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THE EFFECTS OF METALS ON SWIMBLADDER INFLATION OF COMMON CARP (*CYPRINUS CARPIO* L.) LARVAE

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ABSTRACT

The aim of the present study was to evaluate, using the microscope and computer image analysis system MultiScan, the effects of Cu (0.2 mg·dm $^{-3}$) and Cd (0.2 mg·dm $^{-3}$) on swimbladder inflation by common carp (*Cyprinus carpio* L.) larvae under laboratory conditions. The fish were exposed to metals for 30 days from hatching. The results indicate that heavy metals considerably inhibited inflation of the posterior and anterior chamber of swimbladder. Metals affected inflation of each swimbladder chamber in a different way. They reduced the rate of inflation of first (posterior) chamber, delayed the beginning of inflation of the second (anterior) chamber, and inhibited its growth. Metal exposure resulted in differences among the larvae: some of them inflated the anterior chamber, and the others failed to inflate it. At the end of the experiment, 100% of control fish showed this chamber inflated, while in Cu and Cd-exposed groups 24 and 21%, respectively, lacked the anterior chamber. It was also observed that the anterior chamber is inflated with the gas from the posterior one. Swimbladder inflation is necessary for correct development and good condition of fish. The effect of heavy metals on swim bladder inflation indicates their toxic influence on fish larvae.

Key words: carp larvae, heavy metals, swimbladder

INTRODUCTION

The fish swimbladder is a hydrostatic, buoyancy-regulating organ. It plays also a role in hearing, by amplifying sounds registered by Weber's organ.

The swimbladder develops already during embryonic phase from an esophagus wall projection. Later on, it grows towards the tail, under the spine and kidneys, as a thin-walled sac. The sac differentiates into the swimbladder and a narrow pneumatic duct connecting the bladder with esophagus. In the open-bladder fish – *Physostomi*, the duct is present over entire fish life.

The carps are open-bladder fish, and their swimbladders consist of two chambers. During larval development at 22°C, common carp larvae inflate posterior chamber on the 2-3 day after hatching. The posterior chamber is an exclusively hydrostatic organ. Fish swim to the surface, ingest air, and forces it through the pneumatic duct into the bladder. In 12 days from hatching, inflation of the anterior chamber starts. It is inflated with gas from posterior chamber, through a short and narrow interconnecting duct. Correct swimbladder inflation is essential for further fish development.

The inflation of swimbladder may be affected by various environmental factors. Martin-Robichaud, Peterson [13] observed that more larvae of *Morone saxatilis* inflated the bladders at lower light intensity. That was also confirmed by Uotani et al. [22] who reported that *Engraulis japonicus* larvae exposed to intense light (200 lux) failed to inflate the bladders. Inhibition of swimbladder inflation in common carp larvae may result from water acidification to pH 5.0-5.2 [11], or alkalization up to pH 10.2-10.3 [15].

According to various authors, also toxic agents present in the water may adversely affect swimbladder inflation. Inflation inhibition by pesticides was reported by Henry et al. [6], Kim and Cooper [10], and Hamm [5]. Similar action of heavy metals was observed by Holdway [7], Stouthart et al. [21], and Słomińska [19].

In the present study, the effects of copper and cadmium on inflation of each swimbladder chamber in common carp larvae were studied.

