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DIVERSITY AND TOXIN CONTENT OF CYANOBACTERIA IN FISH PONDS (SOUTH MORAVIA, CZECH REPUBLIC) RELATED TO FISHERY MANAGEMENT INTENSITY

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Abstract

KOPP, R., ZIKOVÁ, A., MAREŠ, J., NAVRÁTIL, S., ADAMOVSKÝ, O., PALÍKOVÁ, M.: *Diversity and toxin content of cyanobacteria in fish ponds (South Moravia, Czech Republic) related to fishery management intensity.* Acta univ. agric. et silvic. Mendel. Brun., 2008, LVI, No. 5, pp. 111–118

During the vegetative period of the year 2005 (June–October) we carried out hydrobiological and hydrochemical monitoring of selected ponds inhered in Southern Moravia in relation to different fishery management intensities. Water temperature, dissolved oxygen content, pH, conductivity and water transparency were monitored directly at taking place, $N\text{–}NH_4^+$, $N\text{–}NO_2^-$, $N\text{–}NO_3^-$, $P\text{–}PO_4^{3-}$ content and chlorophyll-a concentration were measured in hydrochemical laboratory. At the same time, water samples for taxonomical analyses of phytoplankton and assessment of toxin content of cyanobacteria were taken. All ponds were characterized by low water transparency, high values of pH, $N\text{–}NH_4^+$, $P\text{–}PO_4^{3-}$ and high chlorophyll a concentration. We detected maximum concentration of 18.7 µg.l^{-1} microcystins in ponds dominated by the species *Microcystis aeruginosa*. Total microcystin concentration in water of all ponds with dominance of pikoplanktonic cyanobacteria were below detection limit (LOD < 0.125 µg.l^{-1}). The dominance of cyanobacteria species shifts from coccal (especially genus *Microcystis*) to small-colonial pikoplanctonic (genus *Aphanocapsa, Aphanothece*) and to single-filamentous (genus *Planktothrix, Limnothrix, Pseudanabaena*) cyanobacteria related to increasing fish stock density.

cyanobacteria, ponds, fish stock, microcystins

Fish ponds represent the most common type of stagnant water habitat in the Czech Republic and play important role in the hydrological system. The management of fish stock ponds did not change much for several hundred years from the middle age until the end of the nineteenth century. Since the 1950s, intensification of fish production started, when liming and fertilization of the fish ponds became a common practice. Thus, these new fish farming practices had an important impact on both the structure and dynamics of the aquatic ecosystem. Management of higher fish stock densities, accompanied by higher nutrient loads, resulted in increasing trophic status, ultimately reaching a state of hypertrophy in fish ponds. The main symptoms of this state are the massive development of phytoplankton and cyanobacterial blooms, great fluctuations in oxygen concentrations and pH, and high values of ammonia nitrogen destabilizing the fish pond ecosystem (Komárková, 1998; Pechar, 2000; Potužák et al., 2007).

Heavy blooms of cyanobacteria are largely associated to eutrophic or hypertrophic waters. Despite objections exist concerning the validity of species indicative of a given trophic level it is evident that the assembly of species in given sites combining physical and chemical properties is far from random. An important regulatory role in the control of the biomass and species composition of the algal assemblage is affected by their consumers, i.e. zooplankton and fish stock density (Komárková, 1998; Masojídek et al., 2001).

Common carp (*Cyprinus carpio* L.) is the main cultivated fish species. High abundance of common carp may potentially affect the primary production and community composition of freshwater systems. Common carp, a bottom feeder, might increase the flux of nutrients from benthic to the pelagial zone of ponds and thus change the dominant primary producers. These changes might especially occur in shallow water (Parkos et al., 2003; Rahman et al., 2008).

MATERIAL AND METHODS

Monitored fishponds are typical shallow, polymictic and hypertrophic ponds for breeding the common carp (*Cyprinus carpio* L.) in South Moravia (mean depth around 1.3 m, bottom containing soft sediment). Data about ponds area, fish production and GPS localization are presented in the Table I.

Water samples were taken from the outlet area of the ponds. Phytoplankton samples were taken into 100-ml plastic bottles from the depth of 0–30 cm by tube sampler. The samples were fixed with Lugol solution and condensed in ultra-filtering equipment. The quantity of phytoplankton was assessed in a Bürker counting chamber.

Basic physico-chemical parameters (oxygen saturation of water, pH and temperature) were measured by a WTW Oxi 340i dissolved oxygen meter and a WTW pH 340i pH meter. Conductivity meter Conmet 1 by an American company Hanna Instruments was used to assess conductivity. The transparency of water was assessed with a Secchi disc. Norm ISO 10260 was used for the assessment of chlorophyll *a* content. Ammonia nitrogen ($N–NH_4$) were determined by the Nessler method, nitrites ($N–NO_2$) by the method using N–(1–naphthyl)–ethylenediamine, nitrates ($N–NO_3$) by the method using sodium salicylate, orthophosphate ($P–PO_4$) by the method using ascorbic acid and ammonium molybdate (APHA, 1981).

