Arch. Ryb. Pol.	Archives of Polish Fisheries	Vol. 8	Fasc. 1	119-128	2000

THE EFFECT OF HEAVY METALS ON POSTEMBRYONIC DEVELOPMENT OF COMMON CARP, *Cyprinus carpio L.*

Iwona Słomińska, Barbara Jezierska

Department of Animal Physiology, Siedlce University

ABSTRACT. Common carp larvae were reared under laboratory conditions, in water containing lead or copper. Development and growth rate, survival, and skeletal deformities were evaluated. Exposure to heavy metals resulted in slowed down development and growth rate, and reduced survival. Exposure to copper inhibited skeletal ossification, while lead caused scoliosis.

Key words: COMMON CARP, LARVAE, LEAD, COPPER

INTRODUCTION

Heavy metals in water are particularly dangerous for fish juveniles and may considerably reduce fish density, or even cause extinction of entire fish population in polluted reservoirs. The data of many authors indicate that heavy metals reduce survival and growth of fish larvae (Holdway 1992). They also cause behavioural anomalies, such as impaired locomotory performance resulting in increased susceptibility to predators (Weis and Weis 1995), or structural damages, mainly vertebral deformities (Eaton 1974).

Lead and copper were chosen for the experiment. Together with mercury and cadmium they belong to the most toxic elements (Hellawell 1989) and often contaminate water bodies due to human activities (Szulkowska-Wojaczek et al. 1992). The effects of lead and copper on living organisms, however, differ. Lead is not involved in any metabolic processes, and copper plays an important role as a microelement necessary for development and growth of the living organisms and it is bound in ceruloplasmin. However, it becomes a strong toxicant in excessive concentrations.

The aim of the present study was to evaluate the effect of lead and copper on postembryonic development of common carp.

MATERIAL AND METHODS

The study was carried out in the laboratory of the Department of Animal Physiology in Siedlce, on common carp larvae incubated during embryonic development in metal-free water. One day old larvae were reared in glass aquaria containing lead and copper solutions. Nominal concentrations of the metals are shown in Tab. 1. Each tank was stocked with 300 larvae for development rate observations, and another 100 were placed in a suspended fine-mesh enclosure for survival calculation. The initial size of the enclosures was 20x18x10 cm, and larger nets were used as the fish grew. Water was constantly aerated using air pumps (DO saturation was maintained at 80-90%). Water temperature was 22±1°C. In series I the fish were reared in 40 dm^3 tanks without flow and water was changed every 3 days. These aquaria were cleaned 3 times a day. In series II and III, flow-through aquaria with water recirculation system of 130 dm^3 were used. Water was filtered and flow rate was maintained at 0.8-1.0 dm^3 min^{-1}. The entire water volume in the system was changed every 12 days in series II, and every 3 days in series III. Laboratory conditions are shown in Tab. 1. The larvae were fed every 2 hours, from 6 a.m. to 8 p.m. For the first 2 weeks brine shrimp nauplii were used together with dry feed, later on – dry feed Ewos was given to satiation.

TABLE 1

Laboratory conditions of rearing

Series year	Nominal metal concentration (mg dm^{-3})	Tank volume (dm^3)	Water exchange (days)	Rearing duration (days)
I	Pb: control (0); 1.0	40	3	35
1995	Cu: control (0); 0.1	40	3	40
II	Pb: control (0); 1.0; 2.0	130	12	40
1996	Cu: control (0); 0.1; 0.2	130	12	40
III	Pb: control (0); 2.0	130	3	40
1997	Cu:control (0); 0.2	130	3	40

Dechlorinated tap water was used in the experiment, of pH 7.8-8.0 and total hardness 167 mg dm^{-3} of Ca. Metal solutions were made of $CuSO_4$ or $Pb(NO_3)_2$. Lead and copper concentrations in the control were 0.076 mg dm^{-3} and 0.018 mg dm^{-3} respectively, but in the discussion metal concentrations in the control were assumed to be zero. Levels of lead and copper were measured daily (for the first 12 days of rearing) using atomic absorption spectroscopy (AAS 30, Carl Zeiss Jena).

