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THE EFFECTS OF OIL-BASE DRILLING MUD AND CRUDE OIL ON DEMERSAL FISH EGGS.

By

Bjørn Serigstad, Ingrid Sværen & Lars Føyn

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

ABSTRACT

The Norwegian spring spawning herring and the Barents sea capelin both with demersal eggs, spawn in relative small and concentrated areas on the Norwegian continental shelf. Besides potential impact on the fish resources from oil pollution, drilling of oil wells can in some cases, where oil-base drilling mud is used, have an impact on the development of demersal eggs.

This paper presents the biotest setup at the Institute of Marine Research in Bergen for testing effects from oil hydrocarbons and other water soluble toxicants on marine organisms. Methods for testing effects of oil and oil-base drilling mud on fish eggs and larvae are discussed. It also shows results from experiments, where capelin and herring eggs/larvae are exposed to oil (200 ppb WSF) and to drill cuttings (1.3 gram/liter) where oil-base drilling mud is used, and elaborate further how the findings can be used in advising the authorities how to avoid possible conflicts from drilling activities.

INTRODUCTION

Off-shore oil activities will always represent a possible threat to marine life in an area near the oil installations. There is a possibility of both large and small oil spills as a consequence of accidents, and there may be continuously leakages of oil hydrocarbons from the installations for example with the production water. These kinds of oil spills will mainly get in touch with pelagic organisms like fish eggs, larvae, larger fishes and plankton. The possible impacts on marine life depends on the magnitude of the spill as well as the effects on the various organisms present.

In order to determine the vulnerable developmental stages, of the marine organisms, biotests have to be performed in a way that the development of the organisms may be followed during the test. With the biotest system presented in this paper we have achieved a method that enables us to follow and to determine the critical stages in marine organisms. The results from these tests and the use of the results in assessment studies are to some extent presented by Føyn & Serigstad 1987 and 1988.

Besides potential impact on the fish resources from oil pollution, drilling of oil wells can in some cases, where oil-base drilling mud is used, have an impact on the development of demersal eggs. The norwegian spring spawning herring and the capelin spawn in relative small and concentrated areas on the norwegian continental shelf. These eggs and other demersal marine organisms may in some cases be exposed to drilling mud. It is therefore of major interest to elucidate the effects of oil-base drilling mud on organisms susceptible to get in touch with it.

MATERIAL AND METHODS

Spring spawning herring (Clupea harengus) were caught by net in Skogsvåg near Bergen in the first part of April. Ripe fishes were sorted out and brought on ice to the laboratory at the Institute

of Marine Research. Single male and female herring were stripped for sperm and eggs. Sperm from two males were mixed with seawater in 30 liters plastic beakers where the bottom was covered with glass plates (size 76 x 26 mm) (Serigstad & Ellingsen 1987). The eggs were stripped directly in to the beaker, were fertilized and stuck to the glass plates.

Capelin (Malotus vilosus) were caught late April by shrimp-trawl on M/S BLOMØY in Bøkfjord, a branch to the Varangerfjord leading in to Kirkenes. Ripe capelin was sorted out. Plastic bags were filled with seawater (about 5 liters, 5°C) and the bottom was covered with glass plates. Sperm from two males were mixed with sea water, and eggs from one female was stripped in to the water. The eggs were fertilized, sunk down and stuck to the glass plates. Twenty different series were started. The eggs were placed in a cold room at Brødrene Aarseter Fish Industry in Vadsø for one day. They were then packed in isolated boxes and send to the lab at the Institute of Marine Research in Bergen. The eggs were rinsed in clean sea water and placed in their experimental facilities about 30 hours after fertilization. The fertilization percent was above 90 percent for all the series, but after 2-3 days the eggs started to die, and in most of the series more than 90% died within the first 5 days. However we had still 4 series with less than 90% death, which were good enough to be used in the experiments. The death seemed to be due to the transport and handling of the eggs. After day 5 the eggs were not sensitive to handling any more, and we had almost no further death.
In the lab. eggs and larvae were incubated at 5°C in the biotest system, or at 8°C in 30 or 10 liters polyethylene beakers.

