Polish J. of Environ. Stud. Vol. 19, No. 1 (2010), 227-230

Original Research

Effects of Copper and Cadmium on Growth and Yolk Utilization in Barbel (*Barbus Barbus* L.) Larvae

Małgorzata Witeska*, Katarzyna Bilska, Piotr Sarnowski**

Department of Animal Physiology, University of Podlasie,
Prusa 12, 08-110 Siedlce, Poland

Received: 31 July 2009
Accepted: 9 October 2009

Abstract

Barbel eggs and fry were exposed from fertilization until 14 days after hatching to 100 µg/l of copper or cadmium. Cadmium, but not copper, significantly reduced larvae survival. Both metals significantly decreased larval growth. The differences in fish body size between the controls and metal-exposed groups increased in time, and were more pronounced in Cd-intoxicated fish. Copper-exposed larvae started exogenous feeding 1 day later than the control. Cadmium-exposed larvae started feeding 4 days after the controls. Both metals reduced yolk utilization rate. The results demonstrated that cadmium was more toxic to barbel larvae than copper.

Keywords: fish, heavy metals, toxicity, development, feeding

Introduction

Waterborne heavy metals are highly toxic to the early developmental stages of fish [1]. Sublethal levels may reduce larval growth [2-5] due to appetite loss [6] or inhibition of digestive tract development [7]. Heavy metals may also reduce the rate of utilization of endogenous nutrient stores of the yolk sac [8-10].

Barbel *Barbus barbus* is a rheophilous cyprinid fish inhabiting rivers throughout Europe, excluding the Italian, Greek and Iberian peninsulas. They are found in deeper, fast-flowing upper reaches of rivers that have stony or gravel bottom. Barbel feed chiefly on benthic invertebrates such as small crustaceans, insect larvae, mollusks, mayfly and midge larvae. Spawning occurs from May to July [11]. In Poland, the barbel is regarded as a threatened species [12]. It is rarely bred and reared in hatcheries, and the data on early development of this species are scarce. A detailed description of embryonic development of this species was given by Ługowska [13]. Nothing, however, is known about the sensitivity of barbel larvae to environmental contaminants such as heavy metals.

Copper is an important essential element, while cadmium does not play any known metabolic role in fish. Both metals are toxic to fish [14]. In unpolluted freshwaters their concentrations are low: Cu levels usually range from 2 to 4 µg/l, while the levels of Cd range from 0 to 13 µg/l. Polluted freshwaters may contain increased concentrations of both metals: Cu – 1-137 µg/l, and Cd – 40-120 µg/l. In strongly polluted waters levels may reach 500-2,000 µg/l, and up to 4,000 µg/l, respectively [15]. Both metals may be leached from bottom sediments under acidic conditions, e.g. during snowmelt, thus the concentrations of free Cu and Cd ions may abruptly increase for a short time, particularly in spring, at the time of fish spawning, and during embryonic and larval development.

*e-mail: wites@ap.siedlce.pl
**e-mail: p_sarski@ap.siedlce.pl

The aim of the present study was to evaluate the effects of copper and cadmium at concentrations of 100 µg/l on growth and yolk utilization of barbel larvae.

Materials and Methods

The study was conducted in spring 2007 (K1, Cu groups) and 2008 (K2, Cd groups). Barbel eggs and sperm were obtained from the hatchery of the Inland Fisheries Institute in Żabieniec. The eggs were fertilized *in vitro*, and incubated in 2 l aerated aquaria in clean dechlorinated tap water (K1 and K2), or in the same water containing 100 µg/l of copper (Cu) or cadmium (Cd). After hatching, 40 larvae from each group were reared under the same conditions as the embryos (except for higher temperature). Water hardness was 162 mg/l $CaCO_3$, pH 7.8-8.0, and dissolved oxygen saturation level ≥ 80%. Concentrations of major ions were (in mg/l): Na^+ – 3.66, K^+ - 1.12, Ca^{2+} - 61.10, Mg^{2+} - 6.1, HCO_3^- - 195.60, Cl^- - 6.40, SO_4^{2-} - 9.17, and NO_3^- - 5.76. Metal solutions were prepared using $CuSO_4 \times 5H_2O$ or $CdCl_2 \times 2½H_2O$, respectively. Water temperature was maintained at constant levels of 18°C for embryos and 20°C for larvae, using aquatic thermostats. Water was renewed daily to maintain a constant level of metals and to remove metabolites. Feces and non-consumed food were siphoned out. Feeding commenced 3 days post hatching, when the larvae were supplied with *Artemia* sp. nauplii 4 times a day *ad libitum*.

