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Original Research

The Effects of Copper and Cadmium in Single Exposure or Co-Exposure on Growth of Common Carp (*Cyprinus Carpio* L.) Larvae

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Abstract

The effects of copper and cadmium in single or co-exposure (each at the concentration of 0.2 mg×dm^{-3}, and in mixture - 0.1 mg×dm^{-3}) on growth of common carp larvae (in terms of body length and perimeter area) during the first 30 days post hatching were evaluated. Body length increased in a similar rate during the entire experimental period, while the increase of body perimeter area became faster after the shift into exogenous feeding, and then during swim bladder inflation. Copper was more toxic to the fish comparing cadmium or a mixture of both metals which indicates a possible antagonism of cadmium against copper toxicity. Body perimeter area was a more sensitive indicator of heavy metal intoxication compared to body length, and may be used as an approximation of body mass for very small fish that cannot be accurately weighed alive.

Keywords: heavy metals, copper, cadmium, growth rate, toxicity, fish

Introduction

Freshwater fish can be used as selective bioindicators of trace metals in freshwater reservoirs [1] since they not only accumulate metals in their bodies but also react to water contamination with alterations of various vital functions. Growth rate of fish is highly variable, and very sensitive to environmental factors. Thus, measurements of growth can be used to provide information on fish performance [2]. The growth of fish is associated with changes in morphometric traits, body shape, and in chemical and biochemical body composition. Fish growth depends on water physio-chemical characteristics, and in polluted waters usually decreases [3-6]. Reduction in growth can be caused by physiological or behavioral stress during exposure to toxicants [5]. Any disturbances in food consumption or energy production induced by toxicants are reflected in fish growth rates. Early life stages are known to be very sensitive to intoxication. Inhibition of larval growth is one of the most distinct symptoms of metal toxicity, cadmium and copper being the most powerful growth inhibitors [7-10].

Most studies of the effects of metals on fish concern exposure to a single metal. Polluted water bodies, however, usually contain elevated levels of various metals. The effect of mixtures of two or more chemicals may be additive, synergistic or antagonistic. The data obtained by many authors presented in the review by Jezierska and Witeska [9] indicate various interactions of various metals, and therefore the effects of their mixtures on fish may also differ from the effect of single elements. It seems that interactions among various metals are related to their competitive uptake from the environment, and different distribution in fish tissues. Thus, a comparison of the effects of metal mixture and single metals on fish growth seems an interesting task.

The aim of the present paper was an evaluation of the effects of copper and cadmium in single or co-exposure on growth of common carp larvae during the first 30 days of their lives.

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Materials and Methods

The larvae of common carp were obtained from a hatchery of the Department of Animal Physiology. One-day-old correctly developed larvae were divided into 4 groups: Control, Cu, Cd and Cu+Cd. The fish were exposed to the nominal concentrations of 0.2 mg×dm^{-3} Cu (as $CuSO_4$), 0.2 mg×dm^{-3} Cd (as $CdCl_2$) or 0.1 mg×dm^{-3} Cu + 0.1 mg×dm^{-3} Cd, while the control group was kept in clean water. Each group consisted of 300 fish stocked into the 60 dm^3 tanks connected with additional 80 dm^3 tanks via the PCV hoses, and water recirculation between the tanks was forced using pumps. Entire volume of water was changed every 3 days to maintain metal concentrations. According to Jezierska and Królak [11], who evaluated the changes of metal concentrations in laboratory rearing tanks, the levels of copper and cadmium in the tanks decreased after 3 days from 0.2 to 0.0987 mg×dm^{-3}, and from 0.2 to 0.1408 mg×dm^{-3}, respectively. Dechlorinated tap water (temp. 22ºC±0.5, dissolved oxygen saturation about 90%, hardness 130 mg as $CaCO_3$×dm^{-3}, pH 6.7) was used. Water was constantly aerated using an air pump. The fish were fed brine shrimp nauplii three times a day (8 a.m. 14 p.m. 20 p.m.), and, from day 25 after hatching, additionally with dry feed. Natural summer fotoperiod was applied.

