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THE EFFECT OF CADMIUM AND CADMIUM/COPPER MIXTURE DURING THE EMBRYONIC DEVELOPMENT ON DEFORMATION OF COMMON CARP LAVAE

Katarzyna Ługowska
Dept. of Animal Physiology University of Podlasie, Prusa 12, 08-110 Siedlce, Poland
e-mail: kongo@ap.siedlce.pl

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Abstract: The aim of present study was to estimate the effect of single and binary combinations of cadmium with copper during the embryonic development on hatching success and quality of common carp larvae. The results were compared to those obtained after copper exposure, during the same experiment (Witeska and Ługowska 2004).
The study was done on common carp newly hatched larvae, the embryonic development of which took place at 0.2 mg/dm^3 of cadmium (Cd group) and 0.2 (0.1+0.1) mg/dm^3 mixture of cadmium and copper (CdCu group), or in clean tap water (control – K group). Newly hatched larvae were counted and inspected, and for 20 days from hatching reared under control conditions. The results show that cadmium (0.2 mg/dm^3) reduced the success of hatching and caused an increase of number of deformed larvae among the newly hatched ones. The results obtained in the same experiment after copper exposure (Cu 0.2 mg/dm^3) (Witeska and Ługowska 2004) indicate that toxic effect of this metal on hatching and quality of larvae was stronger than the effect of cadmium. No differences in effect of cadmium exposure (0.2 mg/dm^3) and it's co-exposure with more toxic copper (0.1 mg/dm^3 Cd+0.1 mg/dm^3 Cu) on hatchability and frequency of deformations were observed. These results indicate metal interaction, which in this case probably was antagonistic. Metal exposure decreased survival of fed and starved normal and deformed larvae. Fish died gradually just after hatching. The comparison of effect of cadmium and Cd+Cu mixture shows that the mixture of both metals was more toxic than cadmium alone, but not stronger than copper alone. These data suggest that effect of metal mixture can be additive. Detailed classification of deformed larvae in the present study showed that cadmium alone and in co-exposure with copper caused the same types of larval deformations: vertebral curvatures, body and yolk sac deformations. Under optimal conditions in laboratory culture, vertebral deformations may recover.

Keywords: cadmium, metal mixture, embryonic development, fish, deformations

Introduction

Cadmium is one of the metals most commonly occurring in polluted natural waters which may affect the early stages of fish development. The data by various authors show the adverse effect of cadmium especially on hatchability (Williams and Holdway 2000, Calta 2001, Gonzales-Doncel et al. 2003, Sikorska and Ługowska 2005) and quality of larvae (Weis and Weis 1977, Woodworth and Pascoe 1982, Witeska et al. 1995, Hallare et al. 2005, Fraysse et al. 2006).

Most studies of the effects of metals on fish concern exposures to single metal. Polluted water bodies, however, usually contain elevated levels of various metals. Many data show that certain metals affect accumulation of the others in fish. It seems that interactions among various metals are related to their competitive uptake from the environment, and different distribution in fish tissues. Interactions among metals may be different, and therefore the effects of their various mixtures on fish survival may also differ. The effect of mixtures of two or more chemicals is commonly referred to either as additive, synergistic or antagonistic (Jezierska and Witeska 2001).

In natural polluted waters cadmium is very often accompanied by copper. Therefore, it is interesting to evaluate combined effect of both metals on survival and development of larvae.

Toxicity of metal mixtures to fish during development and growth was studied by Kazlauskienė et al. (1996), Kazlauskienė et al. (1999), Petrauskienė (1999), and Stoškus et al.(1999), who tried to simulate natural conditions of contaminated water bodies of Lithuania. However, the data on the effects of metal mixtures on embryonic development and hatched larvae of fish are very scarce.

Kazlauskienė and Stasiūnaitė (1999) evaluated effects of heavy metal mixture (Cu, Zn, Cr, Ni, Fe) on the eggs and growth of rainbow trout larvae. Authors revealed that even low concentrations of the toxicant significantly decreased vitality of eggs and larvae. Eaton (1973) exposed *Pimephales promelas* to copper, cadmium and zinc mixture and estimated growth, spawning and hatching success. The mean terminal weight of both males and females was less in the highest metal mixture concentration. Spawning in all higher concentrations (above 0.01 mg/dm^3 of each metal in mixture) was greatly reduced (78% or more) as compared to control results. Author concluded that it was possible to determine whether or not the metals in the mixture were additive in their effects, because each of them had one or more specific and identifiable toxic action.

