



REVIEW ARTICLE

Biochemical parameters of blood plasma and content of microcystins in tissues of common carp (*Cyprinus carpio* L.) from a hypertrophic pond with cyanobacterial water bloom

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Abstract

The aim of this study was to evaluate the dynamics of the blood plasma parameters and the content of microcystins in the tissues of common carp (*Cyprinus carpio* L.) in relation to the toxic cyanobacterial water bloom. Fish (average body mass 2176 ± 697 g) in the hypertrophic pond were exposed to natural water bloom (dominated by *Planktothrix agardhii*, *Pseudanabaena limnetica* and *Limnothrix redekei*), which contained microcystins (concentration in biomass $20\text{--}181 \mu\text{g g}^{-1}$ dry wt, concentration in water $0.3\text{--}9.5 \mu\text{g L}^{-1}$). Biochemical parameters in fish blood plasma were analysed in 89 fish at 14-day intervals during the whole season (nine sampling periods). Our results demonstrated high variability and fluctuations in the investigated parameters. The content of microcystins and density of cyanobacterial cells correlated with some haematological indices as lipase, alanine-aminotransferase, albumin, magnesium and chlorides. The concentrations of microcystins in the muscle and liver of the fish (determined by high-performance liquid chromatography with mass spectrometer) were below the limit of detection during the monitored period [0.31 ng g^{-1} fresh weight (f.w.) for the liver and 0.13 ng g^{-1} f.w. for muscle]. Our study demonstrates that although known cyanobacterial toxin microcystins were not detected in the

fish tissues, several biochemical parameters important for the fish physiology were modulated by natural cyanobacterial bloom.

Keywords: biochemical profile of blood, microcystins, common carp

Introduction

Toxic cyanobacteria pose a serious problem for water supply systems, recreation and agriculture. Cyanobacteria, as photosynthesizing organisms, produce various biologically active compounds that affect aquatic organisms and also the physico-chemical characteristics of water (Chorus, Falconer, Salas & Bartram 2000). The most common and hazardous cyanobacteria in the water bodies of the Czech Republic are the producers of microcystins, MCs, which are peptide hepatotoxins that inhibit regulatory protein phosphatases 1 and 2A (Falconer 1994). Considerable attention has been paid to the impact of cyanobacteria on fish aquaculture (Landsberg 2002), and a review has been published recently by Malbrouck and Kestemont (2006).

The influence of cyanotoxins on fish has been studied by a number of authors using clinical,

morphological, histological, ultrastructural, haematological and biochemical methods. One of the common species producing microcystins is the filamentous cyanobacterium *Planktothrix agardhii*, which occurs in high densities in eutrophied ponds and shallow lakes around the world (Rucker, Wiedner & Zippel 1997). In some cases, *Planktothrix* has been shown to produce extremely high amounts of MCs per dry weight of cyanobacterial biomass (Fastner, Erhard, Carmichael, Sun, Rinehart, Ronicke & Chorus 1999). Besides microcystins, other metabolites of *Planktothrix* such as fatty acids may be toxic to fish and crustaceans (Keil, Forchert, Fastner, Szewzyk, Rotard, Chorus & Kratke 2002). *Planktothrix agardhii* is also a dominant species in about 40% of the Czech fishponds (Hasler & Poulickova 2002).

Some studies demonstrated that cyanobacterial hepatotoxins may accumulate in the fish tissues and may be transferred via the food web (Magalhaes, Soares & Azevedo 2001). Fish, standing at the top of the aquatic food chain, are likely to be affected by toxic cyanobacteria, and the consumption of contaminated fish by humans may pose health risks. In a recent study, MCs were detected for the first time in the serum of a chronically exposed human population of fishermen (average 0.39 ng mL^{-1} MCs) at Lake Chaohu, South China (Chen, Xie, Li & Xu 2009). Indices of hepatocellular damage were observed in exposed persons as well (Chen *et al.* 2009). Most of the toxin accumulates in the liver but MCs have also been detected in other organs and tissues including the intestine, kidney, brain, blood, muscle, spleen, gallbladder and gill (Chen, Xie, Zhang, Ke & Yang 2006). There are several studies regarding the MCs content in different freshwater fish species (Kotak, Zurawell, Prepas & Holmes 1996; Gkelis, Lanaras & Sivonen 2006; Chen *et al.* 2006, 2007; Li, Xie & Chen 2007) including common carp *Cyprinus carpio* (Fischer & Dietrich 2000; Adamovsky, Kopp, Hilscherova, Babica, Palikova, Paskova, Navratil, Marsalek & Blaha 2007).

Majority of the MCs in fish are taken up via the gastrointestinal tract, whereas uptake through the skin or gills is negligible (Tencalla, Dietrich & Schlatter 1994). Intraperitoneal or peroral exposures to MCs (or lysates of cyanobacterial biomass) caused significant changes in the numbers of blood cells and biochemical indices of the liver damage such as elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities (Rabergh, Bylund & Eriksson 1991). Toxic effects following oral administration of MCs are approximately 10-times weaker in comparison

with an intraperitoneal application (Carbis, Rawlin, Grant, Mitchell, Anderson & McCauley 1997).