Development of 25 larvae of each group was registered daily using a MultiScan microscope and computer image analysis system. Photographs were taken from 1 to 30 days after hatching. The photographs were used for measurements of length (*longitudo caudalis*) and body perimeter areas of larvae. Body perimeter area was used instead of body mass in order to avoid fish mortality related to manipulation and blotting. Perimeter area of carp larvae well correlates with body mass [12].

The following growth measures were calculated for three periods: from 1 to 10 (when most fish showed exclusively exogenous feeding), from 11 to 20 (when the fish started to fill anterior swim bladder chamber), and from 21 to 30 days post hatching (when all fish from the control group filled their anterior swim bladder chambers):

GR_L- the rate of body length increase (mm per day):

$$GR_L = (L_t - L_0)/t$$

GR_A – the rate of body perimeter increase (2 mm per day):

$$GR_A = (A_t - A_0)/t$$

where: L_t– final body length after t days, L_0 – initial body length at the begining of measuring period, A_t – final body perimeter area after t days, A_0 – initial body perimeter area at the begining of measuring period, t – number of days between initial and final measurement in each period.

Statistical analysis was done using the Statistica package, and the significance of differences was tested using one-way ANOVA and Duncan's post-hoc test ($p \leq 0.05$).

Results

During the 30-days-long experiment body length of carp larvae increased from 5.98 to 14.61 mm in the control, and to 12.58, 10.94, and 12.46 mm in Cd, Cu and Cu+Cd groups, respectively (Fig. 1A). Larvae exposed to heavy metals were shorter compared to the control, already beginning from day 3 post hatching. Copper-exposed larvae were the shortest, and significantly smaller compared to the control from day 6 post hatching.

The body perimeter area increased faster in older larvae (Fig. 1B), and was lower in metal-exposed fish, particularly in the Cu group, which significantly differred from the control beginning from day 6 post hatching. At the end of the experiment the average body perimeter area of all metal-exposed fish were significantly lower compared to the control, in which it reached 35.11 mm^2, while in Cd, Cu, and Cu+Cd groups 27.53, 16.58, and 26.21 mm^2, respectively.

The absolute daily increase in body length (GR_L) was measured in 3 time intervals: from 1 to 10, from 11 to 20, and from 21 to 30 days of the experiment (Fig. 2A) show that growth rate was significantly slower in all metal-exposed groups, except for the last time interval, when very high variability in growth rate occurred, and despite lower average values (compared to control) growth rate was significantly reduced only in the Cu group.

The absolute daily increase of body perimeter area (GR_A) grew with fish age, particularly in the control (Fig. 2B). The rate of perimeter area of metal-exposed larvae was always significantly lower compared to the control, and from day 11, Cu-exposed fish grew significantly less comparing to Cd and Cu+Cd-exposed ones.

At the end of experiment the mortality in groups K, Cd, Cu, Cu+Cd were 0%, 35%, 55%, and 48%, respectively.