Total microcystin concentration was monitored three times during the vegetative season (July, August and September) in cyanobacterial biomass and water. Concentrations of microcystins in the cyanobacterial biomass were determined by HPLC (Agilent 1100 system, Supercosil ABZ + Plus C18 column) coupled with photodiode array detector. Concentrations of microcystins in the water were determined by ELISA (Home-made Elisa according to Zeck et al., 2001) as previously described by (Bláha and Maršálek, 2003).

I: *Habitat description*

Pond	Pond area ha	Fish production $kg \cdot ha^{-1}$	GPS localization	
			latitude	longitude
Zámecký	30.0	100	48°48'35.492"N	16°48'44.568"E
Hlohovecký	104.0	290	48°46'54.736"N	16°47'17.379"E
Prostřední	52.0	394	48°46'53.839"N	16°48'6.649"E
Nesyt	289.7	506	48°46'41.914"N	16°44'32.267"E
Lužický	24.0	541	48°50'57.391"N	17°4'50.428"E
Mlýnský	108.0	602	48°47'12.25"N	16°49'19.848"E
Dvorský	29.8	951	48°51'17.818"N	17°4'20.452"E
Vrkoč	156.1	1434	48°55'37.187"N	16°33'47.447"E
Novoveský	138.7	1504	48°56'2.992"N	16°31'44.772"E

RESULTS

Values of physical and chemical parameters are presented in Table II and III. All ponds were characterized by low water transparency, high values of pH, ammonia nitrogen, orthophosphate and high chlorophyll a concentration. Differences among values of pH, temperature, nitrate nitrogen and nitrite nitrogen were minimal. Values of dissolved oxygen (fluctuated in great interval) displayed high variability. Transparency declined in dependence of increasing fish stock density. Conductivity mainly depended on richness of ions in bottom layer and their released to water column.

Abundance of main cyanobacteria groups monitored in ponds (June–October) are presented in the Figure I. In phytoplankton of Zamecký pond in 2005 dominated centric diatoms (50%), green algae (40%) and Euglenophyta (10%) during the month June and July. In August the most abundant groups were diatoms (60%), green algae (20%) and first cyanobacteria appear (*Microcystis aeruginosa* 10%, *Planktothrix agardhii* 5% and *Pseudanabaena limnetica* 5%). In September and October diatoms (70%) still dominated, followed by cyanobacteria (*Microcystis aeruginosa* 10%, *Anabaena flos-aquae* 5%, *Planktothrix agardhii* and *Pseudanabaena limnetica* 5%) and green algae (10%).

In Hlohovecký pond during June and July 2005 the highest abundance of cyanobacteria (*Microcystis aeruginosa* 70%, *Microcystis ichthyoblabe* 20% and *Aphanizomenon flos-aquae* 10%) was deteced. During the month August the most abundant groups were green algae (70%), diatoms (10%), Euglenophyta (10%) and pikoplanctonic cyanobacteria *Aphanocapsa* sp. (10%). In September and October Euglenophyta (40%), cyanobacteria (*Aphanocapsa* sp. and *Aphanothece* sp. 30%) and green algae (30%) dominated.

In phytoplankton of Prostřední pond in 2005 green algae (80%) and diatoms (20%) were the most abundant but there was a certain decrease of abundance during the month June and July. In August phytoplankton completely changed, the most abundant group became cyanobacteria (100%), especially genus *Microcystis*. In September and October were dominated Euglenophyta (50%), cyanobacteria (*Microcystis* sp. 40%), green algae (5%) and Cryptophyta (5%).

In pond Nesyt during June and July 2005 the most abundant groups of phytoplankton were pikoplanktonic cyanobacteria (*Aphanocapsa* sp. and *Synechocystis* sp. 75%), green algae (20%) and Cryptophyta (5%). In August the most abundant groups were pikoplanktonic cyanobacteria (*Aphanocapsa incerta*, *Aphanocapsa* sp. and *Synechocystis* sp. 50%) and Cryptophyta (5%). In September and October different kinds of pikoplanktonic cyanobacteria (95%) and Cryptophyta (5%) dominated.

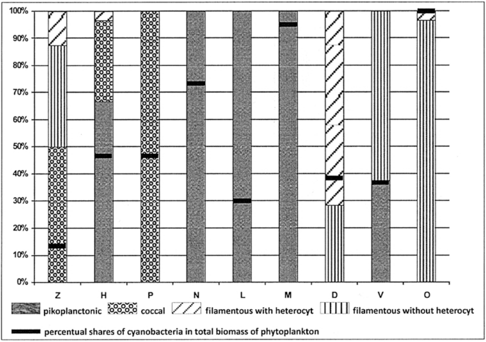
Pikoplanctonic cyanobacteria, mainly genus *Synechocystis* (90%), green algae (5%) and diatoms (5%) dominated in phytoplankton of Lužický pond in June and July 2005. In August phytoplankton completely changed, the most abundant group was diatoms (99%), especially species *Aulacoseira granulata*. In September and October diatoms (40%), green algae (30%), Eugenophyta (20%) and Cryptophyta (10%) were the most abundant.

