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Cadmium uptake by marine fish larvae[1)]

by

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INTRODUCTION

Cadmium as a potentially dangerous heavy metal in river sediments (KOBAYASHI, 1971; BANAT *et al.*, 1972), estuaries (EUSTACE, 1974) and near-shore waters (ABDULLAH *et al.*, 1972; BUTTERWORTH *et al.*, 1972; ABDULLAH and ROYLE, 1974) has recently caused considerable efforts to investigate the toxicity of this metal and its accumulation by aquatic organisms. Numerous experiments have proved the acute and chronic toxicity of cadmium to annelids (BROWN and AHSANULLAH, 1971), molluscs (CALABRESE *et al.*, 1973), crustaceans (O'HARA, 1973; JONES, 1975) and fish eggs (ROSENTHAL and SPERLING, 1974; WESTERNHAGEN *et al.*, 1974, 1975; WESTERNHAGEN and DETHLEFSEN, 1975).

High cadmium levels in the environment are often reflected in the accumulation of this metal by organisms like *Patella vulgata* (PEDEN *et al.*, 1973) *Littorina* sp. (LEATHERLAND and BURTON, 1974), *Nucella* sp. (STENNER and NICKLESS, 1974), and *Crassostrea gigas* (RATKOWSKY *et al.*, 1973). In laboratory experiments ROSENTHAL and SPERLING (1974), WESTERNHAGEN *et al.* (1974), and WESTERNHAGEN and DETHLEFSEN (1975) were able to show that fish

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eggs incubated in cadmium polluted water accumulated this metal up to a factor of 40. Highest accumulation factors were reached shortly after exposure. During the course of incubation cadmium contents of eggs decreased continuously.

Reports on the accumulation of cadmium by fishes are not consistent. Investigations conducted by BROOKS and RUMSEY (1974) and HARDISTY et al. (1974) indicate that some fishes do, at least in kidney and liver, accumulate cadmium, while PORTMANN (1972), TOPPING (1973), WINDOM et al. (1973) and EUSTACE (1974) were not able to confirm accumulation of cadmium by teleosts to higher than usual concentrations.

Although for reproductive success fish larvae are an important and sensiti stage in the life cycle of fishes (SPRAGUE, 1971) there are no data available on the accumulation of cadmium by fish larvae.

This study tries to shed some light on the effects of the toxicant cadmium on newly hatched larvae and postlarvae of inshore fishes and to find out whether there do exist species specific differences in the accumulation of this metal as shown for the eggs of these species by WESTERNHAGEN et al., 1974.

MATERIAL AND METHODS

Larvae of three common inshore teleosts of the Baltic Sea, herring (*Clupea harengus* L.), flounder (*Platichthys flesus* L.) and garpike (*Belone belone*) were subjected to cadmium contaminated water.

Clupea harengus

Eggs of Baltic spring spawning herring from Travemünde (Germany) were artificially inseminated and incubated in clean and cadmium contaminated water (0.1, 0.5, 1.0, 5,0 ppm Cd) of 15.7, 25 and 32 $^{o}/oo$ S at 10°C. Newly hatched larvae of all trials were removed and stored (dried at 80°C) for later cadmium determination. For further experiments newly hatched control larvae of the 15.7 $^{o}/oo$ trial were transferred into 1000 ml jars (1oo specimens/jar) containing clean and cadmium contaminated seawater of 15.8 $^{o}/oo$ S. Cadmium concentrations employed were: 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0 and 4.0 ppm. Water was exchanged every second day. The jars were not aerated. The larvae were not fed throughout the entire experiment.

Platichthys flesus

Eggs of live Baltic flounder of the Fehmarn Belt were artificially inseminated in uncontaminated sea water of 16, 25, 32 and 42 $^{o}/oo$ S at 5^{o}C, and incubated in 800 ml jars containing seawater and test solutions of 0.1, 0.5, 1.0, 2.0, 3.0 and 5.0 ppm cadmium. The larvae were kept at 5^{o}C without aeration and food for two weeks.

