Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 10	Fasc. 2	153-165	2002

THE EFFECT OF TEMPERATURE AND HEAVY METALS ON HEART RATE CHANGES IN COMMON CARP *CYPRINUS CARPIO* L. AND GRASS CARP *CTENOPHARYNGODON IDELLA* (VAL.) DURING EMBRYONIC DEVELOPMENT

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ABSTRACT. The study was conducted on common carp and grass carp embryos and larvae developed under laboratory conditions, at various temperatures and in the presence of heavy metals (Cu 0.20-0.27 mg dm^{-3}, Pb 2.0-4.0 mg dm^{-3}, Cd 0.2 mg dm^{-3}). Heart rate was measured at various developmental stages and was observed to increase along with fish development in all experimental groups. This may be explained by the increase in the metabolic rate of developing embryos. Development was faster at higher temperatures, and the heart rate was usually higher. The results of the present study confirm that heart rate is a reliable indicator of the metabolic rate of developing fish embryos.The embryos and larvae which were exposed to heavy metals had higher heart rates in comparison to those of the control group. This indicates that metal-induced stress caused an increase in metabolic rate. A decrease in heart rate during hatching was observed at non-optimum temperatures and was particularly pronounced in metal-exposed embryos; this indicates that the disturbances were related to the high sensitivity of the fish at this stage.

Key words: COMMON CARP (*CYPRINUS CARPIO*), GRASS CARP (*CTENOPHARYNGODON IDELLA*), EMBRYOS, HEAVY METALS, HEART RATE, TEMPERATURE

INTRODUCTION

Many data obtained for adult fish show that, in addition to oxygen consumption measurements, heart rate is also a reliable metabolic rate indicator (Priede and Tytler 1977, Amstrong 1986, Scharold and Gruber 1991, Lucas 1994, Thorarensen et al. 1996). Brodeur et al. (2001) maintain, however, that cardiac output is a sounder one.

Water temperature affects fish metabolic rate, and fish heart rate increases with increasing temperature (Heath and Hughes 1973, Cech et al.1976, Priede and Tytler 1977, Kolok et al. 1993, Brodeur et al. 2001).

The present study was undertaken to evaluate the reliability of heart rate as a metabolic rate indicator in fish embryos and to determine the effects of temperature and heavy metals on this parameter.

MATERIAL AND METHODS

Heart rate measurements were done on common carp *Cyprinus carpio* L. and grass carp *Ctenopharynogodon idella* (Val.) embryos and larvae developed under laboratory conditions in 1995-2000 in 10 experimental series.

The eggs and sperm were obtained from artificially stimulated spawning at the hatchery of the Inland Fisheries Institute in Żabieniec. The eggs were fertilized using the dry method and placed in plastic sieves (100-200 in each) in 12 dm^3 aquaria equipped with aquatic thermostats to maintain a constant water temperature (± 0.5°C). The experimental set is presented by Ługowska and Jezierska (2000).

Dechlorinated tap water with a total hardness of 167 mg dm^{-3} as $CaCO_3$, and a pH of 7.8-8.0 was used in the experiment. Water without the addition of metals was the control (concentration 0). Lead solutions were made using $Pb(NO_3)_2$, copper solutions - $CuSO_4$, and cadmium - $CdCl_2 \cdot 2\,1/2 H_2O$.

The experimental conditions are presented in Table 1.

TABLE 1

Experimental conditions

Species	Series	Temperature (°C)	Metal concentration (mg dm^{-3})	Development stages at which heart rate was measured	n
Common carp	I	20, 26	0 Cu 0.27 Pb 2.0	2, 5, 7, 9	20
	II	20, 26	0 Cu 0.27	1, 2, 3, 4, 5, 6, 7	5
	III	20, 26	0 Cu 0.27	1, 2, 3, 4, 5, 6, 7	5
	IV	20, 26	0 Cu 0.25	1, 3, 4, 5, 7, 8	10
	V	17, 20	0 Pb 4.0	5, 7	20
	VI	22	0 Cu 0.2 Cd 0.2 Cu+Cd 0.2	1, 3, 4, 5, 7	20
Grass carp	VII	20, 26	0 Pb 3.0	1, 3, 4, 5, 7, 8	10
	VIII	20, 26	0 Pb 4.0	5, 7, 8	20
	IX	20, 26, 30	0	5, 7, 8	20
	X	26	0 Cd 0.2 Cu+Cd 0.2	5, 7, 8	10