OIL EXPERIMENTS

The oil experiments were performed in the new biotest laboratory at the institute of Marine Research. Parts of the biotest setup was built at the University of Bergen under the project: "Fish larval physiology and anatomy. Basic studies and effects of oil". (Fyhn 1986, Fyhn et al 1987). This was a cooperative project

between the University of Bergen and the Institute of Marine Research, financed by A/S Norske Shell. With financial support from the Norwegian Ministery of Oil and Energy, the biotest setup was moved in 1986 to the Institute of Marine Research where it was completed modified and tested out. By this installation considerable improvements are done to the biotest facilities at the institute compared to the old one as described by Johanessen 1983. Fig. 1 shows the schematic drawing of the new biotest system at the Institute of Marine Research.

The biotest setup is a multi-aquarium system with precise temperature control and dosed inputs of water soluble pollutant The system has been built for long term effect studies on marine organisms. The biotest system holds the potential for effect studies of almost any water soluble toxicant on marine organisms within a size rang of 0,1 mg to 1 kg. All fluid flow and oil extraction occur under closed conditions in darkness to minimize evaporation and photo-chemical reactions.

Both herring and capelin were tested for effects of the WSF (water soluble fraction) of crude oil. The different experimental series are shown in TABLE 2. The oil content was determined according to Westrheim & Palmork 1986, and the concentration in the different series are shown in TABLE 1.

TABLE 1:

The different series were exposed to the following average amount WSF (water soluble fraction) of crude oil.

Series	Oil concentration
s187	200 ppb
s387	100 ppb
s587	100 ppb
s288	85 ppb
l188	70 ppb

