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## REPRODUCTION OF FISH IN CONDITIONS DISADVANTAGEOUSLY ALTERED WITH THE SALTS OF ZINC AND COPPER

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**A B S T R A C T.** The study consisted of incubating the oocytes and of fertilised eggs of common carp and goldfish in different zinc and copper concentrations. Also guppies and goldfish were reared in various zinc concentrations, whereas carp were given zinc with feeds. Determinations were performed of an increase of mature oocyte fractions, percentage of normal hatched larvae, gonadotropin (GtH II) level and gonadosomatic (GSI) index. It was found that zinc concentration as low as 3 mg/l and copper concentration 0.2 mg/l decreased hatchability and increased fractions of larval deformations. It was also discovered that rearing of guppies and goldfish in the concentrations which did not inhibit growth nor increased mortality (1.0 and 2.5 mg/l respectively) could result in a statistically significant reduction of progeny number. In the case of goldfish reared in 2.5 mg/l zinc concentration, a significantly lower level of GtH II than in the control was found after 456 days in both sexes. This implies adverse effect of zinc on reproduction processes at the level of hypothalamus and pituitary gland.

Key words: CARP, GOLDFISH, REPRODUCTION, ZINC, COPPER

### INTRODUCTION

There is a large number of publications in the world literature dealing with lethal, and maximum admissible concentrations of toxicants, and with the application coefficients. However, not much has been published on the effect of toxicants on fish reproduction.

With regard to zinc it was shown that doses higher than the organism demand brought about - depending on the concentration and time of exposure - a decrease of fecundity in *Pimephales promelas* (Brungs 1969), a delay of spawning and diminished viability of eggs in *Brachydanio rerio* (Speranza et al. 1977), spermatogenesis inhibition and ovary atresia in *Puntius conchoniuis* (Kuimar, Pant 1984), decreasing of egg size and larval deformation in *Catostomus commersoni* (Munkittrik, Dixon 1989).

As regards copper, it was also found that the supply of this metal exceeding the organism demand caused a delay in hatching of *Brachydanio rerio* (Dave, Xiu 1991)

and in spermatogenesis of *Lebistes reticulatus* (Sehgal et al. 1984), diminished hatchability and increased larval deformities in *Atherinops affinis* (Anderson et al. 1991), inhibited spermatogenesis and induced ovarian atresia in *Puntius conchonius* (Kumar, Pant 1984), diminished egg size and increased larval deformation in *Catostomus commersoni* (Munkittrik, Dixon 1989), and decreased fecundity in *Pimephales promelas* (Pickering et al. 1977), all of these effects being dependent on copper concentration and exposure time.

Increasing heavy metal pollution of fresh waters, including fish ponds (Protasowski 1991, Szulkowska-Wojaczek et al. 1992), as well as incidents of alarmingly high concentrations of these elements in fish induced the Department of Ichthyobiology and Fisheries, Kraków, to undertake studies on the effects of zinc and copper on fish reproduction

The investigations involved incubation of oocytes and of fertilised eggs of common carp and goldfish in various zinc ( $ZnSO_4$ ) and copper ( $CuSO_4$ ) concentrations, rearing of guppies and goldfish in different zinc concentrations, and supplying zinc with carp feeds. Determinations comprised mature oocyte fractions, percentage of normal and deformed hatched larvae, number of progeny per one female, gonadotropin level (GtH II), and gonadosomatic (GSI) index.

## EXPERIMENTS

Eight experiments were carried out as follows:

### INCUBATION OF CARP OOCYTES IN VARIOUS ZINC CONCENTRATIONS

#### MATERIAL AND METHODS

Oocytes after vitellogenesis, obtained from 8 five - years old female carps, were used in this experiment. They were divided into 12 groups. There were 16 replications. Each group was incubated in BSS Cortland fluid in a combination (except the control) with such components as: pituitary gland homogenate (chh), 17a20b progesterone (17a20b P), and zinc in doses of 3, 11 and 50 mg/l. After the incubation, the oocytes were fixed in Serr's fluid and immersed in turpentine oil for transparency. Determinations embraced percentage of the oocytes after the vitellogenesis with the germinal vesicle (G) in the centre, with G shifted peripherally, and those after the G breakdown (GVBD).

## RESULTS

In comparison with the control group, an increase in percentage of oocytes with peripheral G position or after GVBD was observed only in these groups which were incubated in the presence of chh or 17a20b P. There was no relationship between this increase and the presence or absence of zinc in the incubation medium.

