

This paper not to be cited without prior references to the authors

International Council for the
Exploration of the seas

C.M. 1982/E:59
Marine Environmental
Quality Cttee

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

By

Solberg, T.,⁺ Tilseth, S.,⁺ Serigstad, B.,⁺⁺ and Westrheim, K.⁺

⁺ Institute of Marine research, Directorate of fisheries,
5011 Bergen, Norway.

⁺⁺ Zool. Lab., University of Bergen, 5000 Bergen, Norway.

ABSTRACT

Two groups of cod eggs and yolksac larvae were continuously exposed to low levels of the water extracts of a heavy fraction (b.p. > 150°C) of Ekofisk crude oil. Each group was exposed to two different concentrations, 30-50 and 50-150 ppb, and 40-60 and 100-200 ppb, respectively.

Both groups showed a concentration dependant reduction in growth rate, and reduced feeding ability at all concentrations. Larvae exposed to 100-200 ppb showed a reduced oxygen consumption.

The effects are compared to effects found in cod larvae exposed to Ekofisk crude oil and discussed in relation to possible impacts during oilspill situations in open seas.

INTRODUCTION

Tilseth et al. (1981) and Solberg et al. (1982a) reported that cod larvae continuously exposed to low levels of the watersoluble fraction of Ekofisk crude oil during the embryonic and larval stages suffered retarded growth, increased neutral buoyancy, impaired feeding ability and reduced oxygen consumption. The monoaromates benzene toluene and xylene comprised 60-70% of total dissolved hydrocarbons of the waterextract from the crude oil. During oil spill situations in open seas these volatile components will evaporate and disappear relatively fast from the oil slick. In the present study we want to examine whether the reported effects also can be found in larvae exposed to the watersoluble fraction of Ekofisk crude oil from which these volatile components have been removed.

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 transferred to a biotest oil exposure system (Tilseth et al. 1981) and exposed to the water soluble fraction (WSF) of the employed oil. The system includes three subunits, one for each of two selected oil concentrations 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.

Oil and chemical analyses

The oil used in the present exposure experiments was a heavy fraction of Ekofisk crude oil with boilingpoint $> 150^{\circ}\text{C}$. The fraction was provided by the Rafinor oil refinery, Mongstad near Bergen. The chemical analyses were performed the same way as described in Solberg et al. (1982a) during exposure experiments with crude oil.

Growth, feeding and oxygenconsumption

Larval standard length, feeding and oxygenconsumption were measured according to Tilseth et al. (1981) and Solberg et al. (1982a).

Buoyancy

Neutral buoyancy of eggs was determined in a seawater salinity gradient column calibrated with glass balls of known density (Coombs 1981). Fifty eggs were washed with seawater of low salinity, and put on top of the gradient column where they sank down to sea water of corresponding density. After 30 minute's stabilization, the egg positions were plottet on a plastic sheet attached to the face of the column. The egg density was calculated from the egg position relative to the calibrated balls.

Statistics

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

RESULTS

Chemical analyses

The concentrations of dichlormethan extractable hydrocarbons in the exposure aquaria during the experiments are presented in fig. 1. The average concentration at the lowest level of exposure in group A was 43ppb (SD \pm 31ppb) dissolved hydrocarbons, and 81 ppb (SD \pm 42ppb) at the highest level.

During the experiments with group B, the concentration was 54 ppb (SD \pm 19ppb) at the lowest level. Unfortunately, at the highest level of exposure, the concentration varied from 87ppb (SD \pm 13ppb) during the egg stage, to 212 ppb (SD \pm 25ppb) during the larval stage. The concentration of monoaromates was low ($<$ 5%) compared to the 60-70% in the waterextract from crude oil (Solberg et al. 1982a).

Growth

The standard length of cod larvae exposed to the highest level of the WSF of oilhydrocarbons, group A, was significantly shorter compared to the control group larvae (Fig. 2). There was no reduction in size of larvae exposed to the lowest level of contaminated sea water. In group B larvae, however, significant reductions in standard length were found at both concentrations (Fig.2).

