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EFFECTS OF LOW LEVELS OF EKOFISK CRUDE OIL ON EGGS AND YOLKSAC LARVAE OF COD (Gadus morhua L.)

By

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ABSTRACT

Two groups of cod eggs and yolksac larvae were continously exposed to 50-60 and 100-200 ppb of the watersoluble fraction of Ekofisk crude oil.

Both groups showed a concentrationdependant reduction in growth. The highest concentration also caused a reduced larval feeding ability and reduced oxygen consumption. No significant effects were found on heart rate and yolk osmolality and ionic composition.

The results are discussed in relation to larval survival through the first critical stages.

INTRODUCTION

Tilseth et al. (1981) reported that cod larvae continously exposed to low levels of the water soluble fraction of Ekofisk crude oil during the embryonic and larval stages suffered retarded growth, increased neutral buoyancy and impaired feeding ability. In the present study we wanted to test the reproducability of the results and wether effects also could be traced in physiologic parameters such as oxygen consumption and osmo and ionic regulation.

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 washed, treated with antibiotics and incubated according to Tilseth et al.(1981). Ten days after fertilization, about one week prior to hatching, eggs were trasferred to a biotest oil exposure system (Tilseth et al. 1981) and exposed to the water soluble fraction (WSF) of the employd oil. The system includes three subunites, one for each of two selected oilconcentrations and one control.

Two oil exposure experiments were performed with eggs from two different female fish, group A and B. The experiments were terminated about two weeks after hatching. Except for separate feeding experiments, the larvae were not fed during the period of exposure to oil contaminated sea water.

Chemical analyses

The hydrocarbons were extracted from the waterphase with dichlormethan (3 liters of sea water and 50 + 25 + 25 ml dcm). Fluorene in dcm was initially added to the watersample as internal standard. The extract was concentrated at room temperature and reduced air preassure on a rotatory evaporator. The residue was further concentrated under a flowing

stream of dry nitrogen gas. Two ul of the concentrated extract was injected splitless into the capillary column of a HP 5880 gas chromatograph. The column was a 25m and 1D 0.2 mm flexible fused silica capillary column coated with SE-54. Nitrogen gas at 1.5 ml/min was used as carrier gas and the temperature programmed from 40 to 270°C at 8°/min with an initiation time of 4 min.

Bacteriological tests

Bacterial growth in the biotest exposure system was monitored daily by taking 100ml water samples from the stock solution and test aquaria. The samples were preserved with 4 ml glutaraldehyde. Two ml acridine orange were added to each sample (Hobbie et al. 1977), which was filtered through 0.2 um Unipore polycarbonate bacterial filters and the bacteria counted under fluorescence microscope.

Potential bacterial growth on the egg shells was examined both under fluoescence microscope and by measuring the oxygen consumption of eggshells dissected from the embryoes.

Growth

Larval standard length was measured daily. The larvae were preserved on 4% formaldehyde in 10‰ sea water.

Feeding

The feeding ability of larvae was determined in separate feeding experiments. Zooplancton was captured from 15 meters depth by means of an automatic plancton sampler system (Tilseth et al. 1981). Larvae and sea water from the very same exposure aquaria were transferred to 4 liter glass jars and fed zooplancton at 0.5 organism pr. ml. After one hours feeding, the larvae were preserved on 4% formaldehyde in 10 ‰ sea water for later analysis.

Oxygen consumption

The oxygen consumption rate in eggs and larvae were measured daily by means of closed respirometers consisting of 10 ml glass syringes. Five larvae in 5 ml sea water were transferred to each syringe from test and control aquaria and allowed to stand over night in a 5°C thermostat-controlled waterbath and slowly rotated each quarter of an hour by means of a rotatory device. Four syringes with test larvae and 4 with control ones were employd. The larvae were prevented from leaving the syringes by introducing a tephlon catheter 1-2 cm through the syringe opening. The drop in pO_2 was read by pressing a water sample from the syringe on to a Radometer E5046 oxygen electrode placed in a Radiometer D616 thermostat cell.

Heart rate

Larval heart rate was determined by placing the transparant larvae in a glassbeaker covered by a plexiglass housing, flushed with 5.0°C water, and placed under a binocular microscope. The larvae were anaesthetized with MS 222.

