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Combined action of DDT and DDE on early development of embryos of cod, flounder, and plaice

by

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INTRODUCTION

Information on the acute toxicity of DDT on embryos of cod, Gadus morhua; flounder, Platichthys flesus; and plaice, Pleuronectes platessa, was given by DETHLEFSEN (1974).

The present paper represents the second part of these investigations, the results of which are given in the whole by DETHLEFSEN (1975).

The aim of this survey was to investigate the toxic action of two closely related pesticides, DDE being a metabolite of DDT, to compair their toxicity and to gather information on the toxic action of combinations of the two pesticides.

In addition to that information was needed on species specific differences in the response of marine teleost embryos to pesticide-intoxication.

MATERIAL AND METHODS

Spawning fishes were caught by R.V. "Anton Dohrn" and the spawning products were obtained by stripping, fertilization was carried out in plastic containers without water.

20 min. after fertilization aliquots of egg-spermatozoa mixtures were pipetted into temperated (8°C cod and plaice, 6°C flounder) DDT and DDE-spiked glass containers with 300 ml of carefully filtered seawater.

Aeration through plastic pipette tips with CO_2-free compressed air guaranteed fairly equal bubble size and resulted in complete but soft circulation of the eggs in the containers.

Salinity (± 0.1°/oo S) oxygen (± 0.2 mg/l), pH (± 0.2) and temperatures (± 0.1°C) were controlled in regular intervals, always representing optimum conditions.

Due to low natural solubility of DDT and DDE in seawater which is 37 ppb at 25°C (BABERS, 1955) and 5.9 ppb at 2°C (BOWMAN et al., 1960) it was necessary to dissolve the pesticides in an organic solvent (Polyethyleneglycol 200) prior to addition to incubation jars.

Three replicates per concentration (controls, solvent-controls and concentrations) were used.

Test media were renewed in 24 hours intervals by pipetting the embryos into freshly prepared test solutions.

Pesticide concentrations in the test-containers were measured in parallel experiments using hexane-extraction followed by electron-capture gas-liquid-chromatography.

Incubation jars were controlled daily in order to count and remove dead and malformed embryos. Hatched larvae were sampled in 12 hours intervals and it was distinguished between viable, malformed, and dead larvae. Total length of larvae was measured after narcotization with MS 222.

RESULTS

Concentrations of pesticides in incubation jars.

Concentrations of DDT and DDE decreased in the 24 hours intervals investigated (Fig. 1). No differences in the behaviour of DDT and DDE could be detected although it is known that DDE has a somewhat higher vapour pressure than DDT (GOLDBERG, 1975; MAIER-BODE, 1964).

The concentrations of pesticides found immediately after mixing into seawater depended on the starting concentration. Due to high initial losses at high starting concentrations the recovery rates of the pesticides measurable 10 min. after mixing were high at low starting concentrations and lower at higher starting concentrations (Fig. 1).
The decrease of the concentrations of pesticides also depended on the starting concentrations.

Relatively high losses caused by adsorption on the surfaces of the incubation jars and by losses due to codistillation processes lead to rapid decreases of pesticide concentrations in low starting concentrations. Decreases found for higher starting concentrations were less rapid.

These findings are in good agreement with results of BOWMAN et al. (1959, 1960), ACRE et al. (1963) and SØDERGREN (1971).

In order to give uniform concentrations for our trials medium effective concentrations of the pesticides were estimated graphically. The correlation between starting concentrations and medium effective concentrations is given in Fig. 2.

The lower the absolute quantity of pesticide in the incubation jars the higher the relative loss and the lower was the medium effective concentration.

With increasing concentrations the relative loss was lower and the mediums effective concentration increased. When starting concentrations were higher than 0.25 ppm DDT or DDE the medium effective concentrations were found to decrease again. This finding can be explained by the fact that the pesticides are not dissolved in the seawater, they are suspended and precipitation from this suspension must be higher the higher the starting concentrations are.

In the following pesticide concentrations are always given as medium effective concentrations.

Cod, Gadus morhua

Results depicted in Fig. 3 were obtained in the period from fertilization until hatching. The hatching success in the controls was 85.5 % of the initial number of embryos. Malformation rates in controls were 1.5 - 2.2 % embryos and the percentage of viable hatch was 97.5 % of the hatched larvae.

Only 70.0 % of the embryos incubated in 0.0413 ppm DDT were able to hatch, 80 % of those were viable.

When embryos were incubated in 0.090 ppm DDT, hatching success was lowered to 50.4 %, and viable hatch to 54.4 % of the larvae.

76.8 % embryos incubated in 0.0413 ppm DDE were able to hatch and 92.0 % of the hatched larvae were viable.

From the 65.2 % hatched embryos in 0.090 ppm DDE 64.8 % were viable.

Qualitative differences in the action of the pesticides were only found in the parameter hatching time.

Corresponding to the results given in 1974 DDT caused a delay of hatching time compared to that of controls, the hatching period was prolonged from 5 days of controls to 7 to 8 days in 0.090 ppm DDT.

DDE caused an earlier hatching of the larvae, in 0.090 ppm DDT maximum hatch occurred at the second day of the hatching period, in the controls maximum hatch was found at the third day of the hatching period.

Duration of hatching period was shortened under the influence of DDE.

In 0.0095 ppm DDT plus 0.0095 ppm DDE 60.2% of the embryos were able to hatch 75.6 % of the larvae being viable but only 38.1 % hatched in 0.090 ppm DDT plus 0.090 ppm DDE, 12.2 % were viable.

In order to facilitate comparison of the toxic action of the pesticides applied separate or in combination the correlation between concentrations of pesticides and hatching-success and malformation rates is given in Fig. 4.

From this figure it can be seen that DDE is less toxic than DDT applied in the same concentrations (approx. 40 % less).