The relation between oil concentration and % reduction in larval standard length is presented in fig.3. The % reduction is an average for the whole larval period, and is calculated from the daily measured differences between test and control groups (Fig.2). In the linear regression 1) (Fig.3) the oil-concentrations are average values for the whole experimental periods. However, in the most exposed larvae from group B, the waterphase oil concentration increased from approx. 90ppb prior to hatching, to approx. 200 ppb during the larval stage. If the average oil concentration is based on the elevated values during the larval stage, the slope of the regression line will be less, and is given by regression 2).

Oxygen_consumption

The oxygen consumption rate was measured for most-exposed and control larvae of group B, and is presented in fig. 4. The consumption rate shows an initial rise in both groups with a top at day 4 and 5 after hatching, whereupon it steadily drops. The curves are fearly similar, however, at day 4 after hatching the uptake in control larvae is markedly higher than in test ones.

Feeding

In both experimental groups, the larvae exposed to the highest oil concentration suffered a reduced feeding incidence (% larvae with gut content) and feeding index (number of particles ingested pr. larvae with gut content) compared to control larvae (Figs.5 and 6). Also in larvae exposed to the lowest oil concentration the feeding incidence seemed to be reduced compared to control, but not as drastically as in most exposed groups. In group A larvae no clear difference between test and control were found in ability to capture copepod nauplii (Fig.7), while in group B the most exposed ones seemed to suffer a reduction.

Buoyancy

In group A the specific weight of oil-exposed eggs and larvae seemed to be reduced compared to the control group (Fig.8). However, the difference was not significant for the least exposed group. In group B no clear differences were found.

DISCUSSION

The oil induced a growth reduction in all exposed larval groups except at lowest concentration in group A. This low concentration gave significant reduction in group B larvae, and may indicate individual differences between larval groups in response to oil.

As also registered for crude oil (Solberg et al. 1980a) the data indicates a concentration dependant reduction. The present experiments were performed using the same experimental procedure and biotest exposure system as described in Solberg et al. (1982a) during experiments with crude oil. A comparison of the results indicates that the heavy fraction used here has a more potent growth reducing effect than the WSF of crude oil. This can be

seen from the slope of the regressionlines in fig.3 (0.03 - 0.05 %red/ppb) compared to the corresponding value (0.02 %red./ppb) for crude oil (Solberg et al. 1982).

In the present experiments the larval feeding incidence was also lowered at the lowest oil concentrations. In larvae exposed to crude oil, the same nominal concentrations gave no reductions in feeding incidence (Tilseth et al. 1981, Solberg et al. 1982). These results further support the impression that the heavy oil fraction is more toxic than whole crude oil when administered at equal concentrations.

The present results coincide with the reports of Anderson et al. (1974) and Falk-Pedersen (1979), who found higher toxicity in refined oil products such as fuel oils, kerosene and residue than in crude oils.

The oxygen consumption, which was measured only for group B, gave no clear differences between test and control groups except for one day: day 6 after hatching. The general shapes of the curves are fairly similar to the ones found for crude oil exposed larvae (Solberg et al. 1982) which indicated a lowered consumption between day 6 and 8 after hatching. This period corresponds to the time of highest feeding activity (Ellertsen et al. 1980). The registered differences in oxygen consumption therefore might be real, indicating a lowered activity along with the reduced feeding ability.

The differences in buoyancy which were registered between test and control in group A at hatching, were rather small, well within the natural differences registered between groups from different females (unpublished data). The registered effects therefore might be negligible.

The present results clearly indicate toxic effects of the WSF of the heavy fraction of Ekofisk crude oil, and that the toxicity is higher than in the WSF of crude oil at equal nominal concentrations. However, this does not

necessarily imply that the potential harm of oil, spilled during an accident, will increase as the monoaromatics evaporate. The higher toxicity of the remaining, less volatile components, will probably be more than outbalanced by their lower solubility.

ACKNOWLEDGMENTS

We wish to thank Mr. Per Albrigtsen and Mr. Bernt Henning Vagstad at the Rafinor oil refinery for providing the oil necessary to conduct the experiments. We also wish to thank professor Hans-Jørgen Fyhn at the Zoo. Lab. Univ. of Bergen for advice and assistance with necessary laboratory equipment during the experiments. The research work has been supported in part by The Norwegian Marine Pollution Research and Monitoring Programme.