The aim of presented study was to estimate the effect of single and binary combinations of cadmium with copper during the embryonic development on hatching success and quality of common carp larvae. The results were compared to those obtained after copper exposition, during the same experiment conducted on the same fish and presented by Witeska and Ługowska (2004).

Materials and Methods

The study was done on common carp newly hatched larvae, the embryonic development of which took place at 0.2 mg/dm3 of cadmium (Cd group) and 0.2 (0.1+0.1) mg/dm3 mixture of cadmium and copper (CdCu group) or in clean tap water (control – K group). Metal solutions were made using CdCl2·2½ H2O and CuSO4·5H2O. The experiments were done in 3 series.

Newly hatched larvae were counted and inspected. The percent of hatchability was calculated as a number of hatched larvae per initial number of incubated eggs. Among freshly hatched larvae the share of deformed ones was evaluated. Each deformed larva was examined and classified according to the types of malformations using the catalog by Jezierska et al. (2000).

For 20 days from hatching, the larvae were reared under control conditions: dechlorinated tap water, temperature 22°C, oxygen saturation about 80%, pH 7.8, hardness 230 mg/dm^3 as $CaCO_3$.

In all three series, the larvae were reared separately in the 50 cm^3 glass vessels of 7.5 cm diameter. Each vessel was individually marked in order to identify each larva. During the experiments, the fish were starved. Their morphology was examined daily, and mortality was recorded.

The observations were made using a thick slide with a concave chamber, which was filled with clean water. Each larva was carefully placed in the chamber and observed using binocular (magnification 1.5x1.6) connected with camera, and computer image analysis system MultiScan. The observations were recorded in photographs.

Additionally, in series III, the fed (twice a day with *Artemia* sp. *naupli*), and starved larvae were reared in the 100 ml glass vessels, at the density of 20 fish per vessel. Normal and deformed larvae from K group, and deformed ones from Cd and CdCu groups were reared separately. Survival was recorded daily.

Results

Metal exposure caused significant reduction of hatching rate and numerous body malformations (Table 1). No differences in both parameters were observed between Cd and CdCu-exposed groups.

In both Cd and CdCu-exposed groups the same four types of body malformations were distinguished (Table 2). The changes in yolk sac were the most frequent, observed in 13 (Cd group) and 14 (CdCu group) larvae, respectively. In other 9 and 3 individuals from these groups vertebral deformations were observed, and in 5 and 4 fish – body shortening.

Normal fed larvae from the control group (Figure 1), reared under optimum conditions showed very high survival rate, while deformed ones (from control and Cd or CdCu exposure) kept dying over entire experimental period. Only 6 deformed individuals from the control, 3 from Cd-exposed and 2 CdCu-exposed groups survived until the end of experiment. Most of the starved control larvae survived 13 days post hatching, but before the 20 day all larvae quickly died (Figure 2). The deformed larvae from Cd exposure (single or binary with copper) gradually died from the beginning of the experiment. Until the 13 day in group Cd survived up to 5 (Cd group) and 2 (CdCu group) larvae respectively. All larvae from Cd exposure died before 19 and CdCu before 17 day. Detailed observation of individuals provided more information about changes in body morphology of starved deformed carp larvae. In the Cd groups (Figure 2a-c) all the larvae showing G-type deformations died first, and only 1 of them partly recovered. Similarly, also E-type larvae died soon after hatching, and only 2 fish in series I completely recovered, and survived until 15 and 19 day. The B deformation was reversible completely only in 1 series II fish, partly in 2 (series II, II) fish, and B-type larvae survived until the 5 (series I) and 13 (series II) day post hatch. On the contrary, A deformation was reversible in all larvae, and these fish showed the longest survival.

In CdCu exposed groups the larvae showing G and E-type deformations died soon after hatching. Among them, only 3 E deformed fish (1 in each series) partly recovered, and all larvae died until the 14 day from hatching. The B deformation was reversible in only 2 fish (1 complete), while other fish died between 2 and 9 after hatching. In all series, the A deformation was reversible, and larvae survived until 15 (series I) and 17 (series II, III) day post hatching. Figure 4 shows some examples of gradual recovery of deformed larvae observed in the present study. Fish in the Figure 4a and 4c after hatching showed scoliosis.

