International Council for
the Exploration of the Sea

C.M 1987/E:12
Marine Environmental
Quality Committee

# AGE DEPENDENT SENSITIVITY OF OIL ON FISH LARVAE,USED IN ASSESSMENT OF POTENTIAL OIL POLLUTION DAMAGES ON FISH RESOURCES.

BY

Lars Føyn and Bjørn Serigstad

INSTITUTE OF MARINE RESEARCH
P.O. Box 1870, N-5024 Bergen,
Norway

## ABSTRACT

Oil exploration in norwegian waters will probably be extended further north into the Barents Sea. The Barents Sea is the nursery ground for important fish stocks spawning outside the norwegian coast, north of 62°N. Fish eggs and larvae are transported with the current systems northwards ending up as 0-group fish no longer dependent of the transportation provided by the currents.

Some place between the egg/larval stage and mature fish, the fish is not longer vulnerable for oil pollution. This paper discuss the thoughts behind and the use of experiments to establish borderlines for the areas where oil pollution are likely to damage fish resources.

INTRODUCTION.

The continental shelf off Norway, north of 62° N, is of special importance as spawning area for many of the most valuable fish stocks of the North East Atlantic. The environmental conditions composed by the north flowing norwegian coastal current and the intruding nutrient rich Atlantic water are ideal for supporting the early life stages of fish. Fish eggs and larvae are transported northwards, to the nursery area in the Barents Sea, fairly concentrated and in this process they are vulnerable to both naturally occurring events as to man made influence. Of the latter, the search for oil with possible oil pollution, may be a threat to the fish eggs and larvae. Drifting northwards the larvae are developing, and at some stage in the development they will be able to avoid an oil pollution. We have tried experimentally to establish the size/age of the fish larvae of different species on which oil pollution no longer will be hazardous to the larvae. By combining this found size with the distribution of the various sizes of the different fish species we will be able to draw border lines beyond which oil pollution will be of only minor concern for the year class of fish in question.

Norwegian law require a consequence analysis before an area can be opened for oil exploration. The experiments presented here are part of an extensive study initiated by the Ministry of Oil and Energy in the process leading up to an opening of the norwegian part of the Barents Sea, south of 74° 30'N, for oil exploration.

Great efforts has been made to reveal possible effects of crude oil and its constituents on fish development. A short review of the part of the research dealing with metabolism and oxygen transport is given by Serigstad (1986). No effects of oil exposure are found on the ion transport or osmo-regulation in cod eggs/larvae after long term oil exposure to concentrations from 50-280 ppb WSF of Statfjord Crude Oil (Mangor-Jensen 1986). Anatomical studies on the same larvae, using scanning- and transmission- electron microscopy was also negative according to

oil effects (Adoff 1986). No difference was found in protein content or ammonia content of cod eggs or larvae, and no clear effect was found on the free amino acid content (Fyhn et al 1986; 1987). The only clear negative oil effect found on cod, were reduced oxygen uptake of the yolk sac larvae (Serigstad 1986; 1987b; Serigstad & Adoff 1985). The oxygen uptake and thus the metabolic activity of a fish larvae depends on a sufficient oxygen supply from the ambient sea water to the mitochondria in the cells where the aerobic energy production for synthesis, regulatory processes and locomotory activities take place. An impact of oil on the oxygen uptake of yolk sac larvae may therefor have a severe negative effect in the sensitive developmental stage where the larvae are changing from endogenous to exogenous food uptake.

Cod (Gadus morhua L.) is in toxicity tests with different oils and oil products shown to be the most sensitive among several different fish species from the North Sea (Falk-Pettersen & Kjørsvik 1987).

## MATERIAL AND METHODS.

Cod (Gadus morhua L.) used in this study were obtained from the institute's aquaculture station at Austevoll, or from the fish market in Bergen. Eggs and sperm were stripped from single male and female fishes to ensure homogeneity of the groups. The cod eggs were incubated as described by Solberg & Tilseth (1986).

Ripe herring caught by net at Sotra near Bergen, were supplied by Sangolt at Bergen Aquarium. Single male and female herring were stripped for sperm and eggs. The sperm were mixed with seawater in 30 liters plastic beakers with the bottom covered with object glasses. The eggs were stripped directly in to the beaker, were fertilized and stuck to the glass plates. The eggs were washed with clean seawater.

Eggs larvae and postlarvae of both cod and herring were transferred to the biotest exposure systems for continuous exposure to 50-280 ppb WSF of Statfjord crude oil. The oil analyses is described by Westrheim & Palmork (1986)

Juvenile cod, fish larvae and eggs were all kept in 34 o/oo sea water at 5°C.

The biotest system used in the experiments on cod (Gadus morhua) eggs and larvae is previously described by Johannessen 1983; Tilseth et al 1984. Modifications of the system are described by Solberg & Tilseth 1986; Solberg et al 1987. In the studies on herring (Clupea harrengus) eggs and larvae, a new biotest system based upon the flow-through system, but in design, scaling, materials and construction it has chosen completely new solutions (Fyhn et al 1986). Although designed for long term studies of oil (WSF) on marine fish eggs and larvae, the new biotest system has, with minor modifications, the potential for effect-studies of most any water soluble toxicant on a variety of aquatic organisms within a range from less than 1 mg to a few kilos.

Oxygen uptake measurements. Two different principles (Closed and open respirometry) and three different experimental designs were used to fit the different size of the animals, ranging from 450 ug to 600 gram wet weight (Serigstad 1986; 1987a). All the oxygen measurements were performed using a polarographic oxygen electrode (Radiometer E-5046) connected to a meter (Radiometer PHM 73).

## RESULTS.

### Cod.

Eggs: No effects of oil exposure (50-280 ppb WSF) has been found on the oxygen uptake rate of the cod eggs after continuously exposure from day 2 after fertilization (7 series); (Serigstad 1986). A typical set of data is given in fig.1.