Osmolality and ionic distribution

The osmolality of the yolk was measured during the egg stage by determining the yolk freezing point in a Clifton nanoliter osmometer. Yolkmass was drawn from the yolksac with a glass capillary, placed on a platinum plate covered with parafine, and introduced into the osmometer.

Concentration of Na^+, K^+, Cl^- and NPS (ninhydrin positive substances) were determined from the extract of 20 eggs. Ions from the perivitellin space were washed out by flushing the eggs with destilled water for 3 minutes. The eggs were homogenized in 1 ml 5% sulfosalicylic acid. Proteins and peptides were presipitated on a sentrifuge and the supernatant allowed to stabilize at 0°C for 1 hour (Stein & Moore 1946). Na^+ and K^+ were measured on a Pye Unicam spectro-

photometer, Cl^- on a CMT chloride titrator and NPS by the ninhydrin reaction (Blackburn 1968) on a Pye Unicam spectrophotometer. Norleucin was used as internal standard. The NPS comprices substances with a free amino group in α-position to a carboxylic group, mainly free amino acids (Lehninger 1975).

Statistics

The data are treated statistically according to Shefler (1969).

RESULTS

Chemical analyses

The concentration of dichlormethane extractable hydrocarbons in the exposure aquaria during the experiments are presented in fig. 1. The concentrations were 100-150 ppb and 150-250 ppb for the most-exposed larvae of group A and B respectively, and 40-70 ppb for the least-exposed larvae of both groups. The concentrations in control aquaria were negligible. The monoaromates benzene, toluene and xylene constituted 60-70% of total dissolved hydrocarbons.

Bacteriological tests

The bacterial concentrations in the water samples from the biotest exposure system were 10-100 times lower than maximum concentrations registered during natural conditions in open seas (Fuhrman & Azam 1980), and no systematic differences were found between stock solution and rearing aquaria. The values are therefore combined, and the variation given as standard deviation with arrows indicating the time intervals between each clean-up of the biotest exposure system (Fig.2). However, there was a steady bacterial growth on the walls of the exposure system, especially in the stock solution tank. The layer was removed during the weekly clean-up of the equipment.

Eggshells dissected from the embryoes had a traceable oxygen-consumption, but the differences between oil-exposed and

control shells were not significant (Fig.11). Neither did examination under fluorescence microscope reveal any substantial bacterial growth on the shells.

Growth

Larvae exposed to oil-polluted sea water suffered a reduced growth (Fig.3). The reduction in standard length was smallest in larvae exposed to the lowest concentrations. Also larvae exposed to the highest concentration only until hatching or after hatching, suffered a reduced growth, but the reduction was lower than in full-time exposed larvae (group A, fig.3).

The relation between oil concentration and % reduction in larval standard length is presented in fig.4. The % reduction is an average for the whole larval period, and is calculated from the daily measured differences between test and control groups (Fig.3). The oil concentrations are given as average values for the whole experimental periods. The figure also includes data from crude oil exposure experiments reported in Tilseth et al. (1981) as these experiments were performed using the same experimental procedure and biotest exposure system as in the present experiments. The data show a positive correlation (r=0.97) between oil concentration and growth reduction. The data also indicate that growth reduction might occur at lower concentrations than the ones here employd.

Feeding

Both experimental groups were tested in feeding experiments. Group A larvae showed a very low feeding incidence (% larvae with gut content) and no significant differences were found between test and control larvae. Group B larvae exposed to the highest concentration, showed both a reduced feeding incidence (Fig.5) and feeding index (numbers of particles ingested pr. larvae with gut content) (Fig.6). The larvae also suffered a reduced capability in capturing copepod nauplii (Fig.7).

Oxygen consumption

The oxygen consumption rate was measured for larvae exposed to the highest oil concentration and control group larvae of both experimental groups, and are presented in fig.8. The consumption rate incresed steadily during the egg stage with no significant differences between test and control groups. After hatching, however, the oxygen uptake in oil-exposed larvae seems reduced compared to control larvae, which reached a maximum consumption about one week after hatching.

Heart rate

The heart of the cod embryoes started to beat about one week prior to hatching. The rate increased steadily during the embryonic stage, and stabilized to a steady frequency at about 55 beats pr. min. after hatching in both groups (Fig.9). No significant differences were found between test and control larvae.

