

## TOXIC EFFECTS OF ZINC ON FOUR SPECIES OF FRESHWATER FISH

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### Abstract

The toxic effects of  $Zn^{2+}$  on Silver Carp (*Hypophthalmichthys molitrix* C. et V.), Big Head Carp (*Aristichthys nobilis* Richardson), Grass Carp (*Ctenopharyngodon idella* C. et V.), and Blunt Snout Bream (*Megalobrama amblycephala* Yih) are studied. The test results are: (1) There are linear correlations between 24h  $LC_{50}$  and 48h  $LC_{50}$  of  $Zn^{2+}$  for Silver Carp fingerling and temperature. 24h  $LC_{50}$ , 48h  $LC_{50}$  and 96h  $LC_{50}$  of  $Zn^{2+}$  for fry of the four species are also determined; (2) There are logarithmic correlations between the growth rates of the fry and the concentrations of  $Zn^{2+}$  and between expansion of fish egg membranes after absorbing water and concentrations of  $Zn^{2+}$ ; (3) The tolerance of fry of the four species to  $Zn^{2+}$  is in the following order: Grass Carp > Silver Carp > Blunt Snout Bream > Big Head Carp; (4) The safe concentrations of  $Zn^{2+}$  are: Big Head Carp: 0.008 mg/L, Grass Carp: 0.046 mg/L, Blunt Snout Bream: 0.010 mg/L, Silver Carp: 0.012 mg/L, Silver Carp fingerling: 0.09 mg/L.

### INTRODUCTION

Zn is an essential trace element for organisms, but in excessive amount is toxic for organisms. The present wide industrial use of zinc makes it one of the most common pollutants in natural waters. Excessive zinc could inhibit physiological activities of aquatic organisms and even be lethal.

Zinc can be accumulated in organisms and transmitted by the aquatic food chain and finally harm human health (Förstner and Wittman, 1983). Therefore, study on the toxic effects of  $Zn^{2+}$  on fish is very important for protecting human health and aquatic resources and setting water quality criteria for fisheries.

There are some published reports on the toxic effect of  $Zn^{2+}$  on fish (Xu, 1982; Huang et al., 1983 and Wu, 1983) but they did not contain information, especially on the quantitative effect of environmental factors on the toxic effect of  $Zn^{2+}$  on Silver Carp, Big Head Carp, Grass Carp and Blunt Snout Bream. Results of the present study on the relation of temperature to  $LC_{50}$  of  $Zn^{2+}$  for Silver Carp fingerling, the effects of  $Zn^{2+}$  on growth rates of fry of the four species are presented, and safe  $Zn^{2+}$  concentrations for the four fishes are proposed.

### MATERIALS AND METHODS

Cleaned pond water with pH regulated with 0.1 mol/L HCl and 0.1 mol/L NaOH (both A.R. grade) was used. Based on the test requirements, a standard  $ZnSO_4$  solution (A.R.) was added accurately into the water at equal

logarithmic intervals. Each test involved at least a control group and 7 groups containing different concentrations of  $Zn^{2+}$ . Air was continuously bubbled into the water during the test. The test fish and eggs were from the Chongming Breeding Farm. The fry were measured with an OLYMPUS microscope. The test water volumes were 100 L for fingerling and 24 L for fry and egg.

### 1. Determination of the relationships between the $LC_{50}$ of $Zn^{2+}$ for Silver Carp fingerling (S-fi) and temperature

The quality indexes of the test water were:  $Alk=4.31$  meq/L,  $H_T$  (total hardness) =  $4.51$  meq/L,  $pH=7.40$ , S-fi mean body length =  $8.8 \pm 0.6$  cm. Twelve series (temperature 5–24 °C) of the test solutions were prepared, with each series containing 7 groups of different  $Zn^{2+}$  concentrations. The fingerlings in groups of 10 were acclimated by raising temperature before the test. Test solutions were refreshed every other day to maintain a constant  $Zn^{2+}$  concentration and had temperature controlled strictly. Surviving fingerlings were counted at 24, 48 and 96 hours. The values (mg/L) of 24h  $LC_{50}$ , 48h  $LC_{50}$  and 96h  $LC_{50}$  of  $Zn^{2+}$  for S-fi were determined by linear interpolation.

