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MALFORMATIONS OF NEWLY HATCHED COMMON CARP LARVAE

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ABSTRACT

Fertilized eggs of common carp (*Cyprinus carpio* L.) were experimentally exposed to copper or lead at 17, 20 or 26°C. The newly hatched larvae were counted and examined. Body malformations of the larvae were observed. The proportion of defective larvae was assessed, and malformations were classified. The malformed larvae were described in detail, and recorded using the computer image analysis system MultiScan.

Malformations were observed also in control fish but they were scarce (< 10%).

Most common in them (about 80% of all defective larvae) were single vertebral abnormalities. Various malformations observed in the larvae originating from different spawning events point to a possible impact of parents-related factors and of conditions of spawning.

Incubation at temperatures below (17°C) and above (26°C) the optimum range resulted in an increased proportion of defective larvae and the emergence of more complex malformations.

The larval exposure to heavy metals, too, was observed to have considerably increased the frequency of body deformities. Various defects including craniofacial malformations, deformations of the cardiovascular system, yolk sac, and eyes as well as edema were observed.

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The photographic evidence, accompanied by description of various types of larval deformities, allowed to qualitatively and quantitatively assess effects of environmental factors, including heavy metals, on body malformations of the newly hatched common carp larvae.

Key words: fish, larvae, body malformations, metal.

INTRODUCTION

Numerous literature data show that environmental conditions prevailing during the embryonic development may affect frequency of body malformations in newly hatched larvae. Deformities may be induced by both low [25, 27] and high [16] temperature. Malformations induced by low dissolved oxygen content were observed by Tomasik et al. [27] and Keckeis et al. [11]. Water acidification or alkalization may result in larval body defects as well [8, 14]. Shrivastava, Dwivedi [23] observed a high proportion of deformation among fish eggs irradiated by γ-rays.

Exposure to toxic substances, including heavy metals, may also produce deformation of fish embryos and larvae [1, 2, 3, 5, 6, 7, 9, 10, 12, 15, 17, 18, 19, 20, 21, 22, 24, 26, 28, 29, 30].

Most authors focused on the frequency of body malformations; some them only described the deformations they observed and/or indicated the body parts or organs affected. Vertebral, craniofacial, cardiovascular, and yolk sac deformities proved most common, accompanied by edema and haemorrhages. Some authors published photographs or drawings of the deformations observed [3, 13, 20, 24, 25, 29]. It was Munkittrick, Dixon [18] only who described the most common types of defective larvae of *Catostomus commerson,* the larvae being classified as corkscrew, L-shaped, C-shaped, yolk at posterior end, two-headed, and short bodied.

A detailed visual evidence of types of deformed larvae is lacking. Thus, the aim of the present study was to supply such evidence and to classify various types of body malformations of the newly hatched larvae of common carp exposed to copper or lead during the embryonic development.

MATERIALS AND METHODS

The study involved the newly hatched larvae of common carp (*Cyprinus carpio* L.), examined subsequently to their exposure to copper or lead during the embryonic development. The eggs and sperm were obtained from artificially induced spawning at the hatchery of the Inland Fisheries Institute in Żabieniec. Eggs from 3 females and milt from 3 males were assumed representative for each spawning event. The eggs were fertilized dry immediately after the sexual products had been delivered (about 2 h) to the laboratory in a cold box (about 5^{o}C). The embryonic development proceeded under conditions described by Lugowska, Jezierska [15]. Dechlorinated tap water (dissolved oxygen saturation about 80%; $CaCO_3$ hardness 57 mg·dm^{-3} as; pH 7.8) was used. The metal solutions were made up using $CuSO_4$ and $PbNO_3$. The experimental design is shown in Table 1.

Table 1. Experimental design

Series	Metal	Concentration [mg·dm^{-3}]	Temperature [^{o}C]
1	Pb	0; 2.0	20
	Cu	0; 0.2	
2	Pb	0; 2.0	
	Cu	0; 0.2	
3	Pb	0; 2.0	
	Cu	0; 0.2	
4	Pb	0; 2.0	
	Cu	0; 0.2	
5	Cu	0; 0.25	20; 26
6	Pb	0; 3.0	20; 26
7	Pb	0; 4.0	17; 20; 26

The newly hatched larvae were counted and examined. The hatching rate was calculated as a percentage of hatched larvae relative to the initial number of incubated eggs. The proportion of defective larvae among the hatch was assessed and the malformations were classified. Images of the deformed larvae were recorded using the computer image analysis system MultiScan coupled with a still camera.