Yolk osmolality and ionic distribution

Yolk osmolality and yolk distributions of Na^+, K^+, Cl^- and NPS in oil-exposed and control eggs are presented in figs.10, 11, and 12. Unfertilized eggs from both experimental groups taken directly from the gonad, had a yolk osmolality of 330-340 mOsm. After fertilization in sea water the osmolality increased to more than 400 mOsm, whereupon it steadily decreased to about 300 mOsm prior to hatching. Concentrations of Na^+ and Cl^- increased from fertilization to hatching, while K^+ showed a more complex pattern with a minimum concentration during the midle of the embryonic period. However, no significant differences in osmolality or in concentration of Na^+, K^+, and Cl^- were found between test and control eggs. In both experimental groups the concentration of NPS decreased steadily from fertilization until hatching. The data might indicate a reduction in concentration of NPS in oil-exposed embryoes compared to control. The difference is seen between least-exposed embryoes and control ones of group A, and between most-exposed embryoes and control ones of group B.

However, no clear differences were found between most-exposed and control embryoes of group A.

DISCUSSION

The oil hydrocarbons clearly induced a concentration dependant growth reduction in exposed larvae compared to control. Lowest tested concentrations were approx. 50 ppb, but the combined data (Fig.4) indicate that effects might well occur at lower concentrations. Retarded growth in fish larvae in response to oil exposure has been reported for several species. The reduction is thought to be caused by an extra energy demand in the metabolism of hydrocarbons (Johnson et al. 1979, Leung & Bulkley 1979, Lindén 198). However we were not able to detect any increased oxygen consumption in exposed eggs and larvae compared to control ones. On the contrary, the more exposed groups suffered a reduced consumption during the larval stage. The reduction was probabely due to reduced activity, and might be related to the registered impaired feeding activity with reduced capability of catching living prey organisms.

No significant differences in heart rate were registered between test and control groups, which is in agreement with the statements of Anderson et al.(1977) and Sharp et al. (1979) that heart rate in fish emryoes and larvae is a poor indicator of low-level hydrocarbon stress.

Yolk osmolality and concentration of inorganic ions also seemed unaffected by the hydrocarbon stress. Most probabely emryonic and larval life depend on a stable inner ionic distribution, which makes tolerable changes hardly detectable. The registered differences in NPS were inconsistent and further investigations are needed before any conclusions can be drawn. However, a change in NPS concentration might well result from the altered growth pattern rather than imbalance in organic ion concentrations. A closer discussion

of the registered changes in osmotic and ionic concentrations during the embryonic development is beyond the scope of this report.

According to the present results it seems fair to suggest that the increased neutral buoyancy registered in cod larvae exposed to crude oil (Tilseth et al.1981), probably resulted from the altered growth pattern rather than alterations in osmoregulatory mechanisms. However, the buoyancy might also be influenced by accumulation of hydrocarbons of low specific gravity.

The bacteriological tests indicated that the present registered effects most probabely originate from the oil itself rather than bacterial contamination of the test samples. A reduced larval feeding during the first feeding stage would of course be threatening to larval survival. The implications of a reduced growth are more dubious as the full impacts of the biochemical changes involved are not clear. However big larvae are supposed to be better fit for survival during situations of scarce food than smaller ones (Ware 1975), which often is the situation experienced by cod larvae in the first feeding areas (Ellertsen et al. 1981). Therefore long-time exposure to hydrocarbon levels lower than 50 ppb might also be undesireable to the larvae.

