there was a significant reduction both in feeding incidence (percent larvae with gut content) and feeding index (number of food particles per. larvae with gut content) in larvae exposed to the highest concentration of the WSF of oil hydrocarbons compared to unexposed larvae (Figs 7A, B and 8A, B). There proved to be both the effect of exposure to oil polluted sea water and variation within the cod larval groups (egg from different female fish). In group 1 the cod larval feeding incidence was 29% and the feeding index 1.5 particles per. larval gut in cod larvae exposed to 245 ppb WSF oil hydro-carbons, and only 3% feeding incidence and 1.0 particles per. larval gut in the group 2 cod larvae exposed to 265 ppb. The feeding incidence was 70% and feeding index 2.6 in the control group 1 cod larvae, and 60% feeding incidence and the feeding index 1.7 in the control group 2 cod larvae. These result refers to the feeding experiment on day 8 post hatching, when the cod larval feeding activity was at its maximum.

The cod larval swimming activity was tested after exposure to 4.5 ppm and 0.92 ppm the WSF of oil hydrocarbons. The cod larvae shows intermittent swimming, in jerks lasting less than 0.5 seconds abrupted by pauses of variable duration. The swimming activity is measured as the frequency of jerks and the larval swimming speed. The distance per. jerk is then calculated and given as an index of the larval ability to coordinate its swimming pattern. The result of these experiment are presented in Table 2.

Table 2. Cod larval swimming speed (cm/minutes), swimming frequenzy (jerks/minute) and swimming index (cm/jerks) of larvae exposed to 4.5 ppm and 0.92 ppm of the WSF of oil hydrocarbons for one hour prior to recording. Ten larvae were tested in each experiment.

Larval age post hatching	Swimming activity	Control	Cod larvae exposed to 4.5 ppm	0.92 ppm
5	Speed	18.1 (±14.4)	3.9 (±3.1)	
	Frequen.	14.7 (±10.15)	11.9 (±6.2)	
	Index	1.1 (±0.3)	0.3 (±0.1)	
6	Speed	20.0 (±5.0)	4.2 (±2.7)	6.1 (±4.1)
	Frequen.	22.7 (±7.8)	11.9 (±6.2)	8.4 (±3.1)
	Index	1.4 (±0.7)	0.3 (±0.1)	0.7 (±0.5)

The cod larval swimming speed was seriously reduced when the larvae where exposed to oil polluted sea water at 4.5 ppm and 0.92 ppm respectively for 1 hour and 30 minutes. This was also true for the larval swimming index (distance per. jerk), indicating serious disturbance of the larval swimming pattern.

The results of tests on the feeding ability of cod larvae exposed to 0.6 ppm, 0.9 ppm, 4 ppm and 4.5 ppm of the WSF of oil hydrocarbons during, or 1 to 2 hours prior to the feeding experiments, are presented in Tables 3 and 4.

Table 3. Cod larval feeding experiments in oil contaminated sea water (7 days post hatching, group 1). The larvae and food organisms (0.5 organisms per. ml) were simultaniously transferred to the feeding aquaria. The larvae were allowed to feed for 1 hour.

Time, expos.	WSF oil cons.	% Feeding incidence	Feeding index	No., of larvae
1 Hour	0.6 ppm	65	2.6	20
1 "	4.0 ppm	25	1.6	20
Control	0	59	1.6	22

Table 4. Cod larval feeding experiments, in oil contaminated sea water (7 days post hatching, group 2). The larvae were transferred to the feeding aquaria 1 to 2 hours after exposure to oil contaminated sea water (0.5 organism per. ml for 1 hour).

Time, expos.	WSF oil cons.	% Feeding incidence	Feeding index	No., of larvae
2	0.9 ppm	10	1.3	40
2	4.5 ppm	0	0	41
3	4.5 ppm	0	0	20
Control	0	34	1.3	41

Both the time of exposure and the concentration, of the WSF of oil hydrocarbons, effected the cod larval feeding ability. Exposed to 0.6 ppm for 1 hour during feeding, did not seem to cause any disturbances in the larval feeding ability. At 4 ppm, however, only 1/4 of the larval population were able to feed, and when exposed to 4.5 ppm for 1 hour prior to the experiment no larvae were able to capture prey organisms.

The results for the "recovery" experiments are presented in Table 5.

Table 5. The results of feeding experiments on cod larvae 24 hours after exposure to 4.5 ppm, 0.6 ppm and 4.1 ppm of the WSF of oil hydrocarbons for 1 hour.

