Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 9	Fasc. 1	97-104	2001

THE EFFECTS OF AMBIENT MAGNESIUM CONCENTRATION ON THE EMBRYONIC AND PRELARVAL DEVELOPMENT OF MIRROR CARP (*CYPRINUS CARPIO* L., 1758)

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ABSTRACT. In this study, fertilized mirror carp eggs were allowed to develop in fresh water with various magnesium concentrations (0.05, 0.2 and 2 mg l^{-1}). The percentage of dead (eggs+prelarvae) and deformed prelarvae increased sharply to 94% at an ambient magnesium concentration of 0.05 mg l^{-1} compared with 22 and 10% at ambient magnesium concentrations of 0.2 mg l^{-1} and 2.0 mg l^{-1}, respectively. Mirror carp require more than a 0.05 mg l^{-1} ambient magnesium concentration for survival and successful development during the early life stage. The whole body magnesium and calcium concentrations of the developing prelarvae were dependent on the ambient magnesium concentration. The uptake of magnesium by animals decreased and the uptake of calcium increased with decreasing ambient magnesium concentrations. However, the uptake of the sum of these two divalent ions seemed to be independent of ambient magnesium concentration. This indicates that there is competition between magnesium and calcium for uptake into developing embryos.

Key words: *CYPRINUS CARPIO*, MIRROR CARP, EMBRYO, HATCHING, MAGNESIUM, CALCIUM

INTRODUCTION

Magnesium is an essential divalent ion, and it plays a important role in many cellular processes in the eukaryotic cell. For instance, magnesium has been implicated in the activation of a large number of enzymes, including that which transfers phosphate groups (phosphokinase), phosphohydroxylase acetyl coenzyme A (thiokinase) in fatty acid oxidation, and that which activates amino acid synthesis (amino acid synthetase). Magnesium is also essential in skeletal tissue metabolism and neuromuscular transmission (Lall 1989). Freshwater fish derive magnesium ions from active uptake from the environment or from dietary sources.

Fish eggs contain a significant amount of magnesium, and it is thought that some of it is associated with the yolk (Hayes et al. 1946). Therefore, the yolk may serve as a magnesium source for the developing embryo during the early embryonic stage. However, during embryonic development the number of cells increases rapidly before hatching, and it does not seem too imprudent to postulate that developing fish embryo must extract magnesium from the water to supply these cells with this essential element.

Although most fresh water has adequate ambient magnesium (> 5mg l^{-1}) for larval development, soft fresh water has less than 0.5 mg l^{-1} of ambient magnesium. Therefore, the present study addresses the effects of such a low ambient magnesium concentration on whole body ions (Ca, Mg and Na), and the survival of mirror carp is examined during the early life stages of fish that are not yet feeding.

MATERIAL AND METHODS

IN VITRO FERTILIZATION

Adult mirror carp, *Cyprinus carpio* L., weighing 1-2 kg and aged 3-4 years old, were held at 23°C in aerated and dechlorinated tap water containing around 5 mg l^{-1} of magnesium. Carp gametes were obtained through the hormonal induction of ovulation and spermiation by the intramuscular injection of carp pituitary powder (Chaudhuri 1976) suspended in a 0.9% NaCl solution. The suspended carp pituitary was administered at a dose of 0.5 mg kg^{-1} of fish for males and 1.0 mg kg^{-1} of fish for females. Twenty-four hours after injection the gametes of both sexes were stripped and mixed in glass Petri dishes. Fertilization was induced by the addition of water (23°C). After 5 min the eggs were rinsed and the dishes (each containing approximately 300 eggs) were placed in an experimental unit. Each experimental unit had four incubation chambers containing 3 l of medium, in which the Petri dishes containing the eggs were placed, and a reservoir containing another 15 l of medium. The experimental medium from the reservoir was pumped through this experimental unit. The experimental medium in the reservoir was kept at pH 7.8 ± 0.1 by the controlled addition of 0.01 M l^{-1} H_2S0_4 using pH-stat equipment. The water temperature was kept at 23°C and 12L:12D photoperiod was provided for the experimental units.