The results obtained from the four initial series of the experiment allowed to distinguish 8 major types of malformations of common carp larvae, whereby a catalogue of malformities was created. Based on the catalogue, the defective larvae from series 5-7 were subsequently classified.

RESULTS

The data on carp hatch rate and the frequency of deformed larvae in series 1-4 are summarised in Table 2. The data indicate that exposure to the heavy metals reduced the hatch success, while the frequency of body malformations was higher than that prevailing in the control.

Table 2. Hatching rate and percentage of deformed common carp larvae

Series	Hatching rate [%]			Deformed larvae [%]		
	control	Cu	Pb	control	Cu	Pb
1	75	52	60	6	38	22
2	68	44	49	9	21	24
3	68	55	47	7	22	20
4	75	52	59	8	38	18

The detailed examination of the larvae showed vertebral deformations to be most common (usually more than 80% of all defects recorded). Cranial and yolk sac malformations were observed quite often as well. Typical of the defective larvae were yolk sac and heart edema; in addition, patches of extravasated blood were occasionally visible. Considering the fact that a larva could be affected by a number of defects affecting various organs, 8 major types (A-G) of defective larvae were distinguished; those major types were further divided into subtypes. The imagery of various types of defective larvae is presented in Fig. 1, while their description is given below.

Types of body malformation in common carp

A. Larva with axial (lordosis or kyphosis) or lateral (scoliosis) curvature of the spine in the abdominal or caudal region. The curvatures are usually accompanied by slight deformation of the yolk sac.
A1. Lordosis.
A2. Kyphosis.
A3. Axial curvature in the abdominal and caudal region.
A4. Scoliosis in the abdominal region.
A5. Scoliosis in the abdominal and caudal region.
B. C-shaped larva, with deformed tail and spine and considerably distorted, irregularly shaped yolk sac. The body usually shortened.
C. Larva with deformed skull (enlarged lower jaw) and spine. Some individuals showed also the yolk sac edema.
D. Larva with deformed eyes (occasionally accompanied by spinal curvature as well as a shortened and swollen yolk sac).
D1. Four eyes.
D2. Three eyes.
D3. Single eye.
D4. Fusion of the eyes.
D5. Pigment-deficient eye.
D6. Non-pigmented eye.
D7. Lack of the eyes.
E. Larva with deformed yolk sac (occasionally accompanied by edema and spinal curvatures).
E1. Yolk sac edema only.
E2. Yolk sac deformation only; anterior part of the yolk sac enlarged (pear-shaped yolk sac).
E3. Yolk sac deformation; anterior part of the yolk sac enlarged (pear-shaped yolk sac) and edema-affected.
E4. Yolk sac deformation; anterior part of the yolk sac enlarged (pear-shaped yolk sac), accompanied by caudal scoliosis.
E5. Yolk sac deformation; anterior part of the yolk sac enlarged (pear-shaped yolk sac), accompanied by abdominal and caudal scoliosis.
E6. Yolk sac deformation and upward spinal curvature; anterior part of the yolk sac deformed and edema-affected.
E7. Yolk sac deformation and downward spinal curvature; yolk sac edema.
F. Larvae with cardiac edema (often accompanied by spinal curvature and yolk sac malformations).

Fig. 1. Types of deformed larvae (see text for descriptions)

