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Effects of seawater extract of Ekofisk oil on hatching success of Barents Sea capelin

by

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INTRODUCTION

Considerable amounts of oil and oil products are spilled into the oceans every year. Some of this comes from offshore oil-prospecting and production. In Norwegian waters an expansion of oil activity into the northern areas is now under discussion. The Barents Sea capelin, Mallotus villosus, is one of the resources potentially affected by such activity. The capelin spawns in early spring along the Finnmark and Murman coasts. The eggs are demersal and stick to the bottom substratum of pebbles or gravel, mainly in 25 - 75 m depths (Sætre & Gjøsæter, 1975).

These experiments were conducted to investigate the effect of oil hydrocarbons on the hatching success of capelin eggs. Fertilized eggs were exposed to 10 ppb to 2 ppm levels of a seawater extract of crude Ekofisk oil. The extract was continously produced in the laboratory by solution of hydrocarbons from an artificial oil slick.

The experiments lasted more than 9 weeks, throughout the developmental and hatching period of the eggs.

MATERIAL AND METHODS

Naturally-fertilized capelin eggs were sampled by grab in the Finnmark spawning area. The age of eggs at sampling 30 March 1976 was 1 - 7 days after fertilization. Incubation with oil started after ship transportation to the Bergen laboratory.

The experiments were performed in two groups, each consisting of 6 aquaria with oil concentrations from 2 ppm to 10 ppb, plus 2 seawater controls. Group I started 14 - 20 days and group II 23 - 29 days after fertilization. Egg mortality was approximately 3% at the start of group I, increasing slightly to about 7% at the start of group II 9 days later.

Within each group experiments were conducted in both flow-through and stagnant-water aquaria. The volumes were 2000 and 1500 ml, respectively, and about 350 eggs were put into each aquarium. Stagnant water was oxygenated by bubbling air and exchanged every 3 days.

A schematic illustration of the experimental arrangement is given in Figure 1. Crude oil and seawater, in proportion 1 : 4, were continously pumped into an extraction vessel. The oil slick formed on the surface was retained at constant thickness by an overflow system. A magnetic stirrer kept the oil slick and water phase below in gentle motion. Water-soluble hydrocarbons moved continously into the water phase, and the seawater extract of oil thus formed was removed near the bottom of the vessel.

The hydrocarbon concentration in the extract was determined by gas chromatography according to the method described in Grahl-Nielsen et al., 1976. Estimates for total oil hydrocarbons were obtained by including the unresolved envelope of the chromatogram (Medeiros & Farrington, 1974).