Every day 5 fish were randomly sampled from each tank and observed using binocular microscope (at magnification 12.5 x 4) to estimate developmental stage. The fish were classified according to Vasnecov et al. (1957). Survival was calculated daily, and dead fish were removed. Ten randomly harvested fish were weighed using analytical balance and measured every 7 (series I) or 5 (series II and III) days. Ten randomly sampled fish were used on the last day of the experiment for observation of skeletal deformities. The fish were preserved in 10% formaldehyde and stained according to Taylor (1967). Stained skeletons were examined using a microscope. The results are shown in the graphs as arithmetic means.

RESULTS

CHANGES OF HEAVY METAL CONCENTRATIONS IN WATER DURING EXPERIMENTS

Examples of the dynamics of heavy metal concentrations in water during the experiments (measured for the first 12 days of larval development) are shown in Figs. 1 and 2. Lead and copper concentrations decreased considerably, especially at the beginning of the experiment. The values stabilised after 4 days at the level dependent on the initial metal concentration. The results indicate that in series II the fish were exposed for 12 days to decreasing metal concentration, while in series I and III (water changed every 3 days) metal concentrations were fairly constant.

THE EFFECT OF HEAVY METALS ON POSTEMBRYONIC DEVELOPMENT OF COMMON CARP

Lead and copper delayed the beginning of developmental stages from C_1 (exogenous feeding) and caused their extended duration in all series (Figs. 3 and 4). Inhibition of development was observed in the larvae exposed to copper; the fish did not fill one chamber of the swim bladder. After 40 days of rearing in 0.1 mg dm^{-3} of Cu (series I), 30% of the larvae had one-chambered swim bladders, and in 0.2 dm^{-3} (series III) there were 50% of such fish. The remaining fish reached stage D. All the fish filled both chambers of the swim bladder and reached fingerling stage (F) only in series II, although later than in the control.

Survival of control larvae was over 80% until the end of the experiment. Lead and copper reduced fish survival beginning from the first days of rearing (Figs. 5 and 6). The highest mortality of lead-exposed fish occurred at C_1 and C_2 stages (series I and

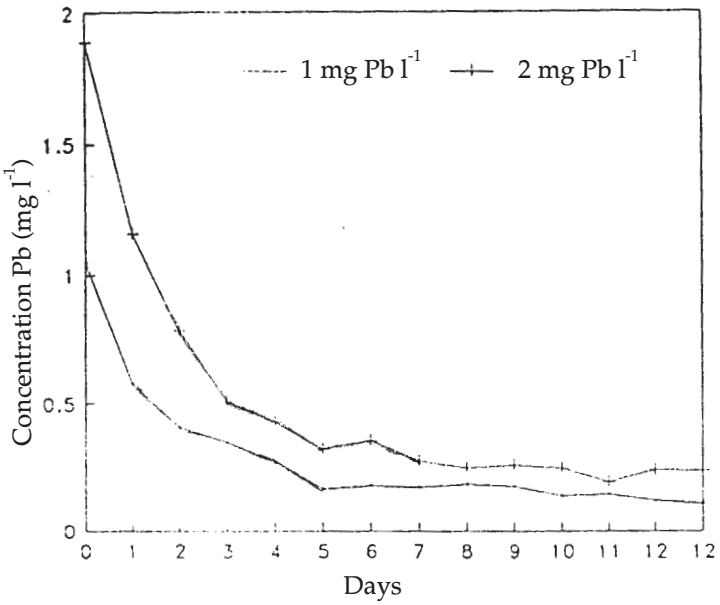

Fig. 1. Lead concentrations in water measured during postembryonic development of common carp.