Larval age	WSF oil cons.	Feeding incidence	Feeding index	No., of larvae
7 days	4.5 ppm	38 %	1.3	21
- " -	Control	68 %	1.4	22
8 days	0.6 ppm	63 %	1.5	21
- " -	4.1 ppm	38 %	1.9	21
- " -	Control	55 %	1.8	20

Larvae exposed to 4.1 ppm or higher concentrations of the WSF of oil hydrocarbon does not seem to have recovered their feeding ability in 24 hours after transfer to clean sea water. Exposed to 0.6 ppm for 1 hour, however, does not seem to effect the larval ability to capture prey organisms 24 hours later.

DISCUSSION

The selected concentrations of dissolved oil hydrocarbons in sea water, in studying sublethal effects in larval cod are within the range that might well be experienced during an oil spill situation (Grahl-Nielsen et. al., 1976, 1979, Law, 1978, Blackman and Law, 1981). In our laboratory experiments, the most volatile components of the crude oil, did not evaporate from the stock solution nor during flow through the doseing system and the biotest exposure system. These components have been observed to evaporate and nearly disappear from the oil slick and the upper surface water layers during the first days of weathering (Grahl-Nielsen et. al., 1976, Petersen, 1979, Riley et. al., 1980, 1981). However, there might well be situations where the concentration of the volatile components of crude oil could reach levels above those reported, for instance during a subsurface blow out. The components benzene, toluene and xylene were most readily dissolved in sea water and constituted approximately 60% of the total WSF of Ekofisk crude oil hydrocarbons in our exposure biotest aquaria. These components are known to be among the most toxic ones of the crude oil (Beuville and Korn, 1977, Morrow et. al., 1975), and could be responsible for the observed sublethal effects in cod larvae.

Exposure of cod larvae to concentrations of the WSF of oil hydrocarbons at levels of 0.9-4.5 ppm for only 1 hour caused serious reductions in larval feeding ability and swimming activity (Tables 2, 4). Effects of the WSF of oil hydrocarbons or oil components at similar levels on other aquatic animals have been reported by Beuville and Korn (1977), Lønning (1977), Rice et. al., (1977) and Moles (1979). However, about 18% of

the larval population exposed to 4.1 ppm for 1 hour did not recovered their feeding ability within 24 hours (Table 5). As cod larvae has proved to be visual feeders at the onset of exogenous feeding (Ellertsen et. al., 1980), the ability to capture prey organisms is consequently a complicated interaction of physiological and behavioural factors, and oil hydrocarbon toxicants could have affected any of these, and thereby permanently reduced the larval feeding ability.

Exposing cod eggs and larvae to 47 ppb, 66 ppb and 245 ppb, 265 ppb of the WSF of oil hydrocarbons for three weeks did not cause differences in mortality between test and control larvae (page 5 and 6), nor did we observe any change in time of hatching. The mass mortality in cod larvae exposed to oil contaminated sea water occurred concomitantly with the control larvae three days past the point of no return (PNR), as described by Tilseth and Strømme (1976), showing that the exposure experiments were run at sublethal concentrations. However, the growth of oil exposed larvae was significantly reduced, both the larval standard length and dry weight (Fig. 3A and B). Also the neutral bouyancy (Fig. 6) of oil exposed larvae became reduced compared to unexposed larvae. Recent experiments (in press) showed no change in the osmotic or ionic composition of the body fluids of exposed larvae, indicating that the reduction in growth and specific weight most probably were caused by a changed capacity in utilization and transformation of yolk to somatic tissue. Retarded growth has been reported in several aquatic larval organisms exposed to oil contaminated water, and this is thought to be caused by an extra energy demand in the detoxification prosess of hydrocarbons (Johnson et. al., 1979), Leung and Bulkley, 1979 and Lindén, 1980).

Morphological deformations following exposure to concentrations of oil hydrocarbons lower than 100 ppb, have been reported for several species; Black Sea flounder (Mazmanidi and Bazhashivili, 1975), spotted seatrout (Johnson et. al., 1979) and in Fundalus

hetroclitus (Linden, 1980). The deformation in the front head upper jaw region of cod larvae (Fig. 4) exposed to 245 ppb and 265 ppb for three weeks was therefore not surprising. The observed reduced feeding ability in these larvae (Figs 7 and 8) was most probably due to this malformation causing reduced capacity in capturing prey organism. In addition, the cod larval visual threshold could have been reduced. This has been reported for spotted seatrout, following exposure to low levels of oil hydrocarbons (Johnson et. al., 1979), which have proved to result in deformation of the eyelense (Hawker, 1980).