EXPERIMENTAL MEDIA

The experimental media contained 4.5 mg l^{-1} KCI, 90 mg l^{-1} $CaCl_2$, 200 mg l^{-1} NaCl and 30 mg l^{-1} $NaHCO_3$ in demineralized water. Three different magnesium concentrations (0.05, 0.2 and 2 mg l^{-1}) in the media were obtained by the addition of $MgS0_4$. The water magnesium concentrations varied within 10% of the nominal concentrations during the incubation period, as determined afterwards by atomic absorption spectrophotometry.

EXPERIMENTAL PROCEDURE

The temperature of all experimental media was kept at 23°C. After the fertilization procedure, unfertilized eggs were removed and the number of fertilized eggs (around 1500) per experimental unit was assessed. Every 24 h until the end of the experiment dead and moldy eggs and, later on, dead prelarvae were counted and removed. As soon as hatching was observed, the number of deformed prelarvae was assessed every 24 h until all eggs had hatched. The identification of deformed prelarvae was based on gross macroscopical appearance. Prelarvae were designated as deformed if they were slightly crescent to almost corkscrew-shaped. The percentage of deformation was expressed as the ratio of the number of deformed prelarvae to the total number of eggs incubated. Throughout the experiment 5 ml water samples were taken from the experimental units every 24 h to assess the magnesium concentration.

In a parallel experiment, the mineral content of embryos under experimental conditions was determined. Petri dishes with about 30 fertilized eggs were placed in each experimental medium. After 6, 12, 24, 48 and 72 h, a dish was removed and 25 eggs were weighed and dried in an oven for 12 h at 60°C, and then weighed again to assess the water content and dry mass of the samples. The dried eggs were digested by 3 ml of concentrated HNO_3. After 24 h, the digested samples were diluted to 25 ml with demineralized water. The total magnesium, calcium and sodium concentrations were measured by an atomic absorption spectrophotometer. Blank probe was also prepared by following the same procedure as above for demineralized water.

The data in the text are presented as mean values ± the standard deviation, unless otherwise stated. The data were analyzed statistically using analyses of variance (ANOVA). Statistical significance was accepted at the 5% level.

RESULTS

The magnesium and calcium concentrations of the embryos after 6 h of exposure to the experimental media did not change significantly (one-way ANOVA, $P > 0.05$; Figs. 1 and 2) between groups and were approximately 710 and 685 μg g^{-1} dry mass, respectively. The concentrations of both ions in the whole body of embryos after 24, 48 and 72 h of exposure to the experimental media changed significantly (one-way ANOVA, $P < 0.05$; Figs. 1 and 2) between groups. Seventy-two hours after fertiliza-

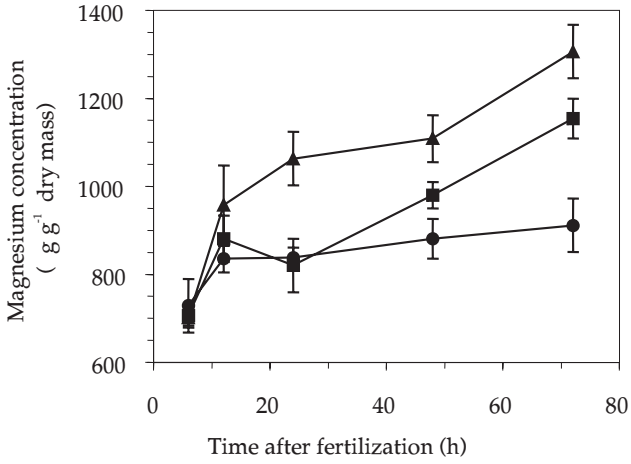

Fig. 1. Magnesium concentration (mean ± SE; N = 20) of mirror carp embryos during development in different ambient magnesium concentrations (● 0.05; ■ 0.2; ▲ 2.0 mg l^{-1}).