Belone belone

Eggs of freshly caught Baltic garpike from Travemünde (Germany) were artificially inseminated in seawater (15, 25 and 35 $^{o}/oo$) at 14.7^{o}C. The eggs then were incubated at 15^{o}C in 800 ml jars containing uncontaminated seawater of the above salinities and test solutions of 0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 ppm Cd^{++} using $CdCl_2$ as toxicant. Newly hatched larvae were transferred into uncontaminated seawater were they were fed and reared until they reached 60 mm in length. Other newly hatched larvae were kept in 50 l basins containing natural and contaminated (0.05 ppm Cd) seawater of 32 $^{o}/oo$ S and 23^{o}C and reared for 34 days. Water in the two basins was exchanged every 5 days.

Cadmium determination in all experiments was accomplished by means of flameless atomic absorption spectophotometry using a Perkin Elmer Type 300 equipped with an electrodeless discharge lamp. For determination of cadmium water samples and larval samples were treated as described by WESTERNHAGEN and DETHLEFSEN (1975).

RESULTS

In Fig. 1 cadmium concentrations found in newly hatched larvae are depicted for all three species. Concentrations found in garpike larvae were of two orders of magnitude lower than those for flounder and herring larvae incubated at the same contamination levels. Maximum values at 5.0 ppm in garpike larvae only reached 0.018 ng Cd/0.1 mg larval dry weight, while flounder and herring larvae at 5.0 ppm contained 1.9 and 2.3 ng Cd/0.1 mg dry weight.

Fig. 2 presents cadmium uptake of newly hatched herring larvae incubated in uncontaminated water and subjected to different cadmium concentrations after hatching. From 0.01 to 0.1 ppm uptake over the 8-day period did not exceed values derived from the control trials. It was only at ambient cadmium concentrations of 0.5 ppm and more that metal concentrations in larvae showed clear differences to controls, at 4.0 ppm reaching 15 times the concentrations (48 ng Cd/0.1 mg dry weight) recorded from control individuals during the study period. Larval survival during the 8-day period was lowest with 57 % in the 4.0 ppm trial increasing over 64 % (3.0 ppm), 72 % (2.0 ppm) and 73 % (1.0 ppm) to 84 % in the controls.

Fig. 3 shows cadmium uptake of newly hatched flounder larvae incubated in cadmium contaminated water and exposed to different cadmium concentrations immediately after hatching. Concentrations of 0.5 ppm and higher caused accumulation of cadmium with exposure time. The same trend is noticeable in Fig. 4, where mean values (ng Cd/0.1 mg larval dry weight) for the combined data derived from 16, 25 and 32 $^{o}/oo$ S are presented. There was a marked tendency only in the higher cadmium concentrations of 1.0 and 5.0 ppm towards increasing larval cadmium contamination with exposure time. Also garpike larvae and juveniles displayed accumulation of cadmium during a prolonged period of exposure to 0.05 ppm cadmium. After 34 days the differences in mean cadmium contents of control and continuously exposed specimens (final total length, 60 mm) were highly significant (control: $\bar{x}$ = 0.00260 ng Cd/0.2 mg dry wt; 0.05:$\bar{x}$ = 0.00971 ng Cd/0.1 mg dry wt) $t_{0.05}$= 3.82).

We were not able to prove any detrimental after effects of cadmium on garpike larvae incubated in concentrations of up to 2.0 ppm. After transfer to unpolluted water newly hatched larvae of contamination levels from 0.05 to 2.0 ppm cadmium fed and grew over a period of more than 30 days, when the experiment was terminated. Initial body curvature of larvae hatched at 2.0 ppm disappeared after about 10 days rearing and the young garpikes at the end of the experiment attained a length between 55 and 62 mm. All of the specimens appeared normal and in good condition. Most probably the recovery of the initially bent larvae could be contributed to the high vitality of Belone larvae, since a cadmium clearance would take longer, as described by GREIG et al. (1974) who showed that cadmium contaminated Tautogolabrus adspersus after reared in clean seawater for one month did not exhibit substantial reductions in cadmium residues of liver and flesh.