Table 1. Hatching rate and quality of newly hatched larvae.

Series	Group	% of hatch	% of deformed larvae
I	K	89.9	14.6
	Cd	73.3*	25.5*
	CdCu	61.7*	24.2
II	K	71.7	14.2
	Cd	46.9*	33.3*
	CdCu	46.7*	28.4
III	K	76.7	4.4
	Cd	58.3*	35.2*
	CdCu	53.3*	30.7*

(* values significantly different from control, U-Mann-Whitney test, $p \leq 0.05$, n=5)

Table 2a. Body deformations of common carp larvae exposed to cadmium during embryonic development.

Series	Number of larvae	Figure
A – curvature of the spine		
I	2	
II	3	
III	4	
	Σ 9	
B – C-shaped larva		
I	2	
II	2	
III	2	
	Σ 6	
E – deformed yolk sac		
I	5	
II	4	
III	4	
	Σ 13	
G shortened body		
I	1	
II	2	
III	2	
	Σ 5	

Table 2b. Body deformations of common carp larvae exposed to cadmium and copper mixture during embryonic development.

Series	Number of larvae	Figure
A – curvature of the spine		
I	1	
II	1	
III	1	
	Σ 3	
B – C shaped body		
I	1	
II	1	
III	2	
	Σ 4	
E – deformed yolk sac		
I	6	
II	4	
III	4	
	Σ 14	
G – shortened body		
I	1	
II	2	
III	1	
	Σ 4	

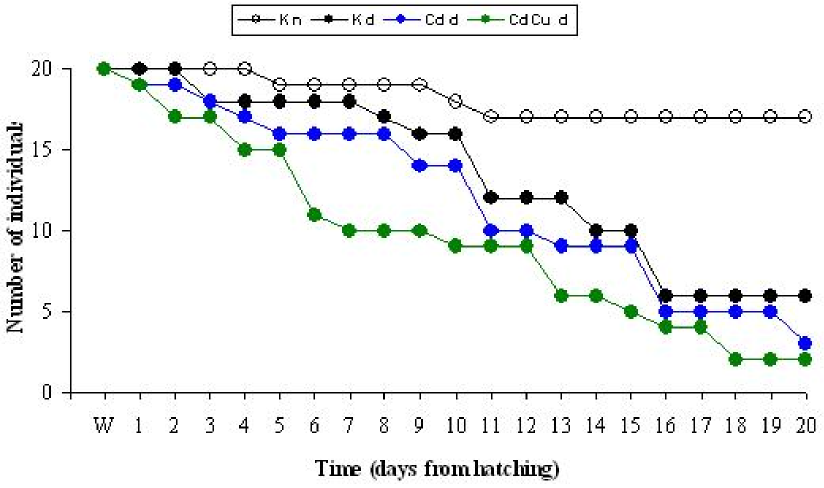

Figure 1. Survival of the fed carp larvae.

H - hatching
Kn – normal larvae from the control
Kd – deformed larvae from the control
Cd d – deformed larvae from cadmium exposure
CdCu d – deformed larvae from cadmium and copper mixture