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FIGURES

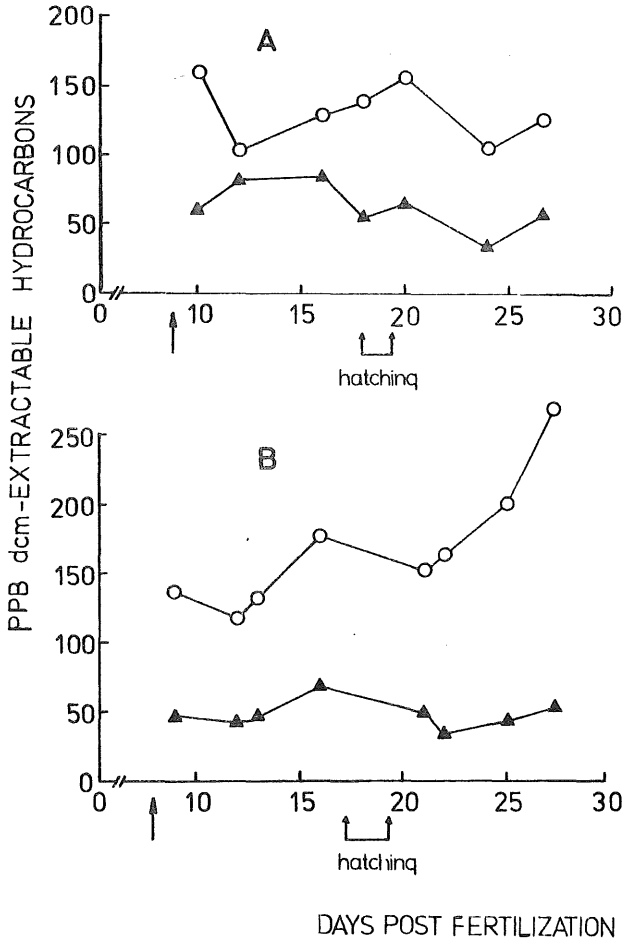

Fig. 1 Concentrations of dichlormethan (dcm) extractable hydrocarbons in more- (O) and less-(▲) exposed aquaria during the experimental periods of group A and B.
↑ start of oil exposure.

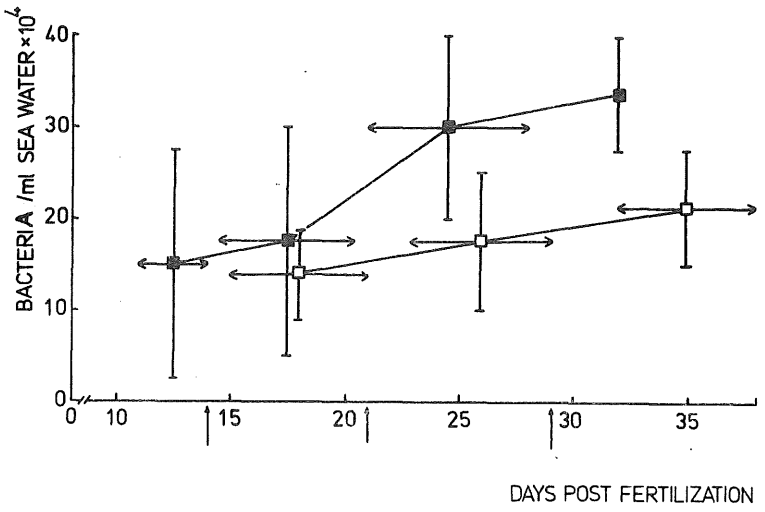

Fig. 2 Bacterial concentrations (± SD) in the biotest exposure system during exposure series A(□) and B (■). The values are averages of samples daily collected during the time interval indicated by the arrows.

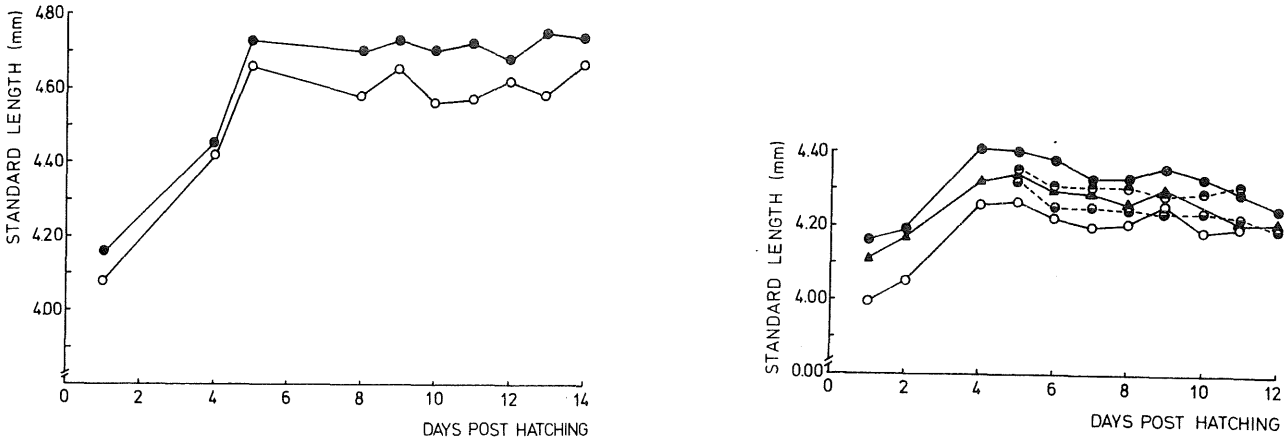

Fig. 3. Standard length of group A and B larvae. O - control larvae, O - more-exposed larvae, ▲ - less-exposed larvae, O - larvae transferred from more-exposed aquaria to control at hatching, O - larvae transferred from control aquaria to more-exposed after hatching. N = 10-20 for each point. SD = 1-3% of the values.