Although a number of controlled laboratory experiments with toxic cyanobacteria and fish were reported, there are still limited field studies addressing the natural variability and dynamics of the toxic water blooms' influence on aquaculture. Monitoring of biochemical parameters in fish populations naturally exposed to toxic cyanobacteria provides important toxicological information (Carbis, Mitchell, Anderson & McCauley 1996). The objective of this work was to study the effects of a natural cyanobacterial water bloom (dominated by *P. agardhii*-producing microcystins) on the biochemical indices in 3-year-old common carp (*C. carpio* L.). With the aim to evaluate the potential risks for human consumption of fish, we have also studied the concentrations of MCs in the fish tissues (liver and muscle) throughout the vegetation season before the fish harvest.

Materials and methods

Characterization of the locality

The fishpond Novovesky (138.7 ha) is a typical shallow, hypertrophic pond for intensive breeding of the common carp (*C. carpio* L.) located in the southern Moravia, Czech Republic ($48^{\circ}56'2.992''\text{N}$, $16^{\circ}31'44.772''\text{E}$). In 2007, the pond was stocked with 3-year-old carp (average body mass $2176 \pm 697 \text{ g}$), and the yearly production was $> 1500 \text{ kg h}^{-1}$. Fish were fed by wheat and a triticale mixture during the vegetative season. The numbers of cyanobacterial cells in the pond were counted every 2 weeks by microscopy using Bürker's chamber. Water bloom was dominated by filamentous *P. agardhii* with subdominant *Pseudanabaena limnetica* and *Limnithrix redekei* with densities ranging $0.3\text{--}21.7 \cdot 10^6 \text{ cells mL}^{-1}$ during the monitored period.

Water quality analyses

A series of physico-chemical parameters [temperature, dissolved oxygen (DO), pH, transparency, conductivity, chlorophyll *a* (Chl *a*), nitrite nitrogen (N-NO_2), nitrate nitrogen (N-NO_3), ammonia nitrogen (N-NH_4), total nitrogen (N_T), phosphate phosphorus (P-PO_4), total phosphorus (P_T) and the total organic carbon (TOC)] were determined every 2 weeks between 9:00 and 13:00 hours during the entire

Table 1 Values of physico-chemical parameters in Novoveský fish pond during the study period in the year 2007

Date	Tem. (°C)	DO (%)	pH	Con. (mS m ⁻¹)	Tra. (cm)	N-NH ₄ (mg L ⁻¹)	N-NO ₂ (mg L ⁻¹)	N-NO ₃ (mg L ⁻¹)	P-PO ₄ ³⁻ (mg L ⁻¹)	Chl <i>a</i> (µg L ⁻¹)	N _T (mg L ⁻¹)	P _T (mg L ⁻¹)	TOC (mg L ⁻¹)
10.7	21.0	102	8.65	84.0	20	0.09	0.010	0.68	0.113	275	5.3	0.50	33.5
24.7	24.1	43	8.27	75.5	25	0.45	0.023	1.58	0.059	370	6.1	0.51	29.1
7.8	22.2	95	8.86	78.3	20	0.11	0.005	1.10	0.035	491	6.2	0.46	34.1
20.8	22.3	50	7.90	73.9	25	0.20	0.020	2.30	0.065	361	6.0	0.45	26.1
6.9	13.6	89	9.26	73.9	15	0.12	0.010	0.40	0.021	616	5.9	0.52	45.6
18.9	16.1	112	9.77	68.5	25	0.08	0.006	1.20	0.040	468	6.6	0.47	28.7
2.10	14.2	91	9.22	66.4	0	0.26	0.012	0.40	0.035	2309	6.0	0.41	84.8
16.10	11.3	104	8.91	72.6	20	1.02	0.016	0.80	0.101	565	7.4	0.62	27.9
14.11	3.0	72	7.71	96.6	10	0.49	0.001	0.36	0.038	79	5.0	0.30	45.6

(Tem., temperature; DO, dissolved oxygen; Con., conductivity; Tra., transparency; Chl *a*, chlorophyll *a*; N_T, total nitrogen; P_T, total phosphorus; TOC, total organic carbon).

2007 vegetation season (Table 1). Water saturation by oxygen, pH and temperature was measured using the portable Oxi 340i meter (WTW, Weilheim, Germany). Water transparency was determined using a Secchi disk. Chlorophyll *a* was analysed according to ISO 10260 (1992), and other chemical parameters were determined using standard methods (APHA 1998).

Sampling and measuring of biochemical parameters in fish plasma

Immediately after catching of the fish by drag or castle net, blood samples were collected and inadequate or haemolytic samples were eliminated. Fish blood was collected by a cardiopuncture using heparinized syringes (50 IU heparin 1 mL⁻¹). The blood was centrifuged at 400 *g* for 15 min at 4 °C, and collected plasma was stored at – 80 °C. Sampling of fish (total *n* = 89) was conducted every second week (nine times, 10 fish per sampling) from 10 July 2007 to the harvest of fish (14 November 2007).