MATERIALS AND METHODS

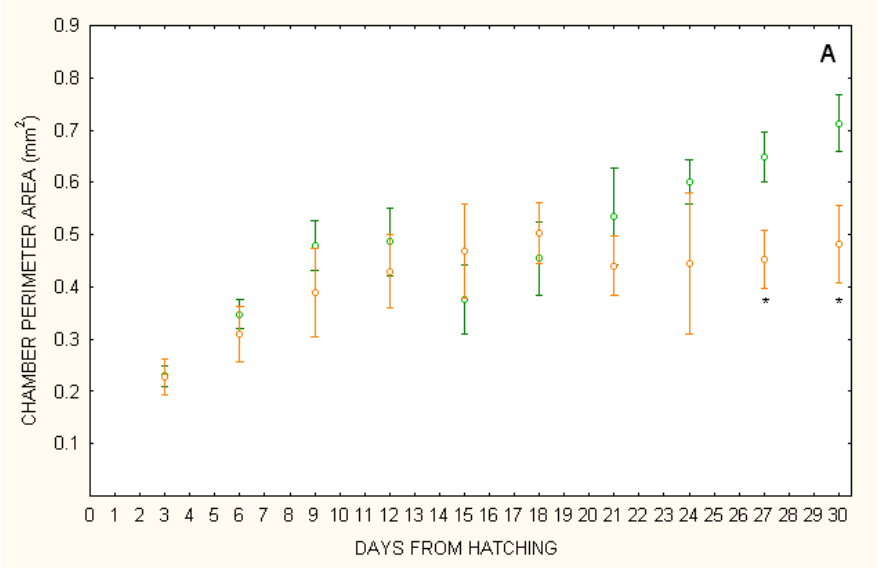
Experiment was done in two series: series I (2002) and series II (2003). Correctly developed fish (incubation took place under optimum conditions described by Ługowska and Jezierska [12]) were kept in the water recirculation tanks. One day old larvae were divided into 3 groups: control, and copper or cadmium-exposed. The fish were placed in tanks with tap water, 0.2 $mg \cdot dm^{-3}$ Cu (as $CuSO_4 \times 5H_2O$), or 0.2 $mg \cdot dm^{-3}$ Cd (as $CdCl_2 \times 2½H_2O$), at the density of 300 fish per 180 l. Metal concentrations used in the experiment were established basing on the results of preliminary experiments and literature data [8, 20, 23], so as they significantly affected the larvae, without causing high mortality. Water was changed every 3 days to maintain metal concentrations in the water. Dechlorinated tap water (temperature 22°C, dissolved oxygen saturation about 90%, hardness 210 mg $CaCO_3 \cdot dm^{-3}$, pH 6.7) was used. The fish were fed brine shrimp *nauplii ad libitum*, three times a day. Fifty fish were randomly sampled every three days from each group in series I, twenty five fish were randomly sampled every day in series II. Their development was registered using the microscope and computer image analysis system MultiScan. Daily examination of fish in series II allowed for detailed analysis of swim bladder inflation. The experiment was terminated on the 30 day from hatching, when all larvae in the control completely inflated the anterior swimbladder chamber. The areas of swimbladder perimeters were measured in the photographs (Fig.1). Statistical analysis was done using the Statistica package, and the significance of differences was tested using one way ANOVA ($p \leq 0.05$). The abbreviations: ACS (anterior chamber of swimbladder) and PCS (posterior chamber of swimbladder) are used in the text.

Fig. 1. The measurement of swim bladder perimeter area

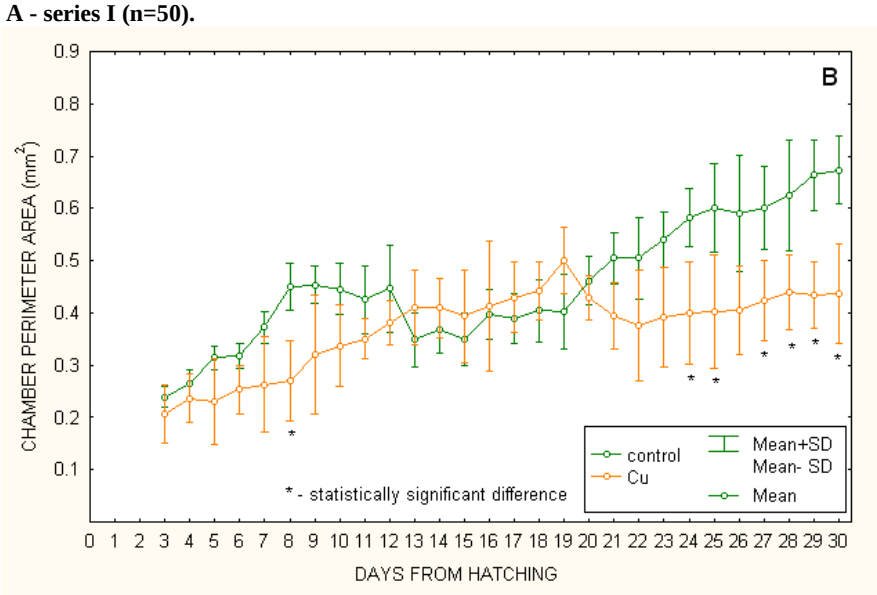
RESULTS

The results show the effect of metals on the size of PCS which was inflated first. Carp larvae started to inflate this chamber on the 3 day from hatching. Begining from this day, PCS in the control group (Fig. 2) increased in size until the 12 day when a temporary decrease occurred. From the 16 day, the length of PCS started to increase again (Fig. 2B). At the end of the experiment, the average perimeter area of the PCS in the control groups was 0.71 mm^2 in series I and 0.67 mm^2 in series II. The metal-exposed fish also started to inflate PCS until the 3 day post hatching. However, from the 5 to 11 day, and from the 21 to 30 day from hatching, the swim bladder of copper-exposed fish (Fig. 2) was significantly smaller comparing to the control. In these larvae, the perimeter area of PCS increased until the 19 day (Fig. 2B). On the next day, the size of this chamber sharply decreased, and remained reduced for 3 days. On the 23 day the chamber started to grow again. High individual variability occurred in this group of larvae, among which the individuals with large and small swim bladders were present. The perimeter area of PCS in copper-exposed fish was 0.48 mm^2 in series I and 0.44 mm^2 in series II, which comprised about 67% of the PCS size in the control fish.

