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Haematological indices are modulated in juvenile carp, *Cyprinus carpio* L., exposed to microcystins produced by cyanobacterial water bloom

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Abstract

This study evaluated the influence of toxic cyanobacterial water blooms on the blood indices of the common carp, *Cyprinus carpio* L. Experimental fish were exposed to a natural population of cyanobacterial water blooms (mainly *Microcystis aeruginosa* and *M. ichthyoblabe*), which contained microcystins [total concentration 133–284 μg g^{-1} (DW), concentration in water 2.8–7.4 μg L^{-1}]. Haematological indices showed marked changes in fish exposed to the cyanobacterial population in comparison with the control group. Statistical evaluation of the influence of cyanobacterial water blooms on biochemical indices of the juvenile carp showed a distinct decrease in albumin, alanine aminotransferase, total bilirubin, calcium, cholesterol, glucose, phosphorus and iron when compared to controls. Values of red blood counts [haemoglobin, haematocrit (PCV), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration] and lactate were significantly increased compared to controls. After exposure to cyanobacterial water bloom, the carp were kept in clean water to monitor the persistence of biochemical indices. The influence of cyanobacterial populations on calcium, cholesterol, glucose, lactate, phosphorus and PCV persisted up to 28 days after conclusion of the experiment. Duration of exposure, toxicity and density of cyanobacterial water blooms had an important impact on individual haematological indices.

Keywords: carp, cyanobacteria, haematology, microcystins.

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Introduction

Eutrophication of aquatic ecosystems is accompanied by mass development of cyanobacteria, causing serious environmental problems. Cyanobacteria as photosynthesizing organisms produce biologically active compounds that may affect the growth and development of other water organisms and the physical and chemical characteristics of the water (Chorus, Falconer, Salas & Bartram 2000). Great attention has recently been paid to the impact of cyanobacterial toxins on fish (Landsberg 2002).

The influence of cyanotoxins on fish following experimental intoxication or the impact of an environment containing cyanotoxins on fish has been studied using clinical, morphological, histological, ultrastructural, haematological and biochemical methods. One of the most common results of cyanobacterial blooms is the production of hepatotoxic microcystins (*Microcystis*) that can occasionally occur in high concentrations in shallow waters where cyanobacteria can accumulate and may induce injury to fish. A complete summary about the effects of microcystins on fish was provided by Malbrouck & Kestemont (2006). The majority of toxins are absorbed into the fish through the gastrointestinal tract, whereas toxin penetration through the skin or gills is negligible

Journal of Fish Diseases

(Tencalla, Dietrich & Schlatter 1994). It has been suggested that phytoplanktonophagous fish species are more affected by cyanobacterial toxins because of their greater digestion of cyanobacterial water blooms (Carbis, Rawlin, Grant, Mitchell, Anderson & McCauley 1997; Vajcová, Navrátil & Palíková 1998).

Intraperitoneal exposure to microcystins or oral application of lysates of cyanobacterial biomass caused significant changes in biochemical indices, red blood cells and plasma enzyme activities. Microcystins cause liver tissue damage in fish, demonstrated by the significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities (Rabergh, Bylund & Eriksson 1991; Navrátil, Palíková & Vajcová 1998; Vajcová *et al.* 1998).

The rate of increase in LDH, ALT and AST depends on the route of administration, characteristics of the material and the amount of toxin. Toxins are synthesized during the growth phase of the cyanobacteria, and large quantities of microcystins are released into the water during the collapse of the bloom or from actively growing cyanobacterial populations (Malbrouck & Kestemont 2006). Chorus & Bartram (1999) showed that 100% of toxins are located in the cells of young populations of cyanobacteria whereas in decaying cells, toxin concentrations in water rose to values of 70–80%.

Exposure of fish to media containing dispersed microcystins demonstrated that toxic effects are time delayed because of their limited penetration into healthy fish. The toxic effect after oral administration is approximately ten times weaker than after intraperitoneal application (Carbis *et al.* 1997).

The long-term impact of cyanobacteria containing microcystins at lower concentrations is relatively difficult to observe in individual fish; therefore, measurements from more individuals of a fish population are desirable. The measurement of aminotransferases (ALT, AST), bile acids, bilirubin, sodium and chloride from blood serum has been described (Carbis, Mitchell, Anderson & McCauley 1996).

Biochemical indices of blood and plasma in fish are affected by many endogenous and exogenous factors. Changes of haematological and biochemical indices in common carp could be caused by chemical factors (Kopp & Heteša 2000; Lusková, Svoboda & Kolářová 2002; Pepeljnjak, Petrinec, Kovacic & Segvic 2003), age, health and body condition (Svetina, Matasin, Tofant, Vucemilo & Fijan 2002), nutrition (Serpunin 1995) or stress (Dobsikova, Svobodova, Blahova, Modrá & Velisek 2006).

The aim of this work was to study the effects of a natural cyanobacterial population with a known amount of microcystins on the biochemical indices of juvenile carp, *Cyprinus carpio* L.