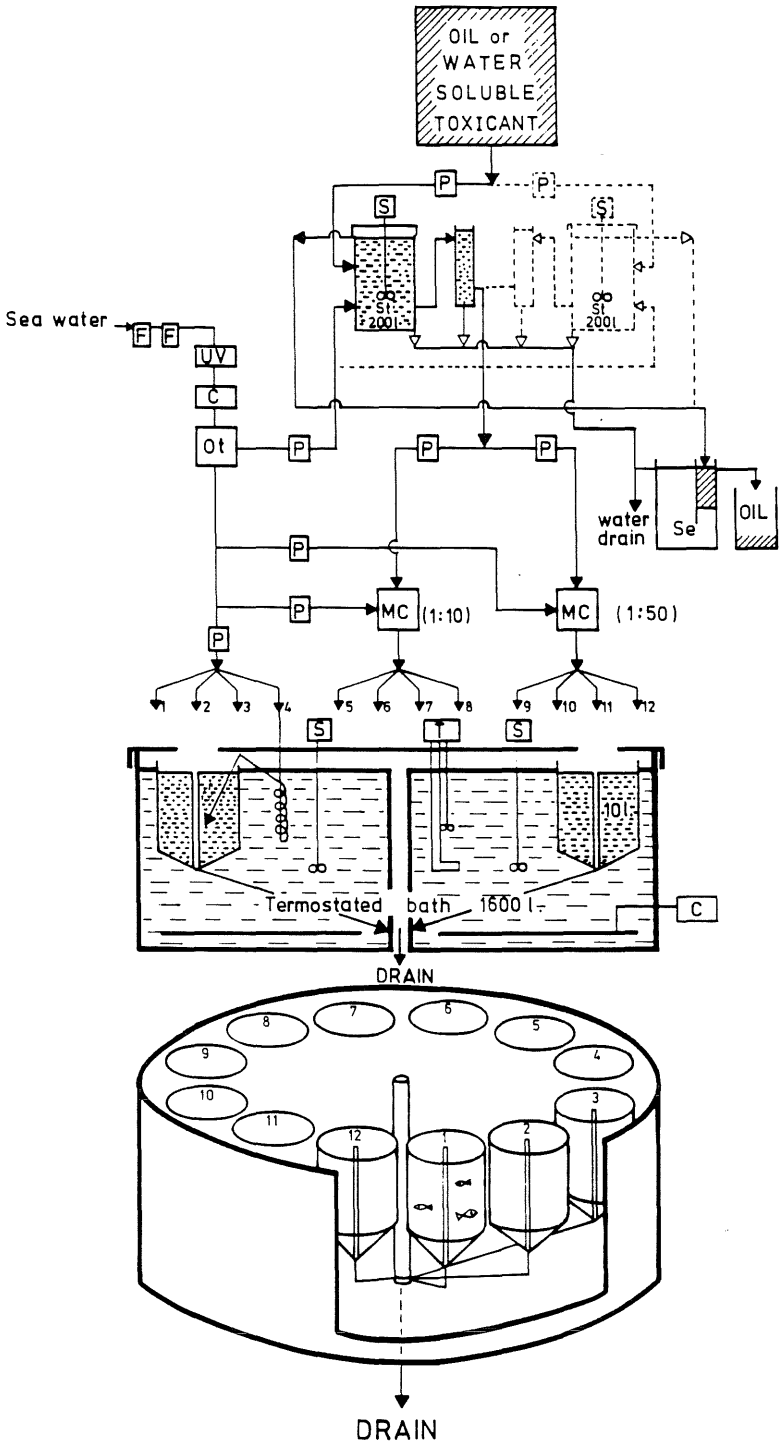

FIGURE 1: A flow diagram of the new biotest system. The components are schematically drawn and identified as follow:

C:cooling unit
F:filter
Mc:mixing chamber
Ot:overflow tank
Uv:uv sterilizer
S:stirrer
Se:oil-water separator
St:stock solution tank
T:thermostat

TABLE 2:

SPECIES	GROUP	FERTILI-ZATION	EXPERIMENTAL-CONDITIONS	ANALYSIS
HERRING	S187	A	Continuously exposure of crude oil	oxygen consumption
HERRING	S387	A	Continuously exposure of crude oil	oxygen consumption
HERRING	S587	A	Continuously exposure of crude oil	oxygen consumption
HERRING	S288	A	Continuously exposure of crude oil	oxygen consumption
CAPELIN	L188	B	Continuously exposure of crude oil	oxygen consumption
HERRING	S388	A	Continuously exposure of drilling mud	Observation of condition,hatching success and death.
HERRING	S388	C	Continuously exposure of drilling mud	oxygen consumption
HERRING	S388	D	No further exposure of pollutant	Observation of condition,hatching success and death.
CAPELIN	L188	B	The test species were kept in seawater until day 18 after fertilization.Then they were moved into a mixture of drilling mud and seawater.There was no further addition of drilling mud after day 18.	Oxygen consumption Observation of condition,hatching success and death.

FERTILIZATION:

A: Sperm and eggs were stripped into clean seawater in 40 l. polyethylene beakers, which bottom was covered with object glasses.

B: Sperm and eggs were stripped into clean seawater in 5 l. polyethylene bags, which bottom was covered with object glasses.

C: Sperm and eggs were stripped into a mixture of drilling mud and seawater in 40 l. polyethylene beakers which bottom was covered with object glasses.

D: Sperm and eggs were stripped into clean seawater in 40 l. polyethylene beakers which bottom was covered with object glasses. Drilling mud was added immediately after fertilization.

EXPERIMENTS WITH OIL-BASED DRILLING MUD

During exploratory drilling, and development of an oil field a fluid is used in conjunction with the rotary system of drilling. This drilling fluid is pumped from the surface down the inside of the rotating drill string, discharged through ports in the bit and returned to surface via the annular space between drill pipe and hole. Modern rotary drilling requires careful manipulation of the drilling fluid in order to reach target depth successfully. According to drilling requirements, drilling fluids can vary from simple mixtures of clay and water to the more complex systems that are chemically conditioned to drill deep holes with the minimum of difficulty. The drilling fluid serves to cool and lubricate the bit, bring drilled cuttings to the surface, consolidate the side of the drilled hole , prevent squeezing-in or caving of formation, control subsurface pressure, suspend drilled cuttings when the column is static and minimize damage to any potential pay zone that might be encountered.

Sometimes, and for several reasons oil-base drilling fluid are used in offshore oil drilling. The drilling fluid are recycled, but stones and smaller particles are separated from the fluid and discharged. This material still contains chemicals (oil) from the drill fluid, and may have an impact on benthic marine organisms, or demersal fish eggs.

In our experiments we tested fish eggs and larvae for effects from oil-base drilling mud used by Statoil on the Gullfaks-A rig. Chemical analysis of hydrocarbons from the drill mud shows that it mainly contains normal and iso-alkanes (Westrheim pers.com.). Hydrocarbon analysis were performed on seawater contaminated with drilling-mud (1.33 g/l). The cuttings were allowed to settle before the water samples was taken. Analysis showed that the water contained 400 ug hydrocarbons/l. Five percent of the hydrocarbons was BTX-components (benzene, toluene and xylenes), while the remaining 95% was made up of heavier components. In contrast to this hydrocarbon composition, the water used in the oil exposure experiments contains approximately 80% of BTX-

components, while only 20% are heavier components.