Fig. 2. The effect of copper on PCS inflation



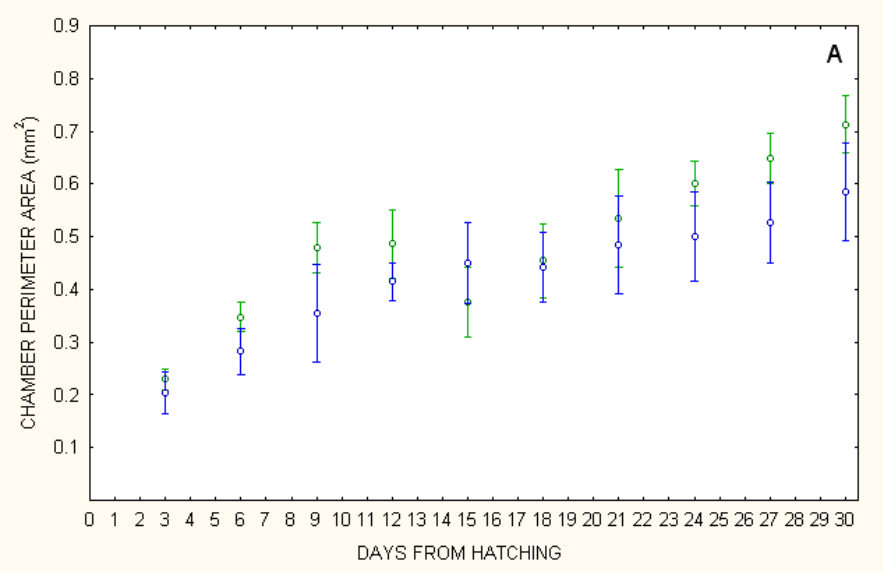
A - series I (n=50).



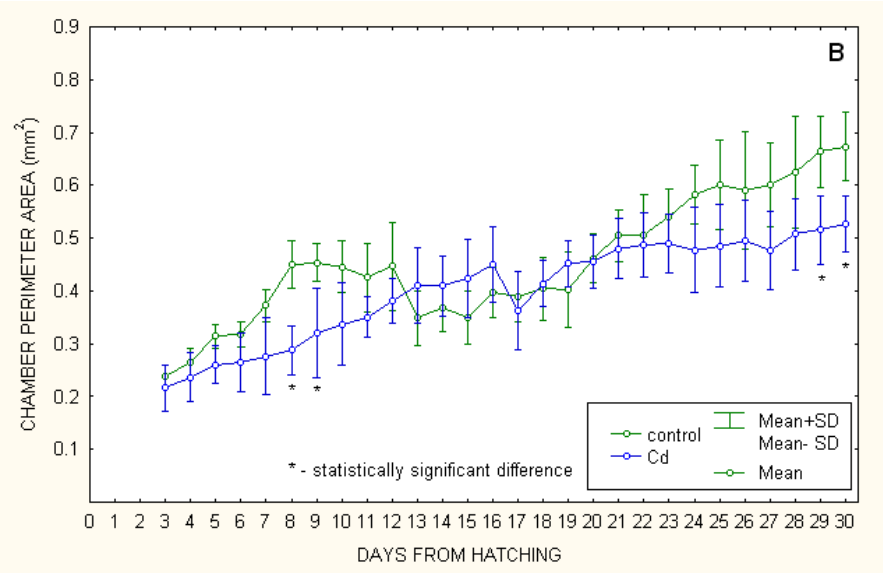
B - series II (n=25).

In cadmium-exposed fish, the PCS grew until the 16 day from hatching (Fig. 3B). On the 17 day the size of chamber decreased, and remained reduced for 2 day. On the 19 day the PCS reached its previous size, then still increased, and at the end of the experiment reached the average area of 0.58 mm^2 in series I and 0.53 mm^2 in series II. It was significantly smaller comparing to the control (comprising about 80% of the control value).