### 2. Determination of $LC_{50}$ values of $Zn^{2+}$ for the fry

Water quality indexes were:  $pH=7.40$ ,  $Alk=2.61$  meq/L,  $H_T=3.52$  meq/L. Mean body lengths of fry of Silver Carp (S-fr), Big Head Carp (Bi-fr), Blunt Snout Bream (Bl-fr) and Grass Carp (G-fr) were  $8.06 \pm 0.50$ ,  $8.37 \pm 0.19$ ,  $7.85 \pm 0.40$  and  $8.75 \pm 0.80$  mm, respectively. There were 20 fry in each group. The test method was similar to that described in Section 1.

### 3. Determination of effect of $Zn^{2+}$ on fry growth

Fry lengths and water quality indexes were the same as described in 2. There were 50 fry in each group of the test solutions. The fry were reared for 17 days, and then their measured body lengths were used to determine the respective growth rates ( $V$ , mm/d) and the relationships between  $V$  and the concentration ( $C_{Z_n}^{2+}$ , mg/L) of  $Zn^{2+}$ .

### 4. Determination of effect of $Zn^{2+}$ on expansion of eggs absorbing water

One hundred newly laid eggs of Silver Carp, Big Head Carp and Grass Carp were put into each group of solutions. The amount of introduced air bubbles was controlled carefully to avoid harming the egg membranes. After the eggs had absorbed water fully, they were taken out from each group randomly. The diameters ( $d$ ) of the egg balls containing embryos, perivitelline fluid and egg membranes were measured to determine the correlations between  $d$  (mm) and  $C_{Z_n}^{2+}$  (mg/L).

## RESULTS

### 1. The correlation between $LC_{50}$ values of $Zn^{2+}$ for S-fi and temperature

Fig.1 shows the correlation between  $LC_{50}$  values of  $Zn^{2+}$  for S-fi and temperature. Statistical analysis indicates that 24h  $LC_{50}$ , 48h  $LC_{50}$  and 96h  $LC_{50}$  values and the temperature have good linear relationship. The correlation equations are as follows:

24h $LC_{50}=49.80-1.72 t$	$n=12$	$r=-0.984$
48h $LC_{50}=39.23-1.31 t$	$n=12$	$r=-0.982$
96h $LC_{50}=29.58-1.07 t$	$n=12$	$r=-0.998$

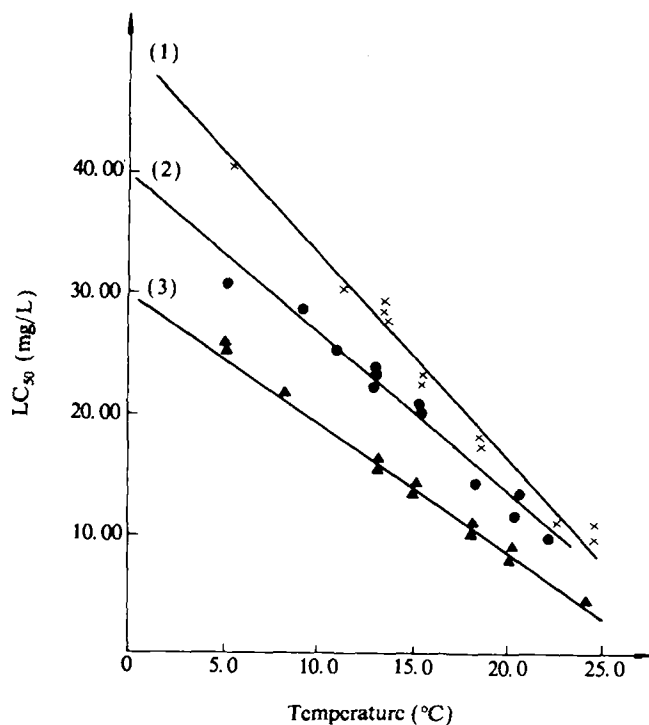


Fig.1 The relationship between the  $LC_{50}$  of  $Zn^{2+}$  for Silver Carp fingerling and temperature (1) 24h, (2) 48h, (3) 96h.