Combinations of both pesticides are more toxic than separately applied pesticides in comparable concentrations.

This finding is confirmed by most of the other parameters tested i.e. rates of malformed embryos, daily coarse of mortalities of embryos etc.

Flounder, Platichthys flesus

Results of the experiments on flounder embryos are depicted in Fig. 5.

Again the period from fertilization until hatching is covered. No major differences to the findings of the cod experiments were noted.

The percentages of postmature embryos, which were unable to hatch and stayed in their egg membranes until the end of hatching was not correlated to the concentrations applied.

Hatching rates in the controls were near to 90 % of the embryos, 90 % of the hatched larvae were viable.

In the highest concentration of DDT (0.090 ppm) only 63.7 % of the embryos were able to hatch, in the same concentration of DDE hatching success was 73.2 %. The same differences were found in the proportion of viable larvae.

Rates of malformed embryos and malformed larvae were relatively high thus indicating a somewhat greater susceptibility compared to that of cod embryos.

Again DDE was less effective than DDT applied in the same concentration, and effects caused by combinations of the pesticides were higher than additive (Fig. 6).

A remarcable finding was the influence of the pesticides on the length of larvae during the time of maximum hatch (table 1).

Larvae reared in DDT were shorter with increasing concentrations, larvae reared in DDE and in combinations of DDT and DDE were up to 0.2 mm longer than controllarvae.

Plaice, Pleuronectes platessa

Hatching rates in the controls were 85.0 %, and the percentage of viable larvae was very high 93.6 % (Fig. 7).
Hatching success was reduced by 0.0413 ppm DDT and DDE to 62 % (DDT) and 72.3 % (DDE).
Malformation rates of hatched larvae were very low, in no concentration exceeding 3.5 % and corresponding to that the percentages of viable larvae were higher than in the preceeding experiments. This finding is indicating that embryos of plaice were less susceptible than those of cod and flounder.

DDE was again less effective than DDT (Fig. 8), but in this experiment the effects of combinations of the pesticides did not differ from the expected additive effects.

CONCLUSIONS

Variations of results in the 3 replicates were 2 - 3 % (variation coeffecients) in low concentrations (0.0095) and controls and near to 5.0 % in higher concentrations (0.090 ppm).

The solvent (Polyethyleneglycol 200) did not have any significant influence on hatching rates and viable hatch.

There was no significant linear correlation between hatching success and numbers of embryos in the incubation jars at the different concentrations tested.

The results on the effects of DDT on embryogenesis of the species tested are in good agreement with those given earlier (DETHLEFSEN 1974,1975).

In most of the parameters tested the effects caused by both pesticides were very similar, and with very few exceptions DDE was less effective than DDT.

This finding is not unexpected when comparing the toxicity data which are available from the literature (MUIRHEAD-THOMSON, 1971; HALSTEAD, 1970; JOHNSON, 1968).

Embryos of the species tested showed differences in the response to the exposition of the pesticides.

Flounder turned out to be the most susceptible species followed by cod and plaice, but differences in the susceptibility of cod and plaice were not very marked.

This finding correlates with the size of the eggs and the thickness of chorion of the three species, eggs of flounder being smallest (Thickness of chorion 1.8 µ, LØNNING and SOLEMDAL, 1971), and those of plaice having a thicker chorion (POMMERANZ, 1972). The chorion seems to protect embryos from the pesticide impact, because embryos were less susceptible than newly hatched larvae.

WESTERMHAGEN and DETHLEFSEN (1975); WESTERNHAGEN et al. (1974) and WESTERNHAGEN et al. (1975) had similar results when investigating the influence of cadmium on embryos of marine teleosts.

The interpretation of qualitive differences of the effects of DDT and DDE on hatching time, duration of hatching period and total length of larvae at hatching may be facilitated by the following model (Fig. 9).
If one assumest hat hatching of larvae of marine teleosts is initiated by a deficiancy of oxygen demand of embryos and oxygen pressure of the ambient water (neuro-humoral-reflex, HAMDORF,1961) one could expect that due to individual tolerances (I in Fig. 9) some embryos are hatching earlier than others.

These embryos are smaller than those hatching later (ALDERDICE and FORRESTER, 1968, 1971 a, b; ROSENTHAL, 1971).
The increase of the intensity of metablosm of normal embryos (A 1 in Fig. 9) as measured by HOLLIDAY et al. (1964); STELZER et al. (1971) and BRAUM (1973) is proportional to the biomass of embryonic material ($A_1 \triangleq A_2$ in Fig. 9).

If the metabolism of embryos is affected by DDT it is probable that they grow slower and hatch later (B_1) than controllarvae.

Major physiological effect of DDT is depression of ATPase activity (MEHRLE et al., 1971; CUTKOMP et al., 1971), the resynthesis of ATP from ADP represents one of the most important energy producing processes in metabolism.

If the oxygen demand of these embryos is intensified in relation to their size (B_2) then they would be smaller at the time of hatching. Duration of the hatching period would be prolonged.

If exposed embryos grow faster than controls they would hatch earlier (C_1) and if their metabolism is depressed in relation to their biomass, like possibly under the influence of DDE, than they would be longer at the time of hatching.
The duration of the hatching period would than be shortened.

This model is rather hypothetic because no information is available on the influence of pesticides on the intensity of metabolism of embryos of marine teleosts.

SUMMARY

Artificially fertilized embryos of cod (Gadus morhua), flounder (Platichthys flesus) and plaice (Pleuronectes platessa) were exposed to different concentrations of DDT, DDE and combinations of DDT and DDE.

DDT affected the hatching success, the percentages of malformed embryos and larvae, the viable hatch, the time of hatching and the duration of the hatching period.

In most of the parameters tested DDE was less effective than DDT applied in the same concentration.