The results presented in the present paper, shows clearly a reduced feeding ability in cod larvae exposed to about 250 ppb of the WSF of Ekofisk crude oil for 14 days. This effect is thought to be most serious considering the survival of first feeding cod larvae. Hjorts (1914) hypothesis for fish larval mortality is based on variable feeding conditions at a critical stage, which is thought to be at the end of the yolksac stage (EYS). Ellertsen et. al. (1976) showed that massmortality in cod larvae occured during a short period of time (8 days) during limited food conditions, starting four to five days past the EYS. Our feeding experiments were run at 500 particles/liter. This density have only been found in patches in sheltered fjords and never in the open sea of the cod larval first feeding areas off the Lofoten islands (Tilseth and Ellertsen, 1981). The observed sublethal effects in cod larvae would most probably have caused heavy mortality in a population of cod larvae in the sea at the onset of exogenous feeding.

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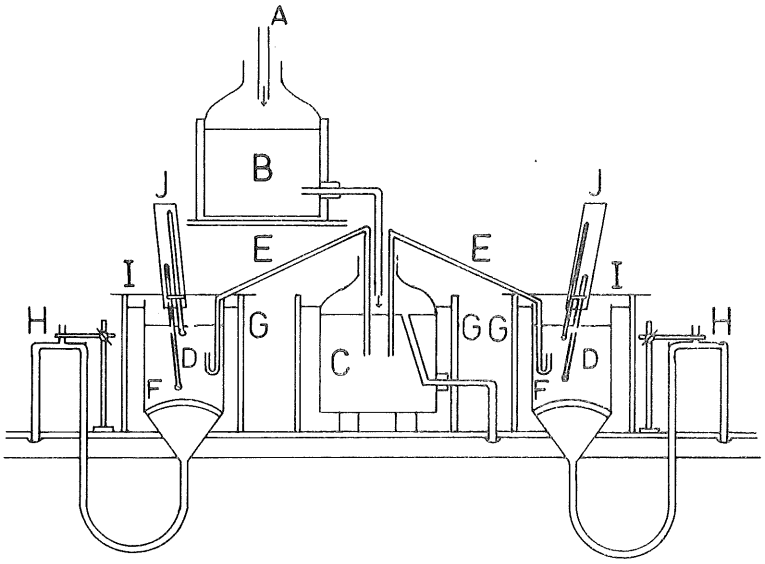

Fig. 1. The biotest exposure system. A, water inlet from the doseing system, B, reservoire, C, overflow reservoire, to keep water level constant, E, siphons, F, glass sinter (100-200 µm), D, 4 liter exposure aquaria, büchner funnels, G, termostat controlled waterbaths (5°C), I, neutral filter (100 lux), J, thermometers. The flow rate through the system is controlled by the difference in levels between the "open" siphon M and the water level in reservoire C.

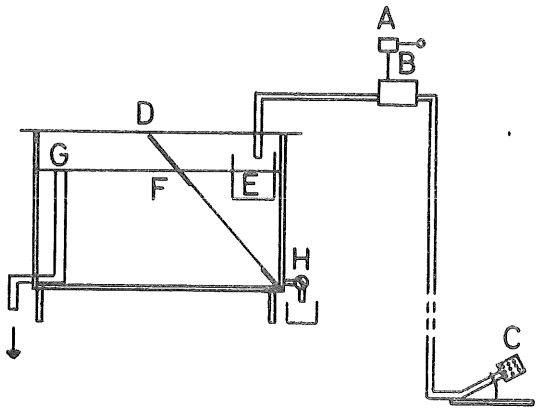

Fig. 2. Automatic plankton sampler. A, time switch controling pump (70 liter/min), B, pumping water from 15 meters depth through the perforated (1 cm in diameter) mouthpiece, C, sea water is filtered through bag, E, (500 µm mesh size) in the 1.5 m^3 sampling tank, F, partition wall with a window covered by 90 µm plankton net. The overflow tube G can be turned 90°, and the concentrated plankton sample (100-500 µm) is collected at M.