## 2. The toxic effect of $Zn^{2+}$ on the fry

$LC_{50}$  values of  $Zn^{2+}$  for the four species of fish fry are listed in Table 1.

Table 1  $LC_{50}$  (mg/L) of  $Zn^{2+}$  for fish fry

Fish	24h $LC_{50}$	48h $LC_{50}$	96h $LC_{50}$
Grass Carp	6.47	6.04	4.60
Silver Carp	3.56	2.63	1.23
Blunt Snout Bream	2.58	2.15	0.95
Big Head Carp	1.48	1.47	0.80
Big Head Carp <sup>1)</sup>	5.24	4.22	2.82

1) Hatched 6 days in advance, water temperature 20 °C.

## 3. The effect of $Zn^{2+}$ on fry growth

Fig.2 giving curvilinear correlations between the growth rates (mm/d) of fry of the four species and the concentration of  $Zn^{2+}$  shows that the curves are very well correlated. The correlation equations are as follows:

$$G\text{-fr: } V = 0.101 - 2.10 \times 10^{-2} \ln C_{Z_n}^{2+} \quad n=7 \quad r = -0.973 \quad S_V = 5.13 \times 10$$

$$S\text{-fr: } V = 3.10 \times 10^{-2} - 1.26 \times 10^{-2} \ln C_{Z_n}^{2+} \quad n=7 \quad r = -0.979 \quad S_V = 2.65 \times 10$$

$$Bl\text{-fr: } V = -1.56 \times 10^{-3} - 4.68 \times 10^{-2} \ln C_{Z_n}^{2+} \quad n=7 \quad r = -0.993 \quad S_V = 4.60 \times 10$$

$$Bi\text{-fr: } V = -3.62 \times 10^{-3} - 2.75 \ln C_{Z_n}^{2+} \quad n=7 \quad r = -0.996 \quad S_V = 2.50 \times 10$$

where,  $n$ , number of sample;  $r$ , correlation coefficients,  $S_v$ , the standard deviations of the regressive equations.

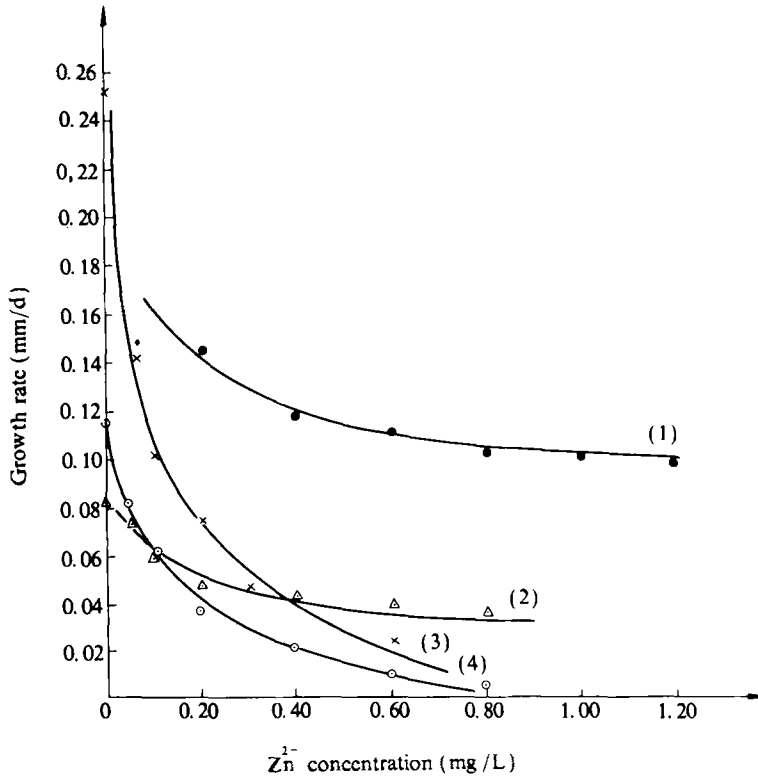


Fig. 2 The growth rates ( $V$ ) of fry of the four species as a function of the concentration ( $C_{Zn^{2+}}$ ) (1) Grass Carp, (2) Silver Carp, (3) Blunt Snout Bream, (4) Big Head Carp.