## INCUBATION OF THE OOCYTES OF CARP GIVEN ZINC IN THE FEEDS

### MATERIAL AND METHODS

Oocytes of carp reared during the growing season preceding the experiments on fodder with an addition of zinc (oocytes of 5 females - group 1) or without zinc (oocytes of 5 females - group 2) were used in these experiments. The females of group 1 had eaten c. 9 g of zinc during the season.

Oocytes were incubated in the same manner as in the previous experiments, either in the BSS Cortland alone or in the BSS Cortland with added chh (100 µg/ml) or in BSS Cortland with 17a20b (1 µm/ml). There were 25 replicates. After 24 h of incubation, the oocytes showing termination of the vitellogenesis were fixed, made transparent, and examined for the percentage of those with G in the centre, those with the peripheral G location, and the others after GVBD.

### RESULTS

Fractions of the oocytes with peripheral G or those after GVBD which originated from the females fed zinc in fodder were significantly lower than the fractions of the same categories of oocytes obtained from the control group 2 (in BSS Cortland + chh, 33.9% and 45.6%, and in BSS Cortland + 17a20b P, 16.0% and 33.9%).

## INCUBATION OF THE OOCYTES OF GOLDFISH (*Carassius auratus*) EXPOSED TO VARIOUS ZINC CONCENTRATIONS

### MATERIAL AND METHODS

In this experiment the oocytes after termination of the vitellogenesis were used. They were taken from 20 goldfish females reared in water containing 0.006 mg/cm<sup>3</sup> zinc (group 1, control), 20 females reared in water with c. 1 mg/l zinc (group 2), and 20 females reared in water with c. 2.5 mg/l zinc (group 3). Oocytes were incubated either in BSS Cortland alone, or in BSS Cortland + chh, at the same doses as in the previ-

ous experiments. The experiment involved 40 replications. The incubation was carried out in the same manner as in the previous experiments. At the end of the incubation not only the percentage of oocytes with various locations of the G was estimated, but also the percentage of resorbing oocytes. Differences between fractions (%) of the oocytes after GVBD in the control group and those in vessels with rearing fluid containing chh were estimated.

#### RESULTS

The percentage of oocytes with G in the central position was significantly higher in group 1 (77.7%) than in the other two groups (44.0 and 49.0, respectively). At the same time fraction of resorbing oocytes was significantly lower (0.42%) than in the other groups (16.9 and 25% respectively).

The difference between the percentage of oocytes after GVBD in the control and that in the rearing fluid with chh corresponded in particular groups to the increase of mature oocytes (after GVBD) owing to chh influence. The greatest increase of oocytes after GVBD was observed in group (53.8%). It was significantly higher than in the remaining groups (30.8 and 26% respectively).

### INCUBATION OF FERTILISED CARP EGGS IN VARIOUS ZINC CONCENTRATIONS

#### MATERIAL AND METHODS

Eggs of 25 female carps were used. They were all fertilised with the same mixed sperm obtained from 3 carp males. The experiments involved incubation of fertilised eggs separately for each female, in Petri dishes with several zinc concentrations: 0.006 -the control, 3, 5, 7, 11, 13 mg/l Zn. Incubation took place in hard, soft and acidic water. Dead eggs were removed every day and counted. After the incubation, percentages of normal and deformed (lordosis, scoliosis) hatched larvae were estimated. Relationships between zinc concentrations and the count and percentage of hatchlings were estimated by means of a linear regression and the General Linear Model using a SAS computer program.

#### RESULTS

The experiments demonstrated that the percentage of normal hatchlings decreased along with increasing concentrations of zinc in hard, soft, and acidic water used for the incubation. Regression coefficients were highly significant. A decline of hat-

chability at increasing zinc concentrations was less pronounced in hard water (regression coefficient = - 2.9) than in soft water (regression coefficient = -3.09) ( Miś et al. 1996b, in press).

## **CARP EGG INCUBATION IN VARIOUS COPPER CONCENTRATIONS**

### **MATERIAL AND METHODS**

Eggs of 24 carp females were used; they were treated in the same way as in the case of various zinc concentrations described above, but copper concentrations (as copper sulphate) were as follows: 0.006 (control), 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mg/l.

### **RESULTS**

The outcome of this experiment conformed very well to that on egg incubation in various zinc concentrations. The hatchability decreased as copper concentration increased and the decrease was more sharp (regression coefficient = -2.76) in soft water than in hard water (regression coefficient = -2.40). Copper proved particularly toxic in acidic water, and at concentrations above 0.2 mg/l no fish hatched (Miś et al. 1996a, in press).