Larvae reared in DDT were smaller than controls at the time of hatching and the duration of the hatching period was prolonged.

When larvae were reared in DDE their total length at the time of maximum hatch was up to 0.2 mm longer than that of control larvae, the hatching period was compressed.

Combinations of the two pesticides gave higher than the expected additive effects.

Embryos of flounder turned out to be the most susceptible followed by those of cod and plaice.

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Table 1: Platichthys flesus, total length of larvae at the time of maximum hatch, larvae were reared in different concentrations of DDT, DDE and combinations of DDT and DDE.

$\bar{x}$ = mean, Sx = standard deviation,

$S\bar{x}$ = error of the mean.

concentration	$\bar{x}$ Sx	$S\bar{x}$	n
K	2.49 ± 0.28	0.036	60
K + PÄG	2.50 ± 0.11	0.014	60
0.0095	2.49 ± 0.13	0.020	40
DDT 0.0413	2.48 ± 0.12	0.016	60
0.090	2.42 ± 0.13	0.017	60
0.0095	2.58 ± 0.14	0.018	60
DDT 0.0413	2.60 ± 0.11	0.014	60
0.090	2.59 ± 0.11	0.014	60
DDT 0.0095	2.62 ± 0.12	0.016	60
+ 0.0413	2.57 ± 0.12	0.025	23
DDE 0.090	2.69 ± 0.12	0.016	60

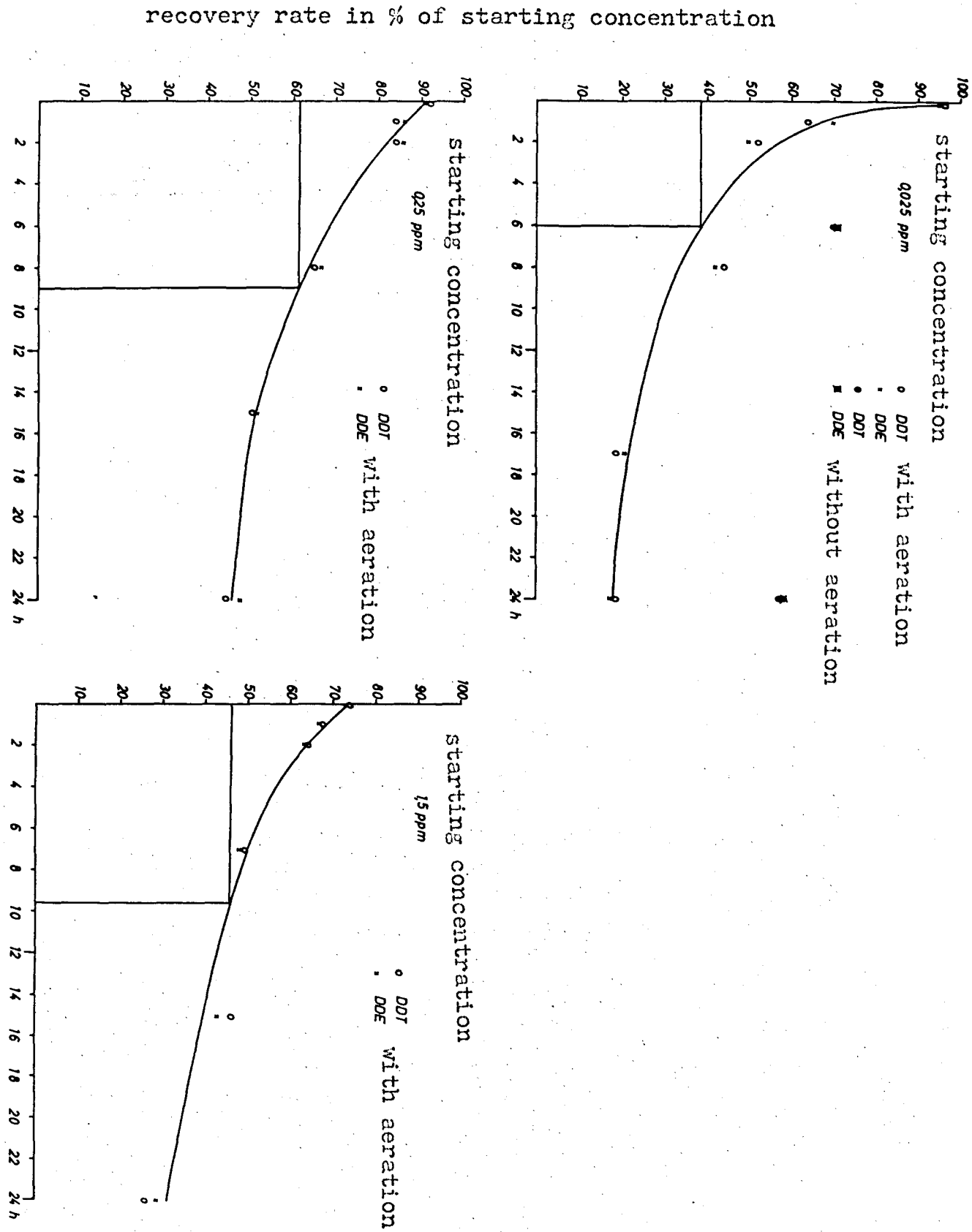

Fig. 1: Concentrations of DDT and DDE in incubation jars (% of initial concentration) in 24 hours, with and without aeration, 300 ccm filtered seawater, 35.3 °/oo S, pH 8, 2, 8°C, measured by gas-liquid chromatography