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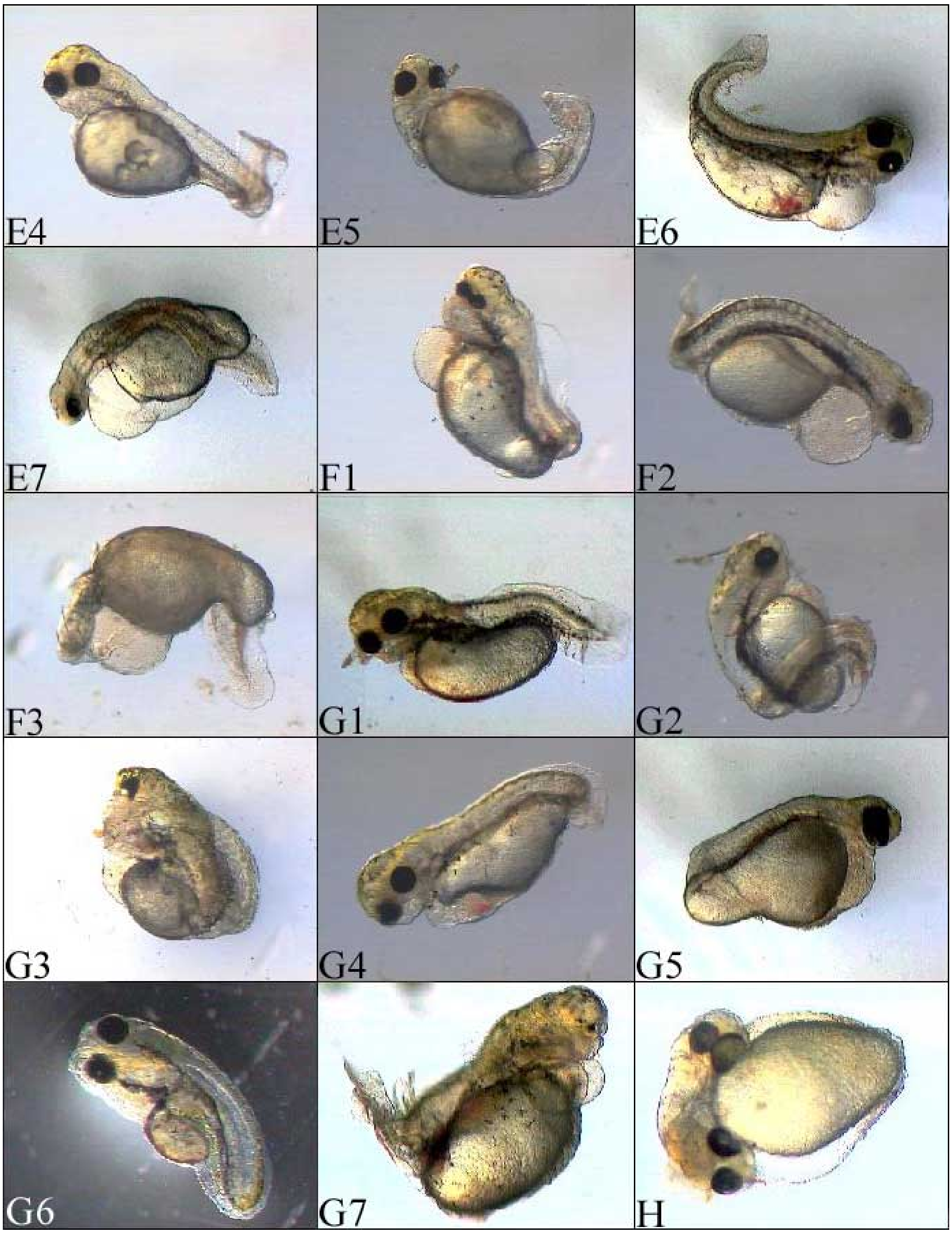
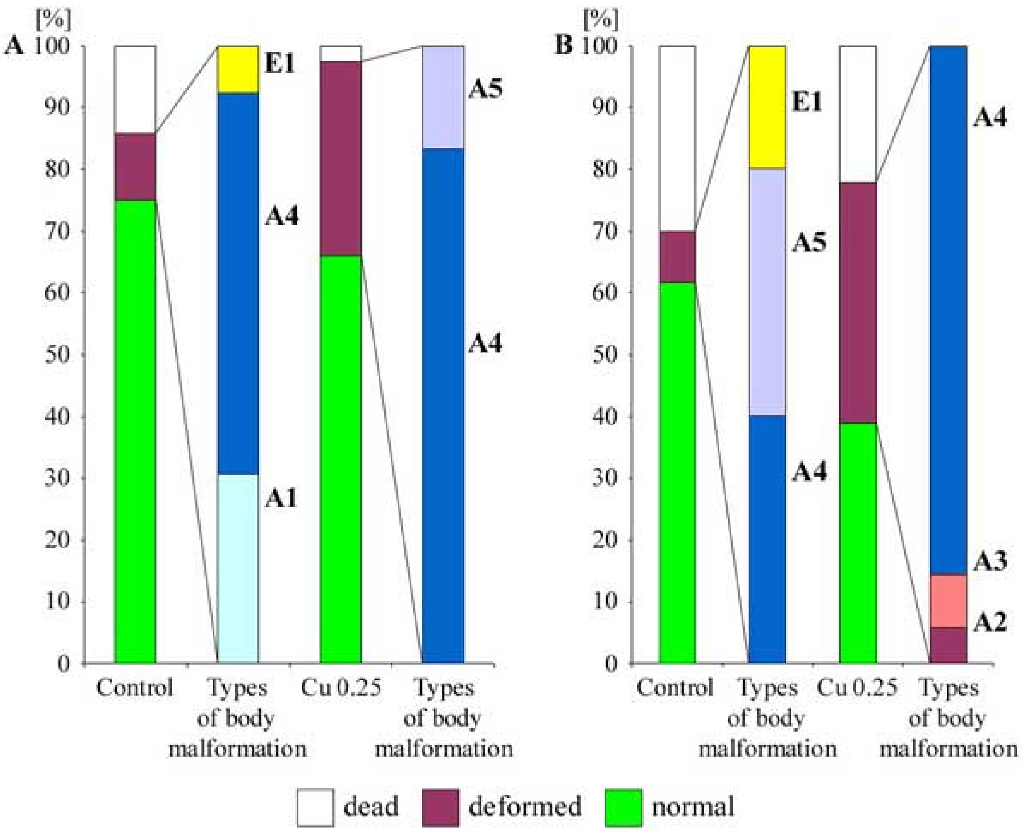