Materials and methods

Fish

Yearling carp (500 individuals) obtained from a single artificial stripping (Fishpond Management Pohořelice) were used for experiments. Fish measured 14.6 ± 1.8 cm and had an average body weight of 47.2 ± 19.3 g. The fish were acclimatised for 2 weeks before the start of the study in natural water without cyanobacteria. Fish were caged and exposed to a cyanobacterial bloom that naturally developed in the breeding pond for 28 days during the months of August to October, 2005. In parallel, control groups of fish were also kept in another pond without any apparent cyanobacterial bloom. Fish were reared under natural conditions without additional feeding. The experimental and control breeding pond had the same water source. After exposure, the carp were placed into two 1000-L tanks containing dechlorinated drinking water (clean water) for 28 days. Fish were exposed to a 12-h light/12-h dark photoperiod, and the tank water was changed daily. Water parameters during the experiments were as follows (given for experimental and control groups, respectively): water temperature 19.2 ± 1.1, 19.6 ± 1.1 °C; dissolved oxygen 10.0 ± 3.2, 11.1 ± 2.8 mg L^{-1}; pH 8.9 ± 0.6, 9.1 ± 0.2; ammonia 0.38 ± 0.14, 0.45 ± 0.14 mg L^{-1} $N\text{-}NH_4^+$; and nitrite 0.058 ± 0.010, 0.070 ± 0.011 mg L^{-1} $N\text{-}NO_2^-$. Oxygen, temperature and pH were measured by a WTW Oxi 340i dissolved oxygen meter and a WTW pH 340i pH meter. Ammonium ions were determined by the Nessler method and nitrites by a method using N-(1-naphthyl)-ethylenediamine (APHA 1981).

Phytoplankton and microcystins

Cyanobacterial and algal biomass were evaluated every week by chlorophyll *a* concentrations (ISO 10260 1992) and by microscopical analyses. The

number of cells was counted in a Bürker's counting chamber. Cyanobacterial biomass (dominated by coccal *Microcystis aeruginosa* and *M. ichthyoblabe*) concentration varied from 198 to 598 μg L^{-1} (3.9–6×10^5 cells mL^{-1}) in the experimental pond. The algal biomass (dominated by chlorococcal green algae – *Scenedesmus* and *Coelastrum*) concentration varied from 216 to 445 μg L^{-1} (1.3–5.4×10^4 cells mL^{-1}) in the control pond.

Concentrations of microcystins in the cyanobacterial and algal biomass were determined by a previously published method using HPLC (Agillent 1100 system, Supelcosil ABZ + Plus C18 column) coupled with a photodiode array detector (Bláha & Maršálek 2003). The concentrations of microcystins in the experimental and control breeding ponds are presented in Table 1. The concentrations are comparable with microcystin levels from other ponds in the Czech Republic (Maršálek, Bláha, Turánek & Neča 2001).

Sampling and measuring of haematological parameters in blood and plasma

Immediately after capture of fish, samples of blood were collected from 20 specimens (ten from experimental and ten from control group). Inadequate and haemolytic specimens of blood were discarded. Sampling of fish was conducted at the beginning of the experiment and every 7th day during and after exposure.

Fish blood was taken by cardiopuncture using heparinized syringes. Heparin at a concentration of 50 IU mL^{-1} was used for blood stabilization. Haemoglobin (Hb), haematocrit (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), erythrocyte (RBC) and leucocyte (WBC) counts were determined by standard methods (Svobodová, Pravda & Paláčková 1991).

The blood was centrifuged at 400 ***g*** for 15 min at 4 °C, and the resulting plasma stored at −80 °C until analysis. Biochemical analyses of blood plasma were conducted using commercially available reagents (Table 2) using the ADVIA 1650 automatic analyzer (Bayer). All serum enzymatic activities were analysed at 37 °C.

Statistical analyses

Data processing was in two stages. Significant differences between every individual experimental and control group were analysed by using the Unistat 5.0 *t*-test.

To determine the total effect of cyanobacteria, blood indices were analysed as follows. First, presumption of data normality and skedasticity was verified, followed by the *F*-test. Data showing abnormality and homoskedasticity was analysed by the Kruskal–Wallis test. Normality or abnormality and heteroskedasticity was transformed before analysis. The significant differences of the total effect of cyanobacterial bloom were analysed using NCSS2000 (NCSS, Kaysville, Utah, USA) and SCAN software (SaTScan, www.satscan.org).

Results

The results of the study are presented in Table 3. The biochemical parameters affected by cyanobacterial water bloom are presented in Fig. 1. Total statistical evaluation of the influence of the cyanobacterial water bloom on biochemical indices of the juvenile carp showed a significant decrease in albumin, ALT, total bilirubin (BIL), calcium, cholesterol (CHOL), glucose (GLU), phosphorus and iron compared to controls. The red blood cell

Table 1 Microcystin concentrations during the experiment

Duration of experiment (days)	Experimental pond								Control
	In biomass (μg g^{-1}) DW		IC (μg L^{-1})		EC (μg L^{-1})		IC + EC (μg L^{-1})		IC + EC (μg L^{-1})
	MC-LR	Total	MC-LR	Total	MC-LR	Total	MC-LR	Total	Total
0	99.0	243.5	3.5	6.9	0.2	0.5	3.7	7.4	<LOD
7	157.0	187.0	–	–	–	–	–	–	<LOD
14	372.1	382.3	3.3	4.6	0.5	1.0	3.8	5.6	0.14
21	317.1	383.9	1.5	1.9	0.5	0.9	2.1	2.8	0.12
28	133.4	133.4	2.8	3.6	0.1	0.1	2.9	3.7	0.23

DW, dry weight; IC, intracellular; EC, extracellular; LOD, limit of detection, 0.05 μg L^{-1}; MC-LR, microcystin-LR.