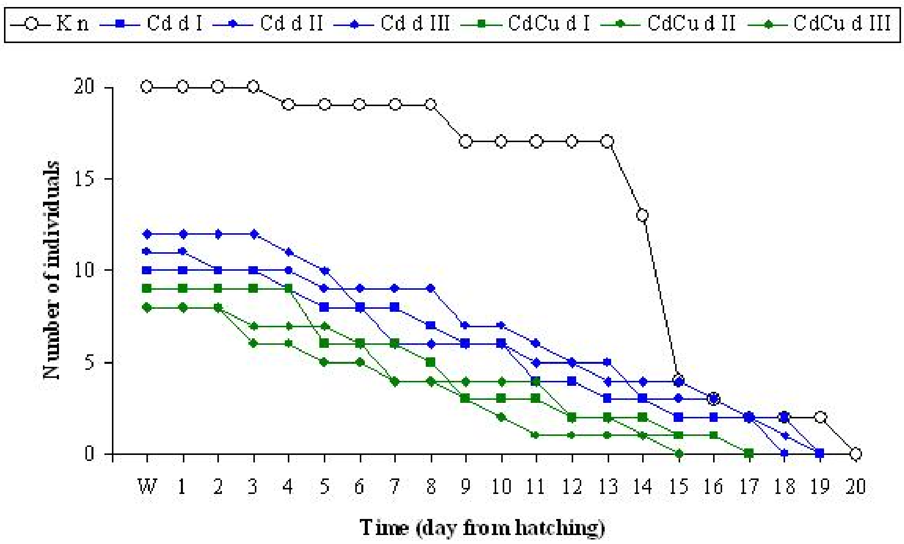

Figure 2. Survival of starved carp larvae.
H - hatching
Kn – normal larvae from the control
Kd – deformed larvae from the control
Cd d – deformed larvae from cadmium exposure
CdCu d – deformed larvae from cadmium and copper mixture
I – series I, II - series II, III – series III

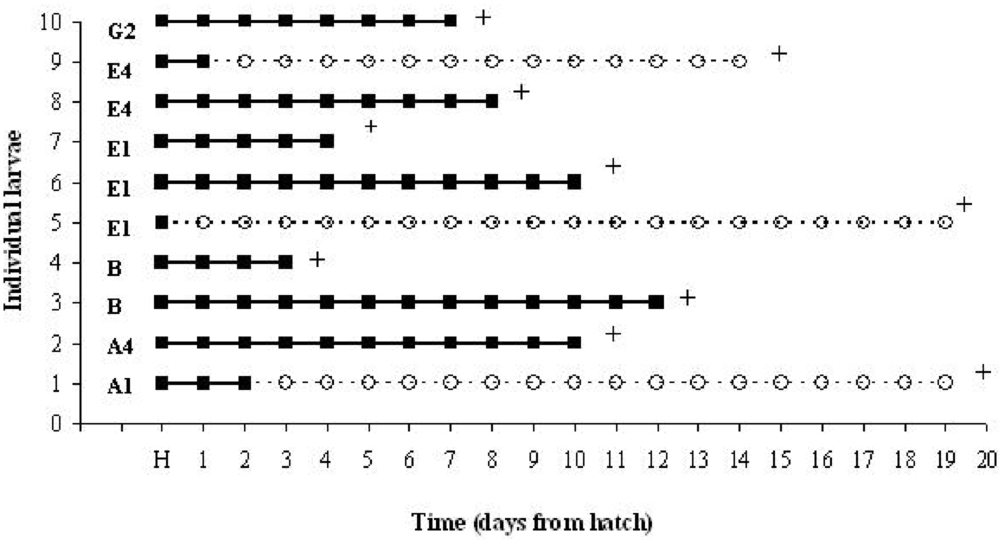

Figure 3a. The changes in body morphology of starved deformed carp larvae.
(Cd, series I)
H – hatching
■ – deformed larva
▲- gradual recovery
○ – normal larva
+ - death

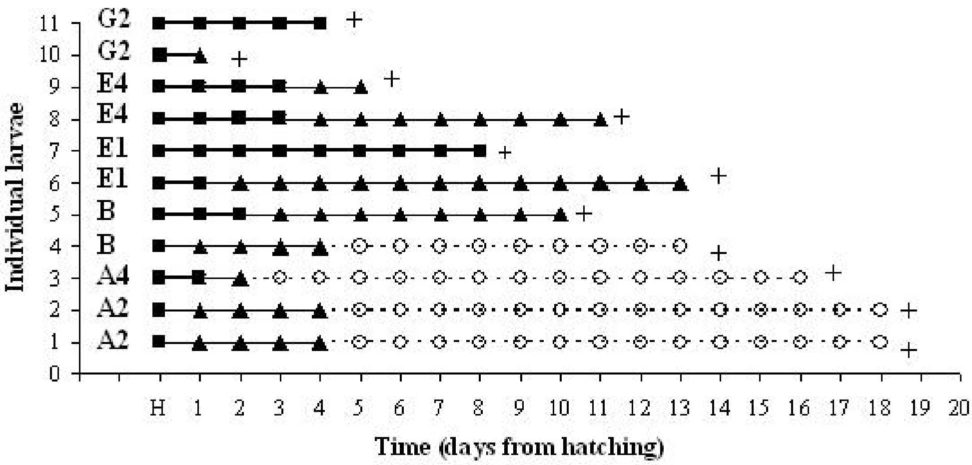

Figure 3b. The changes in body morphology of starved deformed carp larvae.
(Cd, series II)
H – hatching
■ – deformed larva
▲- gradual recovery
○ – normal larva
+ - death