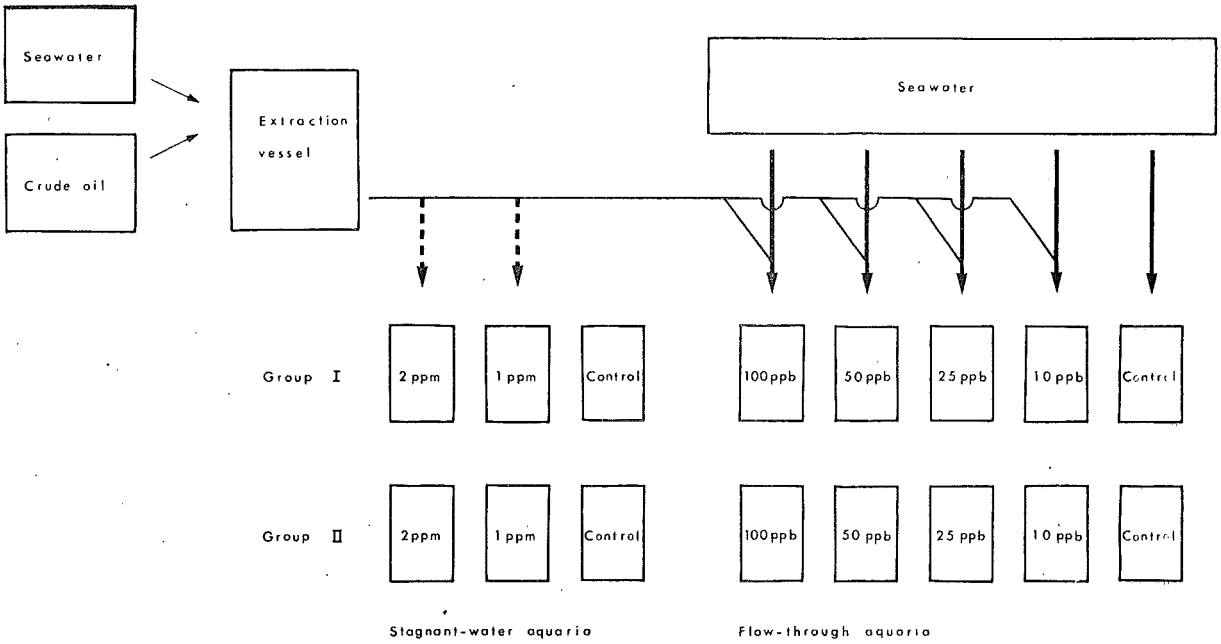

Figure 1. Schematic illustration of the experimental arrangement. See text for explanation.

Samples of the extract was taken at least every 3 days. The concentration was fairly steady throughout the experimental period, staying at a level of about 2 ppm total oil hydrocarbons.

The oil extract was diluted in a dosing system and fed into the flow-through aquaria. The concentrations in these aquaria were approximately 10, 25, 50 and 100 ppb total oil hydrocarbons. Seawater flow was about 100 ml/min. A detailed description of the technical arrangement will follow (K. I. Johannessen, in prep.).

Oil extract was taken out every 3 days for the stagnant water experiments. Here it was used undiluted or diluted 1 : 1 with seawater, giving hydrocarbon concentrations of about 2 ppm and 1 ppm, respectively.

The aquaria water temperature was 6.0 ± 0.5 °C at the beginning, increasing slowly to about 7.5 °C towards the end of the experiment. The stagnant-water temperature was about half a degree lower

than in the flow-through aquaria. To reduce introduction of algae all seawater was filtered through a 10-micron filter before entering the system. Seawater salinity was 34 - 35 o/oo and the oxygen concentration in the aquaria was about 6.5 ml O_2/l.

The number of larvae hatched was counted daily throughout the 6 - 7 week hatching period. In the flow-through aquaria hatched larvae were collected in small traps which filtered the outlet water. In the stagnant-water aquaria new larvae were removed daily by pipette.

Estimates of the relative number of marine bacteria were obtained by inoculating melted-agar dishes with aquaria water samples. The medium was Difco Marine Agar 2216, and the dishes were incubated 8 days at 13°C.

RESULTS AND DISCUSSION

To avoid affecting hatching success, handling of the developing eggs was kept at a minimum. This precluded exact counts of egg mortality. Brief, visual examinations, however, indicated that the mortality stayed low, at least until hatching had started. Acute lethal effects on the embryo therefore had little or no importance for the outcome of the experiments.

The cumulative percent hatching over time was calculated for all experiments. For the flow-through aquaria hatching curves are presented in Figure 2. Evidently, there is a reduction in hatching success when oil hydrocarbons are present. At the highest level, 100 ppb, total hatching was only about 55% in both groups, as compared with about 80% for the controls. Within each group there was a tendency towards progressively lower hatching success with increasing hydrocarbon concentration. This is further illustrated in Figure 3, where the total hatching at each oil concentration is expressed as a percentage of the total hatching in the controls.