Table 2 Methods used for the biochemical serum analyses

Constituent	Method	Kit	References
Acid phosphatase	Enzymatic method, 1-naphtyl phosphate	Roche	Hillmann (1971)
Albumin	Bromcresol green	Skalab	Doumas *et al.* (1971)
Alkaline phosphatase	Enzymatic method, AMP buffer	Bayer	Tietz *et al.* (1980)
Alanine aminotransferase	Kinetic UV	Lachema	Expert panel (1976)
Total bilirubin	Oxidation, Potassium ferricyanide	Skalab	O'Leary *et al.* (1993)
Creatinine	Jaffe, kinetic without deproteination	Bayer	Jaffe (1886)
Aspartate aminotransferase	Kinetic UV	Lachema	Expert panel (1976)
Calcium	Colorimetric method, arzenazo III	Skalab	Ichaylova & Ilkova (1971)
Total protein	Biuret	Lachema	Doumas *et al.* (1981)
Glucose	Colorimetric method, GOD-POD	Lachema	Barham & Trinder (1972)
Magnesium	Colorimetric method, arzenazo III	Skalab	Škavrada (1999)
Lactate	Enzymatic method	Bayer	Shimojo *et al.* (1989)
Lactate dehydrogenase	Enzymatic method, P → L.	Lachema	Hajzer & Jagelková (1988)
Phosphorus	Molybdate UV	Skalab	Kratochvíla & Garčic (1977)
Iron	Ferene, without deproteination	Skalab	Higgins (1981)
Urea	Urease UV	Bayer	Roch-Ramel (1967)
Cholinesterase	Kinetic test, butyryl thiocholine	Skalab	Garry (1971)
Cholesterol	Enzymatic method, CHOD-PAP	Bayer	Roeschlau *et al.*(1974)

Table 3 The effect of cyanobacteria on biochemical blood and plasma indices of carp (average ± SD)

	Exposure under natural conditions		Depuration in clean water	
Constituent	Cyanobacteria	Control	Cyanobacteria	Control
Acid phosphatase, μkat L^{-1}	0.20 ± 0.29	0.37 ± 0.59	0.12 ± 0.03	0.15 ± 0.05
Albumin, g L^{-1}	6.36 ± 0.88*	7.46 ± 1.32	7.71 ± 1.07	8.15 ± 1.40
Alkaline phosphatase, μkat L^{-1}	0.46 ± 0.30	0.47 ± 0.29	0.45 ± 0.40	0.27 ± 0.16
Alanine aminotransferase, μkat L^{-1}	0.29 ± 0.16*	0.58 ± 0.89	0.88 ± 0.59	0.64 ± 0.26
Total bilirubin, μM	0.01 ± 0.03*	2.45 ± 1.50	0.00	0.15 ± 0.31
Aspartate aminotransferase, μkat L^{-1}	4.41 ± 3.02	5.50 ± 4.38	3.95 ± 1.89	4.99 ± 2.69
Calcium, mM	2.21 ± 0.16*	2.48 ± 0.22	2.41 ± 0.18**	2.57 ± 0.22
Cholesterol, mM	3.08 ± 0.21*	4.72 ± 1.16	3.24 ± 0.65**	4.56 ± 1.32
Total protein, g L^{-1}	23.60 ± 2.35	26.77 ± 5.24	28.40 ± 3.04	28.50 ± 3.65
Glucose, mM	2.54 ± 0.50*	3.58 ± 0.72	2.88 ± 1.15**	3.48 ± 0.63
Creatinine, μM	14.29 ± 1.31	13.24 ± 1.76	18.13 ± 5.56	18.02 ± 4.64
Magnesium, mM	0.99 ± 0.11	1.05 ± 0.16	1.04 ± 0.13	1.09 ± 0.18
Lactate, mM	4.22 ± 1.61*	3.66 ± 1.15	5.10 ± 1.87**	5.41 ± 2.17
Lactate dehydrogenase, μkat L^{-1}	16.95 ± 12.02	21.19 ± 21.33	16.03 ± 8.61	19.79 ± 11.06
Phosphorus, mM	1.94 ± 0.37*	3.11 ± 0.56	1.82 ± 0.27**	2.15 ± 0.43
Iron, μM	1.90 ± 2.64*	8.90 ± 6.96	10.61 ± 7.71	14.11 ± 7.08
Urea, mM	0.98 ± 0.26	0.97 ± 0.37	1.18 ± 0.38	1.54 ± 0.57
Cholinesterase, μkat L^{-1}	0.33 ± 0.73	0.10 ± 0.20	1.85 ± 0.84	2.01 ± 0.97
Haemoglobin, g L^{-1}	66.0 ± 14.9*	53.6 ± 11.7	50.1 ± 12.6	50.1 ± 14.9
Haematocrit, L L^{-1}	0.25 ± 0.05*	0.23 ± 0.04	0.25 ± 0.05**	0.23 ± 0.06
Mean corpuscular volume, fL	214 ± 54	206 ± 32	199 ± 37	204 ± 48
Mean corpuscular haemoglobin, pg	54 ± 9*	47 ± 9	43 ± 11	40 ± 12
Mean corpuscular haemoglobin concentration, L L^{-1}	0.26 ± 0.04*	0.23 ± 0.04	0.22 ± 0.10	0.22 ± 0.08
Erythrocyte, T L^{-1}	1.22 ± 0.34	1.17 ± 0.24	1.30 ± 0.18	1.23 ± 0.25
Leucocyte, G L^{-1}	99 ± 33	117 ± 35	118 ± 29	97 ± 31