The embryos (n = 5-20 in various series) used for heart rate measurements were randomly sampled from each sieve at various developmental stages (DS): 1 – first heart movements; 2 – eye pigmentation; 3 – body pigmentation; 4 – blood coloration; 5 – just before hatching; 6 – during hatching; 7 – immediately after hatching; 8 – 24 hours after hatching. The embryos were placed individually on concave glass in a drop of appropriate solution and observed under 12 × 4 magnification. Heart rate was measured for 30 s using a stopwatch, and the number of heart contractions was calculated per min.

The results are presented in graphs as arithmetic means (developmental stages 5 – just before hatching, and 7 – immediately post hatching were marked). The significance of differences between the control group (optimum temperature, without metals) and other temperature or metal-exposed groups were calculated using the t-Student test ($P < 0.05$).

RESULTS

Changes in heart rate during common carp embryonic development at 20 and 26°C are shown in Fig.1 (ABCD). Despite the differences among the series, the heart rates gradually increased during development, with some disturbances at hatching. In series II and III at 26°C, the heart rate was considerably lower after the end of hatching than just before it. The embryos reached each developmental stage earlier at higher temperatures. In series I and IV the heart rates were significantly higher at 26°C as compared to 20°C.

Figs 2 and 3 (ABCD) illustrate changes in the heart rates of the control and copper-exposed fish at 20 or 26°C. Copper-exposed fish had higher heart rates in comparison to those of the control fish. However, during the hatching period itself, disturbances, including a reduction in heart rate, were observed in the copper-exposed fish (Fig. 2 D, 3 C, D).

Lead exposure caused an increase of heart rate at 20°C (Fig. 4 A), which was confirmed by the results obtained in series V when heart rate was measured only before and after hatching. The values were 134.1 and 139.6 counts min^{-1} for the control group and 156.2 and 171.2 in the lead exposure group, respectively.

At 26°C, the heart rate increase was slowed slightly in the lead-exposed fish during hatching (Fig. 4 B) and considerably reduced at 17°C (series V) from 135.3 counts min^{-1} at DS 5 to 118.8 counts min^{-1} at DS 7.

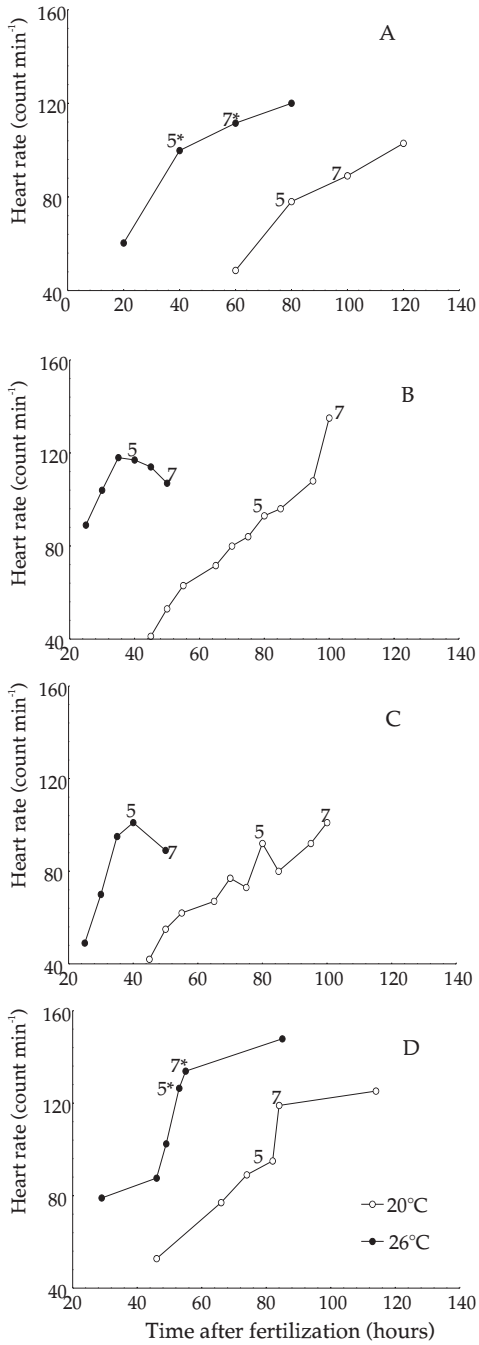

Fig. 1. The effect of temperature on common carp heart rate during embryonic development. A – series I, B – series II, C – series III, D – series IV, 5 – before hatching, 7 – after hatching, * - significantly different from 20°C.