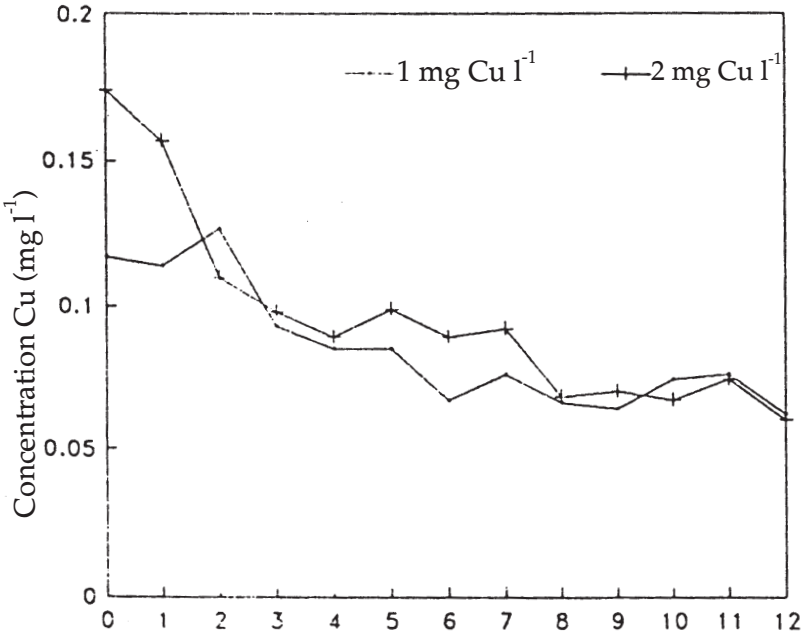

Fig. 2. Copper concentrations in water measured during postembryonic development of common carp.

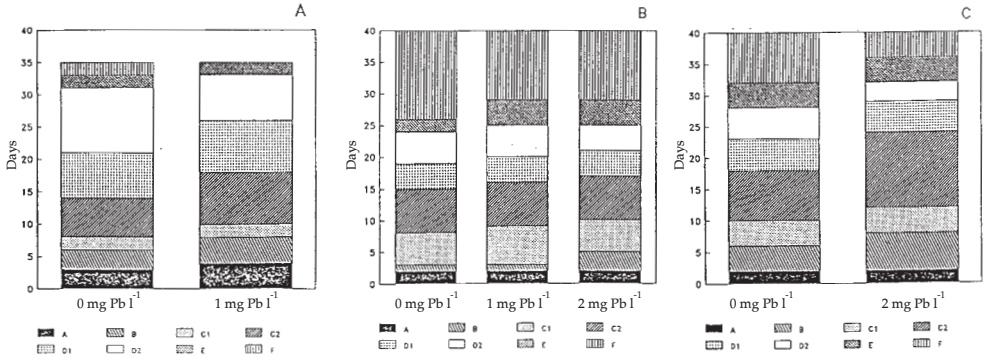

Fig. 3. The effect of lead on postembryonic development rate. A – series I, B – series II, C – series III

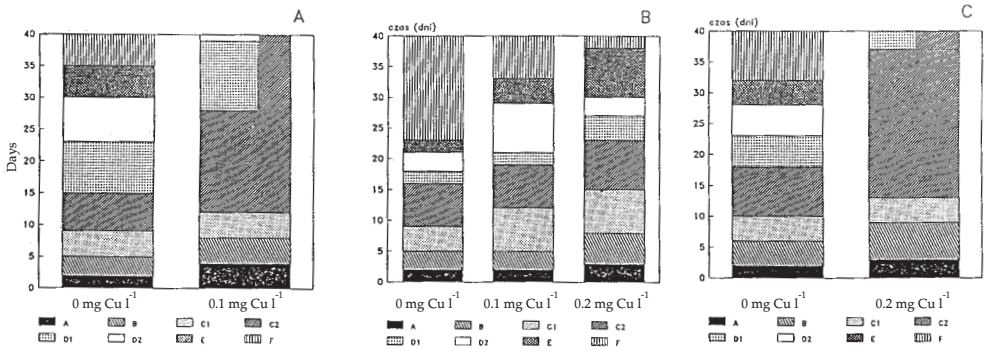

Fig. 4. The effect of copper on postembryonic development rate. A – series I, B – series II, C – series III