Fig. 3. The effect of cadmium on PCS inflation



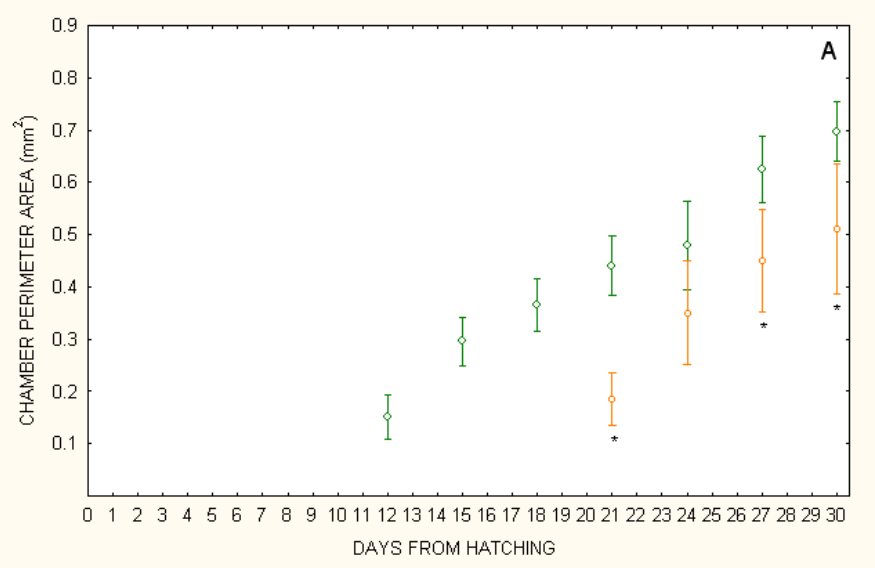
A - series I (n=50).



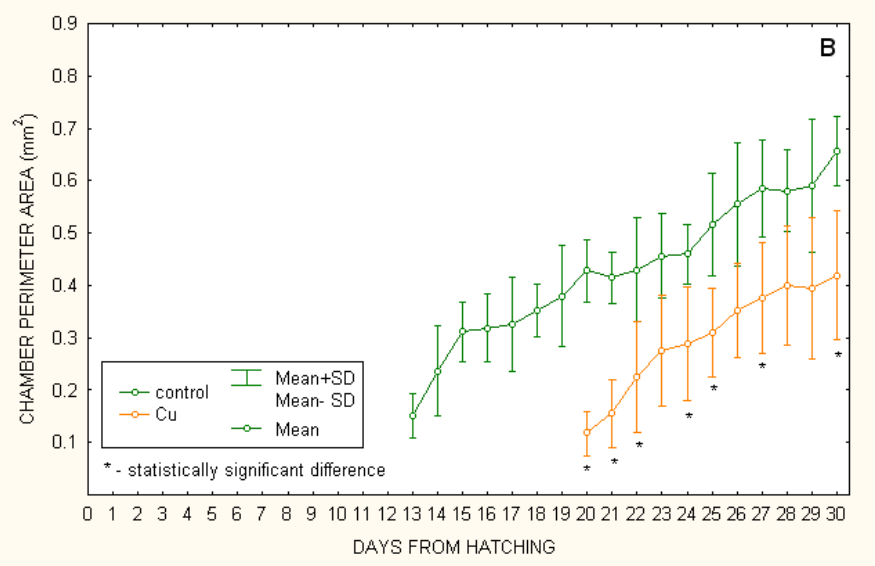
B - series II (n=25).

Heavy metals affected also the perimeter area of ACS. In the control (Fig. 4), fish started to inflate it on the 12-13 day post hatching. Beginning from that day, the size of ACS increased until the 30 day, and at the end of the experiment reached 0.68 mm^2. The Cu-exposed fish started to inflate the ACS from 20 day (Fig. 4B). At the end of experiment its perimeter area was 0.51 mm^2 in series I and 0.42 mm^2 in series II comprising 72 and 63% of the control (statistically significant difference). It seems interesting that in some larvae ACS size was similar as in the control, while the other ones (about 25%) did not inflate it at all.

Fig. 4. The effect of copper on ACS inflation



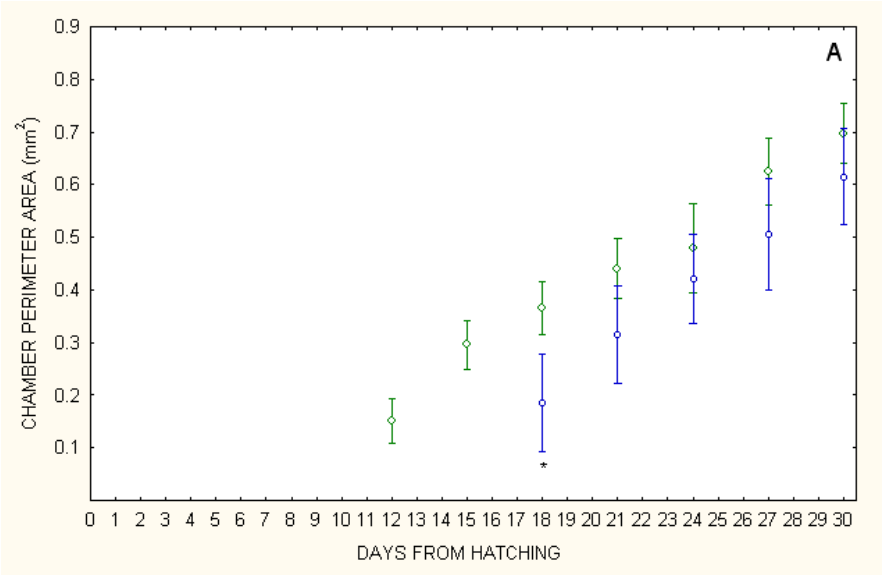
A- series I (n=50).