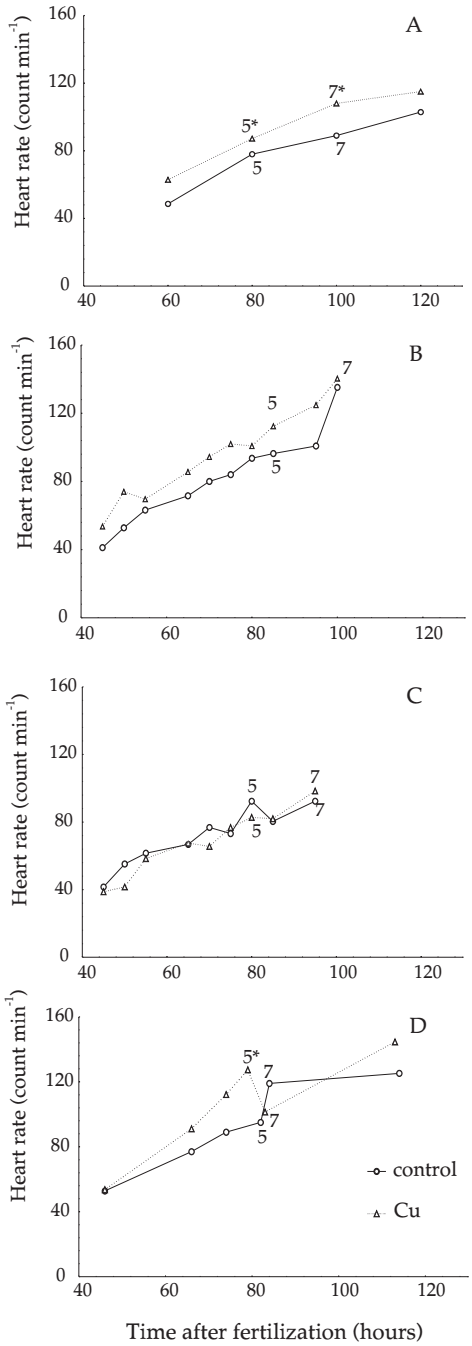

Fig. 2. The effect of copper exposure on common carp heart rate during embryonic development at 20°C. A - series I, B – series II, C – series III, D – series IV, 5 – before hatching, 7 – after hatching, * - significantly different from the control.