**4. The effect of  $Zn^{2+}$  on expansion of fish eggs absorbing water**

Fig. 3 gives the relationship between the egg ball diameter ( $d$ ) of the fish and

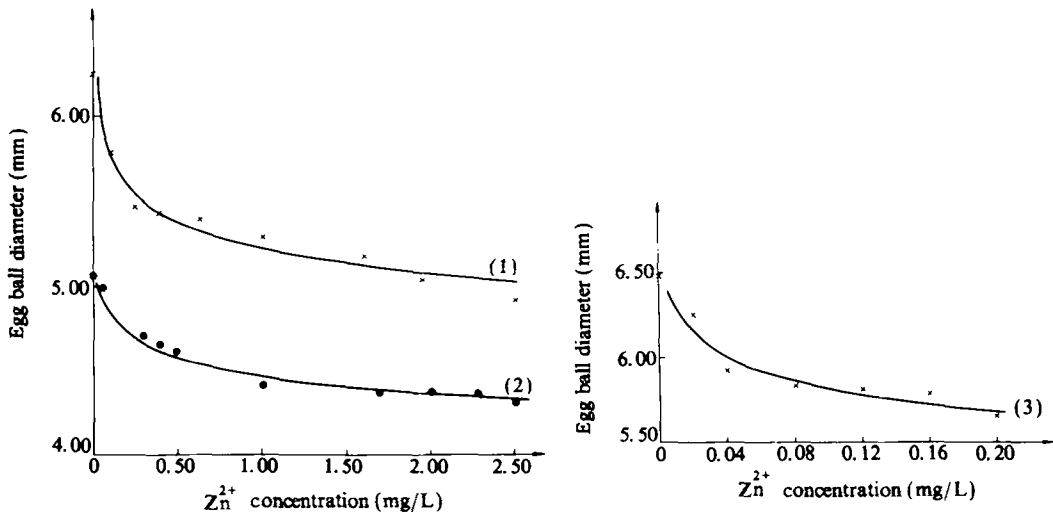


Fig. 3 Egg ball diameter ( $d$ ) of the fishes as functions of the concentration of  $Zn^{2+}$  (1) Grass Carp, (2) Silver Carp, (3) Big Head Carp.

the concentration of  $Zn^{2+}$ , and shows their good correlation. The correlation equations are as follows:

$$\text{Grass Carp egg: } d = 5.32 - 0.214 \ln C_{Z_n}^{2+} \quad n = 9 \quad r = -0.960 \quad S_d = 0.067$$

$$\text{Silver Carp egg: } d = 4.47 - 0.154 \ln C_{Z_n}^{2+} \quad n = 10 \quad r = -0.982 \quad S_d = 0.040$$

$$\text{Big Head Carp egg: } d = 5.23 - 0.136 \ln C_{Z_n}^{2+} \quad n = 7 \quad r = -0.921 \quad S_d = 0.116$$

where,  $n$  and  $r$ , ditto;  $S_d$ , the standard deviations of regressive equations.

## DISCUSSION

### 1. The toxic effect of $Zn^{2+}$ on fish and the factors affecting toxicity of $Zn^{2+}$ to the animals

Table 1 shows that  $LC_{50}$  values of  $Zn^{2+}$  for the four species of fry were different, and that their tolerances to  $Zn^{2+}$  are in the following order: G-fr > S-fr > Bl-fr > Bi-fr. The  $LC_{50}$  values for two groups of Big Head Carp fry of different ages were different. It was also found that the  $LC_{50}$  value difference between Silver Carp fingerling and the fry was much bigger (higher  $LC_{50}$  values for fingerlings, lower  $LC_{50}$  values for fry). These indicate that the tolerance of the fish to Zn increases with age. This agrees with the results of other researchers (Alabaster, 1982; Riberling and Migaki, 1975).