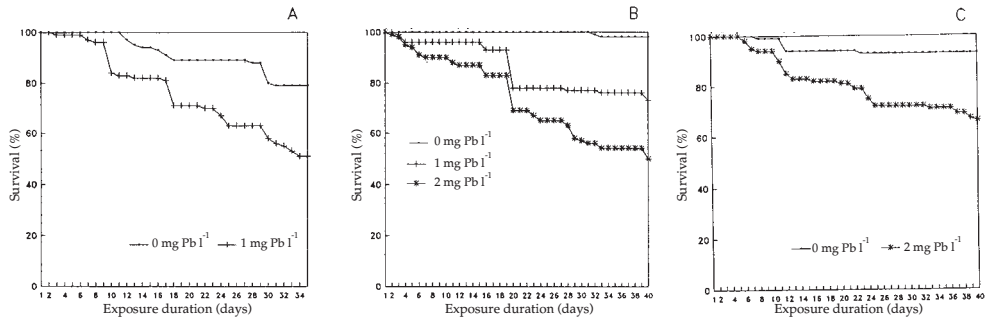

Fig. 5. The effect of lead on larval survival. A – series I, B – series II, C – series III

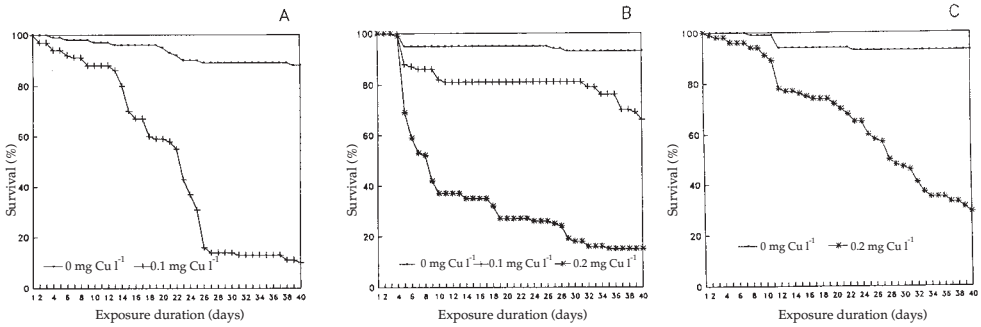

Fig. 6. The effect of copper on larval survival. A – series I, B – series II, C – series III

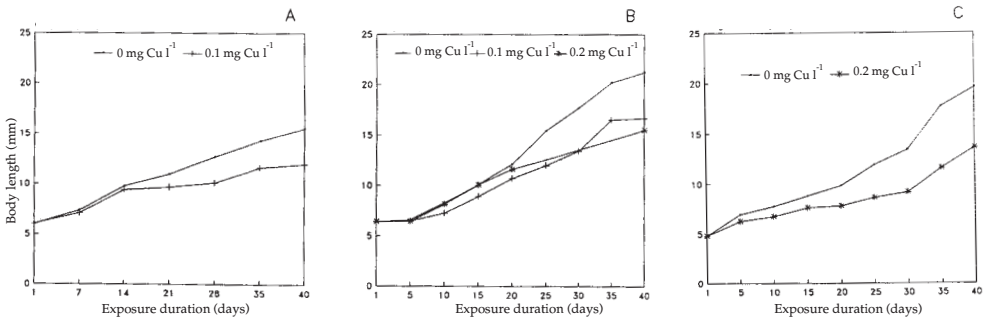

Fig. 7. The effect of lead on larval growth. A – series I, B – series II, C – series III