## **EFFECTS OF FEEDING CARP ZINC SUPPLEMENTED DIET ON SEXUAL MATURATION AND REPRODUCTION**

### **MATERIAL AND METHODS**

In these experiments, 480 three years old carps were used. They were stocked into 4 identical ponds (120 fish per pond in March 1992). Fish in two ponds were fed ground barley (group 1, control); in the other two ponds 2.5 g of zinc sulphate were added per 1 kg of the same feed (group 2). In the latter group each fish consumed c. 9 g zinc during the growing season. The investigation involved: gonadosomatic index, fecundity, response to chh injection, sperm volume, GtH 2 level.

### **RESULTS**

In 1993 there were three females in group 1 and two females in group 2 exhibiting symptoms of spawning readiness. After the injections from 20 to 250 g of spawn were obtained from females of group 1, and 2 - 30 g of spawn each from those of group 2.

In 1994 there were 60% of females showing external symptoms of spawning readiness in group 1 and 58% of such fish in group 2. After the injection, 4 females of group 1 and 5 of group 2 delivered eggs. In group 1, there were from 50 to 150 g of eggs

per 1 kg of body weight, while in group 2 - from 149 to 225 g. In 1993, the average amount of sperm from group 1 males was 3.09 ml per fish, from those of group 2 - 0.31 ml. In 1994, the averages were 1.8 ml per male in group 1 and 0.61 ml in group 2. As per 1 kg of body weight, these volumes amounted to 0.78 ml in group 1 and 0.25 ml in group 2. Differences between the groups proved to be statistically significant.

The gonadosomatic index in group 1 females amounted to 10.5, in group 2 - to 10.4. The average level of GtH 2 in group 1 females was  $12.4 \mu\text{g ml}^{-1}$ , in group 2 females -  $7.9 \mu\text{g ml}^{-1}$ . These two traits (GSI and GtH 2) were statistically insignificant between the groups.

## REARING GUPPIES (*Poecilia reticulata*) IN VARIOUS ZINC CONCENTRATIONS

### MATERIAL AND METHODS

Guppy females and males aged 4 - 5 month, of the mean weight of 1.6 g, originating from one commercial enterprise were used. Two pilot experiments and one final were carried out. The latter involved rearing of 3 groups of guppies over 156 days in water containing: group 1 - 0.26 mg/l zinc, group 2 - 1.121 mg/l, group 3 - 2.59 mg/l zinc. Numbers of progeny from each female, mortality and growth were assessed.

### RESULTS

In group 1, the average offspring number was 46.2 per female, in group 3 - 39.7, and in group 2 - 30.2 fish. Group 3 differed significantly from the control (group 1). There was no disparity among the groups with respect to the growth and mortality of the guppies (Bieniarz et al. 1994).

## REPRODUCTION OF GOLDFISH (*Carassius auratus*) IN VARIOUS ZINC CONCENTRATIONS

### MATERIAL AND METHODS

In this experiment 300 goldfish of both sexes, aged 6 years, the average weight 8 g, were used. The fish were reared in 3 zinc concentrations: group 1 - 0.006 mg/l (control), group 2 - 1.0, group 3 - 2.5 mg/l. Numbers of one day old larvae per 1 g female

body weight, gonadosomatic index, gonadotropin (GtH 2) level, and fecundity were estimated.

## RESULTS

The average number of larvae per 1 g female weight amounted to: 62.8 in the control, 50.2 in group 2, 24.3 in group 3. The latter average differed significantly from the other two. Number of deformed larvae in group 3 was significantly greater than in group 1. There were no significant differences among the groups as regards the gutted weight and GSI. After 459 days of raising the goldfish in various zinc concentrations, the average GtH2 level in group 3 females was  $8.17 \mu\text{g ml}^{-1}$ , in males of this group -  $6.5 \mu\text{g ml}^{-1}$ . It was significantly lower than in females and males of the control group, where the respective levels were  $15.71$  and  $12.78 \mu\text{g ml}^{-1}$ . The GtH2 level in group 2 did not differ significantly from that in the respective sexes of the control (Bieniarz et al. 1996, in press).

## DISCUSSION

The applied doses of zinc in the rearing milieu did not affect endocrine processes related to the last stage of oocyte maturation. This could imply that these stages were less vulnerable to adverse factors than the later ones related to the development and growth of the embryos.

All processes during the last period of oocyte maturation are initiated by the gonadotropin (GtH II) acting on receptors located on the theca (Bieniarz, Epler 1992). Because the oocytes incubated in various zinc concentrations proved to be similarly vulnerable to the effect of hypophysis homogenate, i.e. to gonadotropin, as the oocytes in the control, one can infer that the applied toxicants did not damage the above mentioned gonadotropin receptors. Another conjecture could be that the 24 h incubation period was too short for the applied toxicants to be effective.

