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MALFORMATIONS OF BARBEL (*BARBUS BARBUS*) LARVAE INDUCED BY COPPER AND CADMIUM

DEFORMACJE LARW BRZANY (*BARBUS BARBUS*) POD WPŁYWEM MIEDZI I KADMU

Słowa kluczowe: brzana, miedź, kadm, deformacje larw.
Key words: barbel, copper, cadmium, malformations of larvae.

Zapłodnione jaja brzany (Barbus barbus) inkubowano w wodzie zawierającej 0,1 mg/dm^{-3} Cu, 0,1 mg/dm^{-3} Cd lub w czystej wodzie (grupa kontrolna). Obliczono procent wyklutych larw i odsetek larw zdeformowanych. Larwy zdeformowane dokładnie oglądano i fotografowano za pomocą kamery i komputera z systemem analizy obrazu MultiScan, aby sklasyfikować typy deformacji. Zdeformowane larwy przez 14 dni hodowano w niezanieczyszczonej wodzie. Znaczące zmniejszenie procentu wyklucia odnotowano jedynie pod wpływem kadmu. Oba metale spowodowały znaczący wzrost odsetka zdeformowanych larw, podczas gdy w warunkach kontrolnych były one nieliczne. Oznaczono 5 głównych typów deformacji larw brzany: skrzywienie kręgosłupa, larwa w kształcie litery, deformacja woreczka żółtkowego, obrzęk serca, skrócenie ciała. Po 14 dniach od wyklucia we wszystkich grupach obserwowano nasilanie się deformacji ciała larw, najmniej w warunkach kontrolnych, gdzie jednocześnie najwięcej larw wykazywało ustępowanie deformacji.

1. INTRODUCTION

Body deformations commonly occur in fish larvae, their frequency may be affected by environmental conditions, and they become visible already during embryonic development. Several factors may cause an increase in frequency of body deformations in fish, such as low and high temperature [Stott and Cross 1973, Miś et al. 1995, Lein et al. 1997, Ługowska

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and Jezierska 2000], pH changes [Jezierska 1993, Duis and Oberemm 2000], low dissolved oxygen content [Tomasik et al.1982, Keckeis et al. 1996], radiation [Shrivastava and Dwivedi 1980], and heavy metals [Samson and Shenker 2000, Słomińska and Jezierska 2000, Hallare et al. 2005, Fraysse et al. 2006, Johnson et al. 2007].

Among deformations of fish larvae, different types of spine curvature are the most common [Newsome and Piron 1982, Baumann and Hamilton 1984, Afonso et al. 2000, Słomińska and Jezierska 2000, Calta 2001] followed by yolk sac deformations [Krejčí and Palíková 2006, Nguyen and Jansen 2002]. In our earlier papers we reported detailed description of body malformations in common carp [Jezierska et al. 2000] and grass carp [Ługowska et al. 2002] but detailed visual evidence of body deformations in other fish larvae is still lacking.

The present study describes various types of deformities found in barbel caused by two heavy metals, copper and cadmium, and their possible changes during further larval development.

2. MATERIALS AND METHODS

The experiment was done on barbel larvae exposed during embryonic development to copper or cadmium. Eggs and sperm were obtained from artificially stimulated spawning in the Inland Fisheries Institute in Żabieniec. The material was transported to the laboratory of the Department of Animal Physiology in Siedlce at 5˚C. The eggs were fertilized in about 2 hours after stripping: they were mixed with sperm and a small amount of water. Fertilized eggs were divided into four groups: C (control) – in clean tap water, Cu – at 0.1 mg/l Cu (obtained using $CuSO_4$ solution) and Cd – at 0.1 mg/l Cd (obtained using $CdCl_2$ solution) and incubated at 20^oC. Water was changed every day, and constantly aerated.

Newly hatched larvae were counted and inspected. Hatching rate was calculated as a percentage of hatched larvae in the initial number of incubated eggs. The larvae were divided into two groups: normal – live, motile, without visible abnormalities, and deformed – live, moving erroneously, showing body malformations. The percentage of each group in total number of hatched larvae was calculated. Each deformed larva was examined and classified according to [Jezierska et al. 2000].

Larval development took place in dechlorined tap water (dissolved oxygen saturation about 80%, hardness 167 mg/dm^3 as $CaCO_3$, pH 7.8) for 14 days post hatching. Larvae were kept in 250 ml aquaria placed in water thermostate to maintain constant temperature of 20°C. Water in aquaria was changed daily. Dead larvae were counted and removed daily. Beginning from 3 day post hatching larvae were fed *Artemia salina* naupli. Larvae were observed and photographed daily using computer MultiScan image analysis system and stereoscopic microscope connected with camera. The photographs were used to create the catalog of barbel larvae deformations.