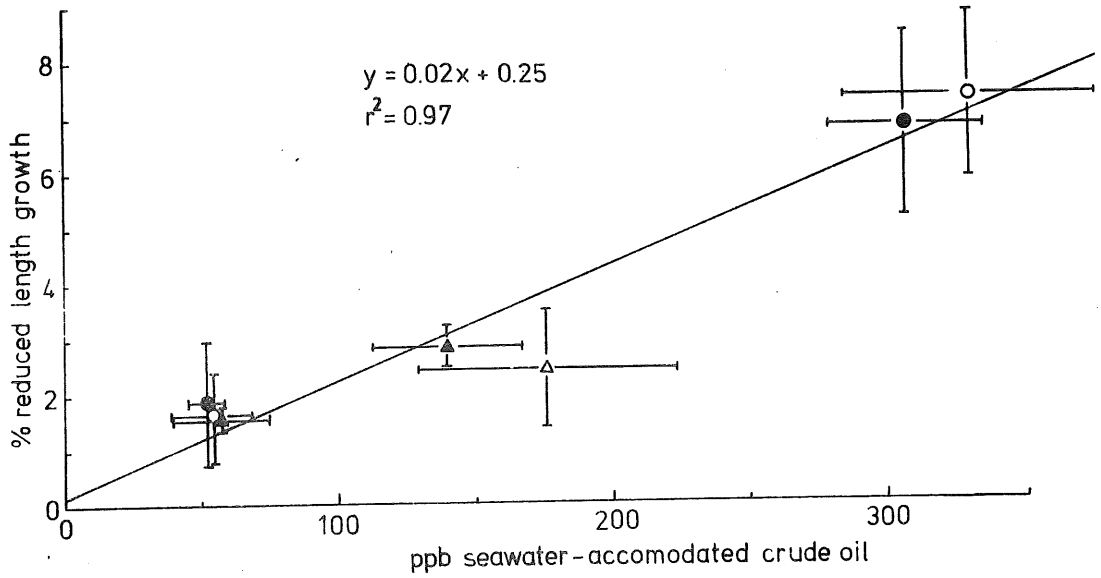

Fig. 4. % reduction in larval standard length versus oil concentration. ▲ - group A, Δ - group B, ●○ - groups from experiments reported in Tilseth et al. (1981).

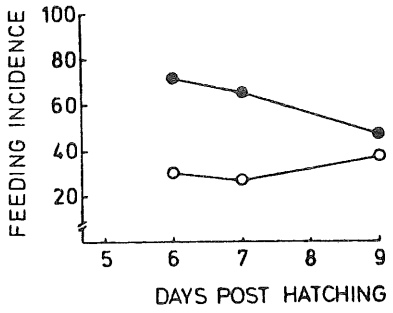

Fig. 5. Feeding incidence (% larvae with gut content) in group B larvae. ● - control larvae, ○ - more-exposed larvae.

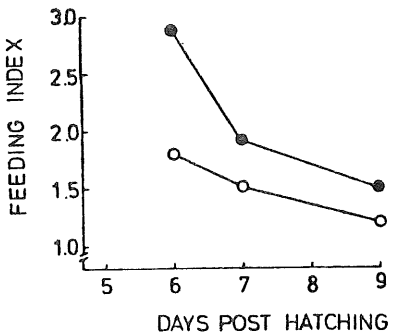

Fig. 6. Feeding index (number of particles ingested pr. larvae with gut content) in group B larvae. ● - control larvae, ○ - more-exposed larvae.

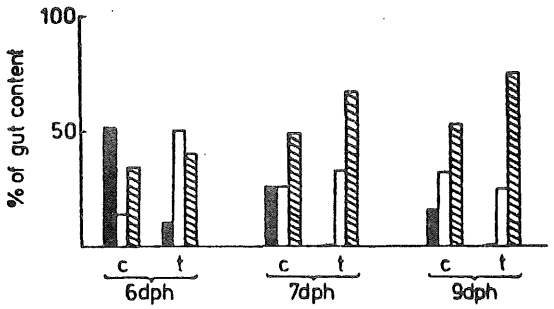

Fig. 7. Composition of gut content from group B larvae. c - control larvae, t - test larvae, ▮ copepodnauplii, ▯ copepod eggs, ▨ unspecified food particles.