Biochemical analyses were performed using the ADVIA 1650 automatic analyser (Siemens, USA) using commercially available reagents. Serum enzymatic activities were determined at 37 °C. Alanine aminotransferase (ALT) activity determination was based on the kinetic assessment of NADPH consumption coupled with the generation of pyruvate (Expert Panel on Enzymes of The IFCC 1976). Aspartate aminotransferase (AST) activity was determined by kinetic measurement of NADH consumption, coupled with the formation of oxaloacetate (Expert Panel on

Enzymes of The IFCC 1976). Lactate dehydrogenase was determined as the formation of NADH during conversion of L-lactate to pyruvate (Hajzer & Jagelkova 1988). Alkaline phosphatase (ALP) was determined by a modification of the enzymatic method using adenosine monophosphate (AMP) buffer (Tietz 1980). Acid phosphatase (ACP) activity was determined using an enzymatic method with 1-naphtyl-phosphate (Hillmann 1971). Cholinesterase (CHE) was determined by a modification of the kinetic test with butyrylthiocholine (Garry 1971).

Total serum protein (TP) was determined by the biuret reaction (Dumas, Bayse, Carter, Peters & Schaffer 1981). Glucose (GLU) concentration was determined using the glucose hexokinase method at 37 °C with an endpoint reading at 340 nm (Barham & Trinder 1972). Calcium (Ca) and magnesium (Mg) concentrations were determined using the modified colorimetric methods with arzenazo III (Ichaylova & Ilkova 1971; Skavrada 1999). Phosphorus (P) was determined using an endpoint method with sample blanking using ammonium molybdenate reagent (Kratochvila & Garcic 1977). Total bilirubin (BIL) was determined by the oxidation reaction with potassium ferricyanide (O'Leary, Pembroke & Duggan 1993). Iron (Fe) was determined using the photometric method with ferene (ferroin-type reagent) without deproteinization (Higgins 1981). The concentration of lactate (LACT) in plasma was measured using the enzymatic method according to Shimojo, Nakajima, Yoshikawa, Okuda and Okada (1989). Albumin (ALB) was determined using the photometric method with bromocresol green (Dumas, Watson & Biggs 1971). Urea (UREA) concentrations were determined using

the kinetic enzymatic method with urease (Roch-Ramel 1967).

Cholesterol (CHOL) was determined using the CHOD-PAP method after enzymatic hydrolysis and oxidation (Roschlau, Bernt & Gruber 1974). Electrolyte levels (Na, K and Cl) were analysed by ion selective electrodes (Eisenman 1967). Amylase (AMS) was determined by the method using ethylidene blocked *p*-nitrophenyl-maltoheptaoside as a substrate (Jensen & Wydeveld 1958). Triglycerides (TRIG) were assessed by the Fossati three-step enzymatic reaction with a Trinder endpoint (Fossati & Prencipe 1982). Lipase (LIP) activity was determined using the enzymatic colorimetric assay according to Neumann *et al.* (1987).

Analyses of microcystins

The concentrations of MCs in the cyanobacterial biomass and water (Fig. 2) were measured using high-performance liquid chromatography (HPLC) as originally described by Lawton, Edwards and Codd (1994) using methods previously established in our laboratories (Babica, Kohoutek, Blaha, Adamovsky & Marsalek 2006). Briefly, extracts of lyophilized biomass were prepared with 50% v/v methanol. MCs in water samples were concentrated by solid-phase extraction using SepPack C18 cartridges (Waters, Millford, MA, USA). Analyses were performed with HPLC Agilent 1100 Series (Agilent Technologies, Waldbronn, Germany) at 30 °C on a Supelcosil ABZ+ Plus column (150 mm; inner diameter 4.6 mm; film thickness 5 µm; Supelco, Bellefonte, PA, USA). The binary gradient of the mobile phase (flow rate 1 mL min⁻¹) consisted of water and acetonitrile (linear increase 20–59% of acetonitrile during 0–30 min; mobile phases contained 0.1% v/v trifluoroacetic acid). Chromatograms at 238 nm were recorded with an Agilent 1100 Series photodiode-array detector, and MCs were identified by the retention time and characteristic absorption spectra at 200–300 nm. Quantification was based on the external calibrations of three MC variants MC-LR, -RR and -YR.

Extractions of fish tissues were performed according to the method originally described by Magalhaes *et al.* (2001). The frozen sample [0.4 g fresh weight (f.w.)] was homogenized with methanol (3 mL), sonicated in an ultrasonic bath for 30 min and centrifuged at 4000 *g* for 10 min. The supernatant was collected and the pellet was re-extracted three times using the same procedure. The methanol fractions

obtained were pooled and repeatedly extracted (three times) with 1 mL of hexane to remove lipids (hexane layers discarded). Methanol extract was evaporated at 50 °C, and the residue was dissolved in water. The concentrations of MCs were measured by HPLC with mass spectrometer (HPLC/MS) system, Agilent 1200, with 6410 Triple-Quad MS using the same separation conditions as described above. MS ion source was in the positive ESI mode using nitrogen as the drying gas (gas temperature 350 °C), gas flow 11 L min⁻¹, nebulizer pressure 50 psi, and capillary voltage 5500 V. Spectra collected in the SIM mode and masses of microcystin-RR, -YR and -LR were scanned.