*Significant influences of cyanobacterial population on biochemical blood and plasma indices of carp ($P < 0.05$, compared to the control).
**Significant influences of cyanobacteria after 28-day exposure of carp in clean water.

(Hb, PCV, MCH and MCHC) and lactate (LACT) counts significantly increased when compared to controls. Changes in acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate aminotransferase, total protein, creatinine, magnesium, LDH, urea, cholinesterase (CHE), RBC, WBC and MCV were not significantly different from controls.

The significance of the differences between fish groups was analysed every 7th day of the experiment and did not always correspond to the total

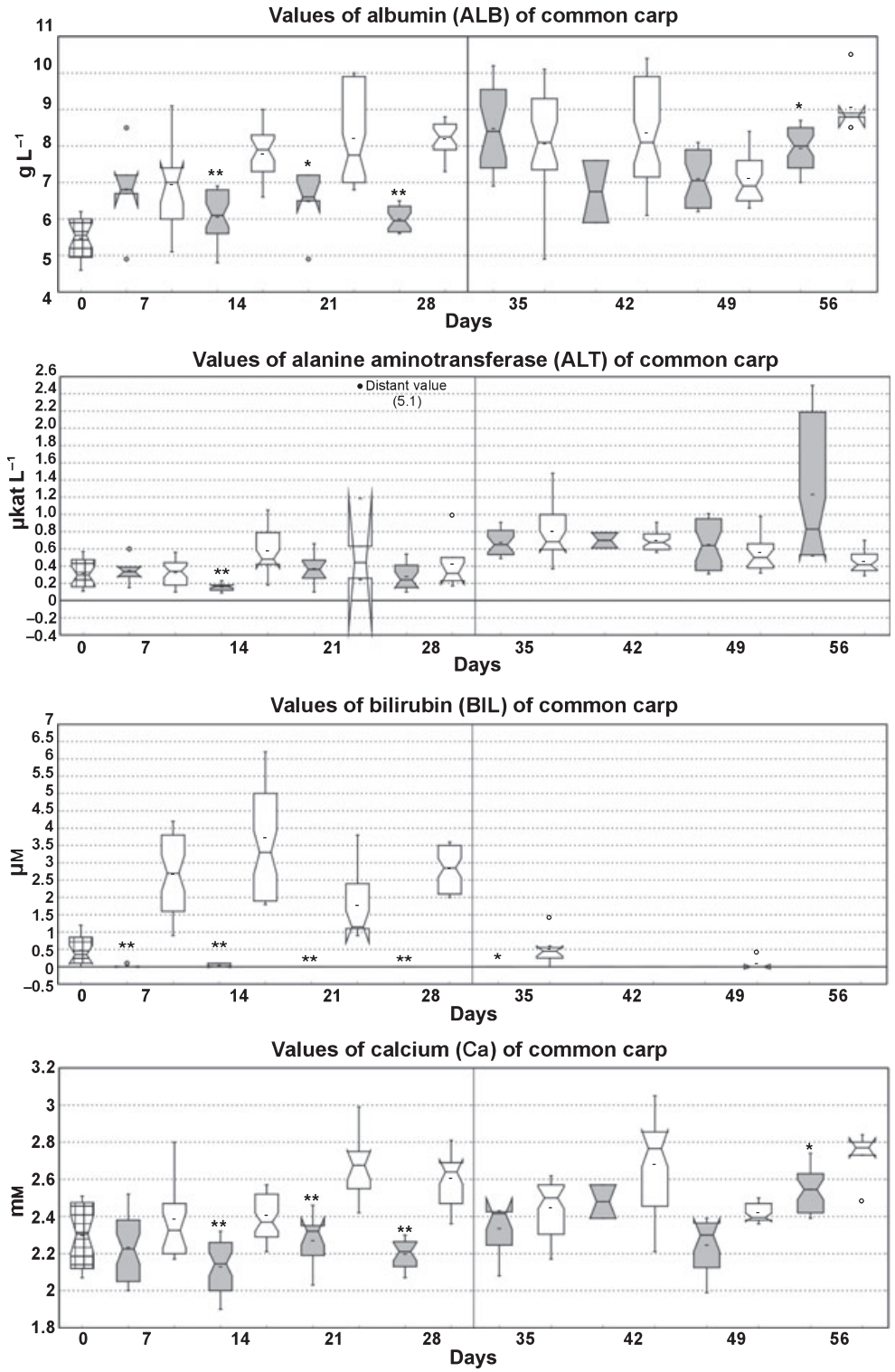

Figure 1 The effect of cyanobacteria on biochemical indices of the common carp. The box plots show the median value, standard error, the upper and lower interquartile range and the 95% confidence interval for the interquartile range. Significantly different indices compared to the control are marked by one asterisk ($P < 0.05$) or two asterisks ($P < 0.01$). Checked boxes indicate input of data. Open boxes indicate control, and tinted boxes indicate experimental groups. Black line in the middle of plots indicates fish transfer to clean water.