Fig. 1 shows that  $LC_{50}$  values decreased with rising temperature. 96h  $LC_{50}$  of  $Zn^{2+}$  for S-fi dropped by 83 percent when temperature rose from 5 °C to 24 °C, though S-fi can live normally in unpolluted water between 5 °C and 24 °C. The above observation that toxicity of  $Zn^{2+}$  to the fishes increases with temperature agrees completely with the toxic character of general poisons (Tamura, 1977; Wu, 1981; Wang, 1983).

### 2. The inhibitory effect of $Zn^{2+}$ on fry growth and expansion of fish egg after absorbing water

Fig. 2 shows that the growth rates of the fry decreased with increasing concentration of  $Zn^{2+}$  and decreased faster at the low concentration range, implying that  $Zn^{2+}$  inhibits fry growth, and that its toxicity to the fishes strengthens with concentration. Alabaster, Conner and Wisely of the United States Environmental Protection Agency suggested that excessive  $Zn^{2+}$  can cause various pathological changes of fish tissues and retard growth and maturity. Fig. 2 also shows that the growth rates of Bi-fr and Bl-fr decreased with  $C_{Z_n}^{2+}$ , faster than that of G-fr and S-fr, and that  $V_{G-fr} > V_{S-fr} > V_{Bl-fr} > V_{Bi-fr}$  when  $C_{Z_n}^{2+}$  was about 0.36 mg/L. This order is the same as the order of tolerance of the fishes to  $Zn^{2+}$ . This result agrees with our deduction from the  $LC_{50}$  values.

Fig. 3 shows that expansion of fish eggs after absorbing water was inhibited strongly by  $Zn^{2+}$ . The strengthened inhibition with increasing  $Zn^{2+}$  concentration indicates that the toxic effect of  $Zn^{2+}$  on the fish eggs is strengthened with increasing concentration of  $Zn^{2+}$ .

To sum up, temperature and  $Zn^{2+}$  concentration are important factors which strongly affect the toxicity of  $Zn^{2+}$  to the fishes.

### 3. The symptoms and mechanisms of $Zn^{2+}$ poisoning

$Zn^{2+}$  is the most toxic form of zinc for aquatic organisms (Wang, 1983). The

test showed that the fish were highly sensitive to  $Zn^{2+}$ . The fish swam wildly and jumped about after they were put into the test solution ( $C_{Zn^{2+}} > 10 \text{ mg/L}$ ). After a while, the gills and the body surface excreted a large amount of mucus; then the fish swam slowly, dashed intermittently against the container walls; the chest fins shivered, the scales dropped off. The fish spat out big bubbles; the respiratory frequency decreased; haemorrhage spots were observed in the eye sockets and gill cover; gill strands became white and were covered with mucus; the fish gradually lost equilibrium, and then died.

The mechanism and chain of reactions in fish to zinc poisoning could possibly be:  $Zn^{2+}$  could have damaged the gill tissues, thus disturbing gas exchange, waste excretion, and osmotic pressure regulation; and could also have reacted harmfully with protein, and therefore prevented the normal function of protein and enzymic activity (Nomiyama, 1981; Pringle et al., 1986; Skidmore, 1970; Ribelin and Migaki, 1975).

#### 4. $Zn^{2+}$ content as water quality criterion for fishery

At present, the safe concentrations of heavy metal ions for fish are commonly based on  $0.01 \times 96 \text{ h LC}_{50}$ . The concentration of  $Zn^{2+}$  in fishery water in the U.S.A. and Japan are limited to less than 0.01 and 0.1 mg/L, respectively (J.F.R.C.A., 1965, 1972; United States Environmental Protection Agency, 1978). In China it is temporarily limited to less than 0.1 mg/L (Wu, 1981). The values of  $0.01 \times 96 \text{ h LC}_{50}$  calculated from the present test results are listed in Table 2. The readers will easily find that values calculated from the results of the present study for the fishes are close to those of the U.S.A..

Table 2 The safe concentrations of  $Zn^{2+}$  for some fresh water (20 °C) fishes

	0.01 × 96 h LC <sub>50</sub>
Big Head Carp fry	0.008
Grass Carp fry	0.046
Blunt Snout Bream fry	0.010
Silver Carp fry	0.012
Silver Carp fingerlings	0.028

### ACKNOWLEDGEMENT

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