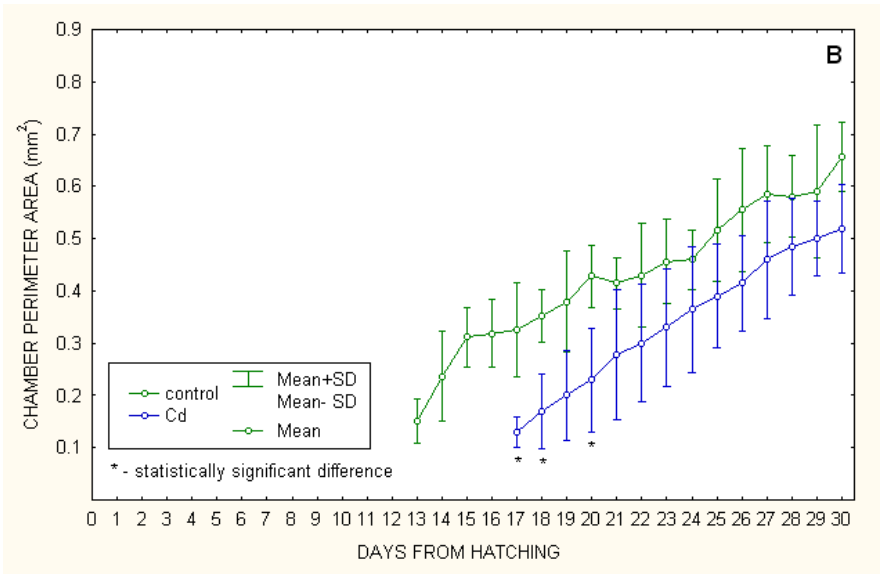
B-series II (n=25).

The Cd-exposed larvae started to inflate their ACS on the 17 day from hatching (Fig. 5). At the end of the experiment, its average size increased to 0.62 mm^2 in series I and to 0.52 mm^2 in series II, comprising about 87 and 78% of the control.

Fig. 5. The effect of cadmium on ACS inflation



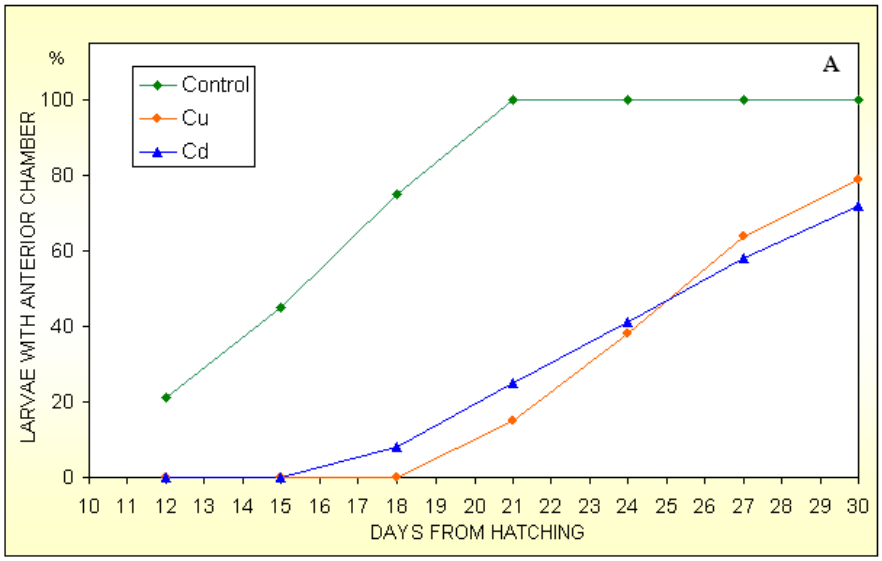
A- series I (n=50).