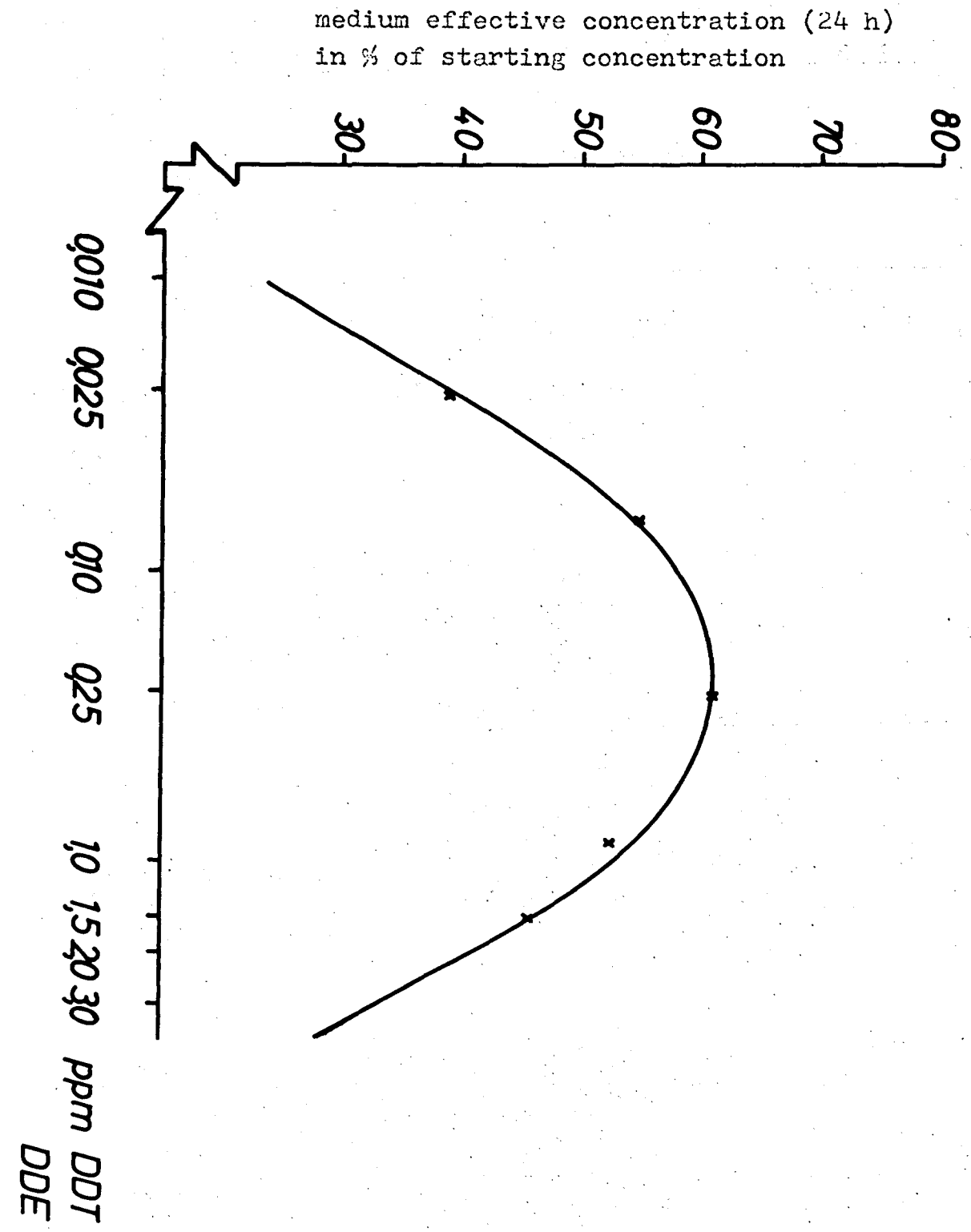

Fig. 2: Correlation between initial concentration of DDT and DDE and the medium effective concentration in % initital concentration