Statistical analyses

The results of biochemical parameters in individual time points were expressed as the median with 25–75% quantiles (data were not normally distributed and arithmetic means would not be representative). The correlations between biochemical parameters and characteristics of the toxic bloom (cyanobacterial cell counts, concentrations of MCs in water, and concentrations of MCs in the biomass) were analysed by Spearman's rank directional correlations (low number of values with not-normal distribution). Statistically significant relationships were further checked for possible partial correlations (controlled for the influence of basic water physico-chemical characteristics such as temperature, pH, etc.). Pearson's partial correlations were calculated on log-transformed data to assure their symmetry (log-normality). *P* values < 0.05 were considered to be statistically significant. All analyses were performed in STATISTICA 8.0 software (StatSoft, Tulsa, OK, USA).

Results and discussion

Environmental conditions

Limnological characteristics during the monitored period are presented in Table 1. The concentrations of biogenic elements (P, N and C) were optimal for the development of cyanobacterial water bloom. The nitrogen/phosphorus ratio was > 10:1, indicating nitrogen-limiting conditions, which do not favour growth of N₂-fixing cyanobacteria (Havens, James, East & Smith 2003). Heavy blooms of non-N₂-fixing planktonic cyanobacteria *Planktothrix*, *Pseudanabaena* and *Limnolthrix* were observed in early spring

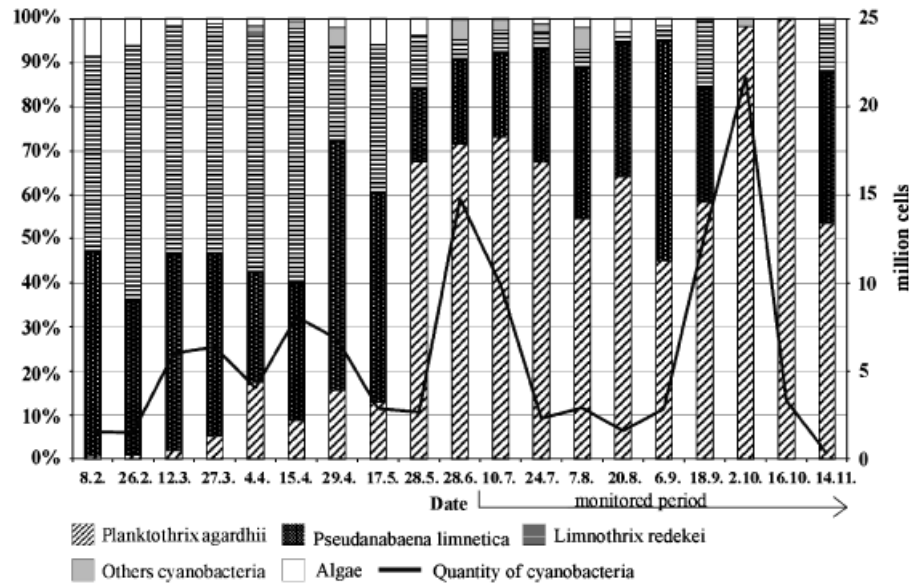


Figure 1 Phytoplankton composition and quantity of cyanobacteria in the Novoveský fish pond during the studied period in 2007 (percentage expression of main species and groups of phytoplankton and counts of cyanobacterial cells).

in the Novovesky pond, and high densities that appeared already in February persisted until late autumn (Fig. 1).

Common carp (*C. carpio* L.), which is the main cultured fish species in the Czech Republic, may affect the primary production in freshwater systems. Carp is a bottom feeder increasing the flux of nutrients from benthic to the pelagial zone, which may affect the occurrence of dominant primary producers. Phytoplankton in ponds with high fish production consists mainly of filamentous cyanobacteria and green algae. Water has low transparency and high fluctuations of dissolved oxygen. Filamentous cyanobacteria (mainly *P. agardhii*, *L. redekei* and *P. limnetica*) are often dominant in turbid ponds and lakes due to their superior ability to survive under low light conditions (Scheffer, Rinaldi, Gragnani, Mur & Nes 1997).