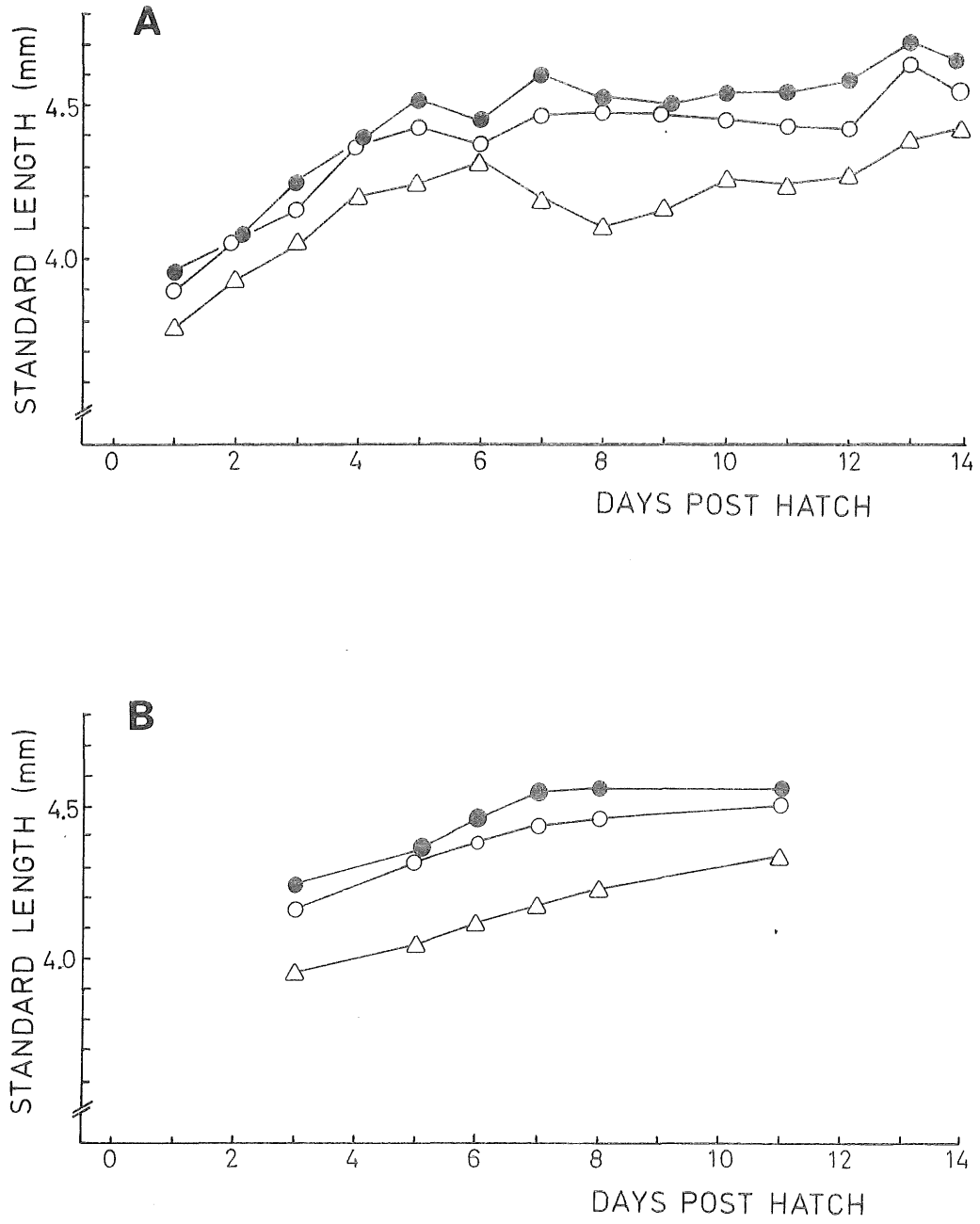

Fig. 3A and B. Cod larval standard length. A, group 1 cod larval exposed to Δ 245 ppb, 0 45 ppb and B, group 2 cod larvae exposed to 265 ppb, o 66 ppb of the WSF of Ekofisk crude oil. The exposure started 7 days prior to hatching. ● unexposed larvae. (Each point is the average SL of 10 larvae).