DISCUSSION

Values of cadmium contamination of newly hatched larvae of flounder and herring incubated in polluted water (Fig. 1) range from 0.7 to > 2.3 ng Cd/0.1 mg dry wt and are in good agreement with the data given by ROSENTHAL and SPERLING (1974) who found about 1.7 ng/newly hatched herring larva incubated at 5.0 ppm Cd. Although the egg membrane as described by ROSENTHAL and SPERLING (1974) and WESTERNHAGEN et al. (1974) acts as a "protecting barrier" that shields the developing embryo against the toxic effects of cadmium, measurable amounts of this metal do penetrate the chorion, which is reflected by the degree of contamination of newly hatched larvae. With increasing cadmium concentration of the incubating medium the cadmium contents of larvae rose. Larvae incubated at 5.0 ppm contained about thrice the amounts of the metal found in control specimens. Why contamination levels of newly hatched garpike larvae were of two orders of magnitude lover than found in herring and flounder larvae is not clear. There might be the possibility that the chorion of Belone eggs is especially efficient in retaining cadmium. We are as jet not certain, but an indication for this assumption is the fact that in the high contamination levels (2.0 - 5.0 ppm) a yellow precipitate developed on the eggs' surface. This was especially evident where the adhesive filaments had been torn off the chorion surface and yellow flakes (possibly CdS?) could be scraped off. Analysis of these flakes showed that they were extremely rich in cadmium.

The experiment show that fish larvae exposed to cadmium contaminated water have reactions differing from those displayed by developing eggs of the same species. While initial cadmium concentrations reached by eggs during the first 24 hrs would usually decrease during incubation, cadmium content of exposed larvae increased with time. Herring larvae exposed to 4.0 ppm for 8 days accumulated up to 48 ng Cd/0.1 mg dry wt (Fig. 2). This was twice the amount of the maximum contamination level of eggs exposed to 5.0 ppm. Flounder larvae with an initial contamination level of about 1.0 ng Cd/

0.1 mg dry wt accumulated up to 7.0 ng Cd/0.1 mg dry wt after 10 to 14 days exposure to 2.0 ppm cadmium (Fig. 3 and 4), which slightly surpasses the maximum concentrations reached by eggs under the same conditions (WESTERNHAGEN and DETHLEFSEN, 1975). The mechanisms with wich the binding of the metal to the larvae is accomplished appear to be different from those assumed to function in the reaction of egg surfaces with cadmium. In the case of metal binding to fish eggs ROSENTHAL and SPERLING (1974) and WESTERNHAGEN et al.(1974) assumed the presence of a complexing agent on or in the chorion, which would account for the rapid metal uptake until a saturation level is reached, depending on the ambient cadmium concentration. The continuous increase in metal contents of larvae with exposure time indicates a mechanism suggested by GOULD and KAROLUS (1974) for the binding of metal in the cunner Tautogolabrus adspersus: the formation of metal-protein. In this connection it might be of interest that metal binding proteins (metallothionein) have already successfully been isolated by OLAFSON and THOMPSON (1974) from the rockfish liver (Sebastodes caurinus) on administration of $CdCl_2$.

Herring larvae in our experiments accumulated more cadmium/0.1 mg dry wt during a certain period of time than did flunder larvae (differences in holding temperatures being neglected). Species specific differences in the ability to accumulate certain metals have already been noticed by HARDISTY et al. (1974). In their investigations, tissues from flounders (Platichthys flesus) from the Severn estuary contained about four times less cadmium than samples taken from Liparis liparis or Pomatoschistus minutus from the same area.

There appears to occur time depending fairly rapid uptake of cadmium by fish larvae exposed to high concentrations of this metal. Experiments conducted by GREIG et al. (1974) with Tautogolabrus adspersus also proved very rapid uptake of cadmium from the surrounding medium. After 4 days of exposure to 3.0 ppm Cd (as $CdCl_2$) cadmium concentrations in liver tissues of experimental fish approximated 15 ppm (wet weight basis). These values are similar to those recorded by BROOKS and RUMSEY (1974) in the liver of wild catches of Polyprion oxygeneios from New Zealand, showing that in their natural habitat fishes are able to accumulate cadmium against an existing ambient concentration. Similar results have been obtained by

PORTMANN (1972) and PRESTON (1973) who could show that cadmium concentrations in fishes from British waters were well above those of the surrounding water (0.12 - 0.18 μg/g wet weight fish; 0.6 - 0.8 μg/l seawater).