Microcystins in biomass and water

Biomass concentrations of total MCs in biomass averaged $65.46 \mu\text{g g}^{-1}$ dry wt, with maximum $180.85 \mu\text{g g}^{-1}$ dry wt (Fig. 2). These concentrations are low to medium in comparison with previously reported MC concentrations in the Czech Republic, with annual median ranging $160\text{--}600 \mu\text{g g}^{-1}$ dry wt (Blahova, Babica, Adamovsky, Kohoutek, Marsalek & Blaha 2008). The concentrations of MCs in

water averaged $3.65 \mu\text{g L}^{-1}$, with a range of $0.29\text{--}9.51 \mu\text{g L}^{-1}$ (Fig. 2). The average values of microcystins in natural waters in the Czech Republic during 1993–2005 were $0.88 \mu\text{g L}^{-1}$, with maxima up to $37 \mu\text{g L}^{-1}$ (Bláhová, Babica, Maršálová, Smutná, Maršálek & Bláha 2007; Blahova *et al.* 2008). Monitoring of 30 shallow eutrophic or hypertrophic lakes in China with dominating *Microcystis* showed average concentrations of microcystins of around $0.27 \mu\text{g L}^{-1}$; maximum of $8.57 \mu\text{g L}^{-1}$ (Wu, Xie, Liang, Wang & Liang 2006). Similar values were reported by Kotak *et al.* (1996) from four lakes in Canada. Slightly higher concentrations ($1.9\text{--}55.4 \mu\text{g L}^{-1}$) were reported by Zheng, Xie, Li, Yang, Wang and Guo (2004) in several eutrophic Chinese lakes during the 2004 season.

Microcystins in fish tissues

In our study, the concentrations of microcystins in the liver and muscle of all investigated fish were below the limit of detection (0.31 ng g^{-1} f.w. for liver and 0.13 ng g^{-1} f.w. for muscle). Previous field studies demonstrated that after ingestion, MCs are first detected in the gut content and then in the liver, muscle and viscera (Magalhaes *et al.* 2001). The accumulation of MCs in the muscles is not always clear. In a subchronic toxicity experiment with silver carp fed

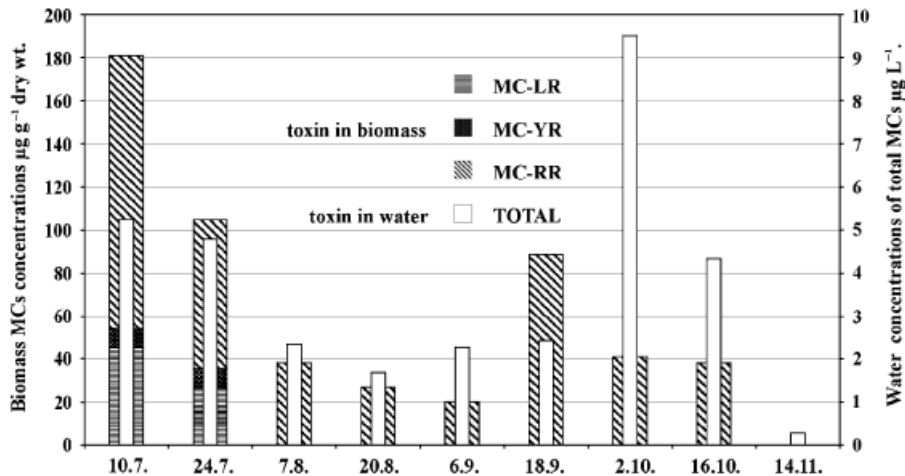


Figure 2 Microcystin (MCs) total concentration in water ($\mu\text{g L}^{-1}$) and in biomass ($\mu\text{g g}^{-1}$ dry wt) in the Novoveský fish pond during the studied period.

with fresh *Microcystis viridis* cells containing MCs (268–580 μg of MC-LR and 110–292 μg MC-RR per gram dry wt), only MC-RR was detected in the liver and muscle (17.8 and 1.77 $\mu\text{g g}^{-1}$ dry wt respectively) but no MC-LR was found, suggesting different bioavailability of MC variants (Xie, Xie, Ozawa, Honma, Yokoyama & Park 2004). Although the relative proportion of MC-RR in the cyanobacterial biomass was higher than MC-LR and MC-YR, our highly sensitive HPLC/MS method was not able to confirm accumulation of microcystins in the tissues of common carp.

Nevertheless, the kinetics of MCs accumulation seems to be species specific. For example, in common carp, a peak in the MCs concentrations was observed after a short exposure period, followed by an apparent decrease after 2 months, and a comparable trend was also observed in the muscle (Adamovsky *et al.* 2007). On the other hand, we have observed continuous accumulation of MCs in the liver of silver carp during a prolonged 2-month period (Adamovsky *et al.* 2007). The differences may be explained by the variable feeding habits of the fish species (phytoplanktivorous silver carp actively ingests cyanobacterial cells; only passive MCs intake can be expected in omnivorous common carp). In addition, common carp is agastric fish, which does not secrete acid into the alimentary canal. This may limit the absorption of MCs as effective digestion of cyanobacterial cells was observed only under acidic conditions (Carbis *et al.* 1997).

Xie *et al.* (2005) mentioned that the MCs content was the highest in the tissues of carnivorous fish, followed by omnivorous fish, and the lowest concentra-

tions were found in phytoplanktivorous and herbivorous species. These findings may indicate the tendency of MCs to accumulate in the food chain. However, the gut content of MCs showed a rather reverse pattern: the highest amounts were found in the phytoplanktivorous fish, followed by the omnivorous and carnivorous fish (Chen, Xie, Zhang & Lei 2007). It seems that phytoplanktivorous fish may be more resistant to MCs due to lower toxin accumulation (Chen *et al.* 2007).