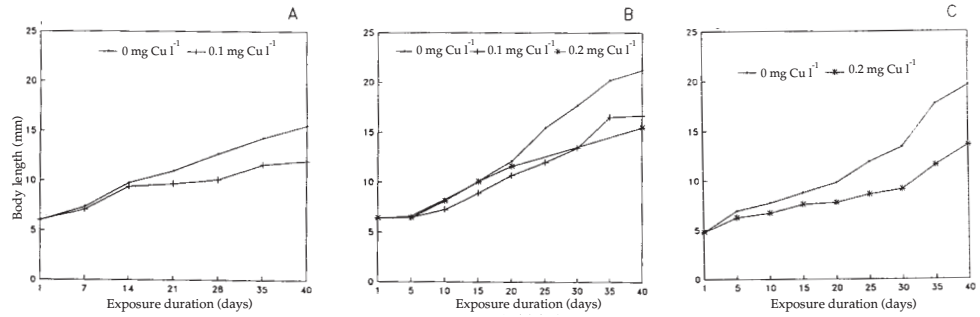

Fig. 8. The effect of copper on larval growth. A – series I, B – series II, C – series III

III), and at D_1 stage (series II). Copper increased fish mortality at C_2 (series I) and C_2 (series II and III).

The most pronounced differences in the growth rate between the control and metal-exposed fish were observed at late larval stages (Figs. 7 and 8). The mean body

length was under 80% of the control fish size after 40 days of exposure in 2 mg dm^{-3} of Pb, and about 70% in 0.2 mg dm^{-3} of Cu.

Fish exposed to lead had scoliosis (lateral vertebral curvature). Slight curvature in abdominal region was noted in 1 mg dm^{-3} of Pb, and distinct malformation of abdominal and caudal regions was observed in 2 mg dm^{-3}. In the larvae exposed to copper skeletal ossification was impaired. Analysis of stained skeletons showed that bones were fully calcified in 40 days old control carps, while skeleton remained cartilaginous in fish exposed to copper. Only in some fish initial ossification occurred in the anterior part of the spinal column.

DISCUSSION

HEAVY METAL CONCENTRATIONS IN WATER AND EXPERIMENTAL CONDITIONS

Concentrations of heavy metals in polluted reservoirs are never constant. They depend on the type and level of pollution, physico-chemical conditions, reservoir size, time from pollution discharge, and composition of the biocenosis. In natural waters soluble metals are quickly bound into insoluble compounds, or are sorbed by mineral and organic fraction of bottom sediment. Metals precipitate from the solution also in laboratory conditions. Metal concentration in the experimental tanks decreases with time due to sedimentation of insoluble compounds on the tank walls. Thus, it is hard to maintain constant concentration of a metal in the experiment.

There are different approaches to this problem. Some authors do not change metal solutions during the experiments and rely on nominal concentrations only (Weis and Weis 1977). Others change water every 24, 48 or 96 hours, attempting to maintain fairly constant concentration (Studnicka 1977). Some studies are performed in flow-through tanks constantly supplied with metal solution (Lin and Dunson 1993), or dosing apparatuses are used (Benoit 1976). The latter seem to produce the most reliable metal concentrations. Not always, however, such apparatuses are available.

Two ways of exposure were applied in the present study. At first, episodic water pollution was simulated (series II): water was changed 3 times during 40-day-long experiment. Figs. 1 and 2 show that metal concentrations considerably decreased in 4 days in 130 dm^3 aquaria. Another way of fish exposure to metals involved fairly constant metal concentration (series I and III). Taking into consideration the effect of tank size on the precipitation rate, and decrease of metal concentrations in water

(Jezierska and Królak 1997), it seems that in series I, in which 40 dm^3 tanks were used, metal concentrations fluctuated more than in series II and III (130 dm^3 tanks).

The results show that different frequency of water exchange affected only copper-exposed fish. Only in series I and III swimming bladder development was inhibited and the larvae did not reach fingerling stage until the end of the experiment (40 days).

THE EFFECT OF HEAVY METALS ON POSTEMBRYONIC DEVELOPMENT OF COMMON CARP

Both metals considerably affected development rate of carp larvae. Larval stages, beginning from C_1 (exogenous feeding), were delayed and lasted longer than in the control. The stage of filling of the second swim bladder chamber (D_1) was delayed most in the fish exposed to copper. Adverse effect of copper on swim bladder filling in common carp was also observed by Stouthart et al. (1996). The authors suppose that the failure to fill the bladder might have resulted from underdevelopment of the upper jaw and inability of air ingestion. They also suggest impaired gas exchange and oxygen resorption and secretion in the swim bladder.