For all 14 days from hatching, the larvae were counted and photographed using a camera connected with binocular and computer image analysis system MultiScan. Each larva, in water, was placed in a concave glass slide for the procedure that lasted no more than 15 s, after which the fish was put back into the aquarium. Measurements of larval body perimeter area (without yolk) and yolk sac lateral perimeter area were done on photographs. The initiation of feeding was defined as the moment when at least 50% of the fish showed food in the digestive tract visible through the transparent body wall.

The differences in fish body and yolk sac size between metal-exposed and control groups, and between both control groups were calculated using Student t-test, at $p \leq 0.05$.

Results

Copper exposure did not reduce the survival of barbel larvae (85% in both, K1 and Cu groups, Fig. 1), while in cadmium-contaminated water most larvae died (survival 100% in K2, and 5% in Cd, Fig. 1).

Initial body perimeter area ranged from 4.48±1.44 mm² in Cd group to 6.29 mm² in K1. Body perimeter area was lower in both metal-exposed groups compared to the controls (Fig. 2) but in Cu group the difference became significant on day 4 post hatching, while the Cd-exposed larvae were smaller compared to K1 already at hatching. The differences in fish size remained significant until the end of the experiment, and increased with time, more in the Cd group in which growth was practically inhibited from day 11 post hatching. At the end of the experiment, final body perimeter area was the highest in both control groups: 31.72±1.30 mm² in K1, and 31.00±1.54 in K2, lower in Cu (26.35±1.25), and the lowest in Cd (19.06±0.00).

Both metals reduced yolk utilization by the fish (Fig. 3). At hatching, yolk size was similar among the controls and metal-exposed fish, ranging from 5.29±0.47 mm² in K2 to 5.60±0.31 mm² in Cu. Beginning from day 4 post hatching, the yolk sacs of control fish were significantly smaller compared to the Cu and Cd-exposed fish, and the differences between the controls and metal-exposed larvae increased with time, and were more pronounced in the Cd group. All fish from K1 group completely resorbed their yolk sacs by day 11 post hatching, while those from K2 had completely resorbed their yolk sacs by day 12. In both metal-exposed groups, complete yolk resorption in all fish occurred by day 14 post hatching.

Fish from K1 were slightly but significantly larger than the K2 larvae from 1 to the 13 days post hatching. On the contrary, their yolk sacs were smaller comparing to the K2 group between days 5 and 8, and larger on day 10.

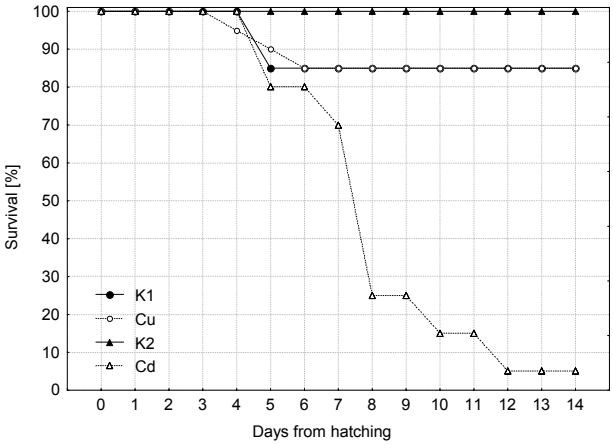

Fig. 1. The effect of exposure to 100 µg/l of Cu or Cd (beginning from fertilization) on survival of barbel larvae (K1 – control for Cu, K2 – control for Cd).

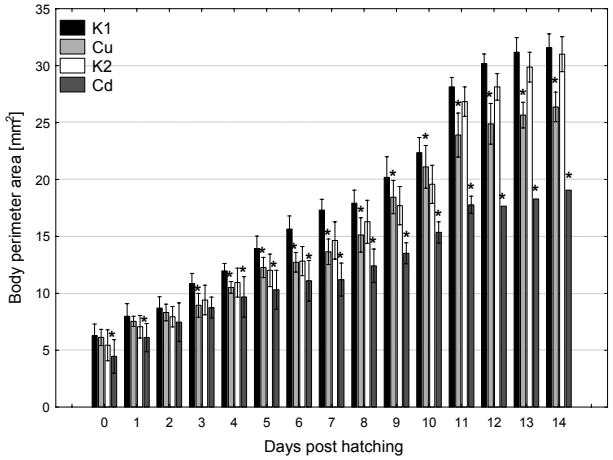

Fig. 2. The effect of exposure to100 µg/l of Cu or Cd on body size of barbel larvae (K1 – control for Cu, K2 – control for Cd, * - values different from the control on particular days).