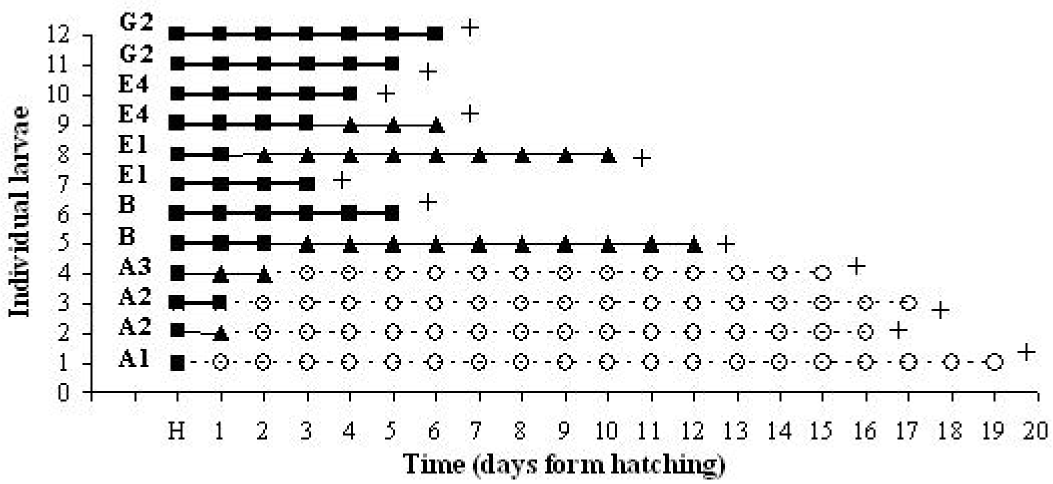

Figure 3c. The changes in body morphology of starved deformed carp larvae.
(Cd, series III)
H – hatching
■ – deformed larva
▲- gradual recovery
○ – normal larva
+ - death

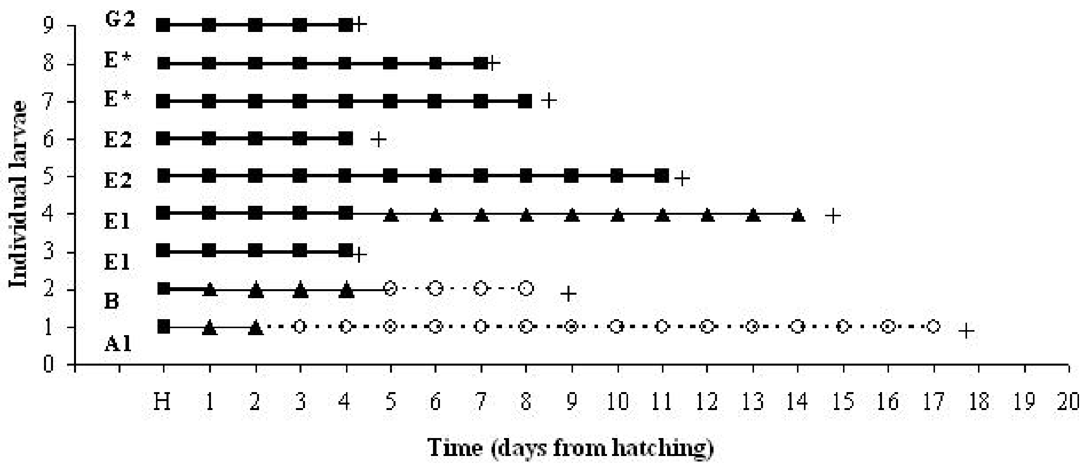

Figure 3d. The changes in body morphology of starved deformed carp larvae.
(CdCu, series I)
H – hatching
■ – deformed larva
▲- gradual recovery
○ – normal larva
+ - death