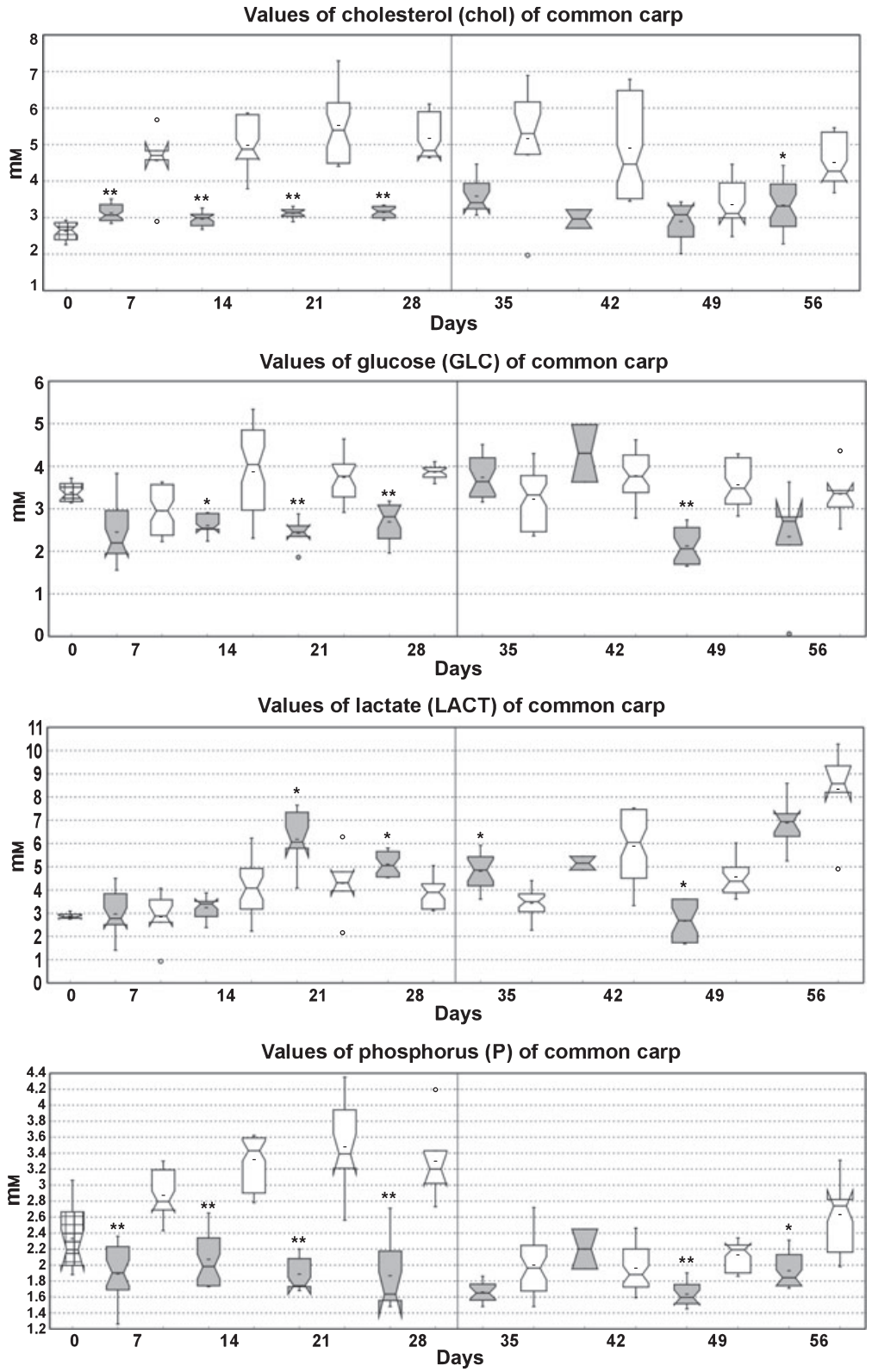

Figure 1 (Continued).