The different experiments with exposure of fish eggs to discharged cuttings, from drilling using oil-base drilling mud, are shown in Table 2.

RESULTS.

Herring

a). Oil

We did not found any significant effects of oil exposure (> 200 ppb WSF) on the oxygen uptake of herring eggs and larvae. Four different series are tested, 3 in 1987 and 1 in 1988 (Fig 2).

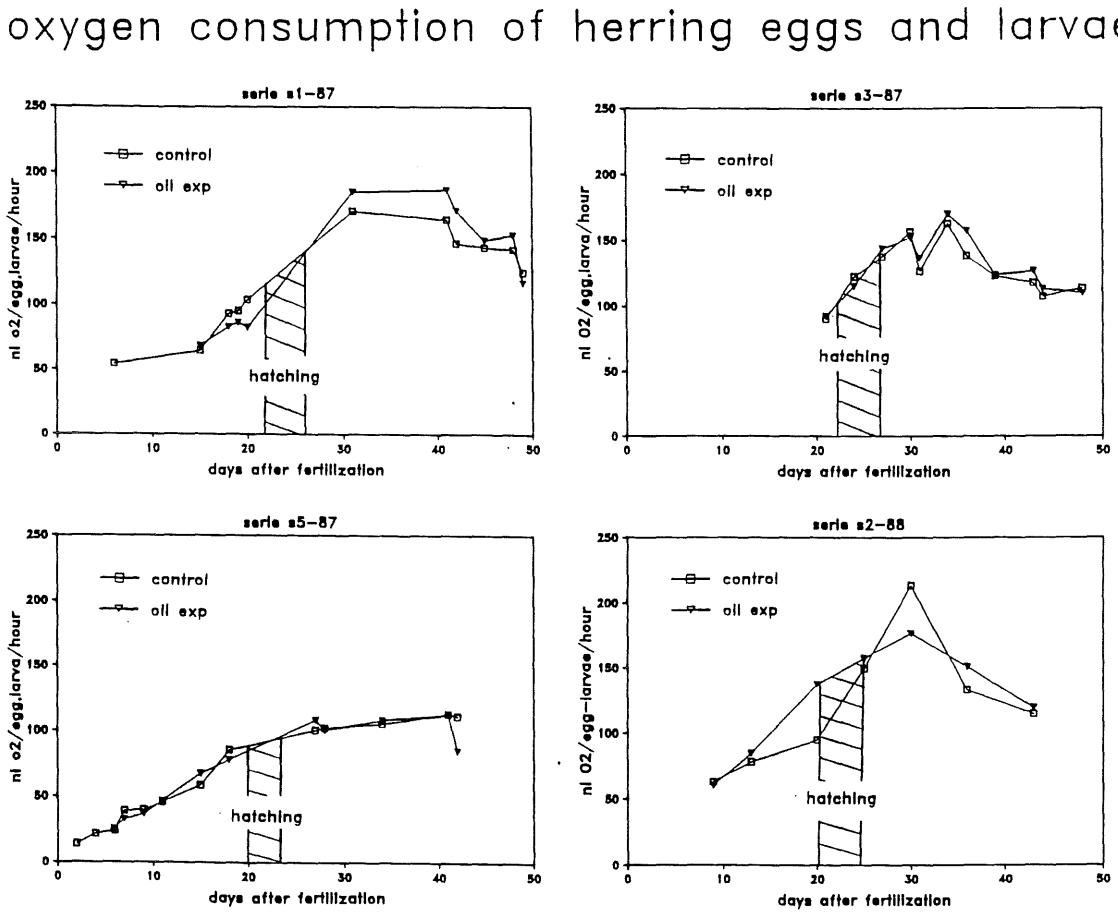

Fig. 2: The oxygen uptake of herring egg/larva as a function of days after fertilization. Each point represent the mean of 20 eggs/larvae. T=5^{o}C, salinity=34 o/oo. Oil concentrations are given in table 1.

All the different series shows a similar shape of the oxygen consumption curve, but there are great deviations in the consumption rate, probably due to differences in the egg and larval size of the different series (Fig 3).