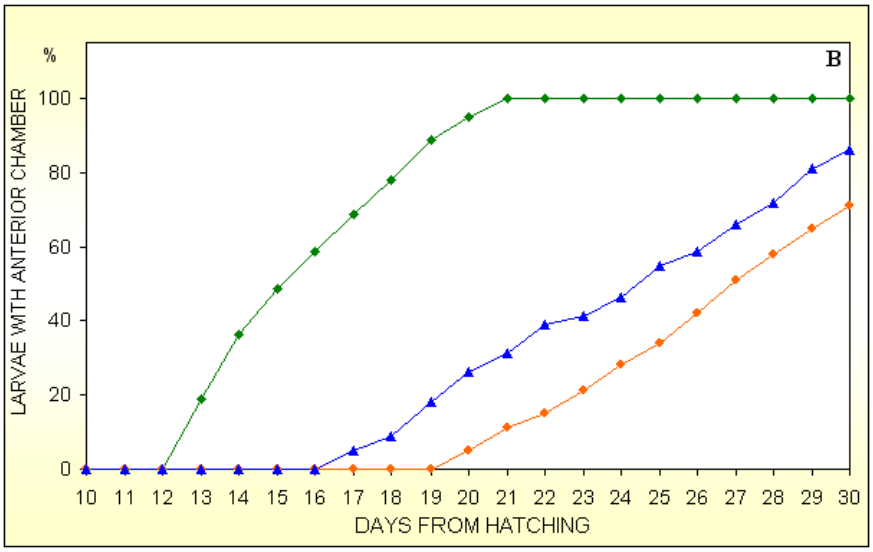
B-series II (n=25).

Fig. 6 shows the percentage of larvae with inflated ACS on the consecutive days post hatching. The results show that not all the larvae started to inflate ACS at the same time. In the control, its inflation started on the 12 or 13 day from hatching (series I or II, respectively). At that time about 20% of control larvae showed two-chambered swimbladder, and within 3 days - 65%. On the 21 day of the experiment 100% of control larvae (in both series) inflated the ACS. The Cu-exposed larvae started to inflate the ACS later – on the 20 day (Fig. 6B), and at that time ACS was present in only 9% of larvae. At the end of the experiment (30 day from hatching) only 78 and 67% (series I and II, respectively) of Cu-exposed larvae had the inflated ACS. The Cd-exposed fish initiated ACS inflation on the 17 day. On the next day only 10% of larvae showed it inflated, and on the 30 day - 72 and 85% (series I and II, respectively).

Fig. 6. The effect of metals on ACS inflation



A- series I (n=50).



B-series II (n=25).

In Fig. 7 a normal, correctly developed 26 days old larva from the control group is shown, and in Fig. 8 – Cu-exposed larva at the same age, that failed to inflate the ACS. This larva was smaller, and lack of anterior chamber resulted in incorrect distribution of internal organs which affected the spine shape in the anterior part of fish body.

Fig. 7. Carp larva twenty six days from hatching

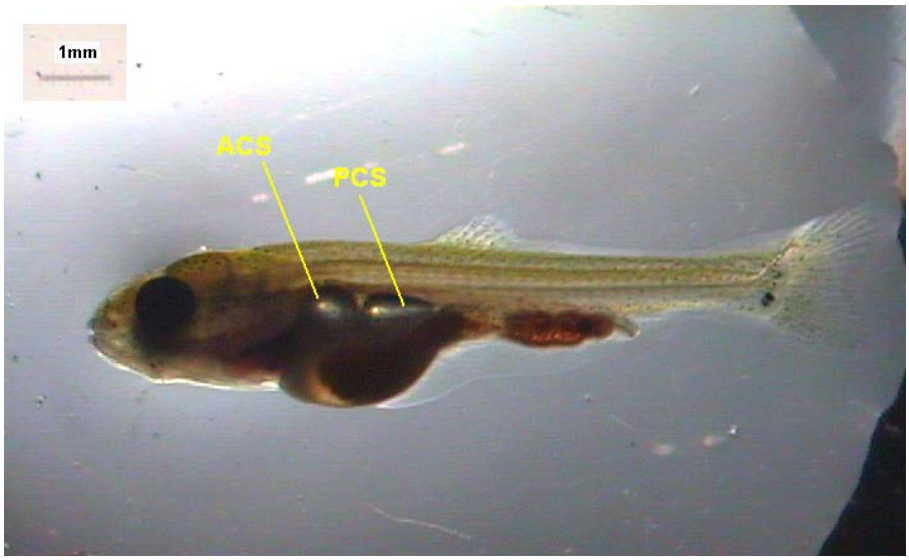
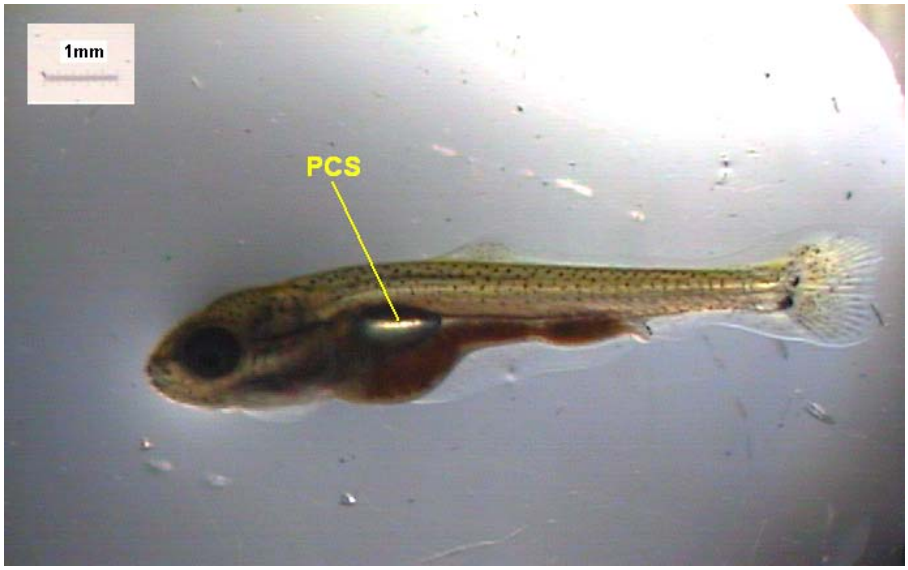