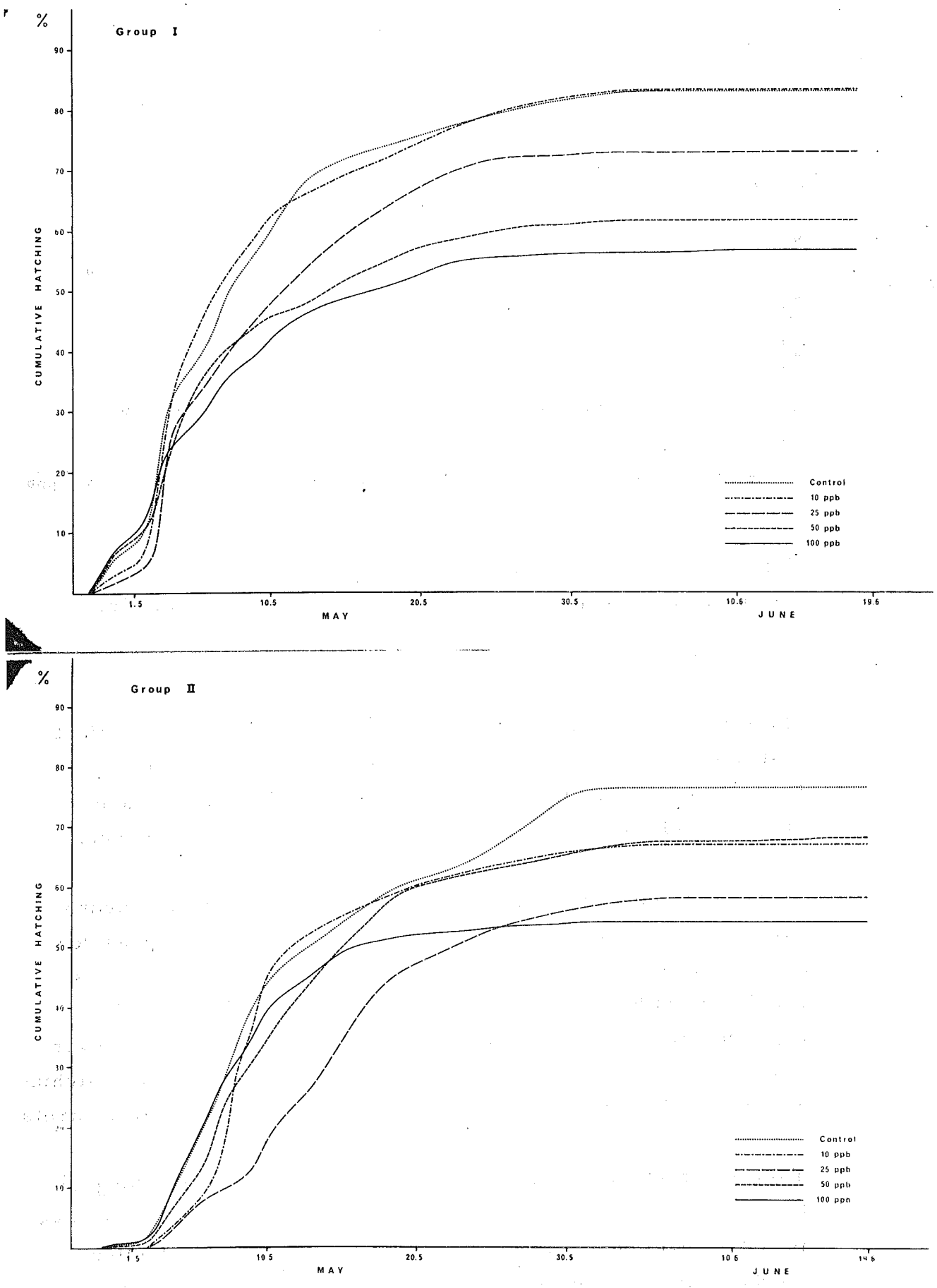

Figure 2. Cumulative hatching curves at different oil hydrocarbon concentrations in flow-through experiments. (Daily observations).