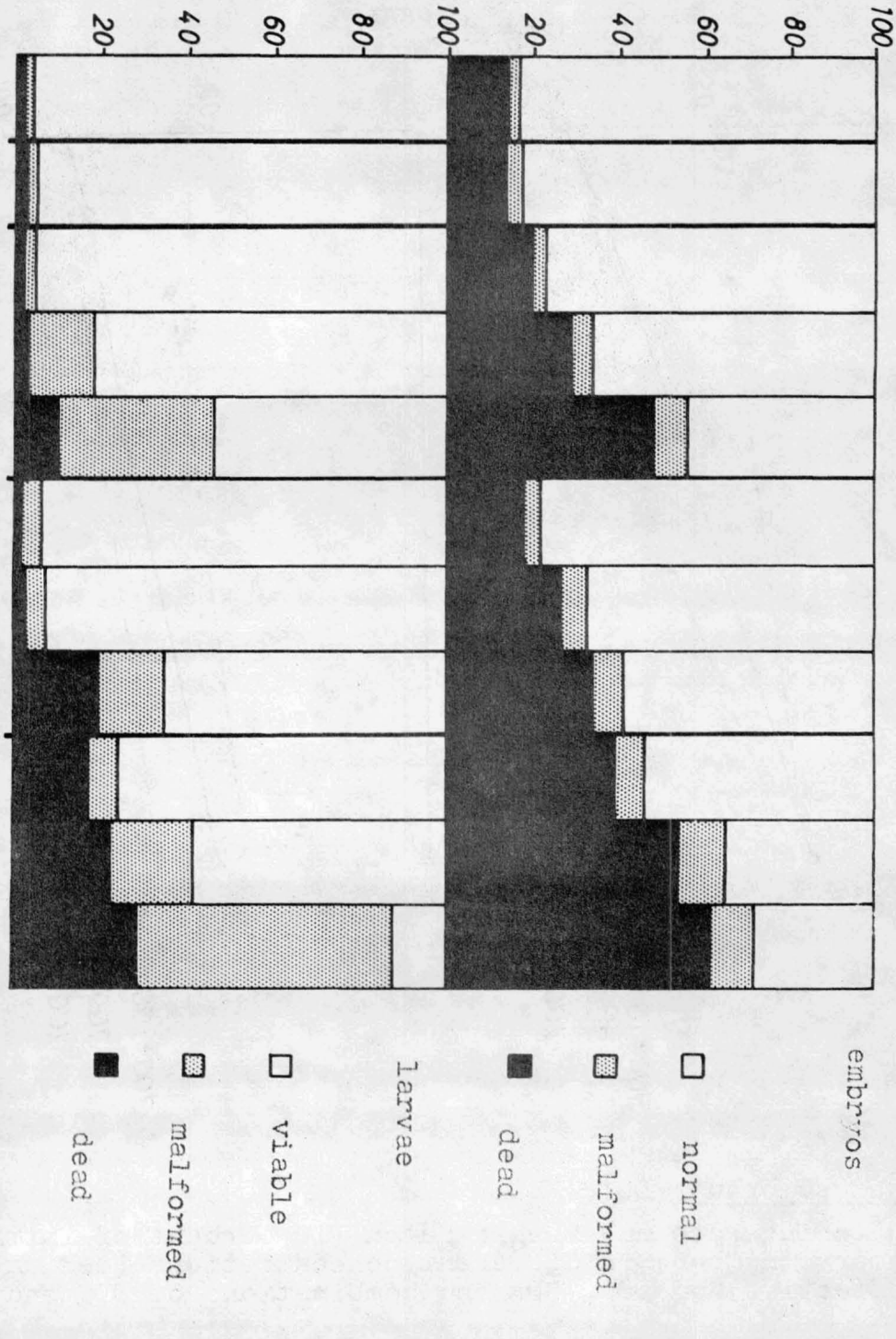

Fig. 3: Gadus morhua, DDT + DDE

Percentages of normal, malformed, dead and surviving embryos, and malformed, dead and viable hatched larvae; reared in different concentrations of DDT, DDE and combination of DDT and DDE, given as medium effective concentrations

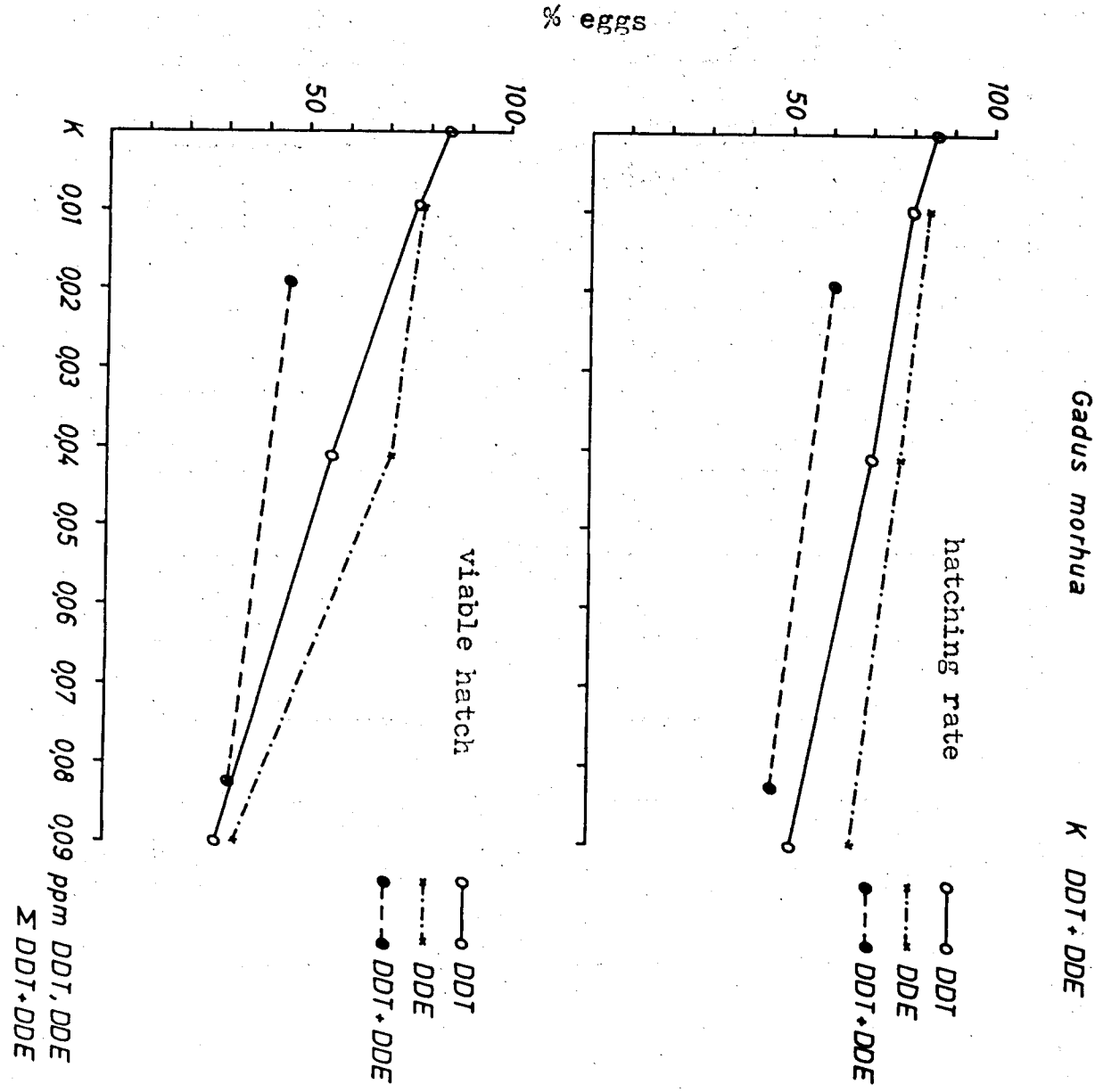

Fig. 4: Gadus morhua, DDT + DDE
Hatching rate and viable hatch (in percent of initial embryo number) in different concentrations (medium effective) of DDT, DDE and combinations of DDT and DDE