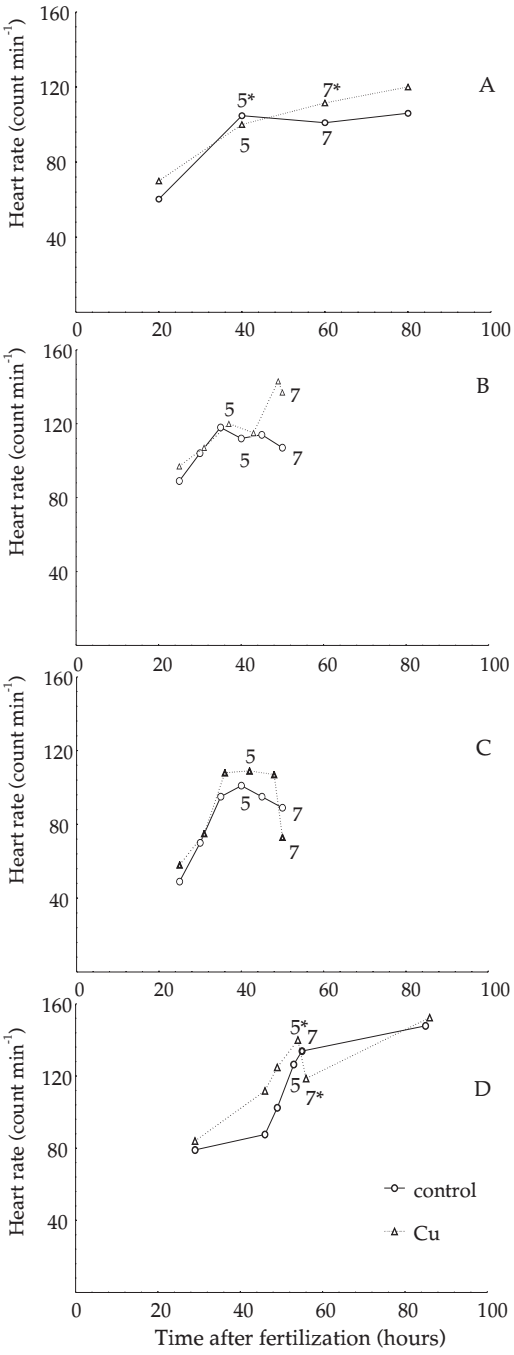

Fig. 3. The effect of copper exposure on common carp heart rate during embryonic development at 26°C. A - series I, B – series II, C – series III, D – series IV, 5 – before hatching, 7 – after hatching, * - significantly different from the control.

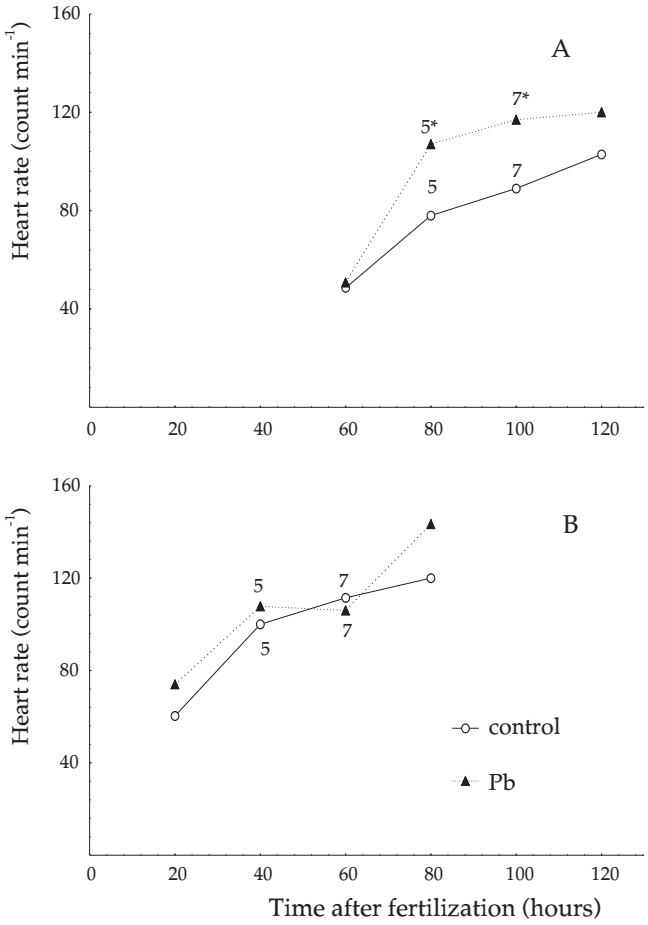

Fig. 4. The effect of lead exposure on common carp heart rate during embryonic development at various temperatures (series I). A – 20°C, B – 26°C, 5 – before hatching, 7 – after hatching, * - significantly different from the control.

Fig. 5 presents changes in the heart rates of common carp embryos under control conditions and under exposure to copper, cadmium and mixed copper-cadmium. In all cases, the heart rates were higher in the metal-exposed embryos. Heart rate was, however, reduced during hatching in the group exposed to copper and after hatching in that exposed to the copper-cadmium mixture.