REFERENCES

- ANDERSEN, J.W., NEFF, J.M., COX, B.A., TATEM, H.E., and HIGHTOWER, G.H. 1974. Characteristics of dispersions and watersoluble extracts of crude oil and refined oils and their toxicity to estuarine crustaceans and fish. Mar.Biol. 27: 75-88.
- COOMBS, S.H. 1981. A density-gradient column for determining the specific gravity of fish eggs, with particular reference to eggs of the mackerell Scomber scombrus. Mar.Biol. 63: 101-106.
- ELLERTSEN, B., SOLEMDAL, P., STRØMME, T., TILSETH, S., WESTGÅRD, T., and ØYESTAD, V. 1980. Some biological aspects of cod larvae (Gadus morhua L.). Fisk.Dir.Skr.Ser.Hav Unders., 17: 29-47.
- FALK-PETERSON, J.B. 1979. Toxic effects of aqueous extracts of Ekofisk crude oil, crude oil fractions, and commercial oilproducts on the development. Sarsia 64(3): 161-169.

SCHEFLER, W.C. 1969. Statistics for the biological sciences.
Addison-Wesley Publ. Comp. Reading Man. U.S.

SOLBERG, T., TILSETH, S., MANGOR-JENSEN, A., SERIGSTAD, B., and
WESTRHEIM, K., 1982a. Effects of low levels of Ekofisk
crude oil on eggs and yolksac larvae of cod (Gadus
morhua L.). ICES C.M. 1982/E:60 14pp. (Mimeo).

TILSETH, S., SOLBERG, T., WESTRHEIM, K. 1981. Sublethal effects of
the water-soluble fraction of Ekofisk crude oil on
the early larval stages of cod (Gadus morhua L.).
ICES CM 1981/E:52, 17pp. (Mimeo).

FIGURES

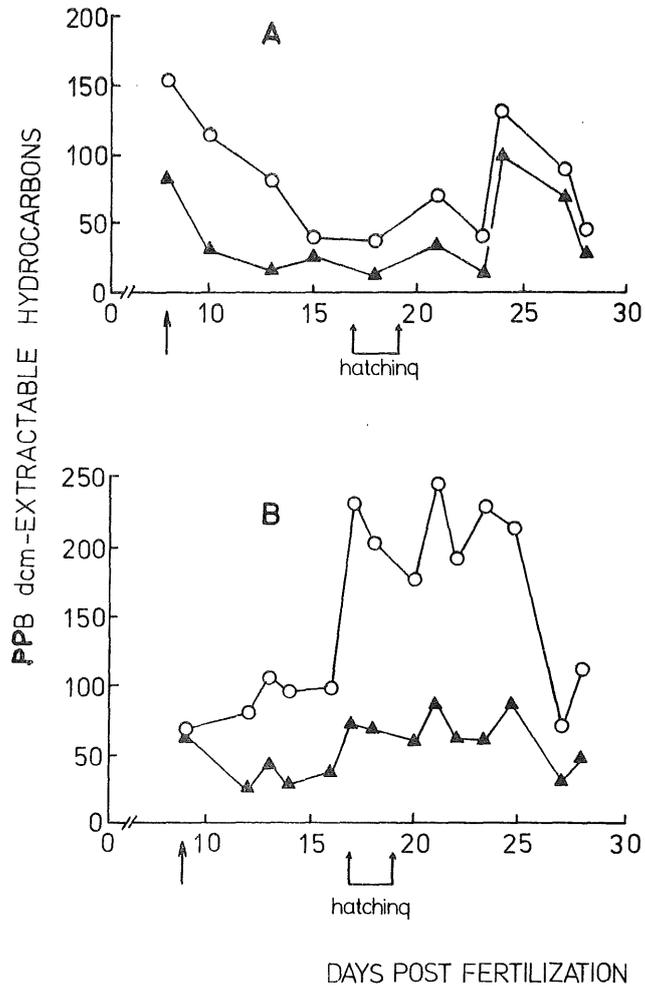


Fig. 1. Concentrations of dichlormethan (dcm) extractable hydrocarbons in aquaria of group A and B. ● - highest concentration, ▲ - lowest concentration. ↑ oil exposure started.