Larvae: The oxygen uptake of long term oil exposed larvae is effected by oil exposure (fig. 1). The exposure started between day 2 and 7 for the 7 different groups. The oxygen uptake is markedly suppressed compared to the controls. The control larvae have a peak value in the oxygen uptake at the time of final yolk absorbtion (day 6-8 post hatching, Fossum 1986). A slight increase but no peak value is observed in the oxygen consumption of the cod larvae during the yolk sac stage. The oil effect on the oxygen uptake is independent of oil concentrations in the range 50-280 ppb of the WSF of Statfjord crude oil (fig. 1.).

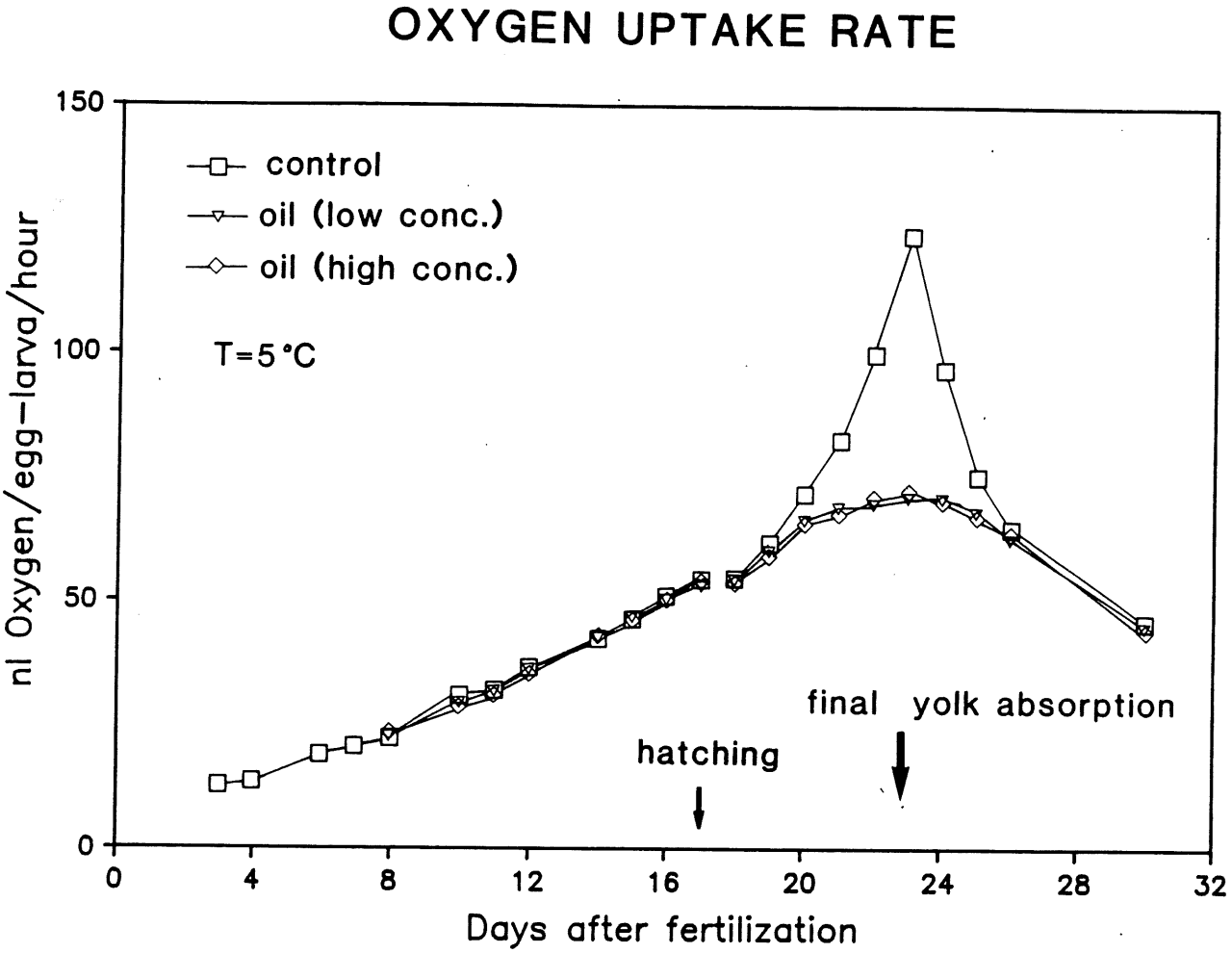


**FIGURE 1. Oxygen uptake of control and oil exposed cod eggs/larvae. Oil exposure at low concentration: 50±20 ppb WSF and high concentration: 230± 110 ppb WSF). Each point represent the mean of 4 parallels wit 10 eggs or 5 larvae each. (SD is less then 5% for all the means). T = 5°C, Salinity = 34o/oo.**

In short term exposure experiments, control larvae of cod were transferred to oil contaminated water (50±20 ppb WSF), on the first day after hatching and on day 4 and 6 post hatching. Measurements on long term oil exposed larvae(230±110 ppb WSF) and control larvae were included in the test. The results show that the oxygen uptake of the short term oil exposed larvae is suppressed to the same extent as that of the long term oil exposed larvae (fig 2).