The results of the effect of temperature on grass carp heart rate are shown in Fig. 6. Similarly as with common carp, differences among various experimental series occurred, and the heart rates increased during and after hatching. The development

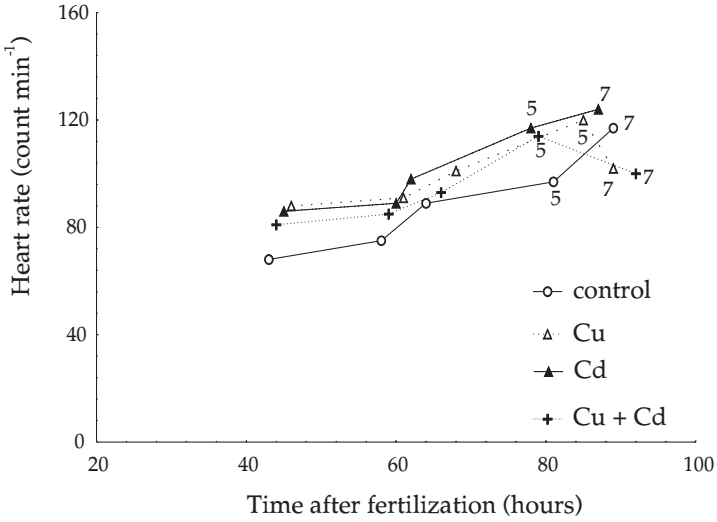

Fig. 5. The effects of copper, cadmium and copper-cadmium exposure on common carp heart rate during embryonic development at 22°C (series VI). 5 – before hatching, 7 – after hatching.

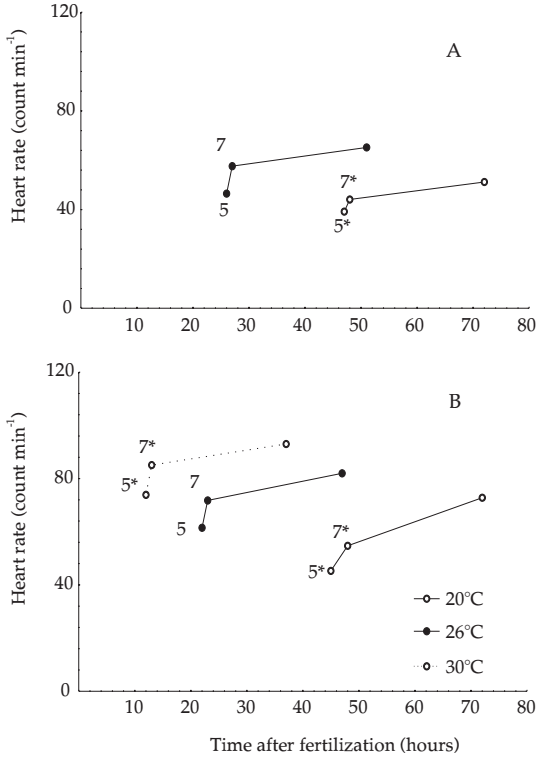

Fig. 6. The effect of temperature on grass carp heart rate during embryonic development. A – series VIII, B – series IX, 5 – before hatching, 7 – after hatching, * - significantly different from 26°C.