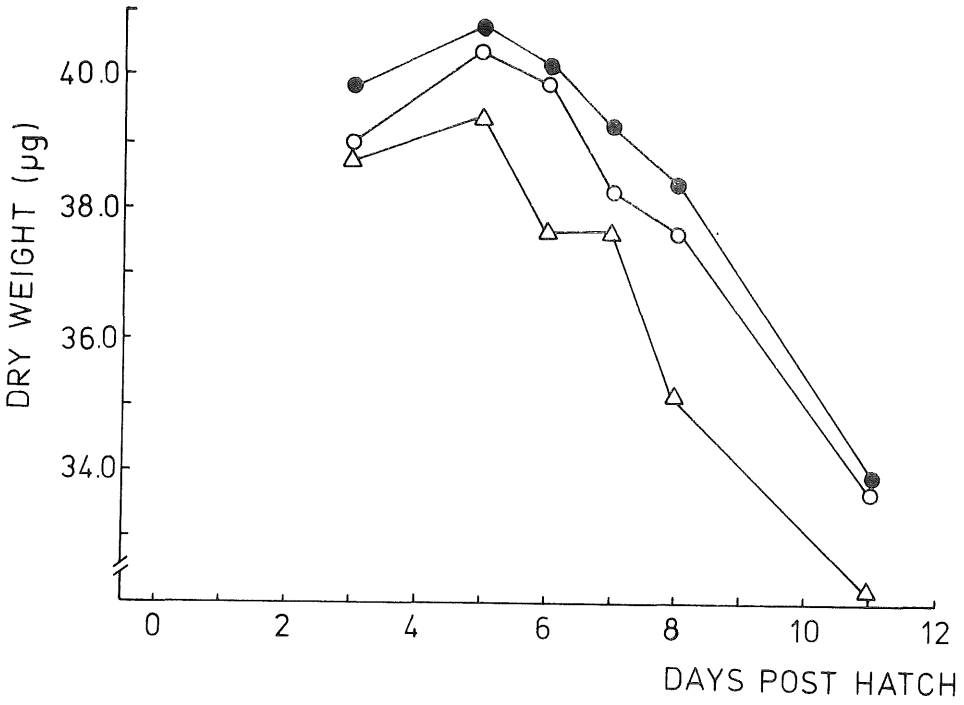

Fig. 4. Cod larval dry weight (group 2). The cod larvae were exposed to Δ 265 ppb and 66 ppb of the WSF of Ekofisk crude oil. The exposure started 7 days prior to hatching. ● unexposed larvae. (Each point is the average dry weight of 10 larvae).

Fig. 5. Schematic drawing of 7 days old cod larvae. Larvae A exposed to 245 ppb of the WSF of Ekofisk crude oil for 14 days, Larvae B unexposed.

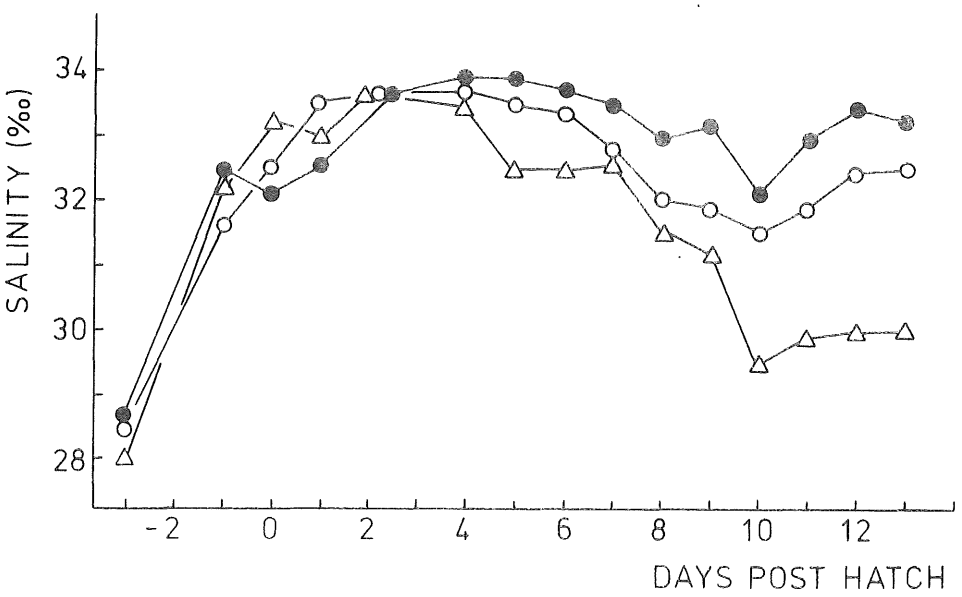

Fig. 6. The salinity of neutral bouyancy of cod eggs and larvae (group 1) exposed to Δ 245 ppb and 45 ppb of the WSF of Ekofisk crude oil. The exposure started 7 days prior to hatching. ● unexposed larvae (n=20 for each point).