Incubation in BSS Cortland with chh addition of the oocytes originating from carp fed zinc-supplemented diet, and of the oocytes from the control group was a different issue. In that case, it appeared that the oocytes from carp fed zinc additive were less responsive to chh than the control carp oocytes. These results suggest that feeding carp zinc-containing diet, at the doses applied here over just one growing season, caused an inhibition of oocyte maturation processes. Considering that a 24 h incubation

of the oocytes exposed to various zinc concentrations did not restrain their last maturation stage, it can be presumed that the adverse effects of zinc manifested themselves at earlier maturation stages of the oocytes. Incubation of the oocytes from goldfish kept in various zinc concentrations in BSS Cortland which chh seemed to substantiate that conjecture. This negative zinc influence on the earlier stages of oocyte maturation can occur not only at the level of gonads, but also the level of hypothalamus and hypophysis. This is evidenced by a considerably lower GTH II level in blood of goldfish kept over 456 days in zinc concentration of 2.5 mg/l than in the control fish, as well as by the results of experiments on perfusion of carp pituitary glands in carp fed zinc additive and in the control fish. It was found that the glands of carp fed some zinc released less gonadotropin induced by GnRH than the glands of the control carp.

Incubation of fertilised carp eggs in diverse zinc or copper concentrations brought about a decrease of normal hatch at increasing concentrations of the metals. The hatchability in soft water was lower in both experiments than in hard water, and it was lower in acidic water than in soft water at the same metal concentration. It was found that in hard water there was a significant relationship only between the percentage of hatch in the control and that in the other zinc concentrations. No such relationship was detected in the case of carp eggs incubated in various copper concentrations. These results suggest that significant correlation between carp hatchability and heavy metal concentration exists only at low content of the toxicants. Therefore, it seems that carp hatchability in the control conditions is directly related to the resistance of its fertilised eggs to heavy metals only in the cases of low toxicity caused either by the external factors (water hardness) or by a relatively low metal concentration (Miś et al. 1996 a,b - in press).

The investigation on carp fed zinc added fodder lasted for a too short period - one growing season - to draw any final conclusions. However, the obtained results seem to support the hypothesis based on *in vitro* experiments that feeding carp zinc-added diet inhibited their sexual maturation. This was indicated by a much lower sperm volume obtained from carp fed zinc added fodder than that from the control fish, although there were no significant differences in GtH II level and in GSI between the respective experimental groups.

The experiments on rearing guppies and goldfish in diverse zinc concentrations confirm the published data showing that elevated zinc content in water (2.5 mg/l) affects negatively the fish reproduction even when it does not influence the survival or



growth.. It is noteworthy that both species revealed strong resistance to relatively high zinc concentration in water.

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## STRESZCZENIE

### ROZRÓD RYB W WARUNKACH NIEKORZYSTNIE ZMIENIONYCH PRZEZ SOLE CYNKU I MIEDZI

Badania polegały na inkubacji oocytów i zapłodnionej ikry karpia i złotej rybki w różnych koncentracjach cynku i miedzi. Oprócz tego gupiki i karasie chowano w różnych koncentracjach cynku i miedzi, a karpię karmiono paszą z dodatkiem cynku. Określono procent dojrzałych oocytów, procent normalnych wylęgniętych larw, poziom gonadotropiny (GtH2) i współczynniki gonadosomatyczny.

Stwierdzono, że cynk już w koncentracji 3 mg/l, a miedź 0,2 mg/l zmniejszają procent wylęgu i zwiększają procent larw zdeformowanych. Stwierdzono też, że chów gupików i karasi w koncentracji cynku (odpowiednio 1,0 i 2,5 mg/l), które nie hamowały wzrostu ani nie zwiększały śmiertelności, ale powodowały statystycznie istotne zmniejszenie liczby potomstwa. W przypadku karasi chowanych w koncentracji cynku 2,5 mg/l przez 456 dni, obserwowano statystycznie istotnie niższy w porównaniu z kontrolą poziom GtH2. U karpi karmionych z dodatkiem cynku obserwowano zahamowanie, w porównaniu z kontrolą, dojrzewania oocytów. Otrzymane wyniki, sugerują, że cynk wywiera ujemny wpływ na procesy związane z rozrodem na poziomie gonad we wczesnych stadiach dojrzewania oocytów oraz na poziomie podwzgórza i przysadki mózgowej.

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