F1. Cardiac edema, caudal scoliosis, and yolk sac malformation.
F2. Cardiac edema, abdominal and caudal scoliosis, and yolk sac malformation.
F4. Cardiac edema, undeveloped head, downward caudal curvature, and yolk sac malformation.
G. Larva with shortened body and severe spinal, head and yolk sac malformation.
G1. Body shortened, spine curved.
G2. Body shortened, abdominal scoliosis, tail curved downwards; yolk sac deformed and swollen.
G3. Body shortened, abdominal scoliosis, tail curved upwards; yolk sac deformed and swollen.
G4. Body shortened, undeveloped tail, edema and deformation of the yolk sac.
G5. Body shortened, tail absent, edema and deformation of the yolk sac.
G6. Body shortened, cranial malformation, tail absent, spinal curvatures; shrunken and deformed yolk sac with edema.
G7. Body shortened, cranial malformation (unpigmented eye), spinal curvatures, deformed yolk sac with edema.
H. Twin form. Double head, two pairs of eyes, lack of tail. Yolk sacs fused.

The proportion of deformed individuals among the newly hatched larvae of series 5-7 was calculated, the defective larvae being classified to malformation types presented above (Fig. 2-4). As seen in the data, the larvae from various series, even the control ones, were affected by different body malformations, proportions of defective individuals differing between the series as well. The lowest and the highest frequency of defects was recorded in series 7 and 6, respectively. At 20°C in series 6 and 7, vertebral malformations (A) were recorded, some of the series 6 larvae showing also the yolk sac edema (E). The proportion of defective larvae was observed to increase among those larvae the embryonic development of which proceeded at a higher temperature (26°C); they showed mainly vertebral curvatures, yolk sac edema (E), and body shortening (G). Similarly, after incubation at 17°C, type E and G deformations were observed (Fig. 4a).

Fig. 2. Effects of copper on newly hatched common carp larvae at 20°C (A) and 26°C (B)



The percentage of defective larvae was higher in the copper exposure (Fig. 2) than in the control, all deformations involving vertebral curvatures (A).
The lead exposure (Fig. 3, 4) resulted in an increased proportion of defective larvae as well. Malformations of type B (C-shaped larva) were recorded. In addition to vertebral curvatures, cranial deformations, yolk sac edema (E), and body shortening (G) were observed.