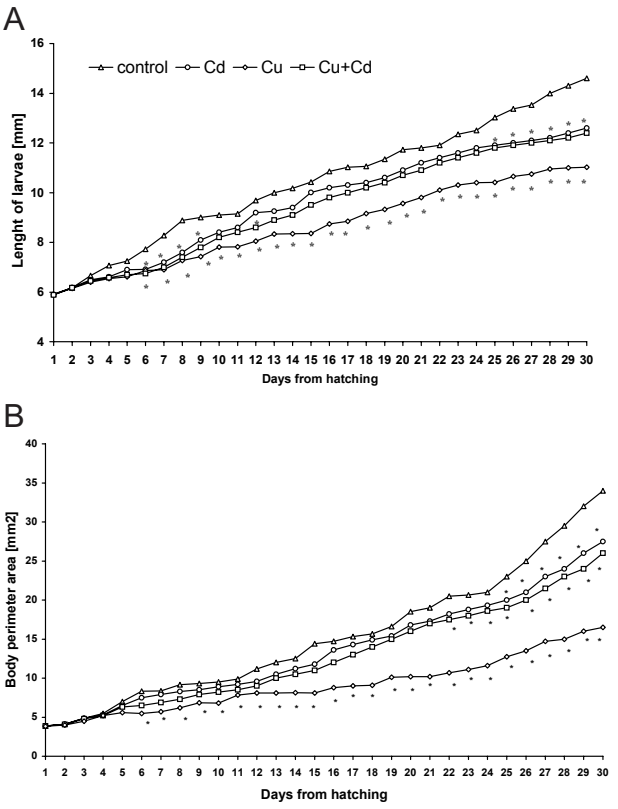

Fig. 1. Growth of carp larvae (A – mean body length, B – mean body perimeter area, n=25).
* - significantly different from the control at $p \leq 0.05$).

Discussion

In the present paper, a significant adverse effect of exposure to heavy metals on fish growth was observed. Similar results of reduced body length increase were obtained by Jezierska and Słomińska [13] for the common carp larvae exposed to Cu over the first 40 days of life. The curves for body length increase are similar to those obtained by [2, 7, 14]. In the present study, the mean body length of 30-day-old larvae exposed to Cd, Cu, and Cu+Cd were respectively: 86, 68, and 85% of the control values. Also, the rate of absolute body length increase (GR_L) of metal-exposed larvae were lower compared to the control. During the first days after hatching growth is related to utilization of yolk, and yolk sac diameter is an indicator of yolk resorption rate. Metal exposure may disturb that process. Hwang et al. [15] reported that *Oreochromis mossambicus* larvae treated with 0.2 mg dm^{-3} of copper for 4 days were shorter and had larger yolk sacs, compared to the control. Also, Morgan et al., [16] and Johnson et al., [17] observed slower growth and yolk resorption by fish larvae. According to Peterson et al., [7], 2 $\mu g \times dm^{-3}$ of cadmium reduced growth and yolk-conversion efficiency in *Salmo salar*. Sarnowski [18, 19] reported that Cu and Cd exposure resulted in a reduced rate of yolk sac resorption of *Cyprinus carpio* larvae.

Body perimeter area was even more affected by heavy metal exposure of larvae than body length. The perimeter area increase was slower in all metal-exposed fish, and the final values were lower (Fig. 1B), in Cd, Cu, and Cu+Cd groups equal to 80.8, 48.5, and 76.5%, respectively, of the values obtained for the control group. These data indicate that the larvae change their shape and become wider after the yolk sac resorption when they feed exclusively exogenously (11-20 days from hatching), and then after swim bladder inflation (21-30 days post hatching) which results in a considerable increase in body perimeter area [13], and an increase in GR_A (Fig. 2B). Similar increase in growth rate (in terms of body mass) in successive 10-day intervals after hatching of *Sparus sarba* was reported by Wong et al. [20]. According to Monteiro [21], who developed the individual growth model for *Engraulis encrasicolus* at successive stages of larval period, older larvae show greater body mass increase. A similar reduction of body weight increase was observed in rainbow trout exposed to Cd and Hg [22]. The absolute daily increase in body perimeter area (GR_A) was significantly lower in all metal-exposed groups compared to the control in all three time intervals (Fig. 2B). Similar reduction of *Sparus sarba* growth rate in terms of weight was observed after copper exposure [20].