The results were subjected to U Mann-Whitney tests to evaluate the significance of differences (at $p < 0.05$).

3. RESULTS AND DISCUSSION

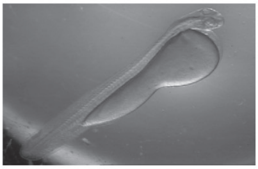
The highest hatching percentage was obtained in the control (50.5 %–73.6 %) whereas in copper exposure it was 51.3 % and 62.5 % (no significant differences comparing to the control). In cadmium exposure hatching percent decreased to 38.9 % which was significantly lower compared to the control (Tab. 1). Calta [2000] observed that only 46.9 % of common carp larvae hatched after exposure to 0.01 mg/dm^3 of cadmium. A decrease in percentage of hatching with the increase of cadmium concentration within the range of 0.001–0.02 mg/dm^3 was reported by [Witeska et al. 1995]. Cadmium at the concentration of 3.3 and 33.3 mg/dm^3 reduced number of hatched *Melanotaenia fluviatilis* larvae [Williams and Holdway 2000]. Similar effect of copper was observed by [Słomińska 1998, Witeska and Ługowska 2004] for common carp and [Ługowska et al. 2002] for grass carp.

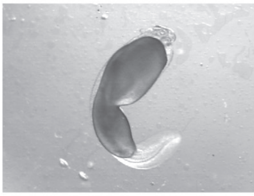
The highest percentage of normal larvae was obtained in the control – about 90 %, and only 9.5 % of deformed ones. Frequency of body malformations increased significantly in copper and cadmium exposures (Tab. 1). An increase in frequency of common carp body deformation rate induced by exposure to cadmium at concentrations 0.002–0.05 mg/dm^3 was reported by [Calta 2001] and at 0.2 mg/dm^3 by [Ługowska 2007]. Similar effect of copper atn concentrations 0.006–1.2 mg/dm^3 reported [Miś et al. 1995, Witeska and Ługowska 2004] for common carp.

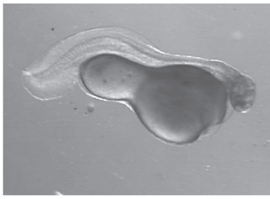
Table 1. Percentage of hatching and quality of newly hatched barbel larvae
Tabela 1. Procent wyklucia i jakość wyklutych larw brzany

Series	Hatching rate, %			Normal larvae, %			Deformed larvae, %		
	control	Cu	Cd	control	Cu	Cd	control	Cu	Cd
I	73.6	51.3	-	87.7	65.6*	-	9.5	19.8*	-
II	69.9	62.5	-	90.0	69.3*	-	4.6	9.5*	-
III	50.5	-	38.9*	84.7	-	52.6*	8.1	-	33.7*

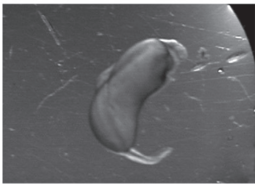
Five main types of malformations of newly hatched barbel larvae were observed (Fig. 1): **A** – spine curvature; A1 – kyphosis, A2 – axial curvature of spine in caudal region, A3 – scoliosis in abdominal region, A4 – scoliosis in both, abdominal and caudal regions; **B** – C-shaped larva, spine curvature and yolk sac deformation, body shortened; **E** – yolk sac deformation, accompanied by edema and spine curvature; E1 – only yolk sac edema, E2 – yolk sac edema , scoliosis in abdominal region, E3 – anterior part of yolk sac enlarged (pea-shaped yolk sac), E4 – edema of pea-shaped yolk sac, E5 – pea-shaped yolk sac and kyphosis, E6 – pea-shaped yolk sac and lordosis, E7 – pea-shaped yolk sac and scoliosis in abdominal region, E8 – pea-shaped yolk sac and scoliosis in caudal region, E9 – anterior and posterior parts of yolk sac enlarged (bean-shaped yolk sac) and its edema, E10 – reduced anterior part and enlarged posterion part of yolk sac with contraction between them, E11 – enlarged posterior part of yolk sac, lordosis, E12 – enlarged anterior part of yolk sac, enlarged and shortened posterior part of yolk sac, scoliosis in abdominal region, E13 – enlarged anterior part of yolk sac, enlarged and shortened posterior part of yolk sac, scoliosis in acaudal region, E14 – changes in consistence of yolk sac (thick yolk in anterior and thin yolk in posterior part of yolk sac); **F** – heart edema and spine curvature usually accompanied by yolk sac malformation; F1 – heart edema and pea-shaped yolk sac, F2 – heart edema and kyphosis, yolk sac malformation (contraction in the middle of yolk sac), F3 – heart edema and yolk sac malformation (contraction in the middle of yolk sac), F5 – heart edema, scoliosis in caudal region; **G** – shortened body accompanied by complex spine curvature and malformation of yolk sac; G1 – shortened body, spine curvature, G2 – shortened body, kyphosis in caudal region, G3 – shortened body, scoliosis in abdominal region, G4 – shortened body, scoliosis in caudal region (tail curved vertically), G5 – shortened body, scoliosis in caudal region (tail curved vertically), malformation and edema of yolk sac, heart edema.