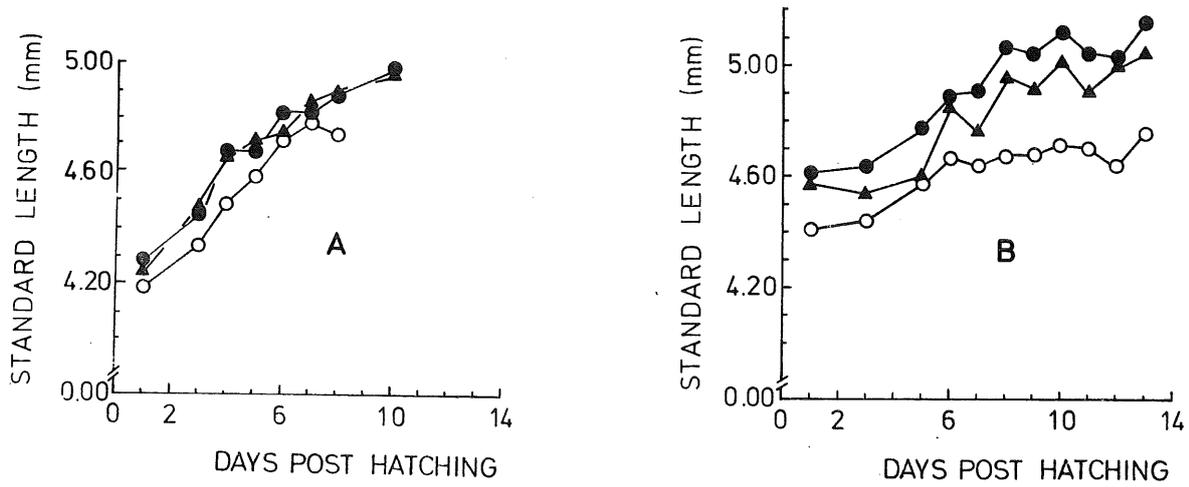


Fig. 2. Standard length of group A and B larvae. ● - control larvae, ○ - more-exposed larvae, ▲ - less-exposed larvae. N = 20-30 for each point. SD = 1-2% of the values.

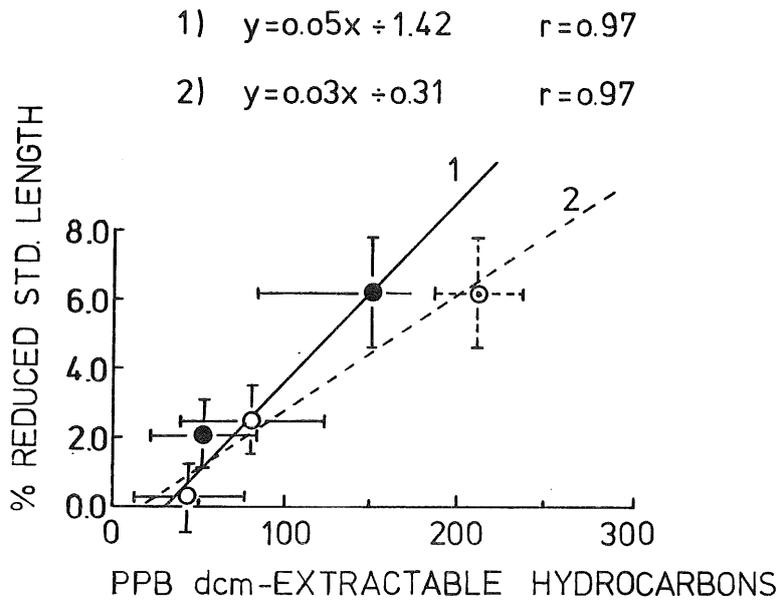


Fig. 3. % reduction in larval standard length versus oil concentration. x - group A larvae, ● - group B larvae. For explanation of regression 1) and 2) see text. ⊙ - reduction in more-exposed larvae of group B when the oil concentrations are based on the elevated values during the larval stage (fig.1.).