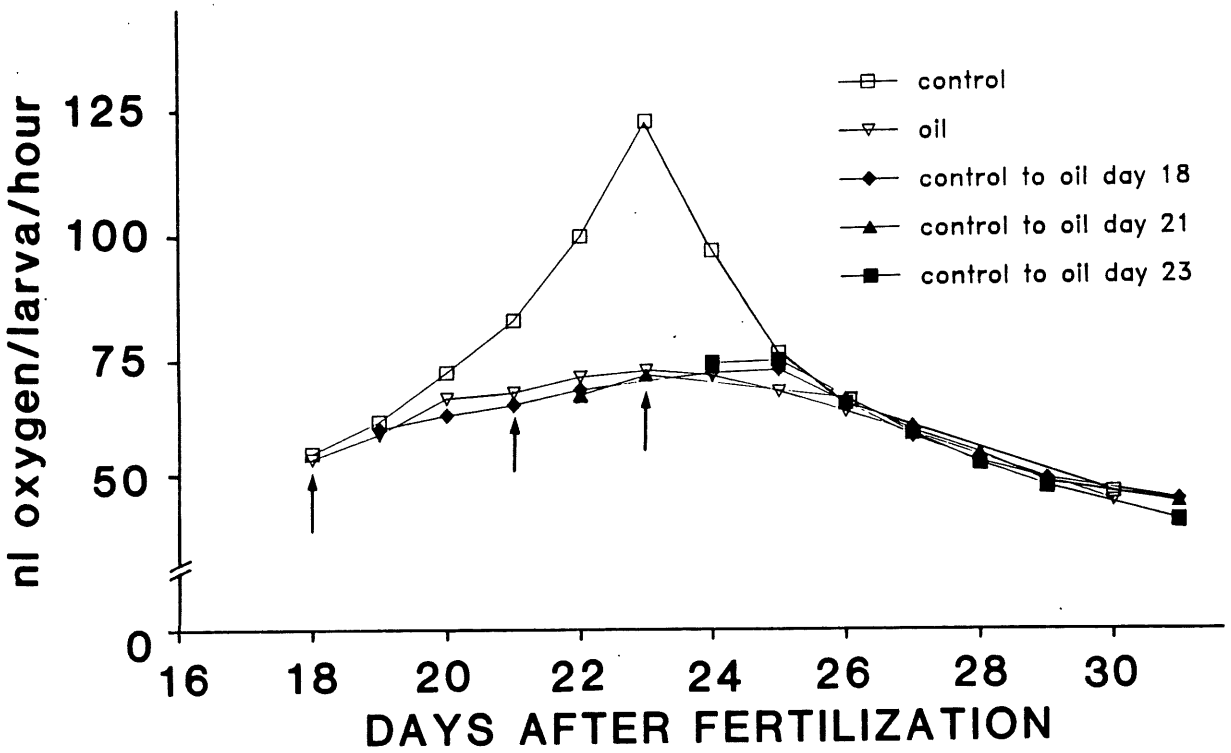


**FIGURE 2. Oxygen uptake of cod larvae during short term exposure experiments. Control and oil exposed (50±20 ppb WSF). Control larvae were transferred to oil water the first day after hatching (day 18), and 4 and 6 days post hatching (arrows). Oxygen uptake was measured 24 hours after transfer and then daily throughout the experiments. Each point represent 4 parallel groups of 5 larvae. (Sd is less then 5% for all the means). T = 5°C, Salinity = 34 o/oo.**

Already within 24 hours of exposure to oil (50 ppb WSF) the oxygen uptake of the exposed larvae is strongly reduced compared to the control larvae. No further reduction occurred over the next days in oil contaminated seawater.

In recovery experiments oil exposed cod larvae (exposed to 230±110 ppb WSF for 10 days before hatching) were transferred

from the oil water to control water in the biotest setup at the first day after hatching and at day 4 and 6 post hatching. Daily measurements of the oxygen uptake were performed under control conditions. No signs of recovery of the oil exposed cod larvae were found (fig. 3). Apparently even 6 days in clean seawater is not enough to remove the suppressive effect of the oil exposure on the oxygen uptake of the cod larvae.