Fig. 8. Cu-exposed carp larva twenty six days from hatching without anterior chamber



DISCUSSION

The data obtained by various authors indicate that waterborne metals may adversely affect fish swimbladder inflation [7, 19, 21]. These authors, however, did not report at which stage the inflation was inibited. The results of the present study indicate that metals affect common carp swimbladder inflation in various ways.

Metals reduce the inflation of posterior chamber (PCS) (Figs 2, 3). At the end of the experiment (30 days from hatching) in metal-exposed fish this chamber was significantly smaller then in the control (measured as perimeter area) – in Cu-exposed fish the PCS size was 68 and 66% of the control (series I and II), and in Cd-exposed one - 87 and 79%.

Metals delay the beginning of inflation of the anterior swim bladder chamber (ACS). Control larvae start to inflate ACS 13 days from hatching, Cu-exposed – 20 days and Cd-exposed 17.

Metals reduce the ACS size (measured as perimeter area) (Figs 4, 5). At the end of the experiment the ACS size in Cu-exposed fish was 72 and 63%, (series I and II) and in Cd-exposed fish 87 and 78% of the control.

The obtained results show also lower differentiation among the control larvae comparing to the metal-exposed ones (Fig. 6). At the beginning of ACS inflation (the 13 day from hatching) 20% of larvae inflated it, and within the 8 days (the 21 day from hatching) all the control larvae showed both swimbladder chambers inflated. In the Cu-exposed group, at the ACS inflation onset (the 19 day from hatching) only 10% of larvae show this chamber, and within 11 days (the end of the experiment) 22.5 and 33% of larvae (series I and II) still lacked the ACS. In the Cd-exposed larvae, ACS inflation lasted over 13 days, and at the end of the experiment 28 and 15% (series I and II) of fish failed to inflate it.

Similar results were obtained by Słomińska [19] who reported that carps exposed from hatching to 0.2 $mg \cdot dm^{-3}$ of copper started to inflate the ACS later comparing to the control. She also found the individuals lacking the inflated ACS among the 40 days old fish.

The comparison of figures (Figs 2, 3, 4, 5) concerning the changes in size of each swim bladder chamber indicates that the decrease in PCS takes place at the same time that the beginning of inflation of the ACS. It indicates that at this moment a large portion of gas passes from the PCS to ACS. Duration of PSC size reduction is related to the successive beginning of ACS inflation by the larvae in each group.

Inhibitory effect of copper on swimbladder inflation was observed also by Stouthart et al. [21] in *Cyprinus carpio.* At low pH of 6.3, fish exposed to low copper concentrations (0.3 or 0.8 $\mu mol \cdot dm^{-3}$) failed to inflate the bladder. Holdway [7] reported that 0.8 $mg \cdot dm^{-3}$ of uranium retarded swimbladder inflation, but later on it developed correctly until the end of experiment, while at 1.5 $mg \cdot dm^{-3}$ completely inhibited inflation in *Melanotaenia splendida* larvae.