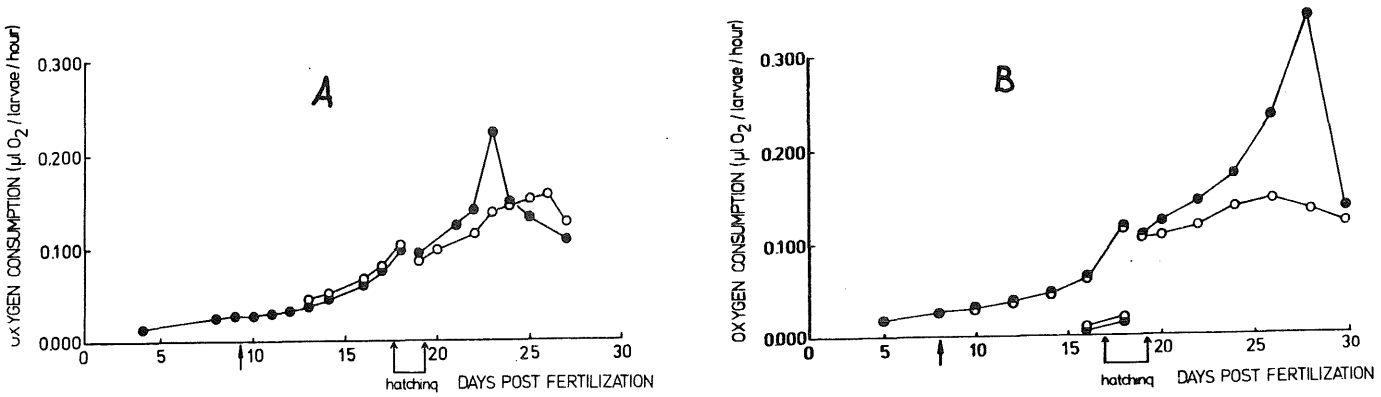

Fig. 8. Oxygen consumption rate in group A and B eggs and larvae, and group B egg shells (day 16 and 18 post fertilization). ● - control group, O - more-exposed group. N = 4 syringes for each point, SD = 3-4% of the values during the egg stage, and 6-8% of the values during the larval stage. ↑ oil exposure started.

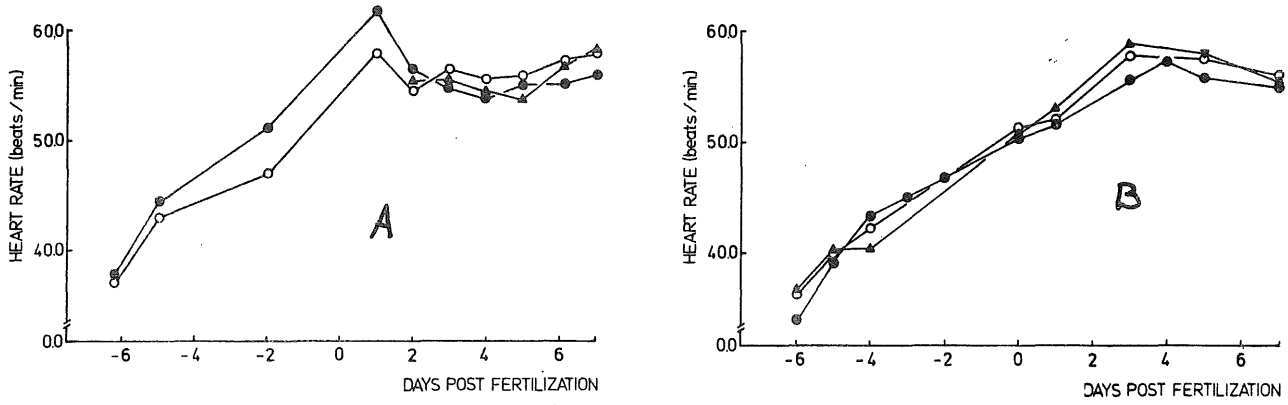

Fig. 9. Heart frequency in group A and B embryoes and larvae. ● - control larvae, O - more-exposed larvae, ▲ - less-exposed. larvae. N = 10 for each point. SD = 0.5-4% of the values.