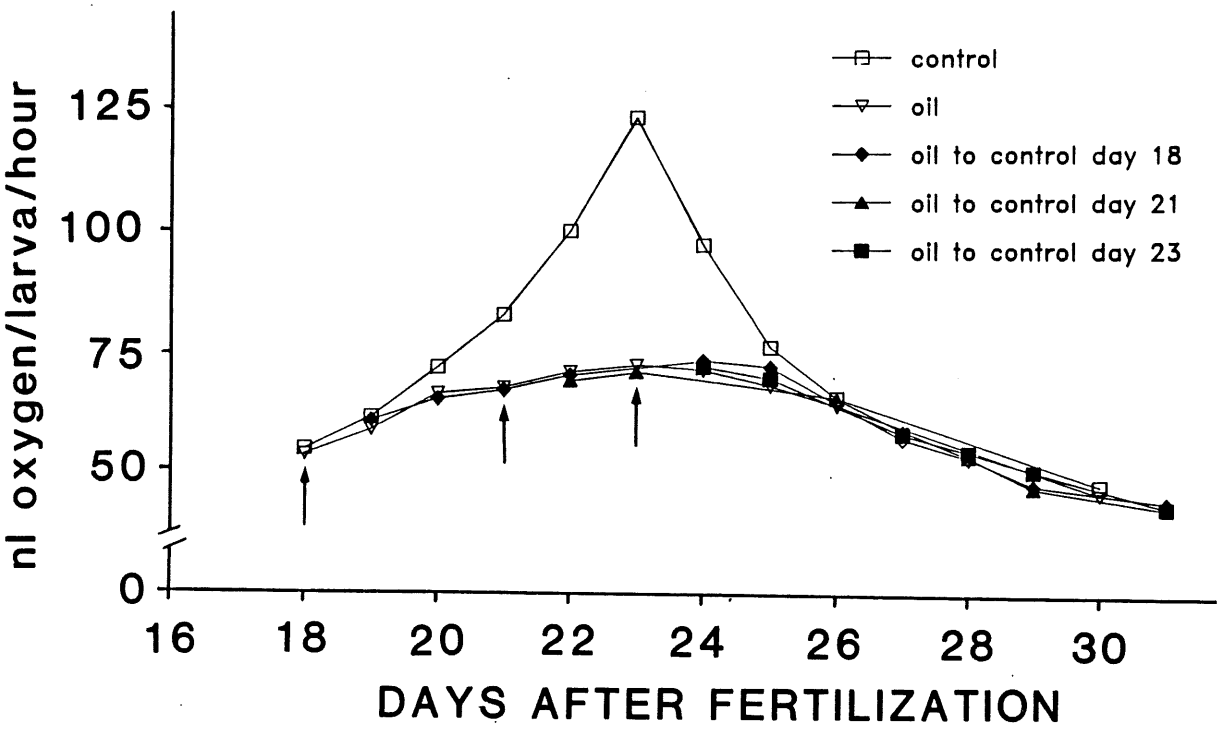


**FIGURE 3. Oxygen uptake of oil exposed cod larvae during recovery experiments in clean sea water. Oil exposed larvae (230±20 ppb WSF) were transferred to control water the first day after hatching (day 18), and at 4 and 6 days post hatching (arrows). Oxygen uptake was measured 24 hours after transfer, and daily throughout the experiment. Each point represent 4 parallel groups of 5 larvae. (SD is less then 5% for all the means). T = 5°C, Salinity = 34 o/oo.**

Postlarvae: The oxygen uptake of growing larvae raised under semi-natural conditions in Hyltropollen,Austevoll (Folkvord et al 1985) has been measured both under control conditions and after oil exposure. Measurements have been done on larvae with a body wet weight ranging from 6 - 1350 mg. A 30 mm long larvae has a body wet weight of about 270 mg. The postlarvae were oil exposed from 3 to 6 days in the biotest system, to an oil concentration of 50±20 ppb WSF (Serigstad 1986). No difference in the oxygen uptake was found between control and oil exposed larvae (fig. 4).

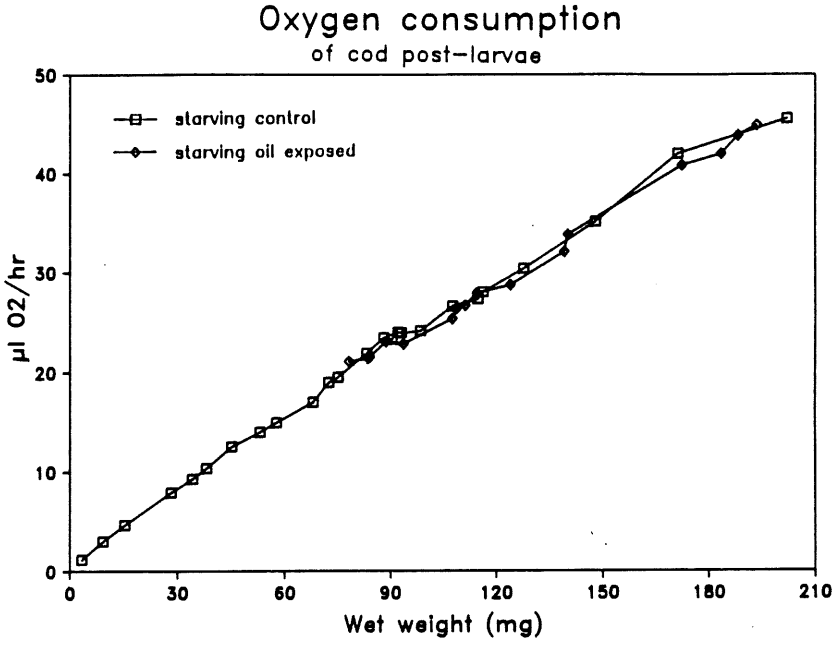


**FIGURE 4. Oxygen uptake of control and oil exposed (50±20 ppb WSF) post-larvae of cod as function of body wet weight. the larvae were not fed during the last 3 days before measurements (6 days for some oil exposed larvae). Each point represent an individual larva. T = 5°C, Salinity = 34 o/oo.**

O-group cod. There were no significant difference in the oxygen uptake of O-group cod exposed to Statfjord crude oil extract (100 ppb WSF for one week), (fig. 5);(Serigstad & Ellingsen 1987). The fish ranged in weight from 24-63 gram.

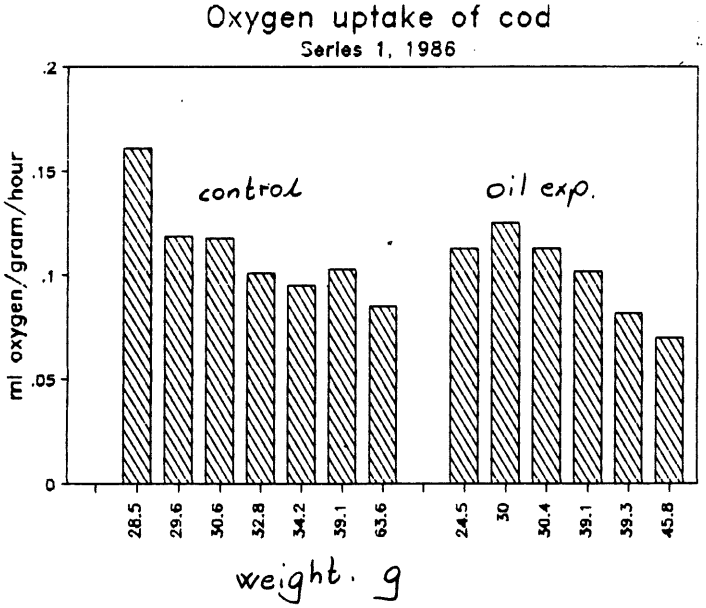


**FIGURE 5. Oxygen uptake of O-group cod (25-65 gram), control and oil exposed (100 ppb WSF for 7 weeks), as function of body wet weight. T=10°C, Salinity = 34 o/oo.**

Experiments with juvenile cod (body weight of about 0.5 kg) showed that upon an abrupt exposure to oil contaminated sea water (100 ppb WSF) they react immediately with a pulse of increased oxygen uptake, followed by a stabilized level below the average oxygen uptake before oil exposure took place (fig. 6).