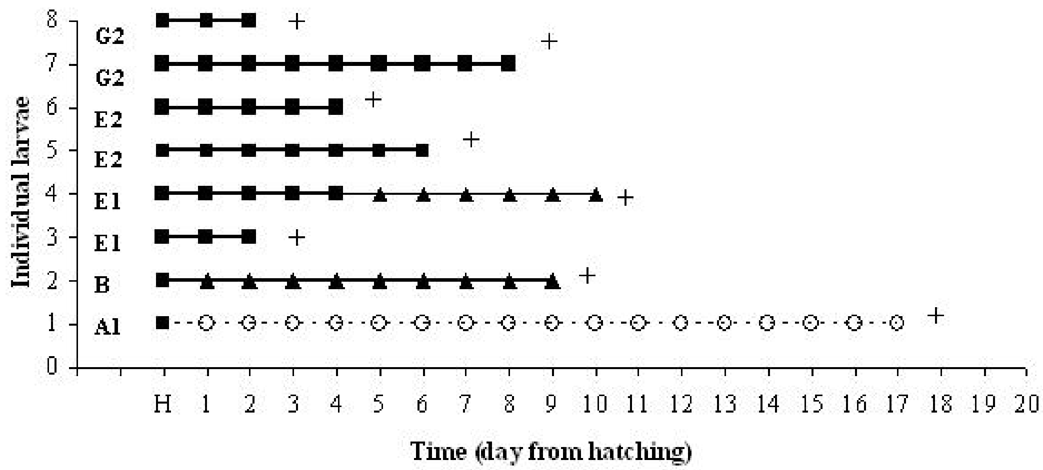

Figure 3e. The changes in body morphology of starved deformed carp larvae.

(CdCu, series II)
H – hatching
■ – deformed larva
▲- gradual recovery
○ – normal larva
+ - death

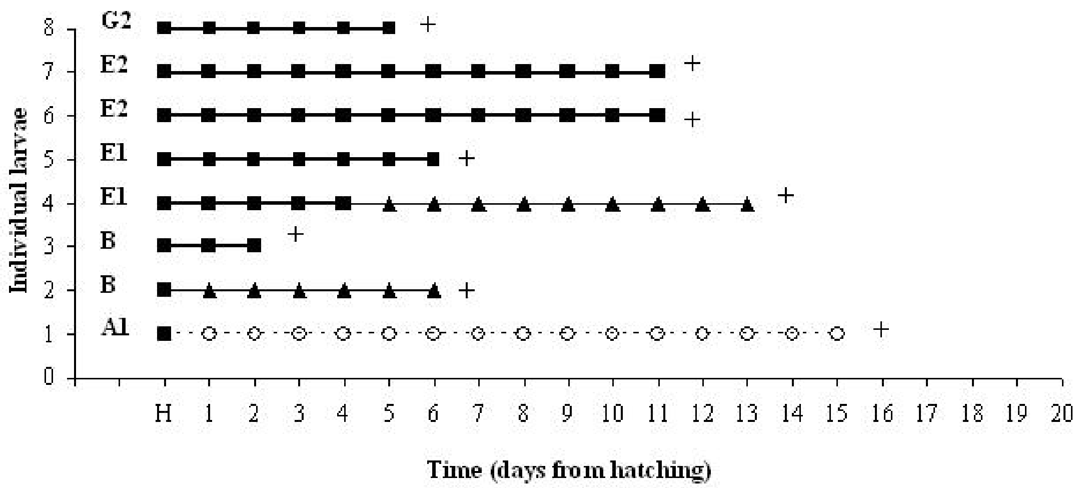

Figure 3f. The changes in body morphology of starved deformed carp larvae.

(CdCu, series III)
H – hatching
■ – deformed larva
▲- gradual recovery
○ – normal larva
+ - death

Figure 4a. Complete gradual recovery of the starved larvae exposed to Cd during embryonic development.

Figure 4b. Partial gradual recovery of the starved larvae exposed to CdCu during embryonic development.

Curvature started to decrease already on the first day post hatching, and disappeared until 6 and 5 day, respectively, when the larvae looked quite normally. But in some cases the recovery wasn't complete. Newly hatched larvae from CdCu (Figure 4b) showed C-shaped body, and their spine started to straighten gradually, beginning from the first day post hatching. On the 3 day fish still sowed slightly curved tail region which remained curved until the death of the larvae.