Exposure of various organisms including fish to toxic cyanobacteria is known to induce detoxification and metabolization mechanisms (Pflugmacher, Wieand, Oberemm, Beattie, Krause, Codd & Steinberg 1998). Our study might indicate that common carp is able to efficiently cope with toxic cyanobacteria, possibly by removal of MCs via detoxification. Further research is needed to fully explore this hypothesis.

Biochemical indices

The results of biochemical analyses in the blood of the common carp are presented in Table 2. Our study demonstrates a large variation in the values of biochemical blood parameters, which are known to be affected by many endogenous and exogenous factors such as age, health condition, nutrition or stress – including chemical stress (Svobodova, Vykusova, Modra, Jarkovsky & Smutna 2006).

We have first studied the relationships between the water bloom characteristics and parameters of water

Table 2 The biochemical variables in blood of common carp [median (25–75% interquartile range)] during the studied period (at last nine fish were analysed at individual sampling date)

Variables	10.7.07	24.7.07	7.8.07	20.8.07	6.9.07	18.9.07	2.10.07	16.10.07	14.11.07
ACP (nkat L ⁻¹)	93.95 (87–110)	97.53 (81–126)	77.28 (70–85)	79.53 (68–85)	71.38 (63–92)	97.13 (78–110)	97.70 (88–105)	428.81 (379–521)	102.32 (66–107)
ALP (μkat L ⁻¹)	1.05 (0.7–2.5)	0.74 (0.6–1.4)	0.75 (0.7–1.0)	1.07 (0.6–1.3)	1.00 (0.8–1.2)	0.78 (0.5–1.0)	1.17 (0.9–1.6)	0.59 (0.4–0.8)	0.37 (0.3–0.6)
ALT (μkat L ⁻¹)	2.18 (1.1–2.8)	0.93 (0.8–1.2)	1.19 (0.9–1.6)	1.23 (0.9–1.5)	1.20 (0.9–1.6)	1.35 (1.1–1.8)	1.99 (1.5–2.5)	1.65 (1.5–2.4)	0.36 (0.3–0.5)
AST (μkat L ⁻¹)	5.12 (2.9–12.0)	1.87 (1.3–2.3)	2.39 (1.9–3.8)	2.43 (1.9–3.8)	2.96 (2.3–5.0)	2.12 (2.0–3.0)	2.35 (1.9–3.1)	2.45 (1.7–6.8)	2.89 (2.0–3.4)
LDH (μkat L ⁻¹)	11.65 (6.1–16.5)	4.71 (3.3–5.7)	2.97 (2.4–6.6)	3.37 (2.9–6.0)	3.62 (2.4–6.2)	3.39 (2.6–4.8)	2.56 (2.0–2.7)	3.90 (3.0–8.9)	5.05 (3.5–7.3)
CHE (μkat L ⁻¹)	13.18 (12.0–15.3)	0.74 (0.3–1.3)	0.49 (0.4–1.1)	0.17 (0–0.3)	1.05 (0.6–1.4)	0.45 (0.3–0.9)	0.59 (0.4–0.7)	0.50 (0.3–0.9)	0.86 (0.5–1.3)
AMS (μkat L ⁻¹)	1.50 (1.2–2.8)	1.69 (1.2–2.3)	1.73 (1.5–2.4)	2.14 (1.7–3.9)	1.66 (1.2–2.1)	1.89 (1.3–3.2)	1.62 (1.2–1.9)	0.43 (0.4–1.3)	0.31 (0.2–0.4)
KREA (μkat L ⁻¹)	17.35 (16–20)	24.35 (23–29)	15.10 (15–16)	16.90 (15–19)	21.65 (19–24)	20.95 (20–24)	20.15 (19–21)	19.40 (19–21)	19.55 (17–22)
LIP (μkat L ⁻¹)	0.110 (0.10–0.11)	0.030 (0.02–0.06)	0.010 (0–0.03)	0.005 (0–0.02)	0.015 (0.01–0.03)	0.030 (0.01–0.03)	0.015 (0.01–0.02)	0.020 (0.01–0.03)	0.000 (0–0.02)
TP (g L ⁻¹)	32.55 (30–33)	37.75 (34–41)	31.05 (28–34)	28.54 (28–32)	31.92 (30–35)	36.28 (35–40)	35.21 (35–36)	37.86 (37–39)	35.02 (30–39)
ALB (g L ⁻¹)	8.90 (8.5–9.2)	9.75 (8.9–11.3)	7.60 (7.0–8.2)	8.15 (7.6–8.4)	8.80 (8.2–9.0)	9.50 (8.4–9.8)	9.00 (8.7–9.1)	8.50 (8.4–9.2)	8.00 (7.5–8.5)
BIL (μmol L ⁻¹)	0.0 (0)	0.0 (0–0.08)	0.4 (0–1.10)	0.0 (0–0.65)	0.0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fe (μmol L ⁻¹)	10.8 (6.6–12.0)	16.7 (11.0–22.9)	5.3 (2.2–10.9)	15.7 (7.4–20.1)	9.5 (1.8–16.9)	7.2 (1.3–12.2)	21.6 (13.5–24.3)	20.5 (13.5–24.1)	9.9 (3.6–15.9)
P (mmol L ⁻¹)	4.46 (4.1–4.6)	4.73 (4.4–4.9)	3.22 (3.1–3.5)	3.24 (3.0–3.5)	2.87 (2.5–3.0)	3.53 (3.3–3.8)	2.16 (2.1–2.2)	1.86 (1.8–2.0)	1.91 (1.6–2.1)
Ca (mmol L ⁻¹)	3.30 (3.1–3.4)	3.48 (3.4–3.8)	2.92 (2.9–3.2)	3.06 (2.9–3.2)	3.25 (2.9–3.5)	3.19 (3.0–3.4)	2.72 (2.6–2.9)	2.39 (2.4–2.4)	2.65 (2.5–2.8)
Mg (mmol L ⁻¹)	1.78 (1.7–1.9)	2.14 (2.1–2.4)	1.69 (1.6–1.7)	1.77 (1.6–2.0)	1.38 (1.3–1.5)	2.04 (2.0–2.1)	1.52 (1.4–1.7)	1.41 (1.4–1.5)	1.60 (1.5–1.7)
Na (mmol L ⁻¹)	151.8 (150–156)	167.4 (153–177)	145.1 (143–149)	148.3 (147–150)	151.1 (151–152)	148.8 (148–151)	150.2 (148–154)	145.7 (144–146)	155.6 (153–157)
K (mmol L ⁻¹)	1.72 (1.6–2.0)	1.08 (1.0–1.4)	1.18 (1.1–1.4)	2.20 (1.9–2.2)	2.47 (2.0–2.6)	1.19 (0.8–1.7)	1.18 (0.8–2.1)	1.80 (1.7–2.7)	1.15 (0.8–1.2)

