

Oceanological and Hydrobiological Studies
Vol. XXXIV, Supplement 3

Institute of Oceanography

(85-92)
2005

University of Gdańsk

Research Article

**THE INFLUENCE OF TOXIC CYANOBACTERIAL WATER BLOOMS
ON THE HEMATOLOGICAL INDICATORS OF SILVER CARP
(HYPOPHthalmichthys molitrix VAL.)**

RADOVAN KOPP^{1*}, JAN MAREŠ¹, ZDENĚK KUBÍČEK²,
PAVEL BABICA³

¹*Department of Fishery and Hydrobiology, Mendel University of Agriculture
and Forestry, Zemědělská 1, 613 00 Brno
Czech Republic*

²*Department of Clinical Biochemistry, Hospital Třinec, Kaštanová 268, 739 61
Třinec, Czech Republic*

³*Laboratory of Cyanotoxins, Institute of Botany
Czech Academy of Science & RECETOX (Research Center for Environmental
Chemistry and Ecotoxicology, Masaryk University, Brno)
Kamenice 3, 625 00 Brno
Czech Republic*

**e-mail: kopp@mendelu.cz*

Key words: cyanobacteria, silver carp, toxins, haematology

Abstract

The aim of the presented work was to evaluate an influence of toxic cyanobacterial water blooms on blood indices of silver carp (*Hypophthalmichthys molitrix* Val.). Silver carp, the body mass 278.8 ± 76.0 g, were used in the experiment. The experimental fish were exposed to natural populations of cyanobacterial water blooms (*Microcystis aeruginosa*, *M. ichthyoblabe* and *M. viridis*), which contained three microcystins (total concentration $425.0 \mu\text{g g}^{-1}$ of dry biomass in

the beginning of the experiment, respective 182.4 $\mu\text{g g}^{-1}$ of dry biomass at the end of the experiment). Hematological indices showed evident changes in fish exposed to the cyanobacterial population in comparison with the control group. The results obtained from these experiments do not always correspond with the literature sources. These data pointed to some hematological indicators that fluctuated in large interval. Toxicity and density of cyanobacterial water blooms had important role at individual hematological indices as well.

INTRODUCTION

Eutrophication of aquatic ecosystem is accompanied by cyanobacterial mass development represents serious environmental problem. Cyanobacteria as photosynthesizing organisms produce biologically active compounds that may affect growth and development of other water organisms and physical and chemical characteristics of water (Maršálek and Turánek 1996). Great attention has recently been paid to the impact of cyanobacterial toxins on fish. Symptoms of poisoning, pathological changes, and influence on blood indices have been investigated as well.

Most of toxins are absorbed into the fish organism through the gastrointestinal tract, whereas toxin penetration through the skin or gills is negligible (Tencalla *et al.* 1994). It is supposed more affection of cyanobacterial toxins on fish organism at higher digestible of cyanobacterial water blooms.

Beveridge *et al.* (1993) reported that the intussusception of *Microcystis aeruginosa* cells by *Hypophthalmichthys molitrix* is significantly depended on toxicity of cyanobacterial population.

Silver carp that digest only cyanobacterial water blooms have a decrease in hemoglobin values and modifications of leukocrit (Šesterenko 1979).

In silver carp, intraperitoneal exposure to microcystin-LR causes significant decrease in values of leukocyte, lymphocyte, haemoglobin, total proteins and significant increase of plasmatic enzyme activity (ALT, AST, LDH) (Vajcová *et al.* 1998).

Intraperitoneal exposure to microcystins causes liver tissue damage in fish that is demonstrated by significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities (Rabergh *et al.* 1991, Navrátil *et al.* 1998).

Toxic effect on fish exposed to media containing the dispersed microcystin is manifested in the time-delay caused by limited penetration into the healthy fish. Toxic effect after the oral administration is approximately 10 times weaker than after the intraperitoneal application (Carbis *et al.* 1996a).

Long-term impact of the cyanobacteria containing microcystins at lower concentrations is relatively difficult to observe in individual fish; therefore, the control of more individuals of the fish population is more useful. The

measurement of aminotransferase (ALT, AST), bile acids, bilirubin, sodium, and chloride from the blood serum is recommended (Carbis *et al.* 1996b).