Discussion

The results show that cadmium (0.2 mg/dm^3) reduced the success of hatching (Table 1). Decrease of hatching rate with increase of cadmium concentration within the range of 0.001-0.02 mg/dm^3 Cd was reported by Witeska et al. (1995), and in 0.2-0.7 mg/dm^3 Cd by Sikorska and Ługowska (2005). Calta (2001) observed that at 0.01 mg/dm^3 of Cd only 46% of mirror carp larvae hatched. Cadmium in concentrations 3.3 and 33.3 mg/dm^3 reduced number of hatched *Melanotaenia fluviatilis* larvae (Williams and Holdway 2000). Gonzales-Doncel et al. (2003) observed a decrease of *Danio rerio* hatching rate at Cd concentrations of 20, 40, and 80 mg/dm^3, and Hallare et al. (2005) – at 0.25-10 mg/dm^3.

Exposure to Cd caused also an increase in the number of deformed larvae among the newly hatched ones. The results of the present study confirm data obtained by other authors. Witeska et al. (1995) reported that 47% of larvae were deformed after exposure to 0.02 mg mg/dm^3 of Cd in comparison to only 1% in control. The proportion of mirror carp deformed larvae increased with increasing of cadmium concentration from 0.002 to 0.05 mg/dm^3 of Cd was observed by Calta (2001).

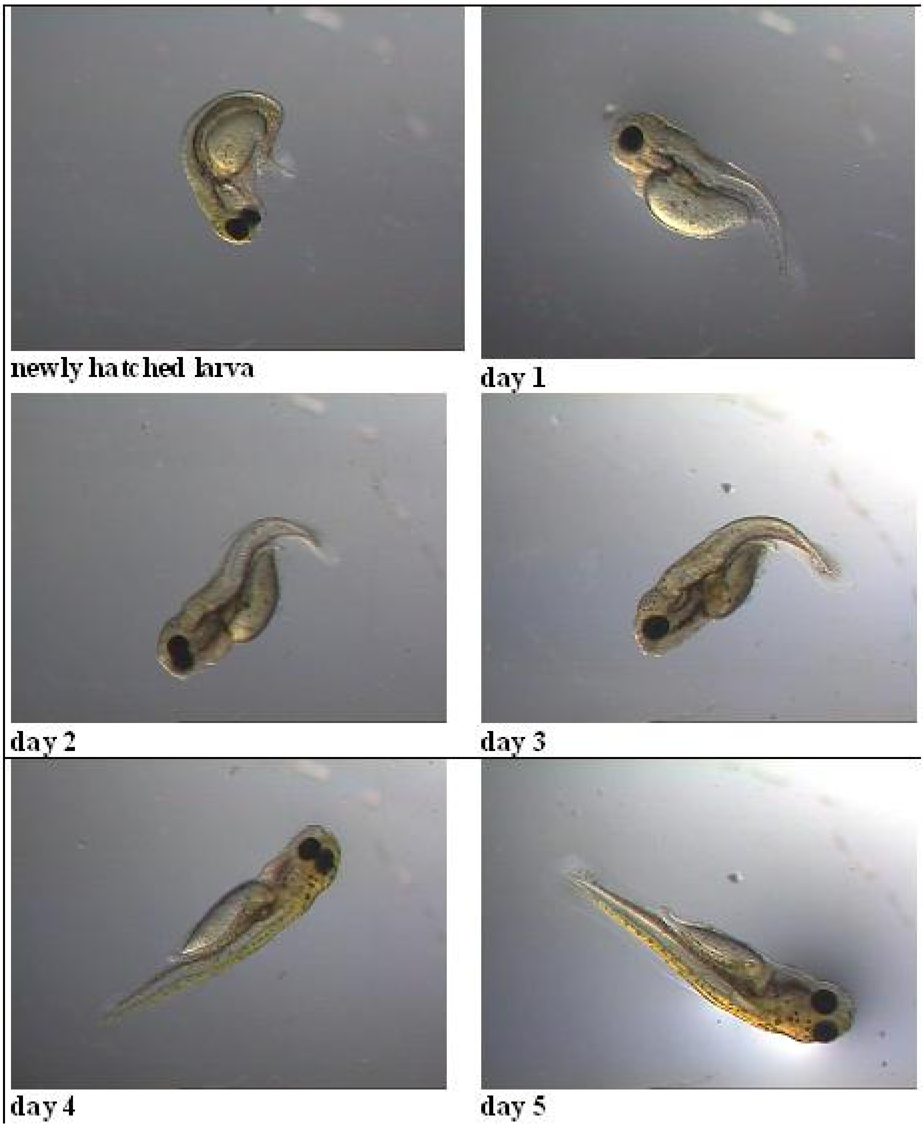

Fig. 4c. Complete gradual recovery of the starved larvae exposed to CdCu during embryonic development.