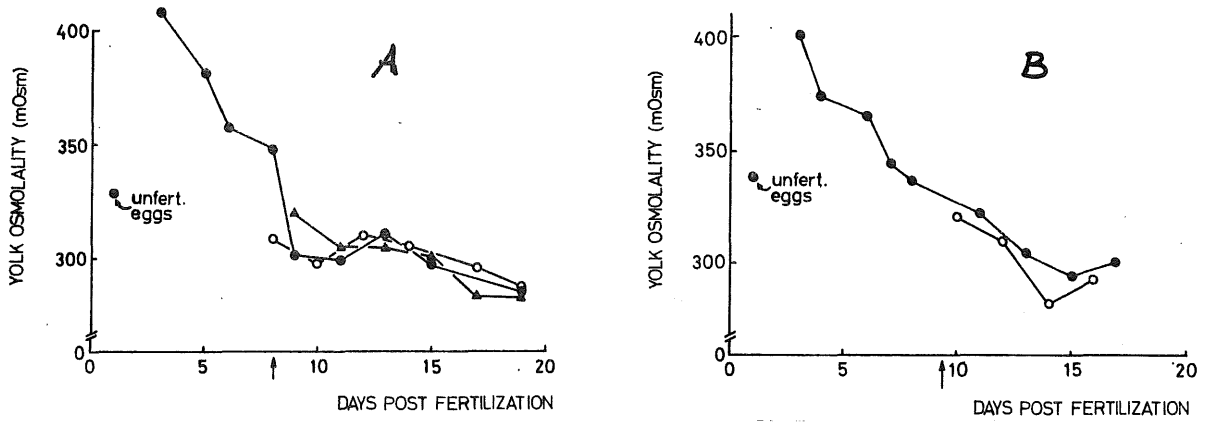

Fig. 10. Osmolality of yolkmass in group A and B eggs. ● - control eggs, O - more-exposed eggs, ▲ -less-exposed eggs. N = 4 for each point. SD = 2.4% of the values. ↑ start of oil exposure.