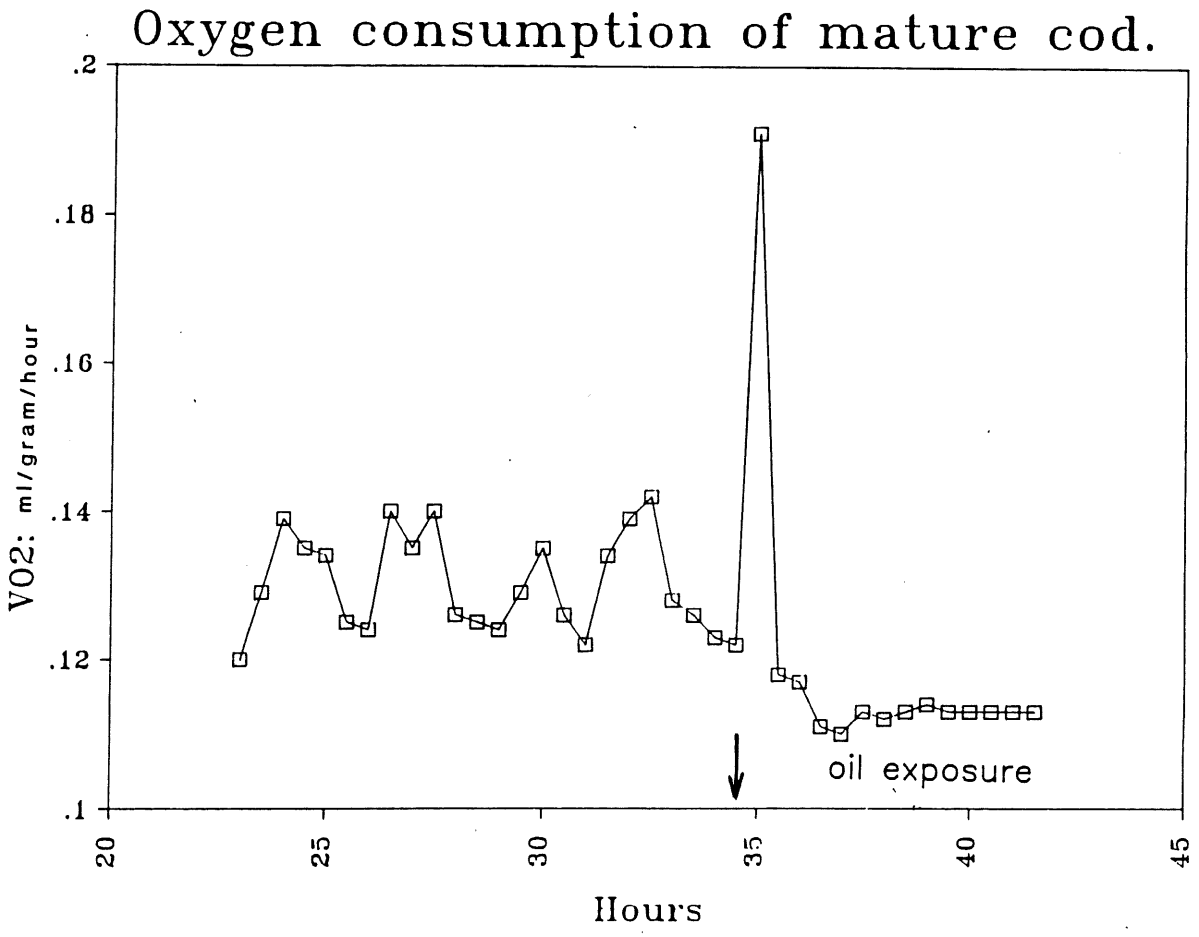


**FIGURE 6. Oxygen uptake of juvenile cod (0.5 kg) before and after introduction of oil contaminated sea water (100 ppb WSF) to the respirometer system at 34.5 hours of incubation. T = 10°C, Salinity = 34 o/oo.**

Herring.

No negative oil effects are shown on herring eggs or yolk sac larvae from 3 different series studied during the spring 1987.

Figure 7 show that we have an increase in the oxygen uptake from fertilization until the yolk is absorbed, then we have a decrease in the $O_2$ uptake of the starving larvae, both control and oil exposed groups. The three groups were exposed to oil concentrations ranging from 80 to 150 ppb WSF for the different series.