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Fig. 3. Effects of lead on newly hatched common carp larvae at 20°C (A) and 26°C (B)

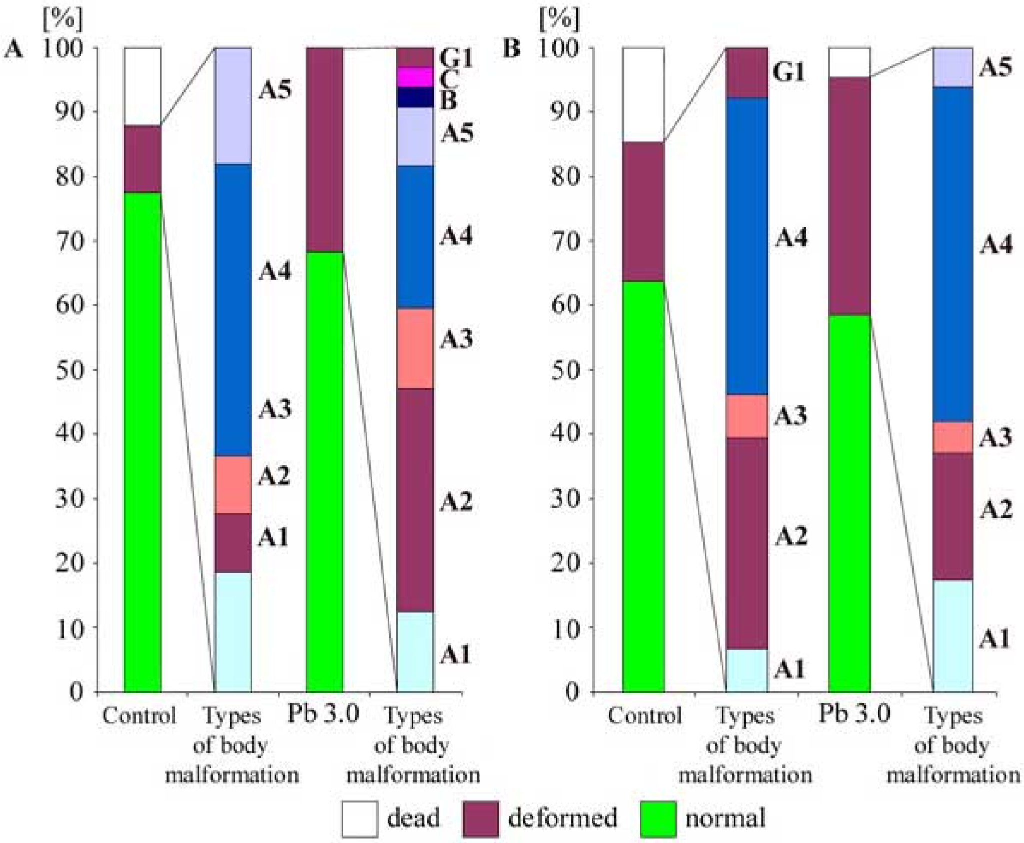
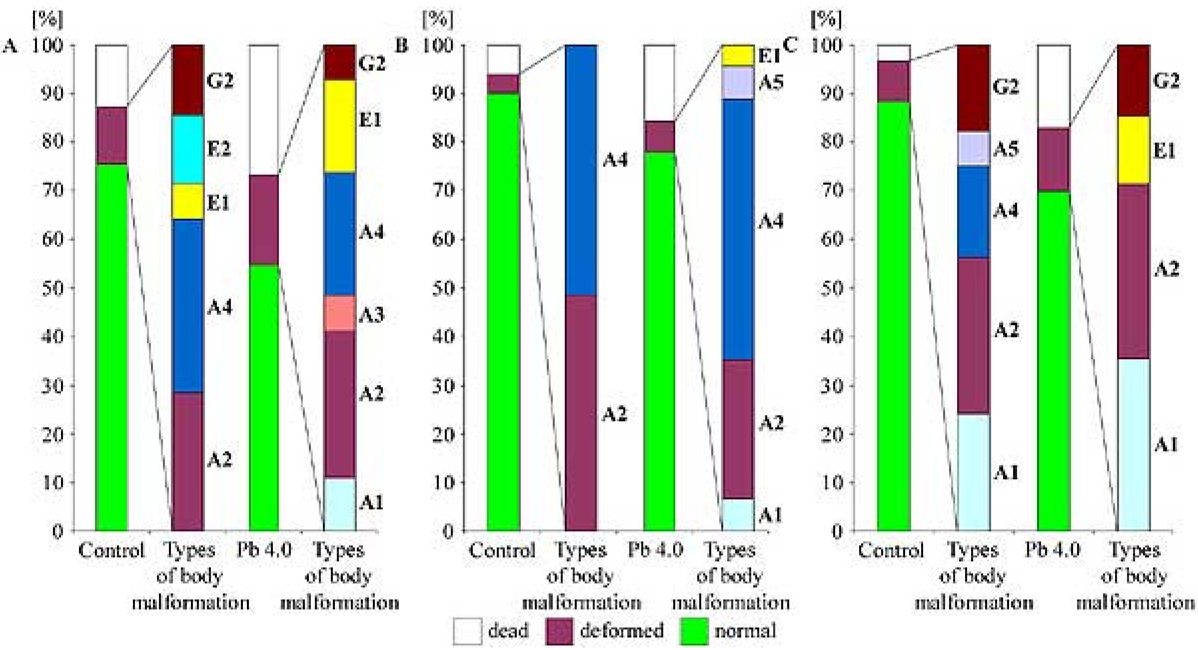