In our experiments larvae exposed to low concentrations of cadmium (0.01, 0.05, 0.1 Fig. 2 and 3) did over a short period of 8 to 14 days not show elevated metal concentrations. Yet longer exposure of 30 days at high temperatur (22°C) did, in rearing experiments with Belone larvae and juveniles yield cadmium concentrations in the experimental fish 4 times higher than determined in control specimens. Also EISLER et al. (1972) were able to prove accumulation of cadmium by Fundulus at concentrations of 0.01 ppm after 21 days of exposure.

As evident from our experiments as of now there are not acute toxic or lethal effects on fish larvae expected to be caused by cadmium in concentrations presently found in the environment (unpolluted waters: $<$0.00001 - 0.00041 ppm, PRESTON 1973; polluted waters: 0.005 ppm, BUTTERWORTH et. al. 1972). Yet the demonstrated ability of fish larvae to extract and accumulate cadmium from the surrounding seawater is reason enough to watch the development of heavy metal concentrations in coastal waters carefully. Further long time experiments would have to reveal whether low cadmium concentrations (0.01 - 0.1 ppb) would be sufficient to cause dangerously high accumulation levels in fish larvae, which due to their high surface/volume ratio are more liable to concentrate heavy metals than adult fish.

SUMMARY

1. Eggs of herring (Clupea harengus), flounder (Platichthys flesus), and garpike (Belone belone) were incubated in cadmium contaminated water (0.05 - 5.0 ppm) and newly hatched larvae analyzed for cadmium contents.

2. Cadmium residues in newly hatched larvae were dependent on cadmium concentrations employed during incubation.

3. Cadmium contents of flounder and herring larvae (0.7 - 2.3 ng Cd/0.1 mg dry weight) was of two orders of magnitude higher than in Belone larvae reared under the same conditions (0.0017 - 0.0185 ng Cd/0.1 mg dry weight).

4. Cadmium contents of herring and flounder larvae held in cadmium contaminated water increased with exposure time.

5. Cadmium contents of herring larvae exposed to cadmium contaminated water for 8 days was of one order of magnitude higher than contamination of flounder larvae kept under similar conditions (max value for herring 48 ng Cd/0.1 mg dry wt; max value for flounder 5.4 ng Cd/0.1 mg dry wt).

6. Cadmium contents of *Belone* larvae and juveniles kept at 0.05 ppm Cd for 30 days were significantly higher than cadmium contamination of control specimens.

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Fig. 2: Cadmium uptake by newly hatched herring larvae exposed to different cadmium concentrations at 10°C and 16°/oo S.