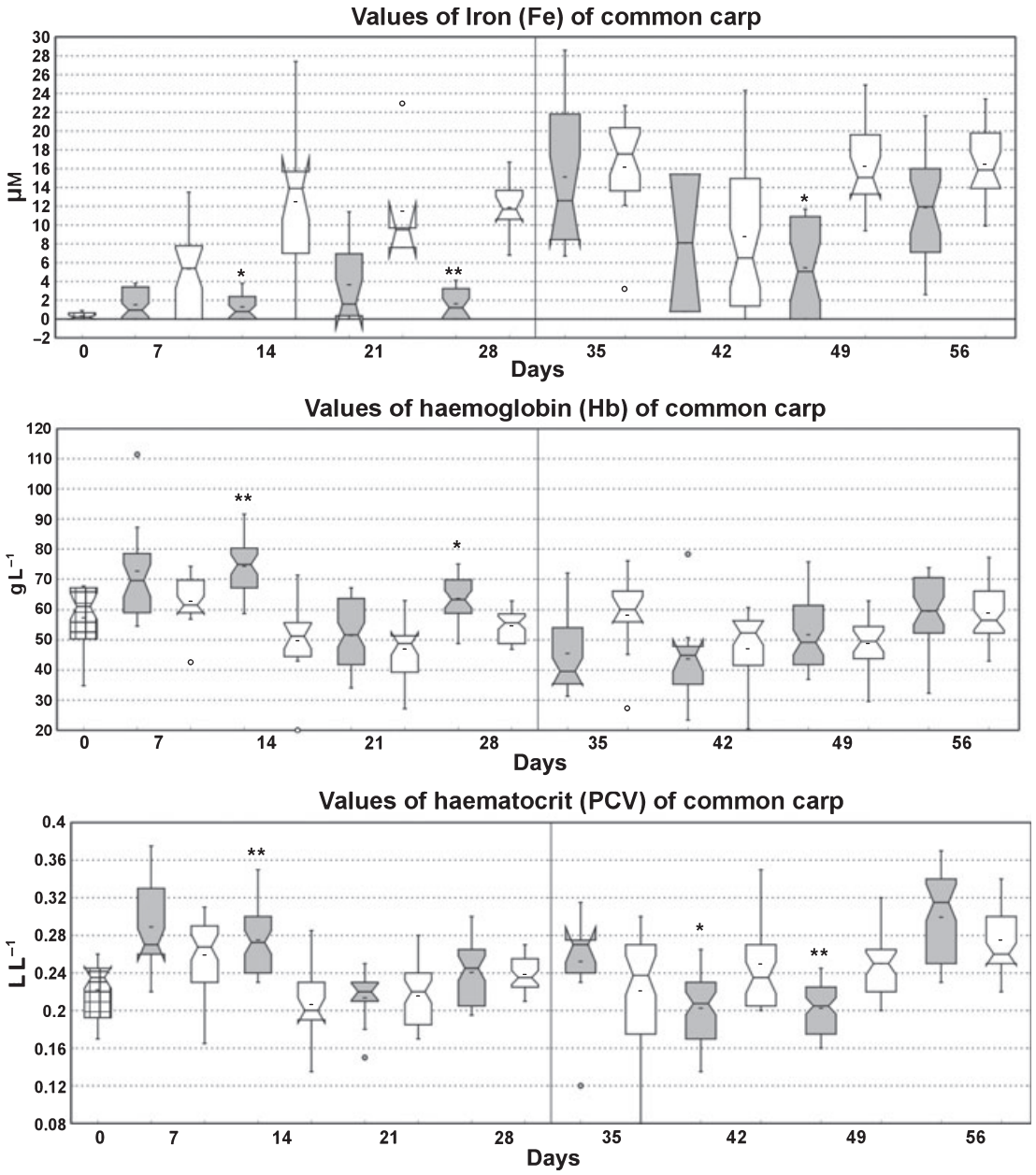

Figure 1 (Continued).

statistical evaluation. Values of BIL, CHOL and phosphorus were significantly different every week of the experiment under natural conditions (Fig. 1), whereas values of ALT, PCV, MCH and MCHC were only significant once in these natural conditions.

After exposure to cyanobacterial water blooms, the carp were placed in clean water to monitor the persistence of biochemical indices. The influence of the cyanobacterial population on the values of calcium, CHOL, GLU, LACT, phosphorus and PCV in the carp was monitored 28 days after exposure. Other indices such as albumin, ALT, BIL, haemoglobin, MCH and MCHC were not significantly different from the control group.

Discussion

The values of haematological indices obtained in this work do not correspond well with the results of

other authors (Rabergh *et al.* 1991; Tencalla *et al.* 1994; Carbis *et al.* 1996, 1997; Navrátil *et al.* 1998; Kopp & Heteša 2000). Some differences are probably mainly due to different ways of administration of toxin and cyanobacterial cell biomass and changes in the physiological status of the cyanobacterial populations.