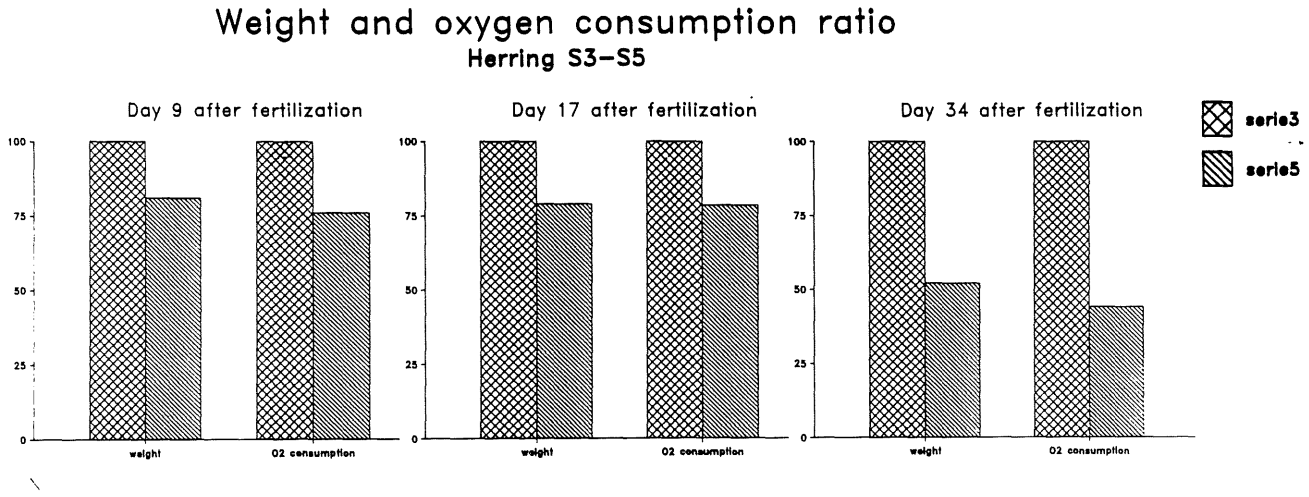

Fig. 3: Weight and oxygen consumption ratio in herring eggs/larvae, series S-3 and S-5. Series S-3 is set to 100% .

Figure 3 shows that the difference in weight between two egg/larvae series is reflected in difference in the oxygen uptake. The weight specific oxygen uptake is about the same for a large and a small herring larvae at the yolk sac stage. Figure 2 shows that there is an increase in the oxygen uptake from fertilization until about 1 week post hatching. After this time the yolk sac is almost depleted, and the oxygen consumption rate levels off, and starts to decrease due to insufficient food supply, in our experiments.

b). Oil-base drilling mud.

Figure 4 shows the oxygen consumption of herring eggs/larvae from experiment S388-C, exposed to oil-base drilling mud.