MATERIALS AND METHODS

Biennial fish of silver carp (*Hypophthalmichthys molitrix* Val.) obtained from single stripping (mean weight 278.8 ± 76.0 g) were used for experiments. Fish were caged and exposed to cyanobacterial bloom (dominated by coccal *Microcystis ichtyoblabe*, *M. aeruginosa* and *M. viridis*, $2.5-26 \times 10^5$ cells ml⁻¹), which were naturally developed in a breeding pond for 67 days during August-October 2004. In parallel, control groups of fish were carried either in a pond without apparent cyanobacterial bloom (phytoplankton dominated by chlorococcal green algae and diatoms).

Concentrations of microcystins in the cyanobacterial biomass were determined by HPLC (Agilent 1100 system, Supelcosil ABZ+Plus C18 column) coupled with photodiode array detector. Previously published method of toxin analyses was used (Bláha and Maršálek 2003).

Basic physic-chemical parameters were monitored during experiment. Dissolved oxygen ranged in all experimental variants between 80-255 % of saturation, pH 7.4-10.1, water temperature 13.8-24.7 °C, ammonia 0.00-1.23 mg l⁻¹ N-NH₄⁺, and nitrite 0.027-0.114 mg l⁻¹ N-NO₂⁻.

Water saturation by oxygen, temperature, and pH were measured by WTW Oxi 340i dissolved oxygen meter and by WTW pH 340i meter.

Ammonium ions were determined by Nessler method and nitrites by method with N-(1-naphthyl)-ethylenediamine and sulfanilic acid (Horáková *et al.* 1986).

Cyanobacterial biomass was evaluated by chlorophyll *a* concentrations (ISO 10260) and by number of cells counted in Bürker's counting chamber.

Blood samples were taken from the head into the heparinised tubes after the termination of the exposure time (30 and 67 days). Additional processing of blood and plasma separation was carried out after Svobodová *et al.* (1986). Values of haemoglobin (Hb) and haematocrit (PCV) were determined by standard methods (Svobodová *et al.* 1986).

Commercial kits were used for the detection of total protein (TP), activities of aminotransferases (ALT, AST), activity of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), cholinesterase (CHE), amylase (AMS), glucose (GLU), lactate (LACT), albumin (ALB), urea (UREA) and electrolyte (Ca, Mg, P, Fe). Mentioned parameters were measurement by ADVIA 1650 (Bayer-USA) analyzer. The analyzer works at photometric principle and forms open analytic system.

Statistical evaluation of results (ANOVA) was provided by software Microsoft Excel 2000.

Table 1

Haematological indices of biennial silver carp under the influence of toxic cyanobacterial water bloom.

Exposition		30 days		67 days		
groups	(n = 10)	Control	Exp.	Control	Exp.	
cyanobacteria	cells ml ⁻¹	-	1.145.833	-	249.167	
microcystins	µg g ⁻¹ DW	-	425.0	-	182.4	
Chlorophyll a	µg l ⁻¹	40.05	451.76	16,02	387.68	
PCV	l l ⁻¹	mean	0.27	0.32	0.28	0.33
		SD	0.04	0,03	0.03	0.03
Hb	g l ⁻¹	mean	58.48	64.27	62.85	74.91
		SD	8.51	6.64	9.37	8.91
ALB	g l ⁻¹	mean	5,9	6.0	6.0	6.1
		SD	0,4	0.6	0.53	0.89
ALP	µkat l ⁻¹	mean	1.95	0.86	0.48	0.38
		SD	0.84	0.25	0.15	0.18
ALT	µkat l ⁻¹	mean	0.98	0.69	0.54	0.35
		SD	0.56	0.33	0.39	0.23
AMS	µkat l ⁻¹	mean	5.41	5.30	3.24	4.53
		SD	1.97	0.64	0.50	2.23
AST	µkat l ⁻¹	mean	2.87	3.16	1.63	2,99
		SD	1.74	1,50	1.53	0,50
Ca	mmol l ⁻¹	mean	2.27	2,56	2.11	1.96
		SD	0.10	0.20	0.10	0.18
TP	g l ⁻¹	mean	24.75	24.98	25.77	26.35
		SD	2.22	2.28	2.90	3.63
GLU	mmol l ⁻¹	mean	4.81	3.36	3.29	3.16
		SD	0.80	1.18	0.41	0.55
Mg	mmol l ⁻¹	mean	1.16	1.15	1.11	0.90
		SD	0.07	0.06	0.07	0.05
LACT	mmol l ⁻¹	mean	4.10	5.29	5.15	4.21
		SD	0.94	1.29	1.18	1.37
LDH	µkat l ⁻¹	mean	12.19	13.17	11.77	13.52
		SD	2.99	2.66	2.11	2.61
P	mmol l ⁻¹	mean	2.51	2.89	1.78	1.71
		SD	0.14	0.30	0.14	0.18
Fe	µmol l ⁻¹	mean	11.3	15.4	8.0	14.0
		SD	4.68	2.18	2.84	2.84
UREA	mol l ⁻¹	mean	0.61	0.70	0.25	0.25
		SD	0.15	0.16	0.17	0.12
CHS	µkat l ⁻¹	mean	84.33	82.75	43.12	66.65
		SD	12.54	9.06	12.24	25.06