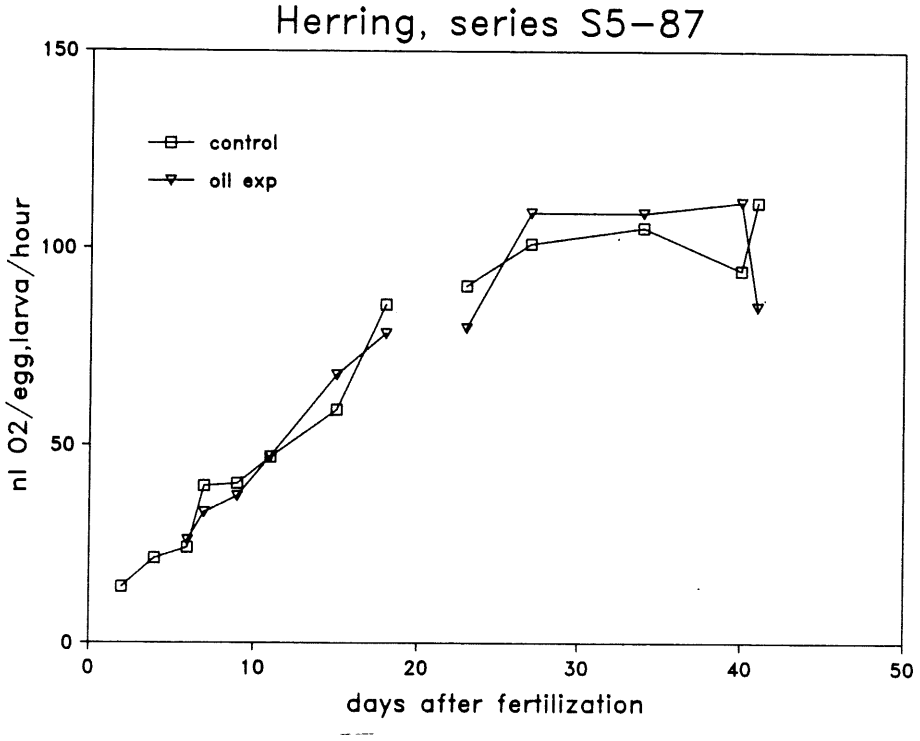


**FIGURE 7. Oxygen uptake of control and oil exposed (150 ppb WSF) herring eggs/larvae as function of time after fertilization. Each point represent the mean of 4 parallels with 10 eggs or 5 larvae each. T = 5°C, Salinity = 34 o/oo.**

## DISCUSSION AND CONCLUSION.

The conclusion of the above presented experiments on cod and herring is, cod eggs and larvae of a size < 30 mm may be harmed by oil concentrations as low as 50 ppb, herring eggs and larvae seems not at all to be effected.

While the conclusion about effects on cod is based on extensive studies over a period of 6 years (Serigstad 1986; Serigstad & Ellingsen 1987) the herring conclusion is based on one seasons experiments and will be followed by further biotests.

In connection with the ongoing consequence study before opening for oil exploration in the Barents Sea it is important to give advises to the authorities. Figure 8 and 9 shows the distribution of cod larvae < 30 mm for July 1986 and July 1987 (Bjørke 1987 pers. com.).