The observed changes and inhibition of various stages of swimbladder inflation might have resulted from metal-induced reduction of locmotory activity of the larvae, and the difficulties in swimming up to the surface to ingest air which is necessary to inflate the swimbladder. Metals may also affect metabolic activity in fish and inhibit gas passage to each swim bladder chamber. The effects of various environmental factors, and direct causes of inhibition of swimbladder inflation are not quite clear and various explanations are possible. According to Stouthart et al. [21], copper reduces air uptake ability of fish, and disturbs secretion and absorption of gas that fills the bladder. Similar hypothesis to explain lack of swimbladder inflation by *Salmo salar* larvae was developed by Poppe et al. [18]. These authors observed the changes in the pneumatic duct used for bladder inflation. The duct opened at the normal point in the esophagus, but it extended caudally along the ventral side of the swimbladder, and entered the caudal pole of the organ. This was in contrast to normal fish, where the pneumatic duct was very short and entered the cranioventral part of the swimbladder. Such a malformation of the pneumatic duct may lead to altered filling of the swimbladder, which in turn may cause the abnormal shape and size of the organ.

Padros and Crespo [17] reported bacteria-induced pathological changes in the *Scophthalmus maximus* L. swimbladder. The main pathological problems observed were: inflation disturbances, malformation of gas gland and *rete mirabile*, pneumatic duct dystrophy, and swimbladder invasion by bacterial cells. These pathologies were described in association with organogenesis disturbances in early stages of development and with the presence of enteric flora. According to Haenen et al. [4], Wuertz and Taraschewski [24], histopathological changes in *Anguilla anguilla* swimbladder might have resulted from *Anguillcola crassus* infection.

The data discussed above indicate that the factors inhibiting swimbladder inflation may act directly or indirectly on epithelial cells of the bladder itself, and of the air duct. That would explain also inhibitory effect of heavy metals. According to various authors cited by Jezierska and Witeska [9], heavy metals cause damage to epithelia in various fish organs: gills, body surface, intestine, or kidney tubules. Typical changes include swelling and proliferation of epithelial cells, followed by necrosis, and sloughing. It is always preceded by heavy mucus secretion. Therefore, it seems that similar changes may be expected in swimbladder and pneumatic duct wall epithelia. Probably, in metal-exposed larvae excessive amounts of mucus may congest the duct. Congestion may also result from histological changes in epithelium, such as hypertrophy. Ostaszewska [16] observed that hypertrophic secretory epithelium reduced the lumen of swimbladder itself, and of the pneumatic duct in pikeperch (*Stizostedion lucioperca* L.) larvae.

Pneumatic duct congestion disturbs or precludes inflation of anterior swimbladder chamber. Therefore, inflation is delayed, and among metal-exposed 19 days old larvae, some individuals showed reduced size of that chamber, while the others completely failed to inflate it. At the same time, all the control fish showed correctly developed two-chambered swimbladder.

The uninflated, or incorrectly developed swimbladder disturbs arrangement of fish internal organs, which often results in vertebral malformations. Andrades et al. [1] reported that juvenile lordotic *Sparus aurata* L. displayed uninflated swimbladders but all lordotic adults possessed an inflated functional swimbladder. Divanach et al. [3] observed that lordosis in *Dicentrarchus labrax* was related to the lack of swimbladder or its delayed inflation. According to Antychowicz [2], fish that suffered from a chronic swimbladder inflammation induced by parasitic protozoans *Sphaerospora renicola* often show skeletal malformations (mainly rib and spine curvatures). Deformed spinal column of these fish looks as if it fitted deformed swimbladder. Disturbances in swimbladder inflation adversely affect fish activity and their feeding abilities. Fish with uninflated bladder show reduced growth rate and survival, and often suffer from severe vertebral malformations [14].

The data discussed above suggest that the factors inhibiting swimbladder inflation may indirectly cause larval malformations. Such malformations and disturbances of swimbladder functions result in altered behavior, reduced locomotor and feeding activity, and in consequence – in reduced growth rate and viability of fish.

The results of present study, and the data obtained by the other authors, concerning the role of swimbladder and the consequences of its noninflation, indicate that heavy metals may disturb that process which considerably reduces viability of larvae. Therefore, inhibitory effect of metals on fish swimbladder inflation may be a reliable indicator of their toxic action.

CONCLUSIONS

1. The exposure of common carp larvae in water containing copper or cadmium reduced the inflation of posterior chamber of swimbladder.
2. Metals delayed the beginning of inflation of the anterior swim bladder chamber of common carp larvae.
3. Metals reduced the size of anterior chamber of swimbladder.
4. Inhibitory effect of metals on fish swimbladder inflation may be a reliable indicator of their toxic action.

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