p ≤ 0.01 p ≤ 0,05

RESULTS

Three microcystin variants (microcystin-RR, -YR and -LR) were determined in the biomass of cyanobacteria at total concentration $540 \mu\text{g g}^{-1}$ d.w. (concentrations of individual variants were 46, 42, $452 \mu\text{g g}^{-1}$) at the beginning of the experiment.

Total concentration of microcystins in the biomass of cyanobacteria was $425 \mu\text{g g}^{-1}$ d.w. (concentrations of individual variants were 52, 110, $263 \mu\text{g g}^{-1}$) at the first extraction of blood (on day 30 of the experiment).

At the end of experiment (on day 67) total concentration of microcystins was $182 \mu\text{g g}^{-1}$ d.w. (concentrations of individual variants were 40, $142 \mu\text{g g}^{-1}$). The concentrations are well comparable with levels from other localities in the Czech Republic (Maršálek *et al.* 2001).

Results of haematological examinations are presented in the Table 1. Statistical evaluation of the influence of cyanobacterial population on hematological indices of the silver carp showed distinct values decrease of ALP and GLU concentrations ($p < 0.01$) after 30 days. These values were also lower than in controls after 67 days, but non-significantly. The Mg significantly ($p < 0.01$) decreased after 67 days exposition, after 30 days the changes were non-significant.

Values of PCV, Ca, P, Fe and LACT significantly increased ($p < 0.01$ or $p < 0.05$) after 30 days exposition compared to controls. The changes of PCV and Fe significantly increased ($p < 0.01$) after 67 days as well. Values of Hb, AST and CHS significantly increased ($p < 0.05$) only after 67 days exposition compared to controls.

The changes of ALB, ALT, AMS, TP, LD, and UREA were non-significantly elevated or decreased compared to controls. The values of bilirubin (BIL), ultra sensitive C-reactive protein (CRP_{us}), transferin (TRF), prealbumin (PALB), and immunoglobulin (IgG) were non-significantly changed or methods of under detection limit were used (data not shown).

DISCUSSION

The values of haematological indices in this work do not always correspond well with the results released by other authors (Rabergh *et al.* 1991; Tencalla *et al.* 1994; Carbis *et al.* 1996a, 1996b; Kopp and Heteša 2000; Navrátil *et al.* 1998; Vajcová *et al.* 1998). Certain differences caused mainly by different ways of toxin and cyanobacterial cell biomass application and by different physiological states of cyanobacterial water bloom populations.

Blood plasma indices appeared as the suitable indicators of toxic effect of cyanobacteria. Liver enzymes (ALT, AST, and LDH) are the most frequently tested enzymes in fish. Rabergh *et al.* 1991 reported that the activity of blood plasma enzymes (ALT, AST and LDH) raise in two hours after an intraperitoneal injection of toxin as a consequence of the hepatocyte necrosis. Tencalla *et al.* (1994) observed a decrease in their activity after 48 h, and interpreted this fact because of damage of the majority of hepatocytes that were not able to release enzymes into circulatory system. Carbis *et al.* (1996b) noted a delay of toxic manifestation in fish exposed to water with immersed microcystin. Our results showed only slightly increase in values of AST after 67 days exposition, values of ALT and LDH were non-significantly changes. Activity of ALT was even slightly decreased compared to control.

Vajcová *et al.* (1998) reported significant decrease in values of Hb, PCV, and TP at silver carp after intraperitoneal application of microcystin-LR. Our observations did not confirm these results. The values of Hb, PCV, and TP increased after 30 also 67 days exposition.

The results indicated large variation in values of haematological indices. Results of blood analyses in fish are affected by many endogenous and exogenous factors. The analysis of fish haematology indices after intraperitoneal injection of toxins or oral application toxic biomass of cyanobacteria showed higher differences values, than exposure of fish to the toxic biomass or soluble microcystins.