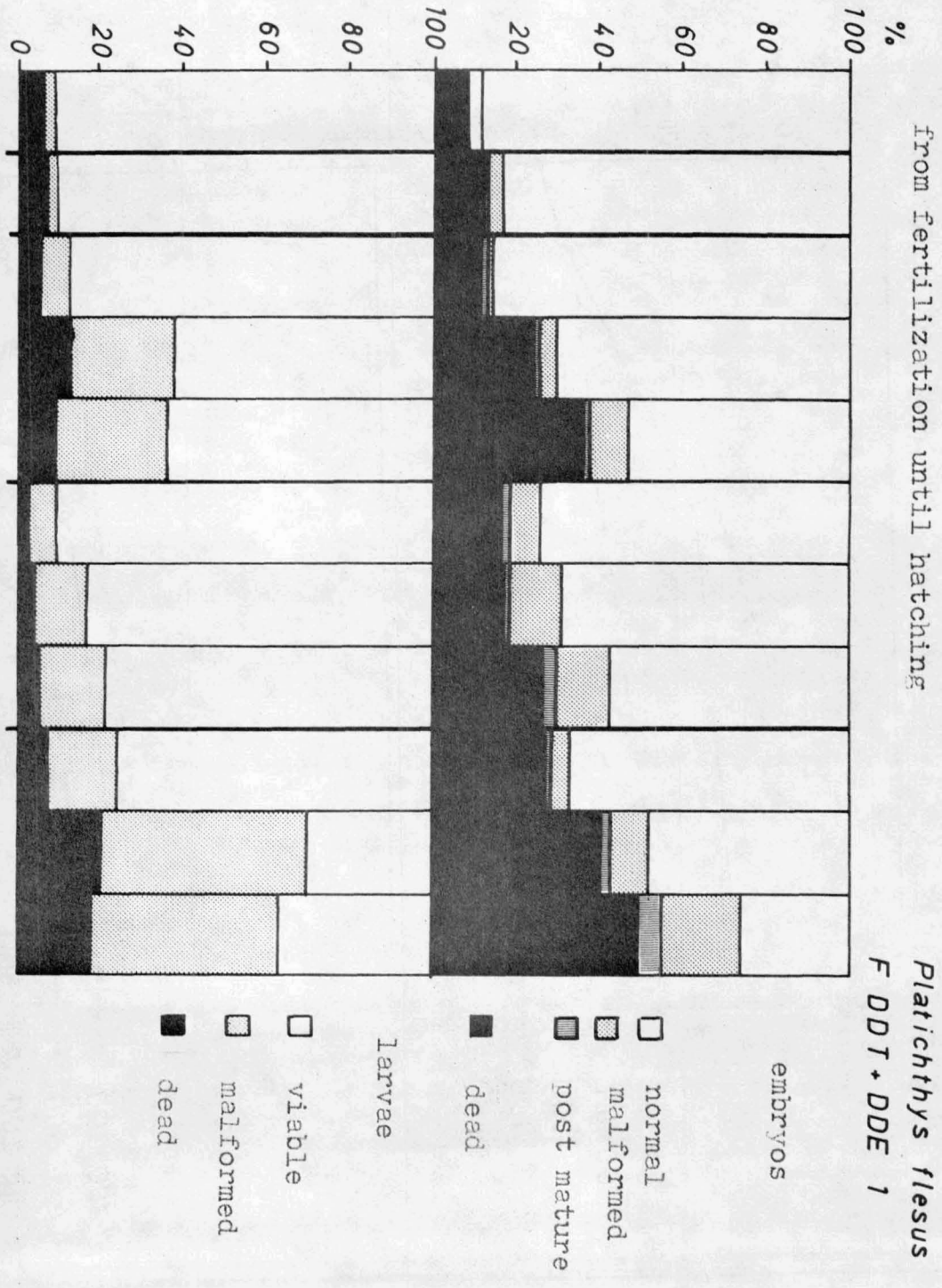

Fig. 5: Platichthys flesus, DDT + DDE

Percentages of normal, malformed, dead and surviving embryos, and malformed, dead and viable hatched larvae; reared in different concentrations of DDT, DDE and combination of DDT and DDE, given as medium effective concentrations