The results obtained in the same experiment after exposure to 0.2 mg/dm^3 of Cu (Witeska and Ługowska 2004) indicate that toxic effect of this metal on hatching and quality of larvae was stronger than 0.2 mg/dm^3 of Cd: the hatching rates in Cu groups were 60, 38.3 and 40%, while in the present study in Cd groups: 73.3, 46.9 and 58.3%, respectively. Percentage of deformed larvae after Cu exposure were: 41, 64 and 66%, while in Cd exposure: 25.5, 33.3 and 30.8%. Also 48 hours LC50 of heavy metals for embryos of *Paralichthys olivaceus* exposed to Cu, Cd, Cr and Pb indicated that copper was the most toxic to embryos (Wu et al. 1990). This indicates that copper is more toxic than cadmium at the same concentration.

Different toxicity of cadmium and copper on embryonic development can be a result of different uptake and accumulation of metals by the eggs. According to Beatie and Pascoe (1978), Michibata (1981), Burinson et al. (2006), the most of cadmium is accumulated in the egg outer shell – chorion, and only small amount in the embryo. However, no data are available on copper accumulation, which as an essential metal, and is probably taken up into the embryo.

No differences in effect of cadmium exposure (0.2 mg/dm^3) and co-exposure with more toxic copper (0.1 mg/dm^3 Cd+0.1 mg/dm^3 Cu) on hatchability and frequency of deformations were observed. These results indicate metal interaction, which in this case is probably antagonistic, as it was suggested by Jezierska and Witeska (2001). Also the results obtained by Ługowska (2005) showed that the effects of cadmium and copper mixture on various parameters of embryonic development (such as swelling, mortality of embryos, deformations of larvae and yolk sac volume) were weaker than the effect of each metal separately.

Detailed classification of deformed larvae in the present study showed that cadmium alone and together with copper caused the same types of larval deformations: vertebral curvatures, body and yolk sac deformations. Exactly the same types of deformations of carp larvae were observed by Witeska and Ługowska (2004) after 0.2 mg/dm^3 copper exposure.

These types of malformations are not cadmium- (or any other metal) specific, and they were observed also in fish exposed to other heavy metals. Similar malformations such as: deformed yolk sac, curved spinal column, enlarged head and edema were described by Jezierska and Słomińska (1997) in copper-exposed common carp larvae. Jezierska and Górzyńska (1998) observed shortening and curvature of caudal part, vertebral malformations, and yolk sac malformations in common and grass carp after copper (0.2 and 0.3 mg/dm^3) and lead (5.0 mg/dm^3) exposures. C-shape body after mercury exposure in *Oryzias latipes* were described by Heisinger and Green (1975). Klein-MacPhee et al. (1984) observed shortened, twisted bodies and yolk sac malformations in winter flounder exposed to silver. Lordosis, twisted tails and inclusions in yolk sac in rainbow trout were reported by Stasiūnaitė (1999) after Ni, Zn, Pb, Cd, Fe and Mn exposure.

In laboratory culture, the hatched larvae were reared in clean tap water. Therefore, observed survival of larvae was affected only by the conditions of the embryonic development, when eggs were exposed to metals.

Metal exposure reduced survival of fed (Figure 1) and starved (Figure 2) normal and deformed larvae. Fish died gradually after hatching. Similar, but slightly stronger toxic effect of copper on survival of larvae was observed by Witeska and Ługowska (2004).

The comparison of effect of cadmium and mixture cadmium and copper shows that the mixture of both metals was more toxic than cadmium alone, but not stronger than copper alone. According to various data presented by Jezierska and Witeska (2001), these results suggest that effect of metal mixture can be additive.

Similar interaction between Cd and Cu in mixture was observed by Jezierska et al. (2006) on feeding activity of common carp larvae. Exposure to copper in mixture with cadmium impaired food uptake much more than in cadmium alone.

Detailed observations and assessment of deformed larvae in laboratory culture (Figure3) showed that complex malformations caused death soon after hatching, and only spine curvatures were reversible. Similar effect after 0.2 mg/dm^3 Cu exposure was observed by Witeska and Ługowska (2004), and recovery was possible only in larvae with vertebral malformations. Thus, under optimal conditions some deformations of larvae, particularly curvatures of the spinal column, may recover.

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