Table Continued

Variables	10.07	24.07	7.8.07	20.8.07	6.9.07	18.9.07	2.10.07	16.10.07	14.11.07
Cl (mmol L ⁻¹)	116.8 (114–118)	120.7 (113–129)	110.9 (110–114)	109.9 (109–111)	109.3 (108–111)	110.3 (106–114)	112.2 (111–113)	114.1 (113–115)	118.3 (113–121)
GLU (mmol L ⁻¹)	4.73 (3.9–6.9)	4.96 (4.0–7.1)	2.50 (2.3–4.5)	5.60 (4.8–6.9)	3.11 (2.6–3.8)	4.40 (4.0–4.5)	2.12 (2.0–2.3)	1.32 (1.2–1.4)	3.32 (3.1–3.6)
LACT (mmol L ⁻¹)	9.16 (8.2–9.8)	7.57 (6.4–11.0)	5.90 (4.6–7.5)	7.16 (6.9–8.0)	11.14 (10.3–11.4)	12.73 (11.5–13.6)	9.87 (7.7–11.1)	3.62 (3.4–4.3)	6.63 (6.4–7.0)
TRIG (mmol L ⁻¹)	6.35 (5.7–6.8)	6.29 (5.1–7.2)	3.88 (3.2–5.7)	6.78 (5.8–7.4)	6.67 (5.4–7.1)	7.23 (7.0–7.4)	7.16 (6.9–7.4)	5.58 (4.2–6.2)	2.08 (1.7–2.6)
CHOL (mmol L ⁻¹)	4.08 (3.5–4.7)	5.24 (4.8–6.3)	3.59 (3.1–4.4)	3.69 (3.4–3.8)	4.31 (4.0–5.0)	5.66 (5.1–6.1)	6.29 (6.2–6.7)	5.93 (5.7–7.8)	3.74 (3.0–5.2)
UREA (mol L ⁻¹)	0.92 (0.8–1.0)	0.94 (0.8–1.0)	1.52 (1.3–1.9)	1.62 (1.4–1.9)	0.89 (0.8–0.9)	1.48 (1.3–1.7)	1.37 (1.3–1.4)	0.77 (0.7–0.9)	0.47 (0.3–0.6)

ACP, acid phosphatase; ALP, alkaline Phosphatase; ALB, albumin; ALT, alanine aminotransferase; AMS, amylase; AST, aspartate aminotransferase; BL, bilirubin; Ca, calcium; CHE, cholinesterase; CHOL, cholesterol; Cl, chloride; Fe, iron; GLU, glucose; K, potassium; KREA, creatinine; LACT, lactate; LDH, lactate dehydrogenase; LIP, lipase; Mg, magnesium; Na, sodium; P, phosphorous; TP, total protein; TRIG, triglycerides; UREA, urea.

quality. The only significant correlation was between the content of oxygen and the number of cells per millilitre (Spearman's $R = 0.69$, $P < 0.05$); other expected trends (e.g. chlorophyll a vs. the numbers of cyanobacterial cells) were not statistically significant (probably due to the low $N = 9$). The major objective was to evaluate the relationships between the fish blood parameters and characteristics of the cyanobacterial bloom (i.e. cell density and MC content in water and in biomass). Several correlations were found to be statistically significant (presented in Table 3), and their robustness was checked for partial correlations with the water quality parameters (Pearson's R at log-transformed data). All correlations presented in Table 3 remained significant after the cross-check for partial correlations; none of the evaluated physicochemical parameters had a significant effect. Some other relationships between blood parameters and water quality were also found to be statistically significant (Spearman's R , $P < 0.05$), but blood parameters that correlated with water bloom parameters (presented in Table 3) were not involved in these relationships, and they are not further discussed.