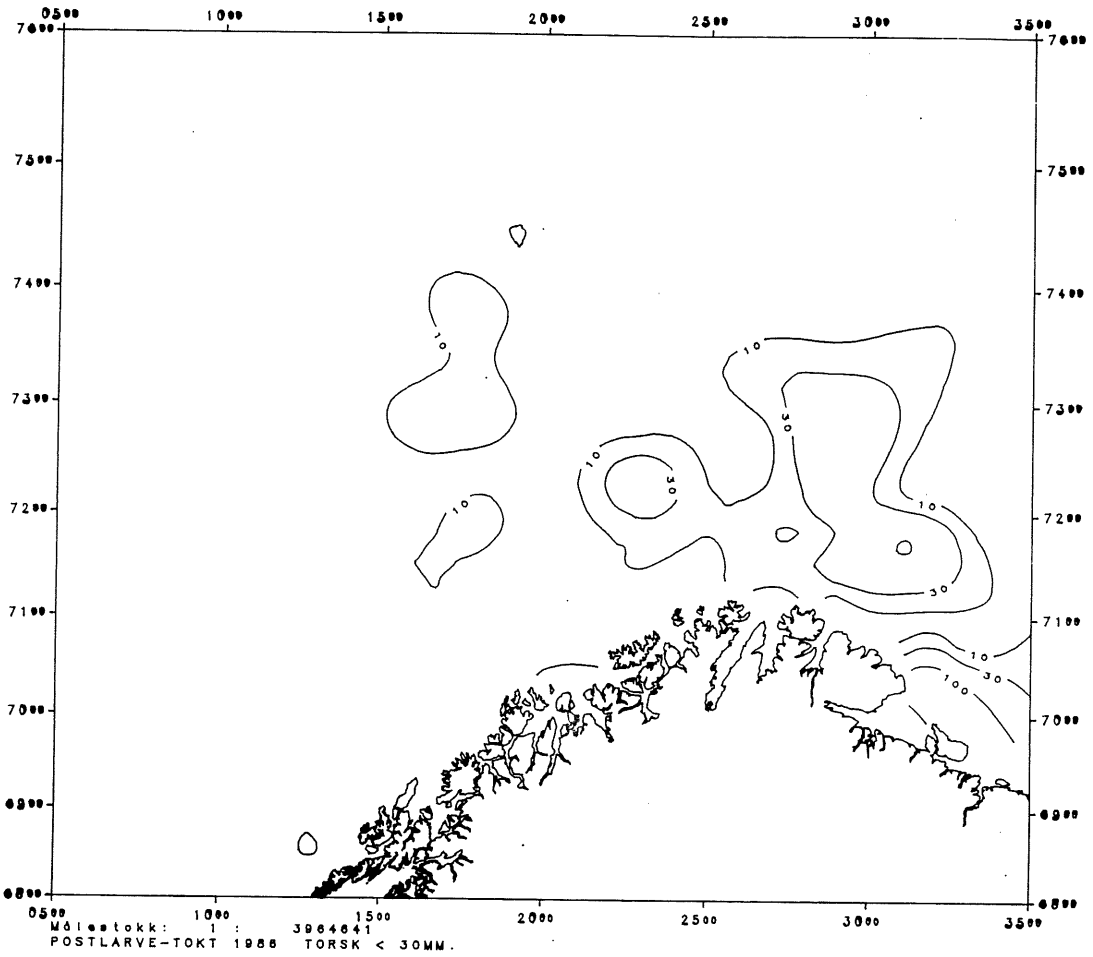


**FIGURE 8. Distribution of cod larvae < 30 mm in July 1986, number pr. trawlhour, after Bjørke pers. com. (1987).**

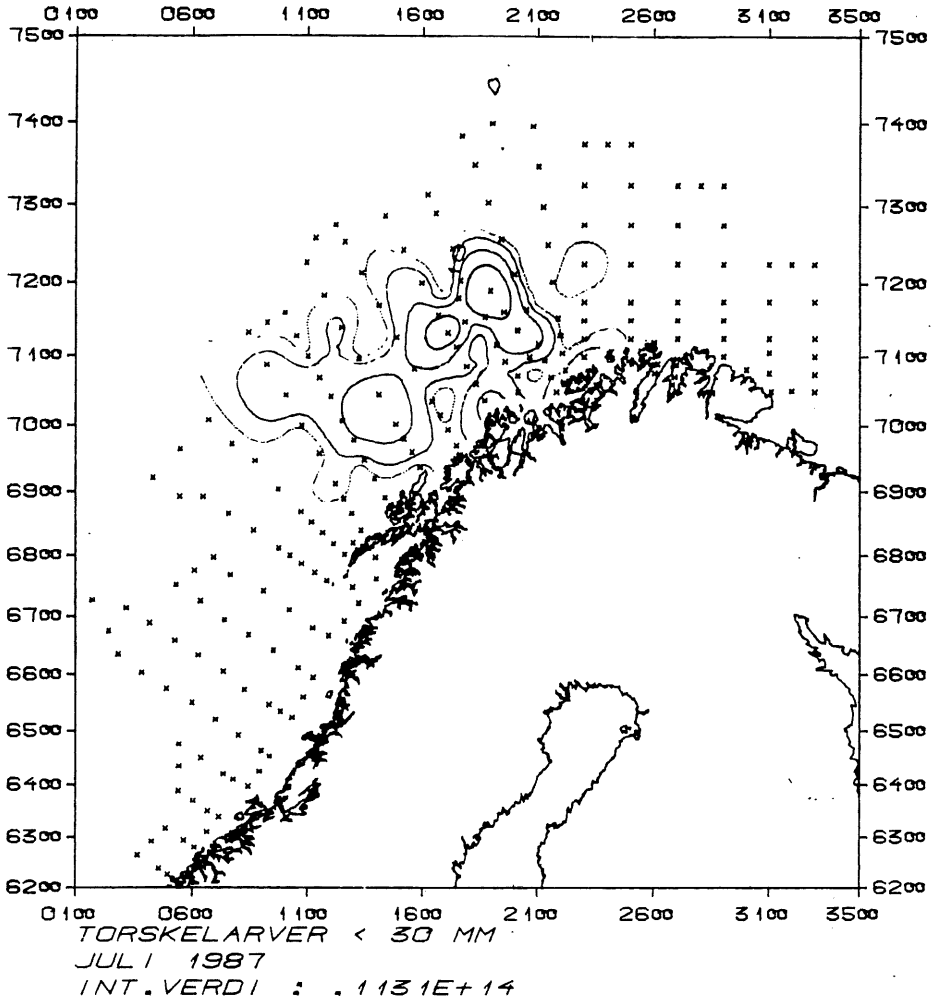


**FIGURE 9. Distribution of cod larvae < 30 mm in July 1987, number pr. trawlhour, after Bjørke pers. com. (1987).**

The distribution represents two extremes compared to the combined data of cod larvae <30 mm from 1977 - 1987 (Bjørke 1987 pers. com.). By using the combined data set (fig. 10) it is possible to draw a line between Bear Island and the North Cape, north and east of which cod larvae < 30 mm is not found. This means that an oil pollution north and east of this borderline is not likely to have any effects on the cod as such. When the cod larvae are moving further east into the Barents Sea they have grown beyond the stage where they may be harmed by an oil pollution.