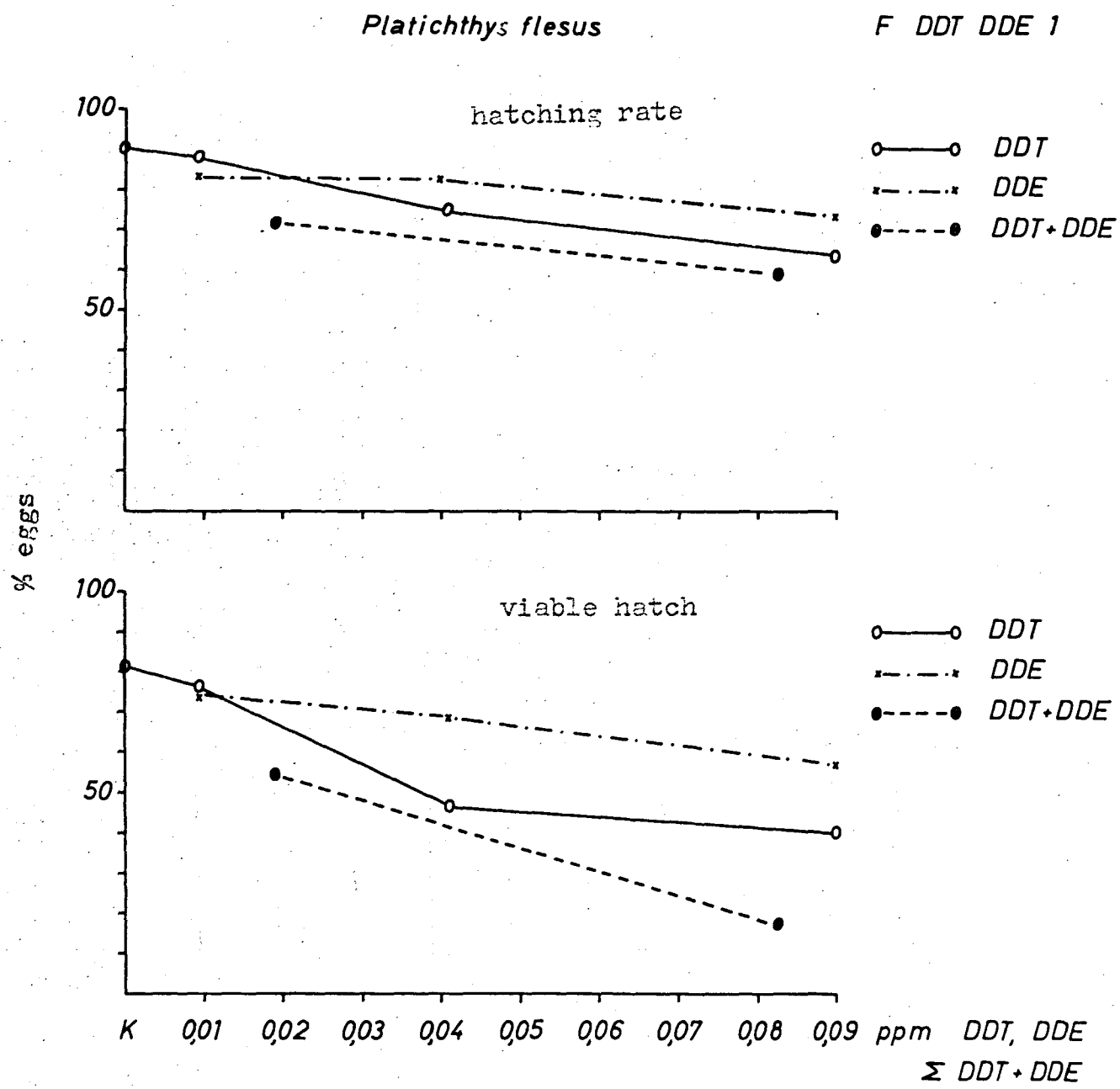

Fig. 6: Platichthys flesus, DDT + DDE
Hatching rate and viable hatch (in percent of initial embryo number) in different concentrations (medium effective) of DDT, DDE and combinations of DDT and DDE