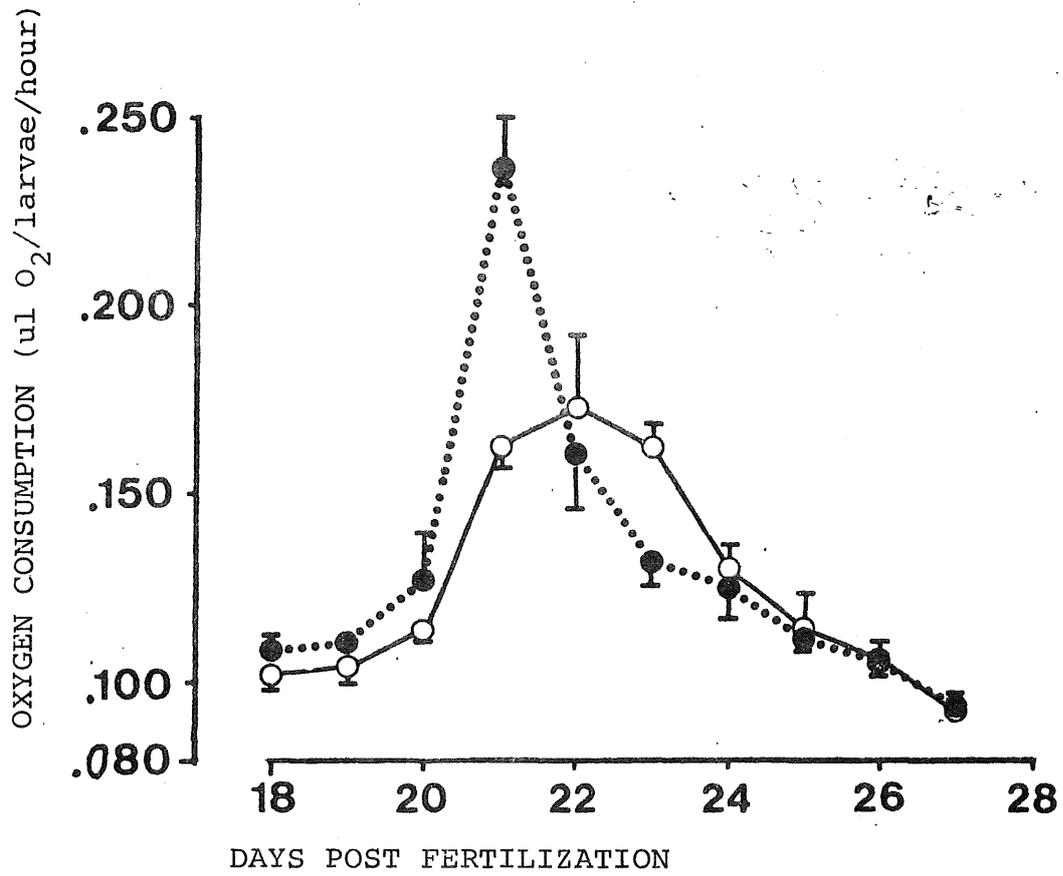


Fig. 4. Oxygen consumption rate in group B larvae.
● - control group, O - most-exposed group. N = 4 syringes for each point.

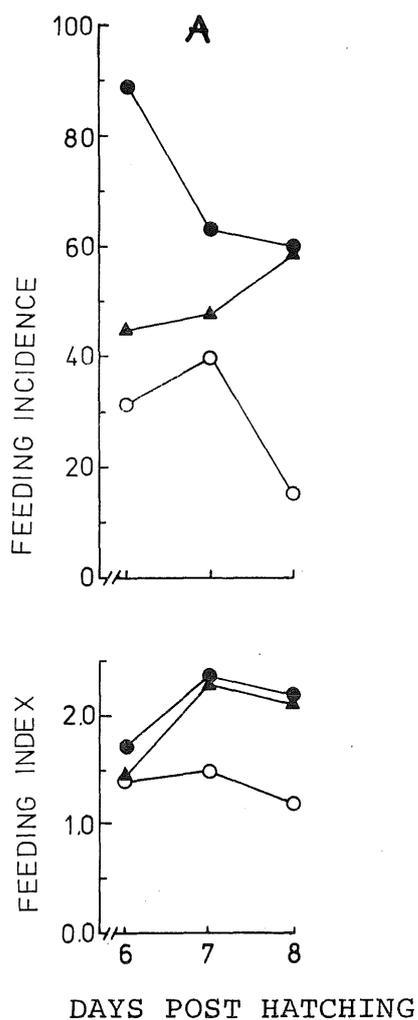


Fig. 5. Feeding incidence (% larvae with gut content) and feeding index (number of particles ingested pr. larvae with gut content) in group A larvae. ● - control larvae, ○ - more-exposed larvae, ▲ - less-exposed larvae. N = 20-30 for each point.