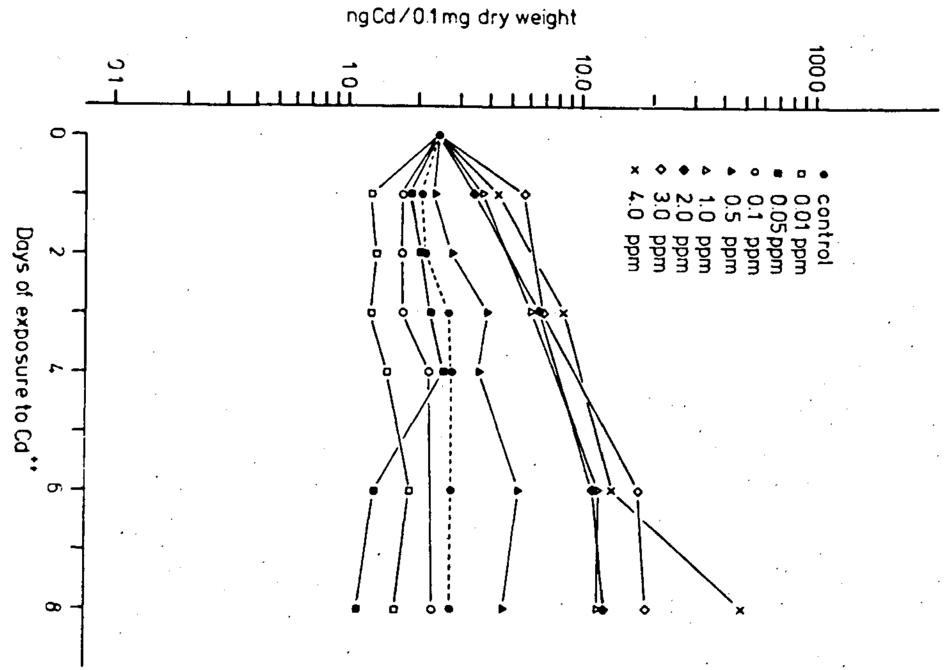

Fig. 1: Cadmium content in newly hatched herring, flounder and garpike larvae incubated at different cadmium concentrations. Combined data from 15°/oo (16°/oo), 25°/oo and 32°/oo) s experiments.

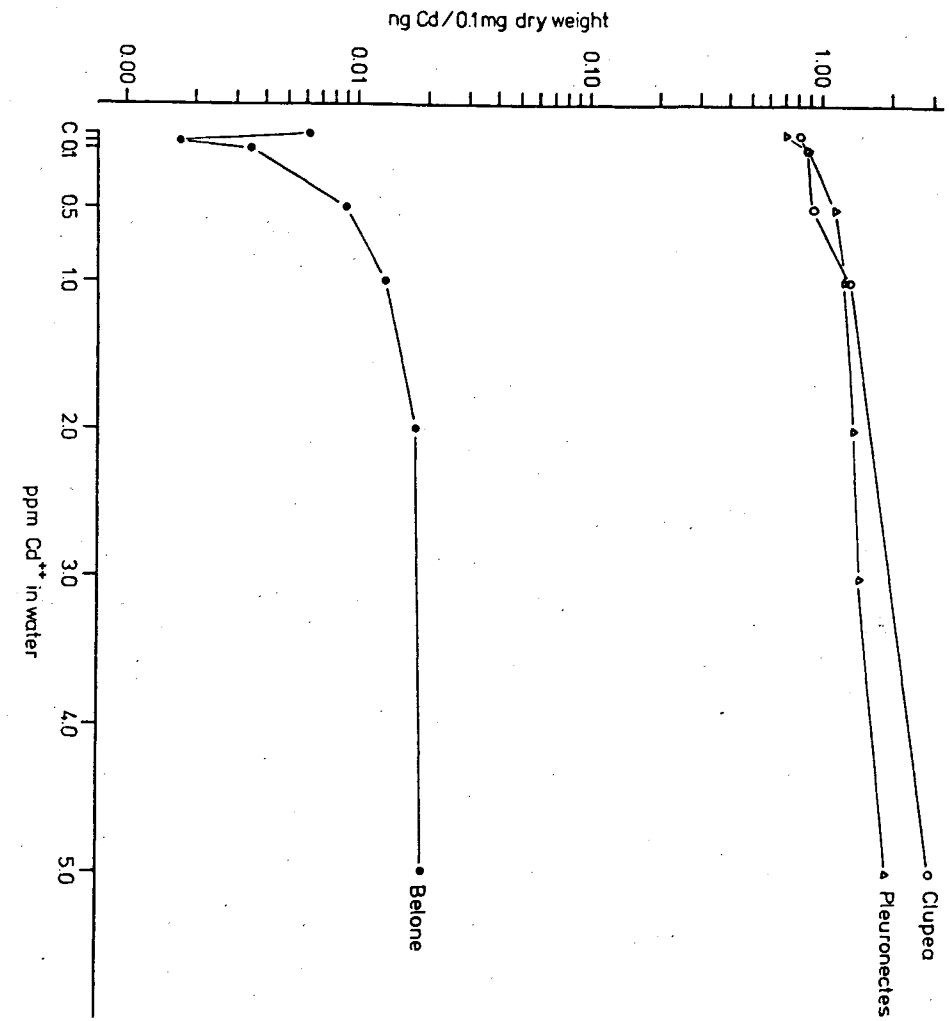

Fig. 4: Cadmium uptake by newly hatched flounder larvae incubated at different cadmium concentrations and exposed to the respective concentrations after hatching at 5°C. Combined data from 16°/oo, 25°/oo and 32°/oo S.