The effect of metals on fish growth rate becomes clearly visible particularly during long-term exposure. The results of various studies presented by Jezierska and Witeska [9] indicate reduced rate of body length or mass increase in long-term metal treatments. The data also show that body mass is more sensitive to metal intoxication than length. According to Shukla and Pandey [23], the growth rate of *Opiocephalus punctatus* was reduced by 11% and 16% for length and mass, respectively, under the stress of cadmium. Some authors reported that metal exposure reduced body mass increase, while body length was the same as in control. Vosyliene and Petrauskiene [24] studied growth of *Oncorhynchus mykiss* at various copper concentrations (0.025-0.2 $mg \times dm^{-3}$), and observed no changes in body length increase, but the increase in body mass was reduced after 2 and 3 months of exposure to 0.1 and 0.2 $mg \times dm^{-3}$. Growth rate reduction is, according to some authors, related to a decrease of food intake [18, 25-30]. Reduction of feeding activity of fish exposed to heavy metals was observed by various authors [28, 31-34]. According to Woo et al. [28], *Oreochromis aureus* at 0.5-20 $mg \times dm^{-3}$ of cadmium were anorectic and lost weight. Holdway [35] observed a decline of body growth rate in two species of tropical fish treated with uranium. They also supposed that the fish lost appetite. Appetite loss and reduced growth rate in copper-exposed *Oncorhynchus kisutch* was reported by Buckley et al. [36]. Reduction of feeding activity may be related not only to appetite loss but also to the difficulties in prey capture. According to Farkas et al. [37], especially initial period of fish larval life depends on available food base and efficiency of food uptake, and any adverse factors that impair feeding efficiency may therefore adversely affect larval growth and development. Also, our own studies indicate that exposure to heavy metals reduced live food

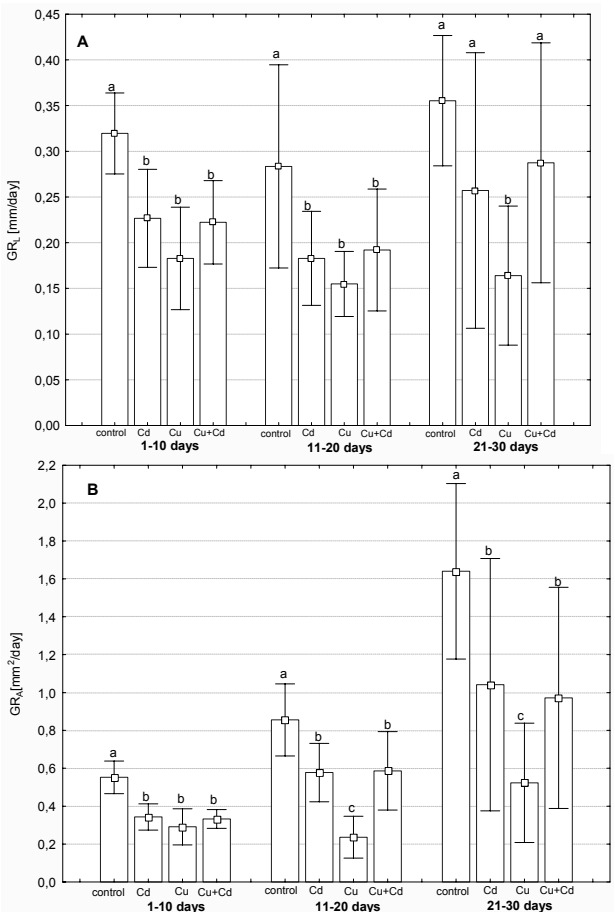

Fig. 2. Absolute growthrate of carp larvae (A − body length, B − body perimeter area, n=25.
a≠b≠c at p≤0.05).

capture and intake by carp larvae [34]. However, Bryan et al. [38] reported that growth was a more sensitive end point than were feeding behaviors, which suggests that reduced food intake was not the only factor contributing to reduced growth rates in *Lepomis macrochirus* exposed to cadmium.

Our results indicate that body perimeter area is more sensitive to metal intoxication than body length. Therefore, this parameter, well correlating with body mass, [12] seems useful for evaluation of the effects of various environmental factors on growth of fish juveniles, and can be used instead of body mass, particularly in cases of fish larvae that are easily damaged due to manipulation during weighing.