Liver enzymes are the most frequently studied biomarkers in fish in relation to the cyanobacterial toxicity. Significant increases in the activities of ALT, AST and LDH were observed after an intraperitoneal or an oral administration of pure microcystin-LR to the carp (Li, Chung, Kim & Lee 2004). Also, in our previous study, the activities of blood plasma enzymes increased after 96 h of exposure of the carp to a natural cyanobacterial population (Kopp & Hetesa 2000). In another study, elevation in AST and ALT serum activities was observed after 7-day exposures of carp to the water with microcystins (Carbis *et al.* 1997). The same study also demonstrated that feral carp (originating from the lake with toxic *Microcystis aeruginosa*) had even higher AST activities in comparison with laboratory-exposed fish (Carbis *et al.* 1997).

Although correlations between the water/biomass toxin concentrations and AST or LDH activities were not significant in the present study, our results showed a significant correlation of ALT with the toxin concentrations in water and the number of cyanobacterial cells (Table 3). Thus, the activity of blood ALT seems to be a sensitive indicator of the fish responses to toxic cyanobacteria, and its role as a biomarker under natural conditions should be investigated.

Our results also showed significant correlations between the lipase activity and MCs in water and biomass (Table 3). It seems that toxic blooms may en-

Table 3 Correlations between biochemical indices and toxic cyanobacteria (numbers of cyanobacterial cells, concentrations of microcystins – MCs – in biomass and water)

	Spearman's correlation (<i>N</i> = 9 sampling dates)		
	MCs in biomass	MCs in water	Number of cyanobacterial cells
Lipase	0.78 (0.02)	0.74 (0.02)	NS
Alanine aminotransferase	NS	0.67 (0.05)	0.8 (0.01)
Albumin	NS	0.67 (0.05)	NS
Magnesium	0.76 (0.03)	NS	NS
Chloride	0.76 (0.03)	NS	NS

Results show two-way Spearman's *R* with the *P*-value in parentheses (presented are only the combinations of parameters that demonstrated statistical significance, *P* < 0.05).

NS, nonsignificant correlation.

hance outlay of energy associated with increased lipid metabolism. On the other hand, cyanobacterial water blooms induce hypoxia (Chorus *et al.* 2000) and depletion of oxygen may have a broad influence on the energy metabolism and biochemical indices in fish (Van Ginneken, Van Caubergh, Nieveen, Balm, Van Den Thillart & Addink 1998). We cannot exclude that modulations of the biochemical parameters observed in the present study resulted from the complex factors related to the cyanobacterial water bloom.

We have also observed a significant increase in the albumin concentrations that correlated with the amount of toxin in water (Table 3). Previous studies with common carp (Carbis *et al.* 1997) and silver carp (Kopp *et al.* 2005) did not report such a correlation. Also, acute intraperitoneal application of MCs did not significantly modulate an albumin in carp (Carbis *et al.* 1996). Thus, our observations may possibly be related to the higher demand on lipid transport in the blood, but direct effects of MCs are less likely.

Our results also showed correlations between selected ions (Mg and Cl) and MC content in cyanobacterial biomass. These findings are in agreement with previous studies with common carp (Carbis *et al.* 1997) or silver carp (Kopp, Mareš, Kubicek & Babica 2005). Electrolytes control the distribution of body fluids, intra- and extracellular acidobasic balance, osmotic pressure and normal neuromuscular activity. Any changes in these basic parameters (decreased or increased values) indicate abnormal physiology in the fish organism. It seems that higher amounts of

MCs may affect electrolyte concentrations but detailed research on the mechanism is needed.

Conclusions

In the present study, the content of MCs in the natural cyanobacterial bloom in the Novoveský fish pond was 20–181 µg g⁻¹ dry wt in biomass and 0.3–9.5 µg L⁻¹ in water. The concentrations of microcystins in the tissues of common carp (liver and muscle) were below the limits of detection, which could be related to the effective detoxification of MCs. Our results demonstrate high variability and fluctuations in various biochemical indices. The content of MCs and the density of cyanobacterial water blooms (number of cells per millilitre) significantly correlated with parameters such as lipase, alanine transaminase, albumin, magnesium and chlorides. Although MCs were not detected in the fish tissues after exposure to natural cyanobacterial water bloom, modulations of several biochemical parameters indicate apparent negative effects of cyanobacteria on fish physiology.

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