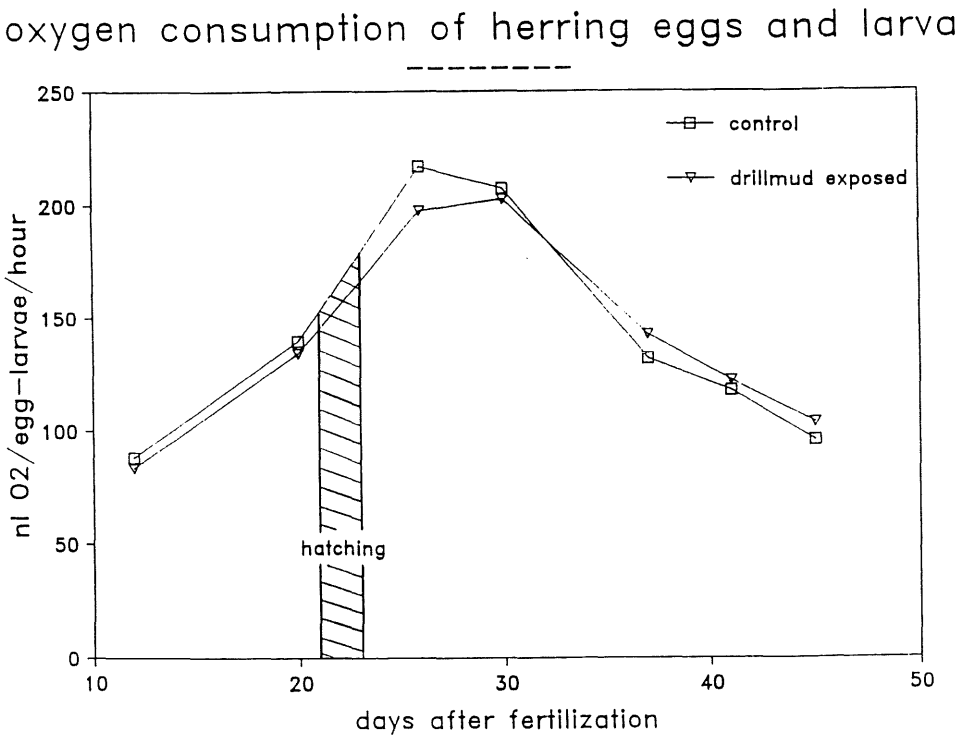

Fig. 4: The oxygen consumption of herring egg/larva as a function of days after fertilization. Each point represents the mean of 12 eggs/larvae. T=5°C, salinity=34 o/oo. Drill cuttings = 1.33 g/l. Oil concentration = 400 ppb WSF.

The oxygen consumption is increasing from fertilization until approximately one week post hatching, then the yolk sack is absorbed and the oxygen consumption decreases due to insufficient food supply in our experiments as reflected in both the exposed larvae and controls. There is no significant difference in oxygen uptake between controls and drilling mud exposed herring eggs/larvae.

CAPELIN

A). Oil

The capelin eggs showed a steady increase in their oxygen uptake from fertilization until hatching (Fig 5).

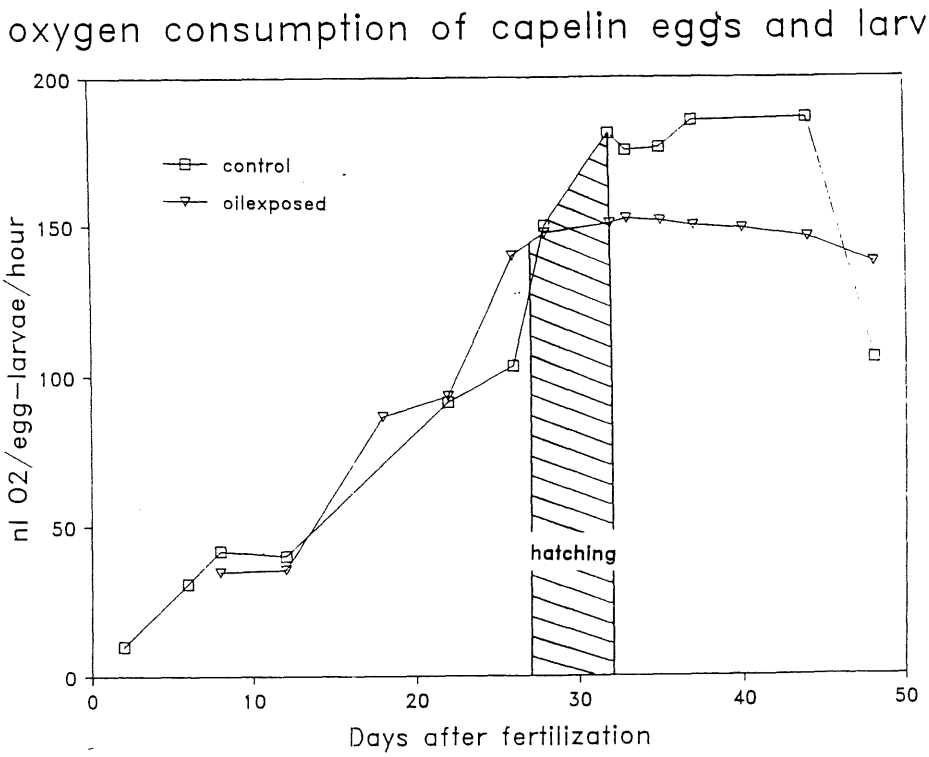

Fig. 5: Oxygen consumption of capelin egg/larva as a function of days after fertilization. Each point represents the mean of 12 eggs/larvae. T=58C, salinity=34 o/oo. Oil concentration = 70 ppb WSF

The hatching took place over a period of 5 days, from day 27 to day 32. After hatching the oxygen uptake rate leveled off, and it was kept stable for approximately 2 weeks even if the yolk sac was empty after approximately 1 week. After day 45 the oxygen uptake decreases. There is no observed difference in the oxygen uptake between controls and oil exposed (70 ppb WSF) capelin eggs. After hatching there is a significantly reduced oxygen uptake of the oil exposed larvae. The difference is approximately 20% and it is kept at that level for about 2 weeks. Then we got a decrease in the oxygen uptake due to insufficient food supply after the yolk sac is depleted.

b). Drilling mud.