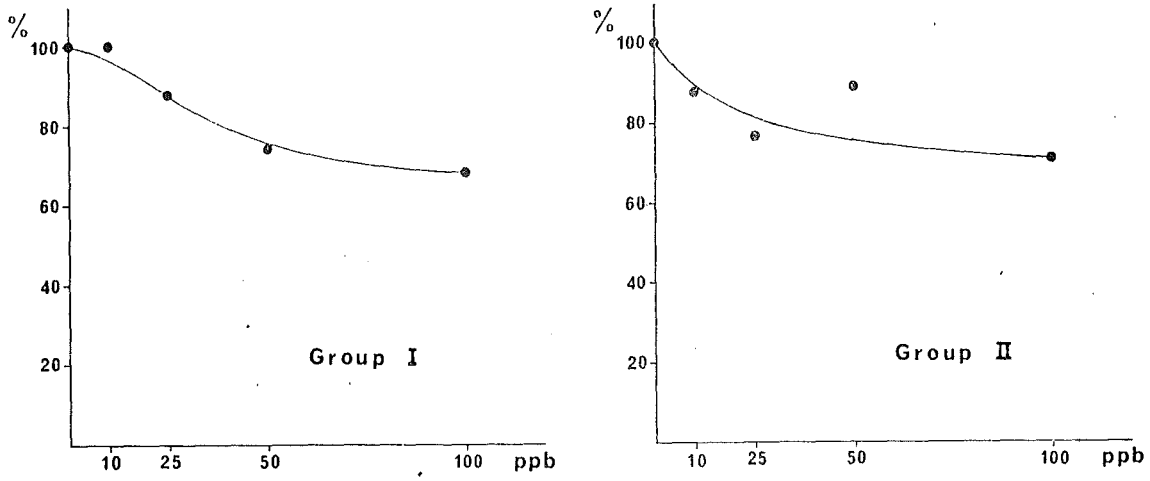

Figure 3. Total hatching at different oil hydrocarbon concentrations in percent of total hatching in the controls.

Thus the flow-through experiments demonstrate adverse effect on hatching at oil hydrocarbon concentrations as low as 10 - 25 ppb. The intensity of the effect tends to increase progressively as the concentration rises from 10 - 100 ppb.

In the stagnant-water experiments (Figure 4) the 2 ppm hydrocarbon concentration was accompanied by total hatching as low as 35 - 40%. This further reduction of hatching success is consistent with the results obtained from the flow-through experiments. The intermediate 1 ppm concentration, however, gave apparently no response in hatching success. Total hatching was 65 - 70%, about the same as in the stagnant-water controls.

This apparent inconsistency and the relatively low hatching in the stagnant-water controls, may indicate that hatching success is not controlled by oil concentration alone. Indirect effect mechanisms, for instance via microbial activity, may also be of importance and will be discussed later.