Reduction of fish growth by heavy metals may be related to their toxic effects on various physiological processes in developing young fish. The data obtained by various authors indicate that fish growth is related not only to food intake but also to the utilization rate, and energy conversion. All these processes may be disturbed by toxic action of metals. Fish growth rate may also be adversely affected by other metal-induced metabolic disturbances, such as disorders in water-electrolyte balance [16, 39-42]. Ion balance is essential for metabolic functions of the organism and any disturbances may result in reduced growth rate. Another important point is a reduction of protein synthesis by heavy metals – protein is one of the main body components, and its level considerably increases at early developmental stages [2]. Relationship between growth rate and the level of nucleic acids, and RNA/DNA ratio, which is an indicator of protein synthesis rate, is stressed by many authors [31, 43]. Cleveland et al. [31] reported that the reduced growth was confirmed by reductions in RNA content, and RNA/DNA ratio in *Salvelinus fontinalis* exposed to aluminum at pH 5.5. Barron and Adelman [44] observed a reduction of RNA and DNA content, accompanied by a decrease in RNA/DNA ratio in *Pimephales promelas* treated with 8-10 $mg \times dm^{-3}$ of chromium for 28 days. The fish showed also decreased rate of mitosis, reduced protein synthesis, and reduced growth. According to Woodward et al. [45], reduction in RNA/DNA ratio in swim-up alevins exposed to 50 $\mu g \times dm^{-3}$ of aluminum at pH 5.5 correlated with lower growth.

Decrease of energy conversion for growth may be related to activation of metal detoxification. That is suggested by Marr et al. [46] who stress the high metabolic cost of this process. The effect of cadmium and copper on fish endocrine system might have been another cause of reduced growth of metal-exposed fish because fish growth is under hormonal control [2]. Jones et al. [47] noted that cadmium delays growth hormone expression during *Oncorhynchus mykiss* development. Lower levels of T_4 and T_3 in fish from water polluted with various toxicants including cadmium and copper, were reported by Hontela et al. [48], and Levesque et al. [49].

The results of the present study show different toxicity of cadmium, copper, and their mixture to carp larvae, copper (0.2 $mg \times dm^{-3}$) being the most toxic, which is indicated by the slowest increase in both body length and perimeter area. The Cd+Cu mix also contained 0.2 $mg \times dm^{-3}$ of metals: 0.1mg of Cd and 0.1 mg of more toxic Cu but growth curves (Fig. 1A, B) for Cu+Cd group are similar to those for the Cd group.

The values of GR_L and GR_A of copper-exposed larvae were significantly lower not only from the control but, for GR_A in 11-20 and 21-30 days intervals, also from Cd and Cu+Cd, while the values for Cu+Cd are very similar to those for Cd (Fig. 2 A, B).

Therefore, the obtained results indicate that copper is more toxic for common carp larvae compared to cadmium. Exposure to Cu caused a significant reduction in both weight and length compared not only to control fish but also to fish exposed to Cd and Cu+Cd. Higher toxicity of copper compared to cadmium for early developmental stages of common carp was reported also by other authors [14, 20]. Stronger inhibition of carp growth by copper compared to cadmium may be related to more pronounced reduction of carp feeding activity – live prey capture by this metal [34].

The obtained results also indicate an interaction between Cu and Cd in mixture and suggest that the presence of Cd reduces the toxic effect of Cu. Thus, it seems that Cd is an antagonist to Cu. Interaction of copper and cadmium toxicity can be related to the interaction of these metals in accumulation in various tissues in which they disturb metabolic processes related to growth. Reduction of toxicity of one metal by another could result from competitive uptake and accumulation by the tissues [50-52]. Pelgrom et al. [53] studied how interaction between copper and cadmium modified metal organ distribution in *Oreochromis mossambicus*, and found that combined Cu+Cd exposure resulted in lower Cu concentration in the liver when compared to the liver Cu concentration of fish exposed to Cu singly. Therefore, it is possible that cadmium-induced reduction of copper accumulation and its toxic effect on metabolic functions might have resulted in moderation of copper-induced inhibition of carp larvae exposed to Cu+Cd mixture.

Conclusions

Copper is more toxic for common carp larvae compared to cadmium, and a mixture of both metals which indicates a possible antagonism of cadmium against copper toxicity.

Body perimeter area is more sensitive to metal intoxication than body length, being a good approximation of body mass for very small fish that cannot be weighed.

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