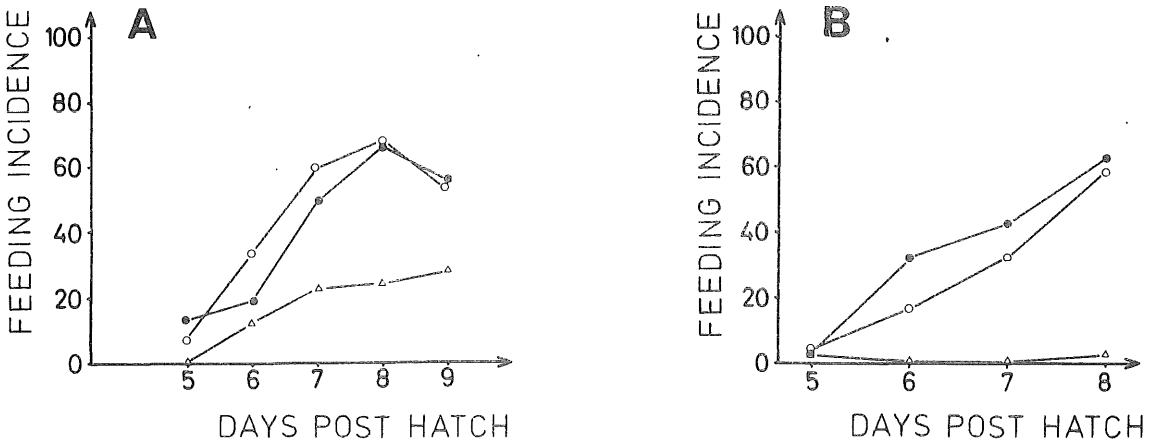

Fig. 7A and B. Cod larval feeding incidence (% larvae with gut content). A, group 1 cod larvae exposed to Δ 245 ppb and o 45 ppb, and B, group 2 cod larvae exposed to Δ 265 ppb and o 66 ppb of the WSF of Ekofisk crude oil. The exposure started 7 days prior to hatching. ● unexposed larvae (n=40 for each point).

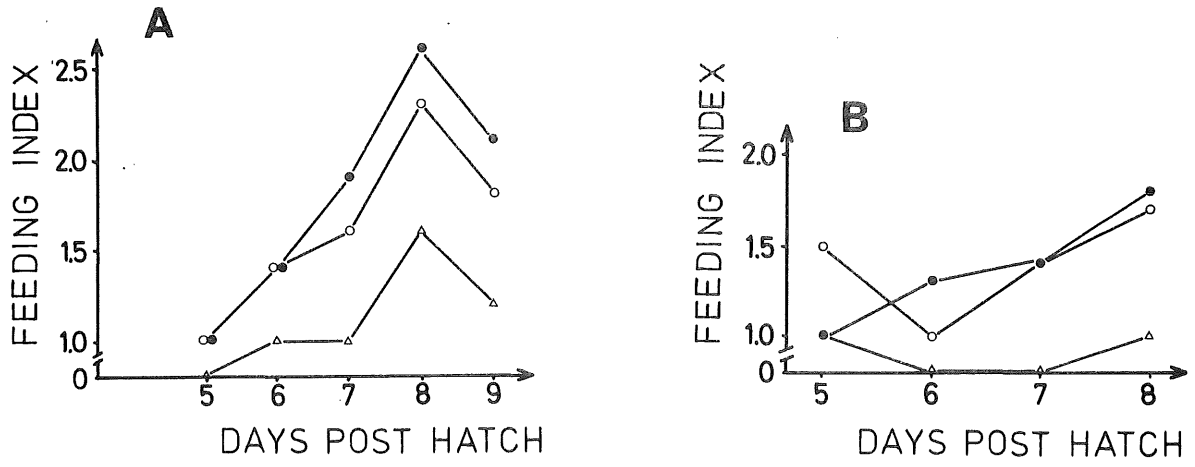

Fig. 8A and B. Cod larval feeding index (number of food particles per. larval gut). A, group 1 cod larvae exposed to Δ 245 ppb and o 45 ppb and B, group 2 cod larvae exposed to Δ 265 ppb and o 66 ppb of the WSF of Ekofisk crude oil. The exposure started 7 days prior to hatching, ● unexposed larvae (n=40 for each point).