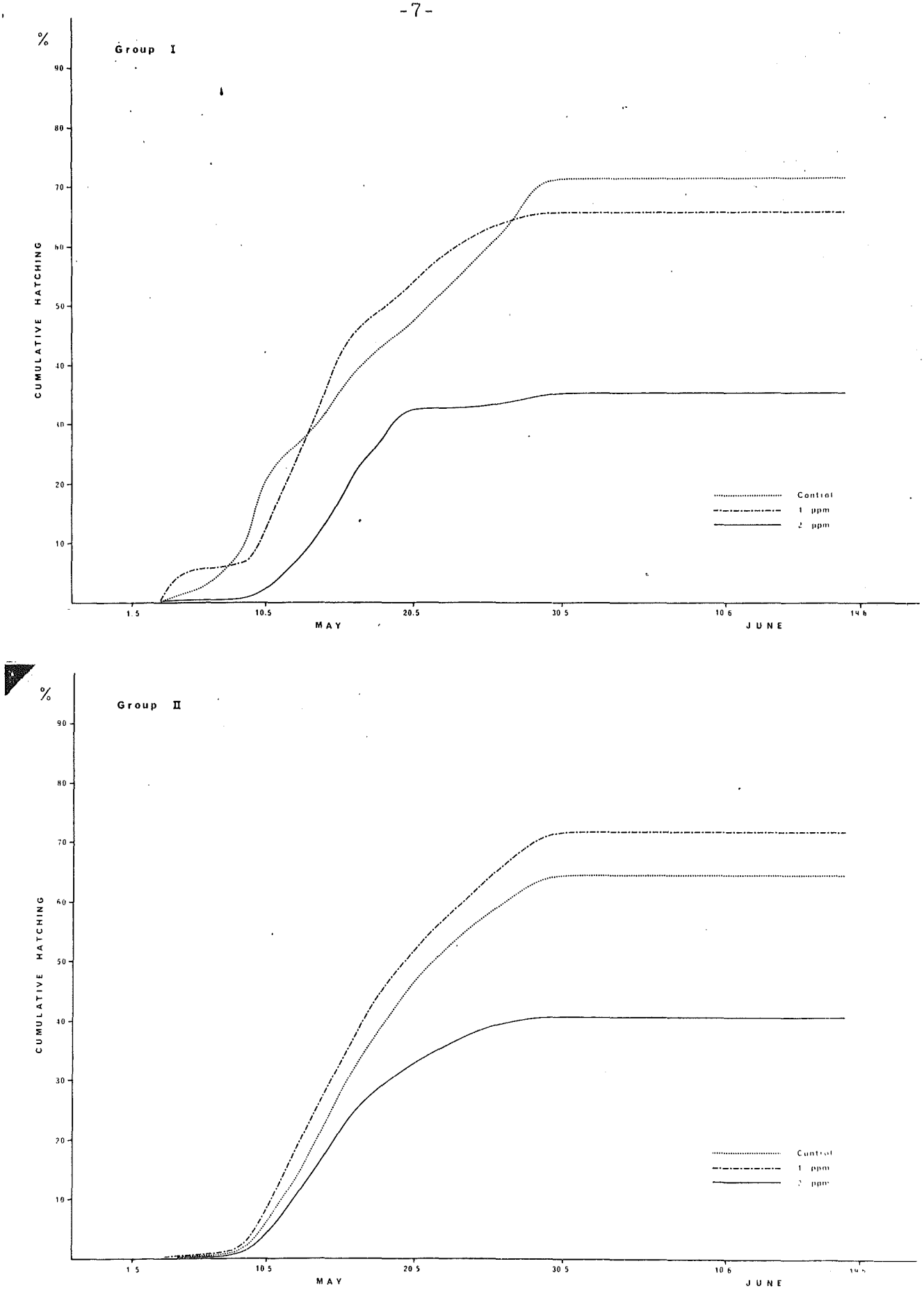

Figure 4. Cumulative hatching curves at different oil hydrocarbon concentrations in stagnant-water experiments. (Daily observations).

Figure 2 - 4 reveal no systematic difference in total hatching between incubation with oil starting 14 - 20 days (group I) or 23 - 29 days (group II) after fertilization. Neither of the two developmental groups appears to be the more sensitive. The possibility of a time-dependent response can not be excluded, however, if exposition starts at an earlier stage, as demonstrated for eggs of cod by Kühnhold (1974).

Cumulative hatching data made up to 100% in each aquarium, were plotted against time for all experiments. The time span for the bulk of hatching, defined as 10 - 90%, was then determined graphically. The results are presented in Table I. The time span tends to be shorter with increasing oil concentrations. The presence of oil hydrocarbons thus appears to restrict the length of time in which hatching can occur.

Different groups of curves in figures 2 and 4 exhibit small variations in the start of hatching. These variations can all be attributed to small temperature differences in the aquaria (Gjøsæter, in prep., Pitt, 1958).

Table I. Time span for 10 - 90 % of the hatching in each aquarium.

		Number of days				
Oil hydrocarbon concentration		Control	10 ppb	25 ppb	50 ppb	100 ppb
Flow-through experiments	Group I	19	18	16	19	18
	Group II	23	15	20	18	12
Oil hydrocarbon concentration		Control	1 ppm	2 ppm		
Stagnant-water experiments	Group I	19	14	8		
	Group II	15	15	13		

During the first weeks of incubation the eggs were light-coloured, transparent and apparently in good condition. Gradually, however, a large fraction of the eggs became dull and darker, often developing a layer of grey-white, wooly fouling. Nevertheless, the embryos were still alive and many of these eggs hatched. As the experiments progressed, a gradient towards more extensive fouling and darkening was observed at the higher oil concentrations (50 - 100 ppb). The same gradient occurred in the stagnant-water aquaria, but here the phenomenon was also observed to some extent in the controls.

Fluorescence microscopy of water samples from aquaria (G.Slaattebræk, pers. comm.) demonstrated a wide varity of microorganisms, quantitatively dominated by bacteria. Estimates of relative bacterial numbers (Table II) reflected the gradient of fouling with increasing hydrocarbon concentrations.

Table II. Relative bacterial numbers in the different flow-through aquaria.

Oil hydrocarbon concentration	Bacteria colonies per ml water sample				
	Control	10 ppb	25 ppb	50 ppb	100 ppb
Group I	$1.3 \cdot 10^2$	$4.8 \cdot 10^2$	$1.1 \cdot 10^3$	$2.3 \cdot 10^3$	$4.3 \cdot 10^3$
Group II	$2.2 \cdot 10^2$	$6.7 \cdot 10^2$	$1.2 \cdot 10^3$	$2.5 \cdot 10^3$	$4.6 \cdot 10^3$

These observations suggest a relationship between oil-degrading bacteria and the development of fouling. The definite nature of the phenomenon, however, remains to be investigated.