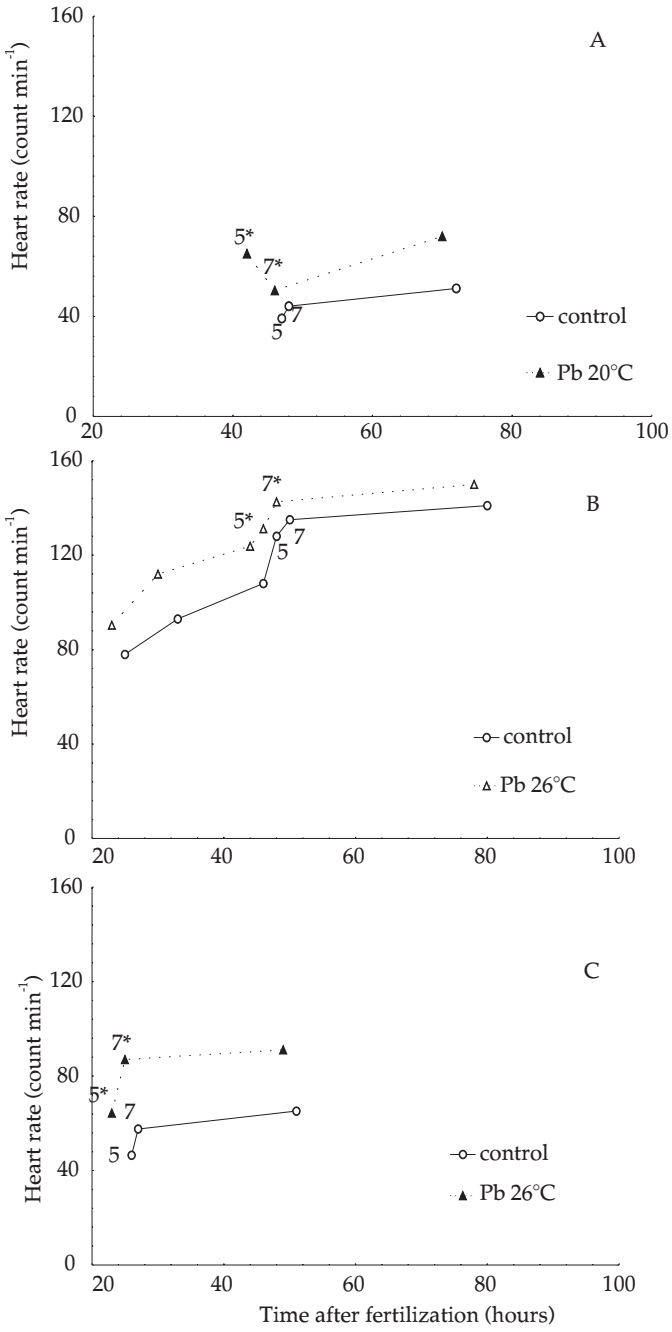

Fig. 7. The effect of lead exposure on grass carp heart rate during embryonic development at 26°C. A - series VIII (20°C), B – series VII, C – series VIII, 5 – before hatching, 7 – after hatching, * - significantly different from the control.

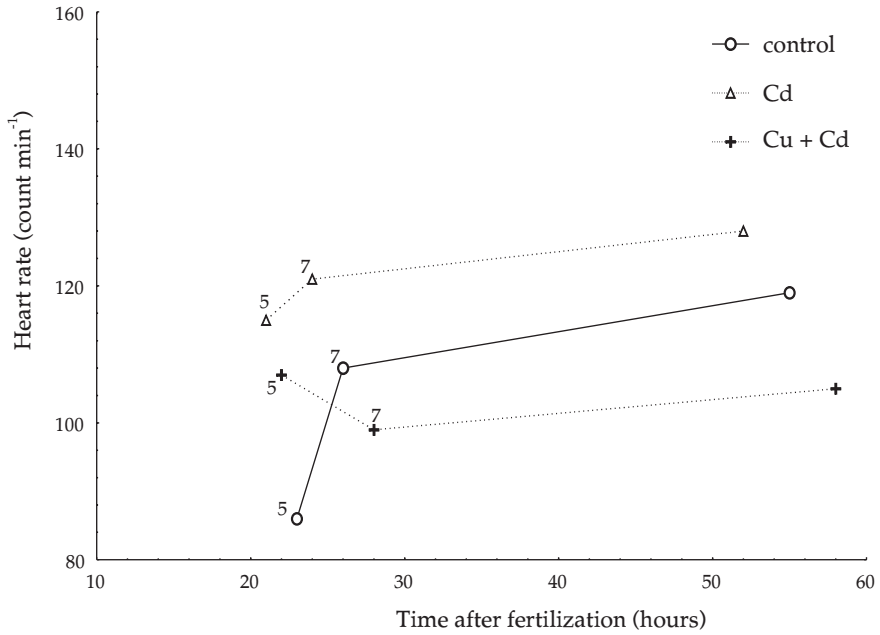

Fig.8. The effect of cadmium and copper-cadmium exposure on grass carp heart rate during embryonic development (series X). 5 – before hatching, 7 – after hatching.

rates and heart rates were higher at higher temperatures. The results shown in Fig. 7 indicate that lead caused an increase of heart rate and a slight increase in the development rates of grass carp embryos. Fig. 7A shows that there was a reduction in heart rate after hatching in lead-exposed fish as compared to the value obtained just before it.

Exposure to the cadmium and cadmium-copper mixtures also resulted in an increase of heart rate immediately after hatching and in the 24 hours following the end of it (Fig. 8).