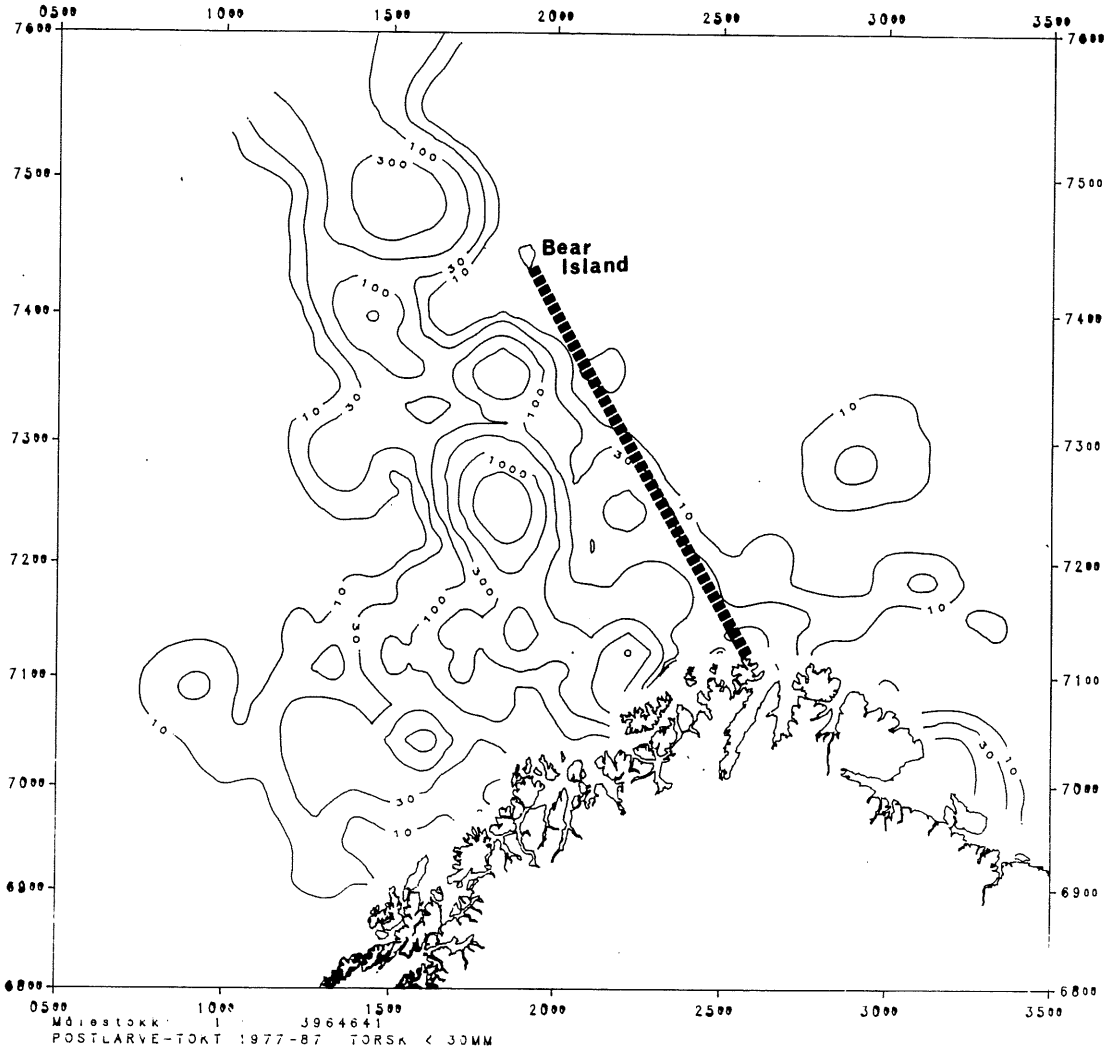


**FIGURE 10. Distribution of cod larvae < 30 mm from the years 1977 to 1987, number pr. trawlhour, Bjørke pers. com. (1987).**

We feel, however, a need to underline that this boarder is drawn only based on effects on the cod larvae as such. We have not considered possible food shortage caused by possible oil effects on the zooplankton in the Barents Sea.

REFERENCES.

Adoff,G.R. 1986. Anatomical studies of developing eggs and larvae of the cod (Gadus morhua L.). -Pp.51-115 in: Fyhn,H.J. (ed.). Fish larval physiology and anatomy. Basic studies and effects of oil. Final report, project no 83:524. University of Bergen, Bergen.

Falk-Pettersen,I.B. and Kjørsvik,E. 1987. Acute toxicity tests of the effects of oils and dispersants on marine fish embryos and larvae. Sarsia 72, in press.

Folkvord,A., Kvenseth,P.G., Petersen,T. and Øiestad,V. 1985. Mass production of juvenile atlantic cod (Gadus morhua L.) in a pond: results and new approaches in 1985. ICES C.M.1985/F:63/Ref.G Mariculture Committee.

Fossum,P. 1986. A staging system for larval cod (Gadus morhua L.). FiskDir. Skr. Ser. HavUnders., 18, 69-76.

Fyhn,H.J., Salhus,H. and Barnung,T.N. 1986. A new biotest system for long term effect-studies of oil on marine fish eggs and larvae. Design, component description and functional tests. -Pp. 265-288 in: Fyhn,H.J. (ed.). Fish larval physiology and anatomy. Basic studies and effects of oil. Final report, project no 83:524. University of Bergen, Bergen.

Fyhn,H.J., Mangor-Jensen,A and Serigstad,B. 1986. Free amino acids in developing eggs and larvae of the cod. Basic studies and effects of oil. -Pp. 167-201 in Fyhn,H.J. (ed.). Fish larval physiology and anatomy. Basic studies and effects of oil. Final report, project no 83:524. University of Bergen , Bergen.

Fyhn,H.J. Serigstad,B. and Mangor-Jensen,A. 1987. Free amino acids as energy substrate in developing eggs and larvae of the cod (Gadus morhua). Sarsia 72, in press.

Johannessen,K.I. 1983. A flow-through extraction and dosage system for long-term bioassay with water-soluble oil hydrocarbons. Mar. Environm. Res. 9, 123-129.

Mangor-Jensen,A. 1986. Osmoregulation in eggs and larvae of the cod (Gadus morhua L.). Basic studies and effects of oil exposure. -Pp. 117-168 in: Fyhn,H.J. (ed.). Fish larval physiology and anatomy. Basic studies and effects of oil. Final report, project no 83:524. University of Bergen, Bergen.

Serigstad,B. and Adoff,G.R. 1985. Effects of oil exposure on oxygen consumption of cod eggs and larvae. Mar. Environm. Res. 17, 266-268.

Serigstad,B. 1986. The effect of oil exposure on the oxygen uptake of eggs and larvae of the cod (Gadus morhua L.). -Pp 203-251 in: Fyhn,H.J. (ed.). Fish larval physiology and anatomy. Basic research and effects of oil. Final report, project no. 83:524. University of Bergen, Bergen.

Serigstad,B. 1987a. Oxygen uptake of developing fish eggs and larvae. Sarsia 72, in press.

Serigstad,B. 1987b. Effect of oil exposure on the oxygen uptake of cod (Gadus morhua) eggs and larvae. Sarsia 72, in press.

Serigstad,B and Ellingsen,T. 1987. Torskens følsomhet for olje, under utvikling fra egg til voksen fisk. Symposium om olje-Naturmiljø. SINTEF avd. Teknisk Kjemi. Trondheim 17-18 februar 1987.

Solberg,T.S. and Tilseth,S. 1986. Biotest exposure system and effect studies on cod eggs and larvae. -Pp 25-50 in: Fyhn,H.J. (ed.). Fish larval physiology and anatomy. Basic studies and effects of oil. Final report, project no 83:524. University of Bergen, Bergen.

Solberg,T.S., Serigstad,B., Tilseth,S., Westrheim,K. and Klungsøyr,J. 1987. Effects of different fractions of North Sea crude oils on eggs and yolk-sac larvae of cod (Gadus morhua L.). In prep.

Tilseth,S., Solberg,T.S. and Westrheim,K. 1984. Sublethal effects of the water-soluble fraction of Ekofisk crude oil on the early larval stages of cod (Gadus morhua L.). Mar. Environm. Res. 11, 1-16.

Westrheim,K. and Palmork,K.H. 1986. Chemical analyses. -Pp. 289-328 in: Fyhn,H.J. (Ed.). Fish larval physiology and anatomy. Basic research and effects of oil. Final report, project no. 83:524. University of Bergen, Bergen.