Fig. 4. Effects of lead on newly hatched common carp larvae at 17°C (A), 20°C (B), and 26°C (C)



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DISCUSSION

Body malformation were observed to happen in the newly hatched larvae even if the embryonic development took in unpolluted and well-oxygenated water, at an optimal temperature (20°C for common carp; Lugowska, Jezierska [15]). In the control, the proportion of defective hatch did not exceed 10%, while at temperatures below (17°C) or above (26°C) the optimum, the frequency of malformations was observed to increase, which is consistent with the results of other authors [15, 25, 27].

The most common malformations in this study involved various vertebral abnormalities which amounted to more than 80% of all the defects recorded in the larvae kept under optimal conditions. Only some of those larvae showed craniofacial, cardiovascular, and yolk sac malformations or edema. Such deformations were more frequent in the larvae kept at 26 or 17°C than in the control. The larvae originating from different spawning events were observed to show differences in their body malformations. The differences were probably related by parental factors and to the conditions of artificial spawning. The results of this study are consistent with data reported by other authors who indicated an increased proportion of deformed larvae in copper and lead exposures [4, 9, 16, 17, 24].

The results show that exposure of fish embryos to copper or lead may bring about a high frequency and complexity of larval malformations which may affect different parts of the body. Malformations may be caused by the disturbances occurring at early developmental stages or may evolve during laborious hatching [24].

Observations on the defective larvae allowed to describe types of body malformations in common carp larvae; they are illustrated in Fig. 1. The most characteristic body malformations included the curved vertebral column (described also by Beattie, Pascoe [1], Holdway, Spraque [5], Woodworth, Pascoe [30], Woock et al. [29], Munkittrick, Dixon [18]), C-shaped curvature of the body (observed also by Klein–MacPhee [12], Munkittrick, Dixon [18]), yolk sac malformation (described by Klein–MacPhee [12]), edema (reported, following selenium exposure, by Gillespie, Baumann [3], Woock et al. [29], Pyron, Beitinger [20]), malformations of the craniofacial part of the head and the cardiovascular system (mentioned by Beattie, Pascoe [11], Perry et al. [19]), blood patches [1, 30], a strong body contraction with deformation of the entire body [18].

The data obtained in series 5-7, in which larval deformities were typified according to the classification presented, showed lead exposure to result in an increased frequency of vertebral abnormalities, C-shaped body, craniofacial malformations, the yolk sac edema, and shortening of the body, i.e., the deformities absent in the copper treatment. On the other hand, the quantitative data obtained failed to show differences between copper and lead in causing larval deformations. Thus, the classification system developed in this study and presented in the catalogue allows to evaluate body malformations in the common carp larvae under various environmental factors not only quantitatively, but also qualitatively. It seems, however, that effects of various metals should be studied at the same time, and under the same conditions on larvae from a single pair of spawners to eliminate possible parental effects.

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