As regards the effects of the fouling, observations indicated a modification of egg-shell rigidity. Larvae hatching from fouled eggs were seen struggling for minutes halfway liberated, while hatching normally is completed in a few seconds. Correspondingly, fouled eggs were difficult to penetrate by dissection. And some of the embryos had abnormally small yolk sacs, apparently because of yolk consumption after normal time of hatching. The fouling thus seems capable of increasing egg-shell rigidity to such an extent that normal hatching is obstructed. Concurrently, the fouling may interfere with oxygen transport to the embryo, thus causing elevated egg mortality (Alderdice et al. , 1958). Increasing fouling gradually causes the hatching rate to decline. This implies both shortening of the period in which hatching can occur (Table I) and reduction of total hatching success (Figures 2 - 4).

The experiments have demonstrated reduced hatching success of capelin eggs in the presence of water-soluble oil hydrocarbons. The mechanism for this effect appears to work at least partly via oil-degrading microorganisms. Oil hydrocarbons stimulate the activity of such organisms, and subsequent microbial fouling on the eggs interferes with the hatching success.

SUMMARY

Fertilized capelin eggs were exposed to an extract of seawater - soluble crude-oil hydrocarbons. Experiments were conducted at hydrocarbon concentrations of approximately 10, 25, 50 and 100 ppb in flow-through aquaria, and at 1 and 2 ppm in stagnant-water aquaria. The solution of oil hydrocarbons was obtained by a continous extraction system. The experiments were performed in two groups, starting 14 - 20 and 23 - 29 days after fertilization of the eggs. Incubation with oil lasted throughout the developmental and hatching period, together more than 9 weeks. Effects on hatching success was monitored by daily observations of hatching in the different aquaria.

Generally there was a reduction in hatching success when oil hydrocarbons were present. The effect was observed at concentrations down to 10 - 25 ppb. In the flow-through controls total hatching was about 80%. The hatching was reduced to about 55% at the 100 ppb level, and further to 35 - 40% at 2 ppm in the stagnant-water aquaria. Acute lethal effects had little or no importance for the outcome of the experiments.

The presence of oil hydrocarbons tended to restrict the length of time in which hatching could occur.

There was no systematic difference in hatching success between incubation with oil starting 14 - 20 or 23 - 29 days after fertilization. Neither of these developmental stages appeared the more sensitive.

Some inconsistency in the results indicated that hatching success is not controlled by oil concentration alone. Visual examinations demonstrated a layer of fouling developing on the surface of the eggs. The fouling increased both with time and hydrocarbon concentration, suggesting that oil-degrading microorganisms were involved. The fouling evidently interfered with the hatching success. The adverse effects of oil therefore apparently work at least partly by stimulating microbial fouling on the eggs.

ACKNOWLEDGEMENTS

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REFERENCES

Alderdice, D. F., W. P. Wickett & J. R. Brett, 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. J. Fish. Res. Bd Can., Vol. 15, pp. 229-249.

Grahl-Nielsen, O., T. Neppelberg, K. H. Palmork, K. Westrheim & S. Wilhelmsen, 1976. Om kontrollerte utslipp av oljehydrokarboner fra produksjonsplattformen på Ekofisk. Fisken og Havet, Ser. B., 1976, no. 3, pp. 1-19.

Kühnhold, W. W., 1974.. Investigations on the toxicity of seawater-extracts of three crude oils on eggs of cod (Gadus morhua L.). Ber. dt. wiss. Kommn. Meeresforsch., Vol. 23, pp. 165-180.

Medeiros, G. C. & J. W. Farrington, 1974. IDOE-5 intercalibration sample: Results of analysis after sixteen months storage. Marine Pollution Monitoring. Nat. Bur. Stand. (U. S.), Spec. Publ. 409, pp. 167-169.

Pitt, T.K., 1958. Distribution, spawning and racial studies of the capelin, Mallotus villosus (Müller), in the offshore Newfoundland area. J. Fish. Res. Bd Can., Vol. 15, pp. 275-293.

Sætre, R. & J. Gjøsæter, 1975. Ecological investigations on the spawning grounds of the Barents Sea capelin. FiskDir. Skr. Ser. HavUnders., Vol. 16, pp. 203-227.