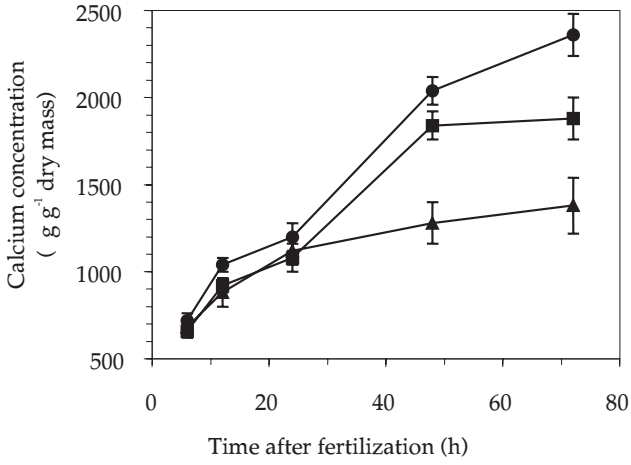

Fig. 2. Calcium concentration (mean ± SE; N = 20) of mirror carp embryos during development in different ambient magnesium concentrations (● 0.05; ■ 0.2; ▲ 2.0 mg l^{-1}).

tion, eggs exposed to 2 mg l^{-1} of magnesium had increased their magnesium concentration to 1306 ± 61 µg g^{-1} dry mass and their calcium concentration to 1380 ± 160 µg g^{-1} dry mass (N = 10). The magnesium increase was around 84%. However, the magnesium uptake of embryos decreased with decreasing ambient magnesium levels (Fig. 1) and was approximately 63% at 0.2 mg l^{-1} of magnesium in the water and 25% at 0.05 mg l^{-1} of magnesium in the water. In contrast, the calcium uptake of embryos

increased with decreasing ambient magnesium levels (Fig. 2). Seventy-two hours after fertilization, the calcium concentration of the embryos at 0.05, 0.2 and 2 mg l^{-1} of magnesium in the water increased 228, 185 and 103%, respectively, compared with the initial concentrations.

The sodium concentration in the embryos after 6 h of exposure was 2800 ± 230 µg g^{-1} dry mass (N = 18); this level increased to 4301 ± 650 µg g^{-1} dry mass (N = 18) after 72 h of exposure. The sodium concentration of the embryos was not affected by changes of ambient magnesium concentration (one-way ANOVA, $P > 0.05$).

The percentage of dead and deformed embryos and prelarvae increased as ambient magnesium levels decreased (Table 1). This increase was statistically significant ($P < 0.05$) between units. At a magnesium concentration of 2 mg l^{-1} in the water, 4% mortality and 6% deformed prelarvae were observed up to 170 h after fertilization. Both the mortality and the number of deformed prelarvae increased as ambient magnesium concentrations decreased, leading to 74% mortality and 20% deformed prelarvae at a magnesium concentration of 0.05 mg l^{-1} (Table 1).

TABLE 1

Number and percentage of dead (eggs and prelarvae), deformed and normal hatched prelarvae after exposure to different ambient magnesium concentrations from fertilization until 170 h after fertilization (initial total number of fertilized eggs in each unit was around 1500)

Time after fertilization (h)	Water magnesium concentration (mg l^{-1})					
	0.05		0.20		2.00	
	Number	%	Number	%	Number	%
24	18	1.2	-	-	-	-
48	20	1.3	12	0.8	10	0.7
72	63	4.2	15	1.0	8	0.5
*96	300	20.0	30	2.0	27	18
	(195)	(13.0)	(180)	(12.0)	(75)	(5.0)
120	450	30.0	18	1.2	12	0.8
	(105)	(7.0)	(60)	(4.0)	(15)	(1.0)
144	165	11.0	11	0.7	3	0.2
170	95	6.3	4	0.3	-	-
Total						
Dead	1111	74.0	90	6.0	60	4.0
eggs	101	6.7	27	1.8	18	1.2
prelarvae	1010	67.3	63	4.2	42	2.8
Deformed	(300)	(20.0)	(240)	(16.0)	(90)	(6.0)
Normal	89	6.0	1170	78.0	1350	90.0

* *the eggs started hatching*

DISCUSSION

The results of the present study show that an adequate level of magnesium in the ambient water is essential for the development of carp embryos and prelarvae. At magnesium levels below 0.2 mg l^{-1}, deformation and death were observed in early life stages. The magnesium concentration increased in developing eggs. Consequently, there is an uptake of magnesium from the water. Apparently, at water magnesium levels below 0.2 mg l^{-1} the accumulation of magnesium is hampered and mortality is increased. Thus, early life stages of carp require magnesium from the water for survival. The elemental concentrations we found for mirror carp eggs (around 710, 685 and 2800 µg mg^{-1} dry mass for magnesium, calcium and sodium, respectively) are close to values found by Van der Velden et al. (1991) for common carp eggs.