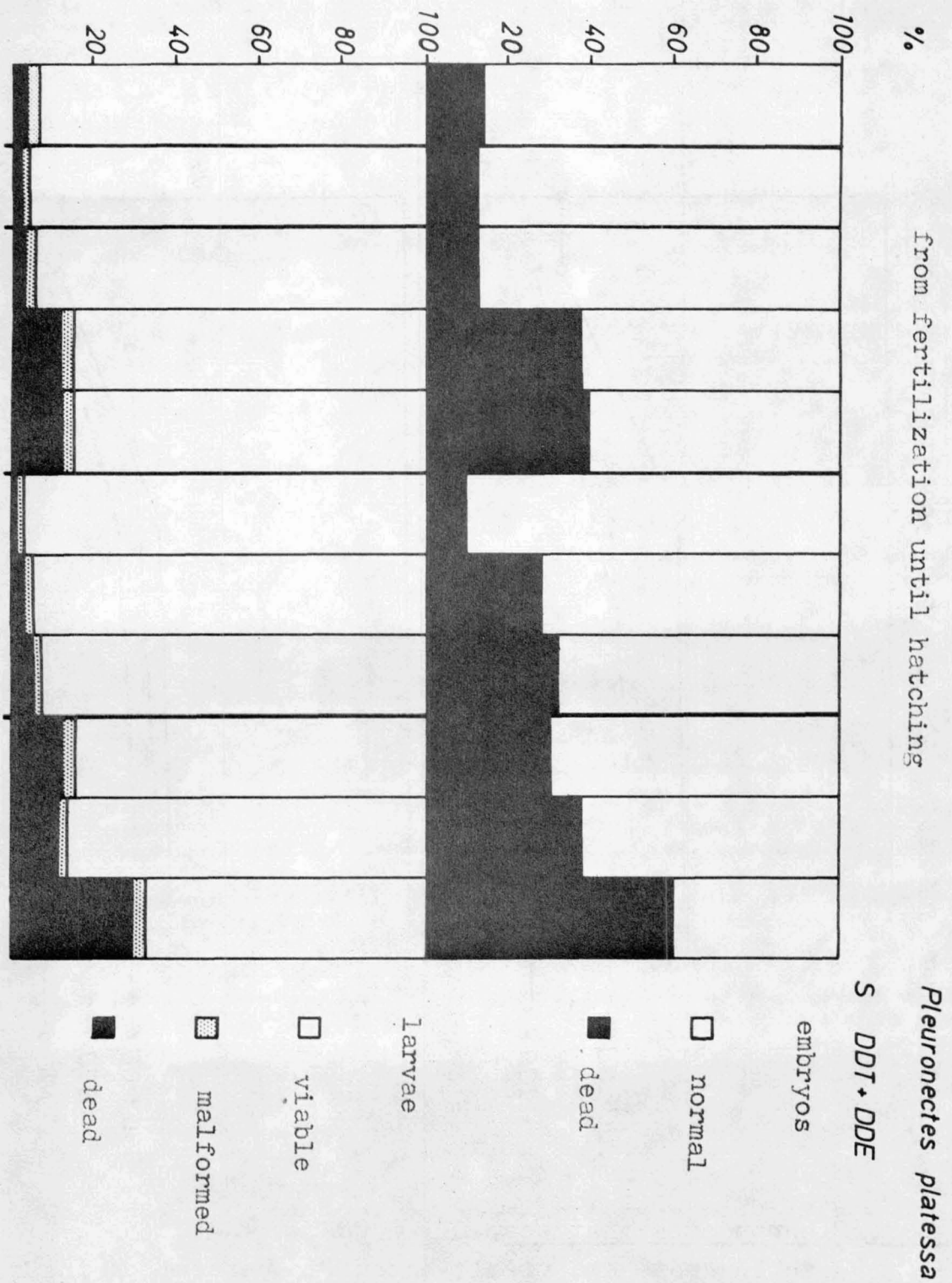

Fig. 7: Pleuronectes platessa, DDT + DDE

Percentages of normal, malformed, dead and surviving embryos, and malformed, dead and viable hatched larvae; reared in different concentrations of DDT, DDE and combination of DDT and DDE, given as medium effective concentrations

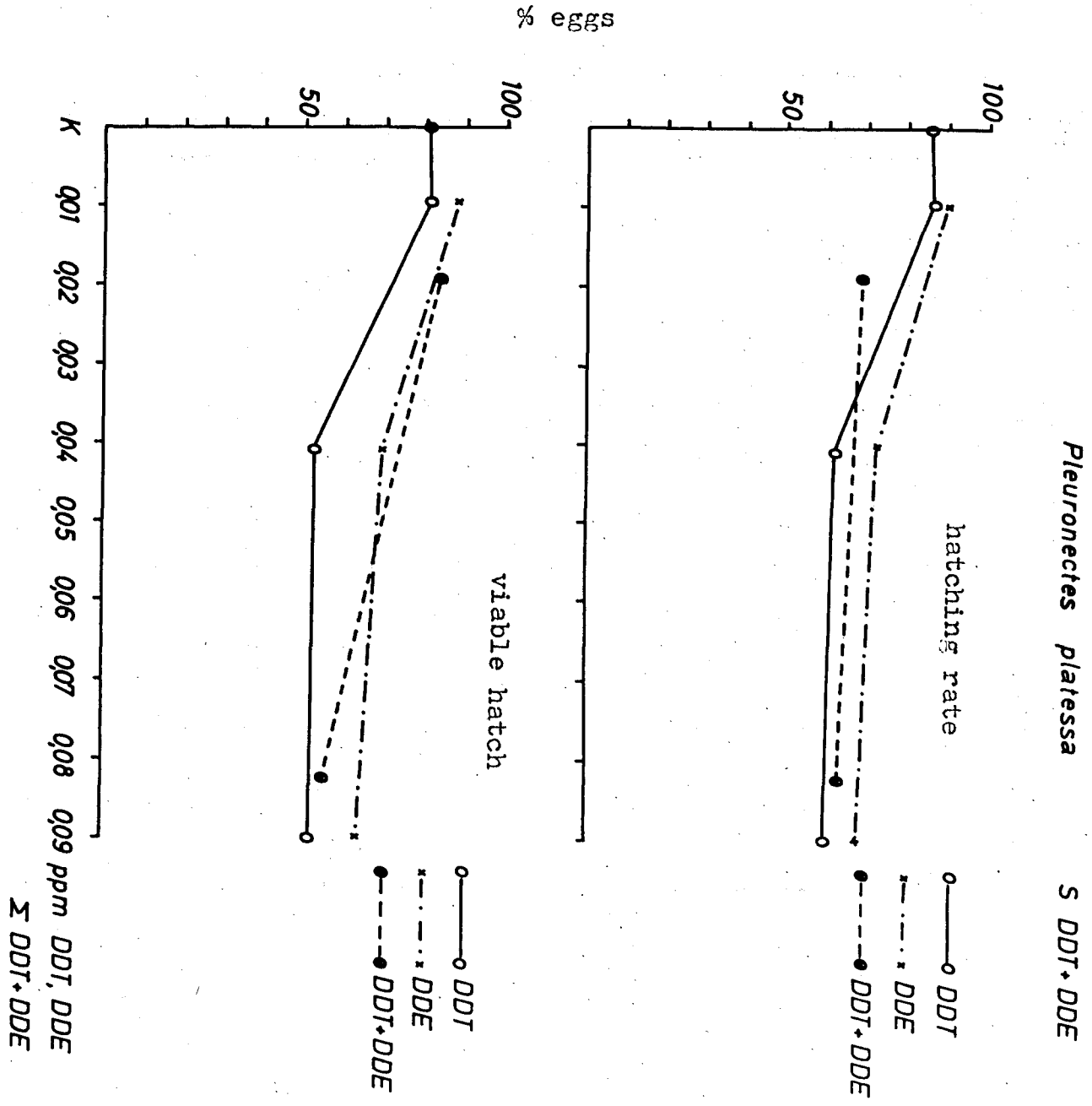

Fig. 8: Pleuronectes platessa, DDT + DDE

Hatching rate and viable hatch (in percent of initial embryo number) in different concentrations (medium effective) of DDT, DDE and combinations of DDT and DDE

Fig. 9: Model on the relations between intensity of metabolism, total length of larvae at hatching time, and oxygen demand of normal and intoxicated embryos.
i = individual tolerances of metabolism. Duration of hatching period with normal growth of embryos (hn) with retarded growth (hr) and faster growth (hf)