DISCUSSION

The results of the present study indicate that there is an increase in heart rate during embryonic development for both common and grass carp. The comparison of the results obtained in various experimental series indicate considerable variability, but the direction of the changes was clear. No other data confirming such changes were found. According to Kamler (1972), however, the oxygen consumption of common carp embryos increased during development which indicates an increase in meta-

bolic rate. The increase in heart rate during development might have been related to the observed increase in embryonic activity. A positive relationship between the activity of adult fish and their heart rate was reported by Stevens and Randall (1967 a, b), Priede (1974), Scharold and Gruber (1991), Lucas (1994), and Brodeur et al. (2001).

Therefore, the data presented indicate that heart rate is also a reliable indicator of metabolic rate in embryos.

Although the differences were not always statistically significant, the results of the present study show an increase in embryonic heart rate. Assuming that heart rate may be an indicator of metabolic rate, it can be concluded that at higher temperatures the intensity of metabolism of common carp embryos increased and resulted in accelerated development.

The results obtained indicate that metal exposure caused an increase in embryonic and larval heart rates. Metal toxicity might have induced stress in fish and enhanced their activity. An increase in metabolic rate that involved a rise in heart rate might have been an adaptive reaction. Other authors also obtained data indicating that environmental stress might induce an increase in heart rate. Davison et al. (1995) studied short-term capture stress in *Trematomus bernacchii* and observed an increase in heart rate. Laitinen and Valtonen (1995) reported increased heart rates in *Salmo trutta* (L.) exposed to aluminum in acidic water.

These relationships may be disturbed during hatching - a critical period of particular sensitivity in fish. It is very important in metal exposures since the egg shell breaks during hatching and no longer protects the fish from toxicity (Cleveland et al. 1986, Słomińska 1998, Kazlauskiene and Stasiunaite 1999).

The sensitivity of hatching larvae probably also depends on parental factors which is indicated by the differences among various experimental series. The results also revealed that the sensitivity of hatching larvae increases under adverse environmental conditions such as non-optimum temperature and metal pollution. Non-optimum temperatures, 17 and 26°C for common carp and 20°C for grass carp (Ługowska and Jezierska 2000), combined with metal exposure disturb metabolic functions during hatching, which results in the reduction of the heart rates of hatching larvae.

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STRESZCZENIE

WPŁYW TEMPERATURY I METALI CIĘŻKICH NA ZMIANY BICIA SERCA PODCZAS ROZWOJU EMBRIONALNEGO KARPIA *CYPRINUS CARPIO* L. I AMURA BIAŁEGO *CTENOPHARYNGODON IDELLA* (VAL.)

Obiektem badań były embriony i larwy karpia oraz amura białego, których rozwój przebiegał w wylęgarni laboratoryjnej w różnych temperaturach i w obecności metali (Cu, Pb, Cd). Na poszczególnych etapach rozwoju wybierano losowo określoną liczbę (n) zarodków, u których oznaczano tempo bicia serca (tab. 1).

Przedstawione na rysunkach (rys. 1-8) zmiany tempa bicia serca pozwalają na stwierdzenie, że wraz z rozwojem częstotliwość bicia serca wzrasta we wszystkich grupach eksperymentalnych. W wyższych temperaturach obserwowano wcześniejsze osiąganie kolejnych etapów rozwoju i na ogół wyższą częstotliwość bicia serca, co można tłumaczyć także wyższym tempem przemian metabolicznych.

Na podstawie otrzymanych wyników można uznać, że tempo bicia serca jest dobrym wskaźnikiem zmian intensywności przemian metabolicznych rozwijających się embrionów.

Ekspozycja w badanych stężeniach metali powodowała wzrost tempa bicia serca embrionów i larw (rys. 2-5, 7, 8). Można to tłumaczyć tym, że toksyczne działanie niewielkich stężeń wywołało stres przejawiający się u ryb zwiększonym tempem metabolizmu.

Stwierdzony w temperaturach pozaoptymalnych, szczególnie po ekspozycji w wodzie zawierającej metale, spadek częstotliwości bicia serca w czasie wykluwania wskazuje na zakłócenia związane z większą wrażliwością ryb na niekorzystne działanie warunków środowiska w tym okresie rozwoju.

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