The hatching success for the drilling mud exposed (1.33 gram/liter) capelin eggs were better than for the control eggs. In the control group there were approximately 80% hatching while the drilling mud exposed eggs had a hatching success of approximately 90%. The drilling mud covered the bottom of the experiment aquarium in a 1-2 mm tick layer. The eggs were therefor completely covered with mud. In the control aquarium the eggs lied uncovered on the bottom. After hatching the control larvae swam around in the aquarium, while the drilling mud exposed larvae were buried down in the mud, and swam up only wh n they were disturbed. Similar observations were done with capelin larvae in the biotest setup. The larvae seemed to prefer to stay in the bottom layer with dead eggs and egg shells.

DISCUSSION

It is difficult to pick out one specific biological parameter and use effects on this parameter as a general indicator of pollution. We have at our institute with success used the oxygen uptake as such a parameter (Serigstad 1987a), and we have also in cooperation with the Biochemical Institute at the University of Bergen, used the induction of the cytochrome p-450 system as an indicator of pollution (Goksøyr et al 1987).

The metabolic activity of a fish larvae depends on sufficient supply of oxygen from the ambient sea water to the mitochondria in the cells, where the aerobic energy production for synthesis, regulatory processes and locomotor activity take place. Oxygen uptake is thus a direct measurement of the aerobic energy production of the fish larvae and a quantitative expression for the rate of consumption of its energy stores. We assume that the normal oxygen uptake of the fish larvae is optimized for its growth and development, and that any deviation from these normal conditions have a serious impact on the survival of the animal. Experiment with oil exposure of cod larvae have shown that the oxygen uptake of oil exposed larvae are depressed compared to the controls (Serigstad 1987a). This effect on the oxygen uptake is seen already after 24 hours exposure to 50±20 ppb WSF of Statfjord crude oil. There is not shown any recovery of the oxygen uptake of oil exposed cod larvae after transfer to clean seawater.

In contrast to these results we found a recovery of the same larvae with respect to the effect on the cytochrome p-450 system. There were a clear induction of the cytochrome p-450 as long as the larvae were kept in oil polluted water, but after about 3 days in clean seawater the p-450 values were almost equal to the control values.

The two methods can not substitute each other. To measure an oil effect as an impact on the oxygen uptake and metabolism of a fish larvae reveals very good control of the experimental conditions,

which means that the best results are obtained in the laboratory. The method using induction of the cytochrome p-450 as an indicator of oil pollution may easily be used in field studies. The problem with this method is that the p-450 values drop to normal within a few days when the source of pollution disappears, even if the larvae are severely hurt by an oil effect as shown on the ability of oxygen uptake. The larvae will die, but we can not see any effect on the cytochrome p-450.

We know that cod eggs accumulate oil hydrocarbons in the yolk sac (Solbakken 1984). This accumulation is rapidly. Some hydrocarbons are concentrated with a factor of about 300 after one hour exposure to water containing oil hydrocarbons while the discharge rate is much slower. Fish eggs exposed to oil hydrocarbons may therefore already bee damaged by oil, even if this is not registered on the oxygen consumption of the eggs. As our experiments shows (Serigstad 1987b, Serigstad & Føyn 1987), effects will not be detectable as a reduced oxygen uptake until the eggs are hatched, and the larvae starts to swim searching for food.

The purpose of our experiments is to find the developmental stages of fish most vulnerable to oil pollution, or exposure to oil-base drilling mud. We are interested to determine possible effects to a fish egg or a larva that has been exposed to oil or oil-base drilling mud, both short time effects and those of more permanent character. On this basis the oxygen uptake and metabolic studies are the only known methods that can give us the answer to: Will the larva die or is it reasonable to think it will survive the pollution.