The uptake of ions from the water may be driven by the perivitelline potential (Peterson and Martin-Robichaud 1986, Eddy et al. 1990), by ion exchange (Shephard 1987), directly by a transporting enzyme or by any combination of these.

A remarkable phenomenon observed was the stimulation of calcium uptake in eggs by decreasing ambient magnesium levels (Fig. 2). The decrease of magnesium uptake was almost compensated by the increase of calcium uptake. In other words, the increase of the sum of magnesium and calcium contents appeared to be independent of the magnesium content of the ambient water. This indicates competition between calcium and magnesium for uptake into developing embryos. In higher vertebrates hypomagnesaemia (as a result of magnesium deficiency) may result in hypercalcaemia (George and Heaton 1975, Geven et al. 1988). On the contrary, in adult tilapia *(Oreochromis mossambicus* Peters) a high magnesium concentration in the ambient water resulted in hypermagnesaemia and hypocalcaemia (Wendelaar Bonga et al. 1983, Bijvelds et al. 1997).

The observation that the uptake of the combined divalent ions Ca^{2+} and Mg^{2+} is not affected by ambient magnesium levels argues against the latter possibility, assuming that both ions interact with these fixed anions. Ca^{2+} and Mg^{2+} follow the same pathway for uptake into the developing embryos and prelarvae. The rate of uptake of Mg^{2+} and Ca^{2+} appears to depend on the total divalent ion concentration in the water and is dictated by the electrochemical potential difference between the chorionic fluid and the water. The high mortality and deformation at low ambient magnesium in water indicated the dependency on water magnesium concentration of early life stages of carp.

It has been shown that a low water pH and toxic metals also inhibit the uptake of ambient ions by early life stages of carp (Stouthart et al. 1994, 1995) and brown trout , *Salmo trutta* L. (Reader et al. 1989, McDonald et al. 1989, Çalta 1996). A decrease in heart rate and pigmentation was reported for early life stages of freshwater rainbow trout, *Oncorhynchus mykiss* (Walbaum) exposed to a low pH or to a low ambient calcium concentration (Nelson 1982). Clearly, the ionic composition of the ambient water is of importance for the development of fish during the embryonic and larval stages. This holds true for the magnesium concentration as well as for the calcium and proton concentrations of the water.

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STRESZCZENIE

WPŁYW MAGNEZU NA ROZWÓJ EMBRIONALNY I PRELARWALNY KARPIA LUSTRZANEGO (*CYPRINUS CARPIO* L., 1758)

Zapłodnioną ikrę karpia lustrzanego inkubowano w wodzie zawierającej dodatek magnezu w koncentracji 0,05, 0,2 i 2 mg l^{-1}. Najwyższą śmiertelność ikry i larw, wynoszącą 94%, zaobserwowano w grupie doświadczalnej przetrzymywanej w wodzie o koncentracji magnezu 0,05 mg l^{-1}. W kolejnych grupach, koncentracje 0,2 i 2 mg l^{-1}, śmiertelność była znacznie niższa i wyniosła odpowiednio 22 i 10%. Stwierdzono więc, że do prawidłowego rozwoju karp potrzebuje powyżej 0,05 mg l^{-1} magnezu. Zaobserwowano, że koncentracja magnezu i wapnia w ciele świeżo wyklutych larw była zależna od zawartości magnezu w wodzie. Pobór magnezu przez ryby zmniejszał się wraz ze spadkiem koncentracji tego pierwiastka w otoczeniu. Z kolei pobór wapnia wzrastał. Uzyskane wyniki wskazują na istnienie pewnego rodzaju współzawodnictwa pomiędzy tymi pierwiastkami w czasie ich wchłaniania przez rozwijający się embrion karpia.

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