Adverse effect of metals is also revealed in vertebral malformations of the larvae.

Copper caused inhibition of skeletal ossification which might have resulted from impaired ionic regulation. Reader et al. (1989) noted that copper reduced calcium uptake and bone calcification in *Salmo trutta*. Carp larvae exposed to lead developed scoliosis. Similar effect of lead was observed by Holcombe et al. (1976) in *Salvelinus fontinalis*. The authors suggest that lead inhibits enzymatic decomposition of tryptophan and may also cause inhibition of other enzymes due to its affinity to amino, imino and sulfhydryl groups of the enzymatic active centres.

Heavy metals present in the environment caused also reduction of fish growth rate (Figs. 7 and 8). Impaired growth was also observed by Benoit (1976) in chromium--exposed *Salmo trutta*, by Rombough and Garside (1980) in cadmium-treated *Salmo salar*, and by Holdway (1992) in two Australian tropical fishes exposed to uranium.

Impaired growth of larvae exposed to metals might have been due to reduced appetite. Woodward et al. (1989) noticed that at pH 6.0 aluminum reduced by 67% feeding rate in *Oncorhynchus clarki* swim-up larvae. It is also probable that the larvae suffered from impaired ionic balance. Hwang et al. (1995) observed significant decrease of water, calcium and potassium content in *Oreochromis mossambicus* larvae exposed to cadmium. In case of copper treatment, growth inhibition might have been related to underdeveloped swim bladder which impaired fish activity and feeding.

Heavy metals considerably reduced carp survival. Similar results were obtained by Hazel and Meith (1970) who observed concentration-related decrease in *Oncorchynchus tshawytscha* exposed to copper. McKim and Benoit (1971) reported 24% mortality of *Salvelinus fontinalis* after 3 months in 32.5 μg dm^{-3} of Cu, while all fish died after 4 months. In the present experiment fish survival differed between metal treatments and among the series (Figs. 5 and 6). Generally, however, lead and copper caused considerable fish mortality after yolk sac resorption. This confirms the results of Coyle et al. (1993) for *Lepomis macrochirus*. These authors explained increased mortality with the failure of exogenous feeding after yolk resorption. It is possible that in polluted environment, individuals most affected by a toxicant may be too weak to feed and die from starvation. Body malformations may also reduce ability of fish to capture food, which results in death.

CONCLUSIONS

The results of present study show that heavy metals in the environment cause:

- reduction of the development and growth rates
- developmental anomalies (skeletal ossification inhibited by copper , and scoliosis caused by lead)
- reduction of fish survival, especially at the beginning of exogenous feeding

REFERENCES

Benoit D.A. 1976 – Toxic effect of hexavalent chromium on brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) – Water Res. 10: 497-500

Coyle J.J., Buckler D.R., Ingersoll G., Fairchild J.F., May T.W. 1993 – Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*) – Environ.Toxicol.Chem. 12: 551-565

Eaton J.M. 1974 – Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinescue) – Trans. Am. Fish.Soc. 103: 121-124

Hazel Ch.R., Meith S.J. 1970 – Bioassay of king salmon eggs and sac fry in copper solution – Calif.Fish.Game. 56: 121-124

Hellawell J.M. 1989 – Biological indicators of freshwater pollution and environmental management – Elsevier, London and New York, 546

Holcombe G.W., Benoit D.A., Leonard E.N., McKim J.M. 1976 – Long - term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*) – J.Fish.Res.Board Can. 33: 1731-1741

Holdway D.A. 1992 – Uranium toxicity to two species of Australian tropical fish – Sci.Total.Environ. 125: 137-158

Hwang P.P., Lin S.W., Lin H.S. 1995 – Different sensitivities to cadmium in tilapia larvae (*Oreochromis mossambicus*; Teleostei) – Arch.Environ.Contam.Toxicol. 29: 1-7

Jezierska B., Królak E. 1997 – The effect of various factors on metal concentrations (Cu and Pb) in the water under laboratory conditions – Conf.Inter. Metals in Environment, Wilno 5-8.10.1997