Experiments with oil exposure (<300 ppb WSF) of herring eggs and larvae did not show any effects on the oxygen uptake, neither on the egg stage nor on the yolk sac stage. Four different herring series are tested for effects, 3 series in 1987 and 1 series in 1988 and all the series shows the same, no observed negative effects. The difference in oxygen uptake between controls and oil exposed cod larvae observed just after hatching (Serigstad 1987b)

when the larvae still lives on the yolk sac, but has to start exogenous feeding may be due to differences in activity. Cod larvae feed mainly on crustacean nauplii, rotiferes and other invertebrate larvae (Fyhn & Serigstad 1987). Those zooplankton food organisms are swimming and the cod larvae need to do fast attack to catch their pries. Observation of cod larval behavior shows that the larvae have a rapid increase in activity at the developmental stage where we find the difference in oxygen uptake between controls and oil exposed larvae. We assume that the oil exposed cod larvae are unable to increase their oxygen uptake, and thus also the locomotor activity at this important period of development, resulting in non or reduced feeding success and later on death. The herring larvae which does not show any reduction in oxygen uptake may have an other feeding pattern than the cod. If it is not feeding on fast swimming organisms an effect on the ability to take up oxygen, or any kind of metabolic problems could bee masked by low swimming activity in the early life. Even if no effects is shown in our experiments, there may bee a hidden damage that will be discovered when the larval activity increases at a later occasion. In fact there is reported an exogenous uptake of free amino acids from sea water by herring eggs/larvae (Siebers & Rosenthal 1977). No such uptake is found in cod eggs/larvae (Mangor-Jensen 1986).

There is a large effect of oil exposure on cod larvae. The oxygen uptake is reduced with 40-50% for all the 7 experimental series (Serigstad 1987a), while there is no effects seen on any of the 4 herring series. From our experiments we may anticipate that in assessment of pollution damages, cod and herring may serve as the lower and upper limits, respectively, for effect registrations on fish.

Capelin larvae showed a significant effect of oil exposure. The oxygen uptake is reduced with 20% compared to the control larvae.

REFERENCES.

Fyhn,H.J. 1986. Fish larval physiology and anatomy. Basic studies and effects of oil. Final report, project no 83:524. University of Bergen, Bergen.

Fyhn,H.J.,Salhus,H & Barnung,T.N. 1987. A biotest system for long term effect-studies of oi. on marine fish eggs and larvae. Design, component description and functional tests. -Sarsia 72> 321-328.

Fyhn,H.J. & Serigstad,B. 1987. Free amino acids as energy substrate in developing eggs and larvae of the cod Gadus morhua. -Marine Biology 96: 335-341.

Føyn,L. & Serigstad,B. 1987. Age dependent sensitivity of oil on larvae, used in assessment of potential oil pollution damage on fish resources. ICES C.M. 1987/E:12. Marine Environmental Quality Committee.

Føyn,L & Serigstad,B. 1988. Oil exploration in new offshore fields. Fish larvae as the critical component in the assessment of potential consequences for the fish resources. ICES C.M 1988/E:18. Marine Environmental Quality Committee.

Goksøyr,A., Serigstad,B., Solberg,T.S. & Stegeman,J.J. (1987). Response of cod (Gadus morhua L.) larvae and juveniles to oil exposure detected with anti-cod cytochrome p-450c IgG and anti-scup cytochrome p450E MAb 1-12-3. Marine environ. 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 of eggs and larvae of the cod (Gadus morhua L.). Basic studies and effects of oil exposure. /Pp117-168 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, Norway.

Serigstad,B. 1987a. Respiratory studies on cod (Gadus morhua L.) with special reference to effects of oil exposure on eggs and larvae. Dr. Scient. thesis, University of Bergen Norway.

Serigstad,B. 1987b. Effects of oil exposure on the oxygen uptake of cod (Gadus morhua L.) eggs and larvae. Sarsia. 72: 401-403.

Siebers,D. & Rosenthal,H. 1977. Amino acid absorbtion by developing herring eggs. Helgolander Wiss. Meersunters. 29, 464-472.

Solbakken,J.E., Tilseth,S. & Palmork,K.H. 1984. Uptake and elimination of aromatic hydrocarbons and chlorinated biphenyl in eggs and larvae of cod (Gadus morhua L.). -Mar. Ecol. Prog. Ser., 16:297-301.

Westrheim,K & 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, Norway. 328 pp.