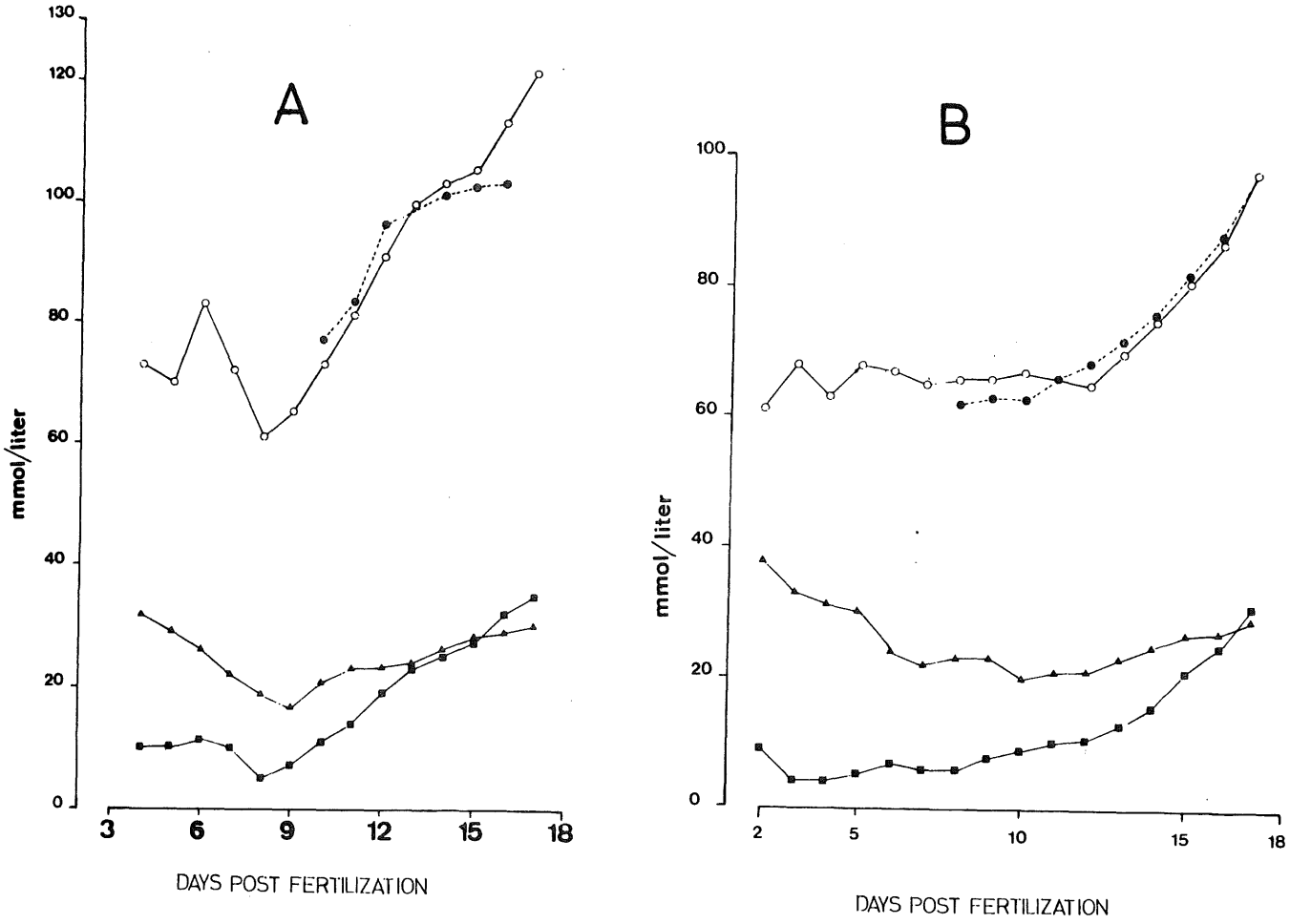

Fig. 11. Content of Cl^- ,Na^+, and K^+ in group A and B embryonic total homogenat. O - concentration of Cl^- in control eggs, ● - concentration of Cl^- in more-exposed eggs. ■ - concentration of Na^+ in control and most-exposed eggs. ▲ concentration of K^+ in control and more-exposed eggs N = 20 for each point.

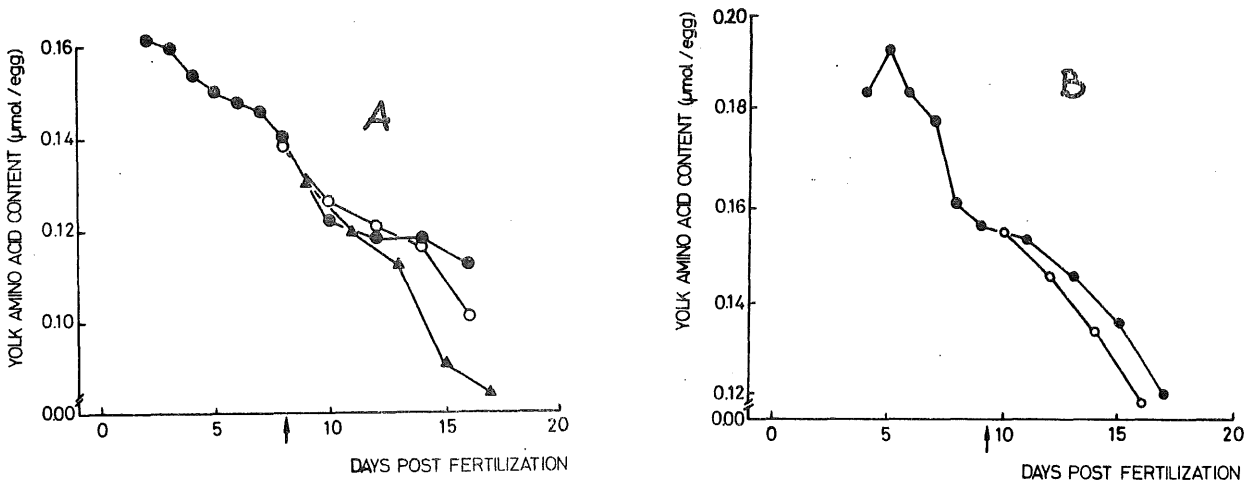

Fig. 12. NPS content in embryonic total homogenat from group A an B. ● - control eggs, O - more-exposed eggs, ▲ - less-exposed eggs. ↑ start of oil exposure. N = 20 for each point.