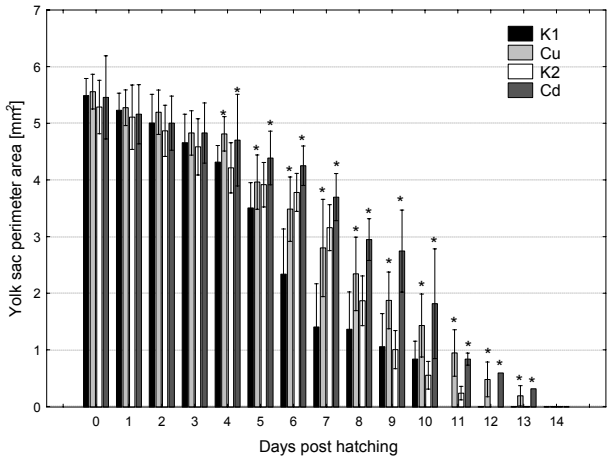

Fig. 3. The effect of exposure to100 µg/l of Cu or Cd on yolk sac size of barbel larvae (K1 – control for Cu, K2 – control for Cd, * - values different from the control on particular days).

Fish in both control groups (K1 and K2) started exogenous feeding on day 7, Cu-exposed larvae on day 8, and Cd-exposed fish on day 11.

Discussion

The results in fish size, yolk size, and beginning of exogenous feeding between control groups K1 and K2 probably resulted from parental factors, since in each season the larvae originated from different spawners. According to Pakkasmaa et al. [16], metabolic rates of *Salvelinus alpinus* significantly differed among the offspring of various parents.

The results show that copper and cadmium adversely affected the growth, yolk utilization, and feeding ability of barbel larvae. Cadmium was more toxic compared to copper. Survival of barbel larvae in the control groups and Cu treatment were similar to those obtained under normal controlled conditions [17]. Cadmium was more toxic to barbel larvae than was copper. Our earlier studies on common carp (*Cyprinus carpio*) revealed higher toxicity of the latter [3, 18] which suggests different metabolisms of metals in these species.

Cadmium was not only more toxic to barbel larvae, compared to copper, but also reduced growth, yolk utilization, and inhibited commencement of exogenous feeding. Reduction of growth due to copper or cadmium intoxication may result from various metabolic disturbances. According to Couture and Kumar [19], both metals reduce oxidative metabolism in fish due to a direct inhibition of mitochondrial enzymatic activity, and increase protein metabolism. A decrease in oxygen consumption in copper-exposed common carp were reported by De Boeck et al. [20]. Both metals also disturb ion balance in fish, which may result in growth reduction due to the increased metabolic cost of compensatory osmoregulation. According to McGeer et al. [21], rainbow trout larvae exposed to copper or cadmium showed significantly reduced body sodium and calcium levels. Another possible cause of growth reduction by heavy metals are metabolic costs of detoxification. Giguere et al. [22] reported that most hepatic Cd and Cu in *Perca flavescens* were found in the heat-stable cytosolic peptides and protein fraction including metallothioneins, the synthesis of which is induced by metal presence in the organism [23]. The difference in body size between K1 and Cu became significant 3 days post hatching, while in the Cd group it was already significant at hatching. This shows that cadmium was probably more efficient at penetrating barbel eggs. Cadmium was also more toxic to the embryos than was copper.

Metal-exposed fish showed lower rates of utilization of yolk sac nutrient stores as indicated by slower yolk sac absorption. Yolk is the exclusive source of nutrients to early larval stages of fish, and yolk material is partitioned between growth and metabolism [24]. According to various authors, heavy metals may delay yolk absorption in fish larvae. Wu et al. [8] reported reduced yolk utilization rate in *Oreochromis mossambicus* larvae exposed to 30-400 µg/l of copper, while Hwang et al. [25] observed the larvae of the same species, treated with 200 µg/l of copper, had not only larger yolk sacs but were also shorter in length compared to the control. Yolk sac absorption rate in *Danio rerio* larvae subjected to 50-1,090 µg/l of copper was reduced in a concentration-related way [9]. Sarnowski [10] reported delayed yolk sac absorption in common carp treated with 200 µg/l of copper or cadmium. According to Peterson et al. [5], cadmium reduced growth and efficiency of yolk utilization by *Salmo salar* larvae. The reduction of the yolk utilization rate under conditions of heavy metal intoxication might have resulted from reduced metabolic rate or from a direct effect of metals on yolk, such as coagulation of yolk material [7] that rendered nutrients unavailable to fish.

The later onset of exogenous feeding in metal-exposed groups of barbel larvae might have resulted from lowered appetite [4, 20, 26, 27], impaired ability to catch prey [10, 28], or disturbances in development of the digestive tract [7]. Delayed exogenous feeding contributed to the increase in difference of body size between the controls and metal-exposed groups at the end of the experiment.

Under natural conditions, smaller and slower growing larvae are more susceptible to predation, and are less efficient in food competition. Therefore, even a short-term water contamination episode with copper, and especially cadmium, during early barbel development may considerably threaten the population. Cadmium is particularly toxic to barbel larvae and, besides the adverse effect on growth and feeding, it considerably reduces larval survival.

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