Liver enzymes (ALT, AST and LDH) are the most frequently tested enzymes in fish for the indication of cyanobacterial toxicity. Rabergh *et al.* (1991) reported that the activity of blood plasma enzymes (ALT, AST and LDH) was raised in 2 h after an intraperitoneal injection of toxin as a consequence of hepatocyte necrosis. Tencalla *et al.* (1994) observed a decrease in their activity after 48 h and interpreted this as a result of damage of the majority of hepatocytes that were not able to release enzymes into the circulatory system. Significant increase in the activities of ALT, AST and LDH after intraperitoneal or oral administration of microcystin-LR to carp has been reported (Bury, McGeer, Eddy & Codd 1997; Navrátil *et al.* 1998; Malbrouck, Trausch, Devos & Kestemont 2003; Li, Chung, Kim & Lee 2004). Kopp & Heteša (2000) stated that the activity of blood plasma enzymes was increased after 96-h exposure of carp to a natural cyanobacterial population. Carbis *et al.* (1996) noted a delay of toxic manifestation in fish exposed to water with dispersed microcystin. Serum activities of AST and ALT increased 7 days after the carp were exposed to water that contained microcystins. Feral carp from a lake, where toxic *Microcystis aeruginosa* was dominant, had higher activity of AST in serum (Carbis *et al.* 1997). Malbrouck *et al.* (2003) reported that the activities of plasma enzymes (ALT, AST and LDH) completely recovered 21 days after the intraperitoneal injection of microcystin-LR.

Our results showed a significant decrease ($P < 0.05$) in values of ALT after exposure to cyanobacteria. Values of AST and LDH did not significantly change during the experiment. There was also a significant decrease ($P < 0.01$) in ALT compared to controls but this was only after 14 days of exposure. Daily low-dose exposure of fish to cyanobacteria containing microcystins caused non-significant changes in serum, ALT, AST and LDH activities. The absorption of common concentrations of microcystins in natural water through oral, dermal or branchial pathways may be limited in healthy fish. Acute toxicity of microcystins is unlikely to occur in feral carp, and chronic injury will probably not be detected by changes of enzyme (AST, ALT and LDH) activity in the blood plasma. Values of ALT in control and experimental fish did not differ after transfer to clean water.

Higher values of bilirubin may indicate hepatic injury. Toxic substances increase values of bilirubin in the common carp (Pepeljnjak *et al.* 2003). Concentration of bilirubin rises 8 h after intraperitoneal injection of microcystins (Carbis *et al.* 1996). Higher concentration of toxic cyanobacteria in a natural lake caused an increase in bilirubin concentration in serum of feral carp (Carbis *et al.* 1997). Our results showed a significant decrease in bilirubin values. The values were under the limit of detection (LOD < 0.03 μM) after 14-day exposure of the carp to cyanobacteria. The amount of microcystins which caused the rise of bilirubin concentration reported by Carbis *et al.* (1997) was much higher than in our experiments. Control values of bilirubin decreased after the transfer of carp to clean water, analogous to experimental fish values in natural water. Experimental and control values were under the LOD after 14-day exposure of carps to clean water. The decreased values of bilirubin were probably not caused by the influence of cyanobacteria.

Compared to the control fish, exposure to cyanobacteria had no effects on serum ALP, ACP and CHE activity. An increased value of ALP indicates the abnormal secretion of bile. ACP values were elevated in haemolytic specimens of plasma and when blood had coagulated. The decrease in CHE values may indicate chronic damage of the hepatopancreas (Masopust 1998). Kopp, Mareš, Kubíček & Babica (2005) showed that serum ALP activities significantly decreased and activities of CHE significantly increased in silver carp exposed to a toxic cyanobacterial population. The ALP and ACP activities of carp did not change in water that contained the pesticide diazinon (Lusková *et al.* 2002). The activities of alkaline and ACPs and CHE in our study support the assumption that the liver tissue of the experimental fish was not markedly affected. Likewise, the practically identical values of creatinine, observed in the control and experimental groups, indicate that the toxic population of cyanobacteria did not damage parenchymatous tissues or skeletal musculature. An increased value of creatinine is an indicator of kidney damage, muscular dystrophia and activity of the organism (Masopust 1998).

Bury, Eddy & Codd (1996), in brown trout, and Ernst, Hoeger, O'Brien & Dietrich (2006), in whitefish, observed slightly increased levels of GLU in fish exposed to cyanobacteria, but changes were not significant. An increase in GLU is a general response of fish to acute pollutant effects and stress (Lusková *et al.* 2002; Svobodová, Vykusová, Modrá, Jarkovský & Smutná 2006). Consequently, the stress, as shown by plasma GLU level, does not appear to be associated with liver pathology. The significant decrease in GLU in our experiment may be because of a high cyanobacterial cell concentration in the water, causing respiratory difficulties in the fish, a decrease in the availability of nutrients and an increased consumption of energy for basic metabolism. The values of GLU did not differ 7–14 days after transfer to clean water. Differences between control and experimental values of GLU following transfer (21–28 days) could be caused by other factors.

Blood cell parameters Hb, PCV, RBC, MCV, MCH and MCHC usually decrease after the application of pure microcystins or toxic cyanobacterial biomass as a consequence of extensive haemorrhage in the skin, eyes, hepatopancreas and swim bladder (Navrátil *et al.* 1998; Vajcová *et al.* 1998). Our results showed a significant overall increase in Hb, PCV, MCH and MCHC, but significant changes in individual weeks were not large. RBC and MCV did not increase significantly. Natural populations of cyanobacteria in water do not cause extensive injury to fish but change other environmental conditions (e.g. dissolved oxygen and ammonia). Cyanobacterial biomass causes fluctuations in oxygen saturation of the water, and as a possible compensation for the oxygen deficiency, the fish had higher haemoglobin and RBC counts. Hb, PCV, MCH and MCHC did not markedly change after transfer to clean water. Small differences among biochemical indices in the fish population were normal.