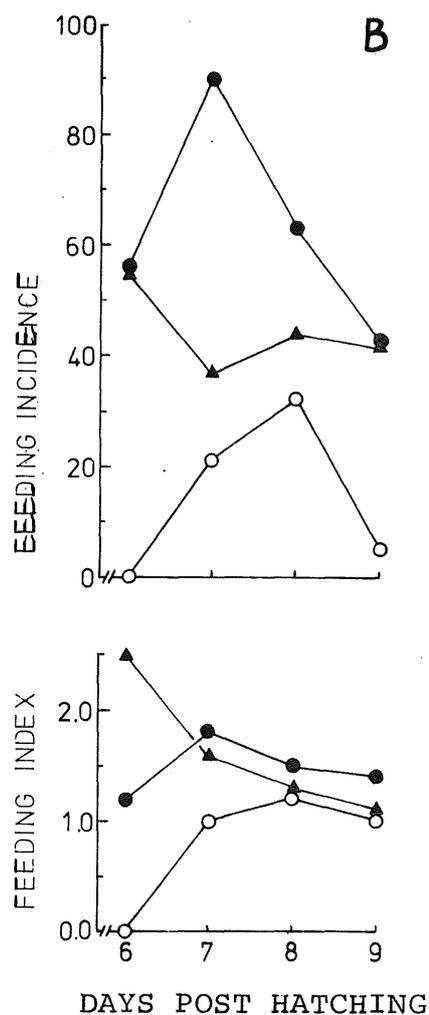


Fig. 6. Feeding incidence (% larvae with gut content) and feeding index (number of particles ingested pr. larvae with gut content) in group B larvae. ● - control larvae, ○ - more-exposed larvae, ▲ - less-exposed larvae. N = 20-30 for each point.

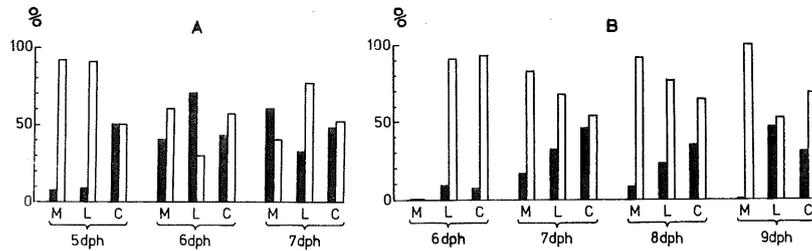


Fig. 7. Composition of gut content from group A and B larvae. C - control larvae, H - most-exposed larvae, L - less-exposed larvae. ■ copepod nauplii, □ unspecified food particles. dph - days post hatching.

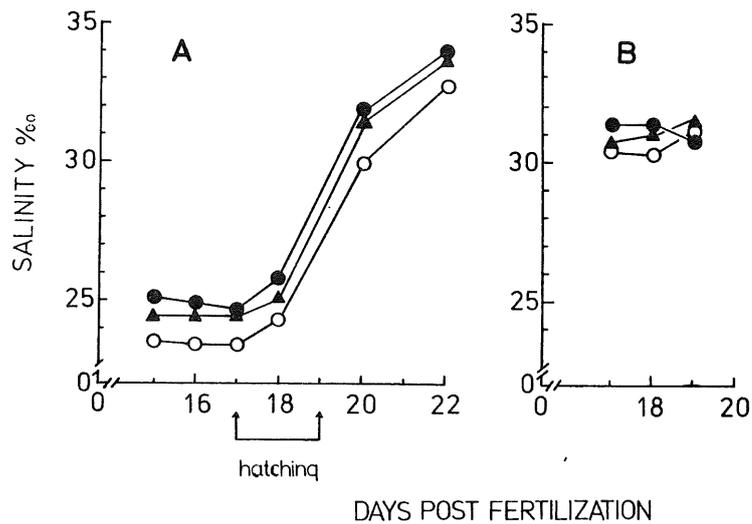


Fig. 8. Neutral buoyancy in group A eggs and larvae and group B eggs. ● - control eggs and larvae, O - most-exposed eggs and larvae, ▲ - less-exposed eggs and larvae. In group A SD = 3-6% of the values. In group B SD = 3-5% of the values. N = 30-50.