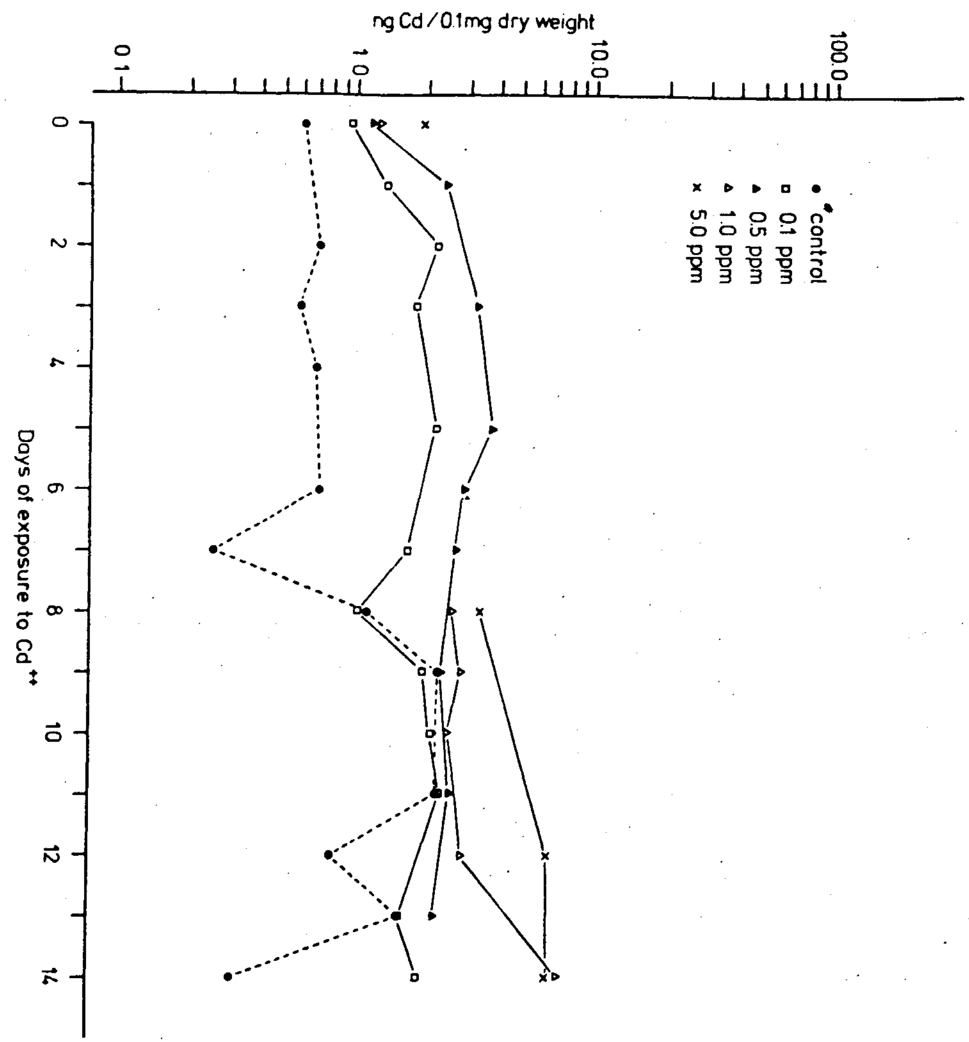

Fig. 3: Cadmium uptake by newly hatched flounder larvae incubated at different cadmium concentrations and exposed to to respective concentrations after hatching at 5°C and 42°/oo S.