A significant decrease in total leucocyte counts (WBC) was observed after intraperitoneal or oral administration of microcystin-LR in carp (Palíková, Kovářů, Navrátil, Kubala, Pešák & Vajcová 1998). Our results showed a decrease in the values of total WBC counts, although this value was not significant. It should be noted that the leucocyte counts in blood were higher in the control and experimental groups of carp in our experiment compared to other authors (Svobodová *et al.* 1991; Palíková *et al.* 1998; Tripathi, Latimer & Burnley 2004).

In previous experiments, values of total protein significantly decreased after intraperitoneal application of pure microcystin-LR into common carp (Navrátil *et al.* 1998) and silver carp (Vajcová *et al.* 1998) but did not change in a study by Carbis *et al.* (1996). Total protein under the influence of natural cyanobacterial populations was reduced in common carp (Kopp & Heteša 2000) but was not changed in silver carp (Kopp *et al.* 2005). The values of total protein concentration in our experiment were in normal ranges for the serum biochemical indices of the common carp provided by Svobodová *et al.* (1991) and Carbis *et al.* (1997). Similarly, the almost identical values of urea, observed in the control and experimental carp groups, indicate that protein metabolism was not damaged by the toxic population of cyanobacteria. On the other hand, our results showed a significant decrease in values of albumin compared to the control. Significant changes in individual weeks occurred 14 days after the commencement of the experiment; values of albumin continued to differ even after fish had been transferred into clean water. Lower albumin values may indicate reduced liver activity, but the decline in values may be caused by other factors. The values of albumin were within the normal ranges found during the study of carp (Carbis *et al.* 1997) and silver carp (Kopp *et al.* 2005) under the influence of microcystins. Changes in values of albumin in carp were not observed even after the intraperitoneal application of microcystins (Carbis *et al.* 1996). Thus, microcystins alone do not influence albumin values and the decline of values may be affected by another cyanobacterial metabolite.

Most authors have reported an increase in plasma LACT concentration in various fish following stress and as a result of the acute effects of toxic substances including bacterial toxins (Dabrowska, Dabrowski, Meyer-Burgdorff, Hanke & Gunther 1991; Kakuta, Namba, Uematsu & Murachi 1991; Williams, Nelson & Heisler 1997; Kopp *et al.* 2005). On the other hand, Lusková *et al.* (2002) reported a decrease in plasma LACT concentration in carp in water containing the pesticide diazinon. In our experiment, a significant increase in LACT concentration in blood plasma of experimental fish compared to controls may indicate a lower metabolic rate of LACT in the hepatopancreas and/or be a result of stress from the effect of the cyanobacteria.

The negative effect of different pollutants at sublethal concentrations and of stress of fish may be shown by a decrease in CHOL values (Gluth &

Hanke 1985; Svobodová *et al.* 2006). The decrease in CHOL levels in our study may indicate slight damage to the hepatopancreas, analogous to the significant increase in LACT levels in our experiment. Significant changes at individual weeks began 7-days post-exposure, and CHOL value gradually became similar after fish transfer to clean water.

Our results showed a significant decrease in electrolyte values (Ca, Fe and P) 7–28 days after the exposure of the fish to cyanobacteria. Values of Mg decreased, although not significantly when compared to the control. Kopp *et al.* (2005) reported a significant decrease in values of Ca, and Mg and a significant increase in Fe and P in silver carp under the influence of a natural cyanobacterial population. Lusková *et al.* (2002) reported that levels of Ca and P in blood plasma of carp decreased in water containing pesticide. The basic function of electrolytes in the body lies in controlling fluid distribution, intra-and extracellular acid–base balance, maintaining osmotic pressure of body fluids and normal neuromuscular function. Calcium and phosphorus ions functionally participate in maintaining normal function of the heart, muscles and nerves, as well as the selective permeability of cell membranes. Magnesium and iron are important for normal function of the kidneys, liver and proteosynthesis. Decreased or increased values of electrolytes in blood plasma indicated the abnormal function of the fish. The values of electrolytes were not significantly changed 7–14 days after transfer to clean water. Subsequently, significant differences between individual groups of fish were seen. Differences in values of electrolytes (21–28 days after transfer) may have been caused by other factors.

Biochemical indices of blood and plasma in fish are affected by many endogenous and exogenous factors. Liver enzymes (ALT, AST and LDH) are the most suitable indicator parameters in fish of the toxicity of cyanobacteria after intraperitoneal or per-oral application. The toxic effect of cyanobacteria on fish under natural environmental conditions is much weaker than after intraperitoneal or per-oral application. In the case of chronic exposure, cyanobacteria will most likely not be detected by enzyme activity changes (ALT, AST, ALP, ACP, CHE and LDH) in the blood plasma. Our results show that liver damage was not so severe as to cause changes in the activities of liver enzymes. Other parameters, Ht, LACT, Ca, P, CHOL, BIL and GLU in particular, were influenced by the natural cyanobacterial water bloom together with other active substances and changes in water chemistry. The measurement of albumin, CHOL, LACT and electrolytes in blood serum is therefore recommended under natural conditions.

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