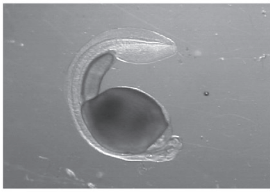

Normal larva


A1

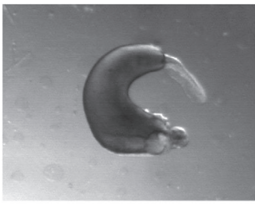

A
2


A3


A4


B


E1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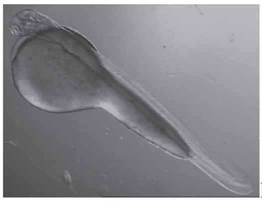
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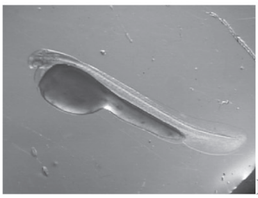
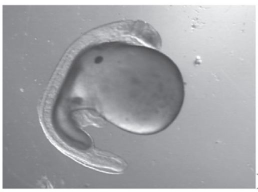

E4


E5


E6

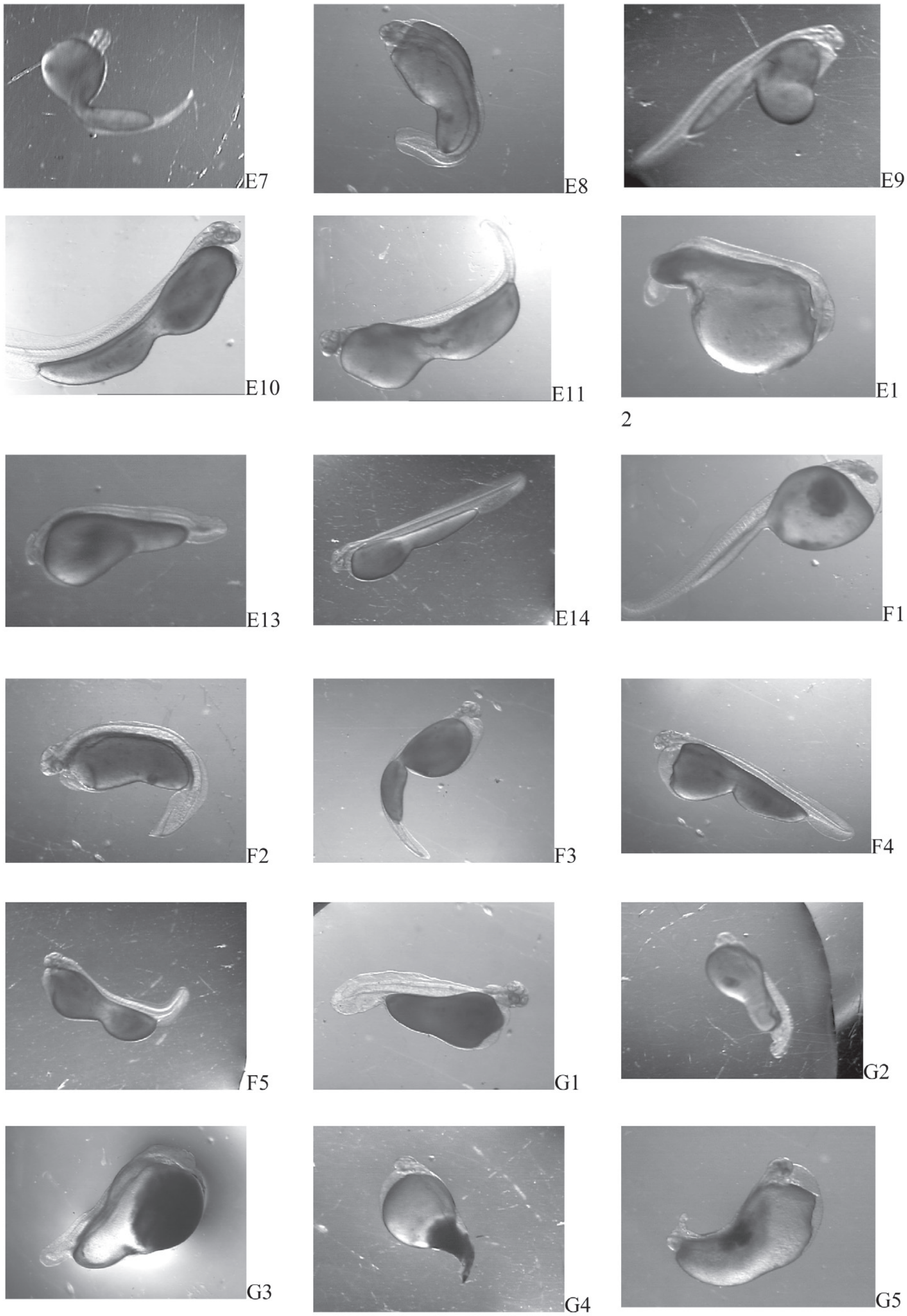


Fig. 1. Types of barbel larvae malformations

Rys. 1. Typy deformacji larw brzany

In barbel the most common type of body malformation was yolk sac deformation (E), observed in 60.95–82. 5% of deformed control fish, 78.3 %, 90 % of deformed copper-exposed and 95% of cadmium-exposed fish. Under control conditions spine curvatures (A) and heart edema (F) were quite often observed, while less than 10% of larvae showed body shortening (G) and C- shaped body (B) (Tab. 2). In metal-exposed fish deformations A, B, F and G were observed in less than 10 % fish. These types of malformations are not copper, cadmium (or any other metal) specific, and they were observed in fish exposed to different heavy metals, for example: different types of spine curvature [Holcombe et al. 1976, Weis and Weis 1977], C-shaped body [Heisinger and Green 1975, Munkittrick and Dixon 1989], body shortening [Klein-McPhee et al. 1984, Sarnowski and Jezierska 1999], yolk sac deformations [Jezierska et al. 2000, Ługowska et al. 2002, Witeska and Ługowska 2004].

Table 2. Percentage of types of barbel deformations

Tabela 2. Procentowy udział różnych typów deformacji larw brzany

Series	Control					Cu					Cd				
	types of deformations, %					types of deformations, %					types of deformations, %				
	A	B	E	F	G	A	B	E	F	G	A	B	E	F	G
I	8.7	8.7	60.9	17.4	4.3	4.3	0.0	78.3	8.7	8.7	-	-	-	-	-