There is no information in the literature on changes of AMS, Ca, Mg, P, Fe, LAKT, UREA, and CHS in fish after the administration of microcystins or cyanobacterial biomass. The most interesting value is ferrum. The changes of Fe were significantly increased after exposition of cyanobacteria, probably under the thumb of liver damaged, consistent of the exposure to microcystins.

Continuous grazing on toxic cyanobacteria cells by silver carp may affect hepatic function but it may be difficult to detect these changes by analyzing the blood or serum chemistry of individual fish. However, the analysis of fish serum should be useful for evaluating microcystins toxicosis in a designated population of fish.

ACKNOWLEDGMENTS

Research on cyanotoxin effects in silver carp is supported by grant GAČR 206/02/D031.

REFERENCES

- Beveridge M. C. M., Baird D. J., Rahmatullah S. M., Lawton L. A., Beattie K. A., Codd G. A., 1993. *Grazing rates on toxic and non-toxic strains of cyanobacteria by Hypophthalmichthys molitrix and Oreochromis niloticus*, Journal of Fish Biology 43: 901 - 907
- Bláha L., Maršálek B., 2003. *Contamination of drinking water in the Czech Republic by microcystins*, Arch Hydrobiol 158: 421-429.
- Carbis C. R., Mitchell G. F., Anderson J. W., McCauley I., 1996a. *The effects of microcystins on the serum biochemistry of carp, Cyprinus carpio L., when the toxins are administered by gavage, immersion and intraperitoneal routes*, Journal of Fish Diseases, 19: 151 - 159
- Carbis C. R., Rawlin G. T., Mitchell G. F., Anderson J. W., Mccauley I., 1996b. *The histopathology of carp, Cyprinus carpio L., exposed to microcystins by gavage, immersion and intraperitoneal administration*, Journal of Fish Diseases, 19: 199 - 207
- Horáková M., Lischke P., Grünwald A., 1986. *Chemické a fyzikální metody analýzy vod*. SNTL, Praha, 392 s. (in Czech)
- ISO 10260, 1995. *Měření biochemických ukazatelů – Spektrofotometrické stanovení koncentrace chlorofylu-a*. Praha, 12 s.(in Czech)
- Kopp R., Heteša J., 2000. *Changes of haematological indices of juvenile carp (Cyprinus carpio L.) under the influence of natural populations of cyanobacterial water blooms*, Acta Vet. Brno, 69:131-137
- Maršálek B., Bláha L., Turánek J., Neča J., 2001. *Microcystin-LR and total microcystins in cyanobacterial blooms in the Czech Republic 1993-2000*. [in:] *Cyanotoxins - Occurrence, Causes, Consequences*, I. Chorus. Berlin, Germany, Springer-Verlag: 56-62.
- Maršálek B., Turánek J., 1996. *Biologicky aktivní látky produkované sinicemi vodního květu*, [in:] *Vodní květy sinic*. (ed.), Maršálek B., Keršner V., Marvan P., Nadatio flos - aquae, Brno, 86 – 100 (in Czech)
- Navrátil S., Palíková M., Vajcová V., 1998. *The Effect of Pure Microcystin LR and Biomass of Blue-green Algae on Blood indices of Carp (Cyprinus carpio L.)*. Acta vet. Brno, 67, 273-279
- Rabergh C. M. I., Bylund G., Eriksson J. E., 1991. *Histopathological effect of microcystin LR a cyclic polypeptide from the cyanobacterium Microcystis aeruginosa on common carp (Cyprinus carpio L.)*. Aquatic toxicology, 20, 131-146
- Svobodová Z., Pravda D., Palačková J., 1986. *Jednotné metody hematologického vyšetřování ryb*. Edice metodik VÚRH ve Vodňanech, Vodňany, 36 s. (in Czech)

- Šesterenko A. E., 1979. *Nekotoryje gematologičeskije pokazateli rastitelnojadnych ryb pri skarmivanii im sinezelenych vodoroslej*, Rybn. choz., Kiev, 28: 29–33 (in Russian)
- Tencalla G. F., Dietrich R. D., Schlatter CH., 1994. *Toxicity of Microcystis aeruginosa peptide toxin to yearling rainbow trout (Oncorhynchus mykiss)*, Aquatic Toxicology, 30: 215–224
- Vajcová V., Navrátil S., Palíková M., 1998. *The Effect of Intraperitoneally Applied Pure Microcystin LR on Haematological, Biochemical and Morphological Indices of Silver Carp (Hypophthalmichthys molitrix Val.)*, Acta vet. Brno, 67: 281-287