Lin H.C., Dunson W.A. 1993 – The effect of salinity on the acute toxicity of cadmium on the tropical, estuarine, hermaphroditic fish, *Rivulus marmoratus* – a comparison of Cd, Cu and Zn tolerance with *Fundulus heteroclitus* – Arch.Environ.Contam.Toxicol. 25: 41-47

McKim J.M., Benoit D.A. 1971 – Effects of long - term exposures to copper on survival, growth and reproduction of brook trout (*Salvelinus fontinalis*) – J.Fish.Res.Board Can. 28: 608-616

Reader J.P., Everall N.C., Sayer M.D.J., Morris R. 1989 – The effects of eight trace metals in acid soft water on survival, mineral uptake and skeletal calcium deposition in yolk – sac fry of brown trout, *Salmo trutta* L. – J.Fish.Biol. 35: 187-198

Rombough P.J., Garside E.T. 1980 – Cadmium toxicity and accumulation in eggs and alevins of Atlantic salmon *Salmon salar* – Can.J.Zool. 60: 2006-2014

Stouthart A.J.H.X., Haans J.L.M., Lock A.C., Wandelaar Bonga S.E. 1996 – Effects of water pH on copper toxicity to early life stages of common carp (*Cyprinus carpio* L.) – Environ.Toxicol.Chem. 15: 376-383

Studnicka M. 1977 – Badania nad zawartością w tkankach i toksycznością rtęci dla ryb – Rozprawa naukowa 47, AR Lublin

Szulkowska-Wojaczek E., Marek J., Dobicki W., Polechoński R. 1992 – Metale ciężkie w środowisku stawowym – Zesz.Nauk. AR Wrocław, Zoot. 37: 7-25

Taylor W.R. 1967 – An enzyme method of clearing and staining small vertebrates – Proc.U.S.Natl.Mus. 122: 1-17

Vasnecov V.V., Eremceva E.F., Lange N.O., Dmitrova E.N., Braginskaja R.J. 1957 – Etapy razvitija promyslovych poluprochodnych ryb Volgii Dona – lesca, sazana, vobly, tarani i sudaka – Tr.Inst.Morf.Živ. 16: 27-35

Weis J.S., Weis P. 1977 – Effects of heavy metals on development of the killfish, *Fundulus heteroclitus* – J.Fish.Biol. 11: 49-54

Weis J.S., Weis P. 1995 – Swimming performance and predator avoidance by mummichog (*Fundulus heteroclitus*) larvae after embryonic or larval exposure to methylmercury – Can.J.Fish.Aquat.Sci. 52: 2168-2173

Woodward D.F., Farag A.M., Mueller M.E., Little E.E., Vertucci F.A. 1989 – Sensitivity of endemic Snake river cutthroat trout to acidity and elevated aluminium – Trans.Am.Fish.Soc. 118: 630-643

Yulin W., Hongru Z., Lanying H. 1990 – Effects of heavy metals on embryos and larvae of flat fish *Paralichthys olivaceus* – Oceanol.Limnol.Sin. 21: 386-392

STRESZCZENIE

WPŁYW METALI CIĘŻKICH NA ROZWÓJ POSTEMBRIONALNY KARPIA (*Cyprinus carpio* L.)

Zbadano wpływ ołowiu i miedzi na rozwój postembrionalny karpia. Analizowano tempo rozwoju i wzrostu, przeżywalność oraz zmiany w szkieletach. Metale ciężkie powodują opóźnienie rozwoju (od etapu C_1) i wzrostu, spadek przeżywalności, szczególnie podczas przechodzenia na egzogenne odżywianie, oraz zmiany w szkieletach. Miedź powoduje zahamowanie kostnienia, a ołów rozwój skoliozy.

ADRES AUTORA:

Dr hab. Barbara Jezierska
Wydział Rolniczy, Zakład Fizjologii Zwierząt
Akademia Podlaska
08-110 Siedlce, ul. Prusa 12
E-mail: jezbar@ap.siedlce.pl