II	7.5	0.0	82.5	5.0	5.0	2.5	0.0	90.0	2.5	5.0	-	-	-	-	-
III	20.0	0.0	67.5	5.0	7.5	-	-	-	-	-	2.5	0.0	95.0	0.0	2.5

After 14 days of rearing in non-contaminated water some fish in all groups showed advancement in body malformation, while most of them recovered. In the control less than 20 % initially deformed fish showed malformations (A and G types) (Tab. 3). At the same time, in copper-exposed group deformed fish showed only spine curvature (30–50 %) whereas in cadmium-exposed group 58 % of fish showed body shortening. Remaining fish in all groups – especially those showing light and simple deformations – gradually recovered. Gradual recovery of fish exposed to heavy metals during embryonic development was observed and described in our previous paper by [Witeska and Ługowska 2004].

Table 3. Changes in body malformation of barbell larvae after 14 of rearing

Tabela 3. Zmiany deformacji larw brzany po 14 dniach chowu

Series	Body malformation, % / type								
	complete recovery			part recovery			no change or advancement		
	Control	Cu	Cd	control	Cu	Cd	control	Cu	Cd
I	100	50	-	-	7/A	-	-	43/A	-
II	80	70	-	5/A	10/A	-	15/A	20/A	-
III	80	-	42	5/A	-	4/G	15/G	-	54/G

4. CONCLUSIONS

1. Hatching success of barbel was reduced by both metals, but only the effect of cadmium was significant.
2. Copper and cadmium increased frequency of larval body malformations
3. The most common malformations of barbel larvae induced by both metals were yolk sac anomalies
4. Body deformations may advance (resulting in most cases in fish death) but most larval malformations gradually recover.
5. Frequency of larval body malformations is a good but non-specific indicator of water quality.

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