In pond Mlýnský from June to October 2005 the most abundant groups of phytoplankton was pikoplanctonic cyanobacteria (*Aphanocapsa* sp. *Synechococcus* sp. and *Synechocystis* sp. 95%). Remaining part of phytoplankton was formed by green algae (5%).

Green algae (55%), especially species *Golenkinia radiata* and cyanobacteria (*Anabaenopsis elenkinii* 20%, *Planktothrix agardhii* 10% and *Anabaena flos-aquae* 5%) dominated in pond Dvorský during June and July 2005. During the month August the most abundant groups were cyanobacteria (*Anabaenopsis elenkinii* 40%, *Cuspidothrix issatschenkoi* 15%) and green algae (40%). In September and October green algae (75%) and cyanobacteria (*Anabaena* sp. 10%, *Planktothrix agardhii* 10%, and *Anabaenopsis elenkinii* 5%) were dominant.

In phytoplankton of Vrkoč pond during June and July 2005 green algae (60%), diatoms (20%), cyanobacteria (*Planktothrix agardhii* 10%) and Cryptophyta (10%) were the most abundant. In August green algae (50%) together with cyanobacteria (*Planktothrix agardhii* 35% and *Aphanocapsa* sp. 5%) dominated. Remaining part of phytoplankton was formed by diatoms (5%) and Cryptophyta (5%). In September and October cyanobacteria (*Aphanocapsa* sp. 50% and *Synechocystis* sp. 10%), green algae (20%) and diatoms (20%) were dominant.

The most abundant group of phytoplankton in Novoveský pond was filamentous cyanobacterium *Planktothrix agardhii* (95%) from June to October 2005. Remaining part of phytoplankton was formed by other kinds of filamentous cyanobacteria *Cuspidothrix issatschenkoi* and *Limnothrix redekei* (5%).



1: *Abundance of main cyanobacteria groups monitored in ponds (average values of vegetation season June–October). Ponds are ranged by increasing fish production. (Z – Zámecký, H – Hlohovecký, P – Prostřední, N – Nesyt, L – Lužický, M – Mlýnský, D – Dvorský, V – Vrkoč, O – Novoveský)*

Total microcystin content was determined using the biomass of cyanobacteria at a maximum concentration of 3234 µg·g^{-1} d.w (Prostřední pond). High concentration of microcystins in biomass was detectable only in ponds with coccal cyanobacteria (Zámecký, Prostřední, Hlohovecký), where average concentration was 1269 µg·g^{-1} d.w.

II: *Physical parameters in ponds during the vegetative period (June–October) of the year 2005. (average values ± SD, D. O. – dissolved oxygen)*

Pond	Transparency cm	Conductivity mS.m^{-1}	pH	Temperature °C	D.O. %
Zámecký	67 ± 5	52.3 ± 0.1	9.00 ± 0.14	20.3 ± 0.4	102 ± 10
Hlohovecký	67 ± 31	143.8 ± 1.8	8.67 ± 0.08	19.6 ± 0.9	76 ± 7
Prostřední	65 ± 60	139.5 ± 0.9	8.66 ± 0.25	21.0 ± 1.8	77 ± 34
Nesyt	35 ± 4	131.7 ± 1.8	8.72 ± 0.18	18.9 ± 1.4	72 ± 10
Lužický	45 ± 11	85.8 ± 1.9	8.55 ± 0.55	20.6 ± 0.8	109 ± 84
Mlýnský	25 ± 4	135.7 ± 4.0	8.67 ± 0.19	20.9 ± 2.0	83 ± 27
Dvorský	25 ± 4	63.6 ± 0.8	9.42 ± 0.08	20.5 ± 1.0	120 ± 13
Vrkoč	23 ± 5	74.0 ± 1.4	7.67 ± 0.28	20.1 ± 0.6	27 ± 10
Novoveský	35 ± 4	95.1 ± 2.1	8.17 ± 0.45	20.1 ± 0.8	74 ± 47

Total water-soluble microcystins in all ponds with only pikoplanktonic cyanobacteria (Nesyt, Lužický, Mlýnský) was below detection limit (LOD < 0.125 µg.l^{-1}). Higher concentrations of microcystin in water were detected in ponds with filamentous cyanobacteria and high fish stock density (Dvorský, Vrkoč, Novoveský). Maximum concentration was 5.6 µg.l^{-1} (Novoveský pond); average concentration in these ponds was 1.35 µg.l^{-1}. The highest concentrations of microcystin in water were determined in ponds with coccal cyanobacteria and low fish stock density (Zámecký, Prostřední, Hlohovecký). Maximum concentration was 18.7 µg.l^{-1} (Prostřední pond); average concentration in these ponds was 3.75 µg.l^{-1}.

III: *Chemical parameters in ponds during the vegetative period (June–October) of the year 2005. (average values ± SD)*

Pond	N-NH4 mg.l^{-1}	N-NO3 mg.l^{-1}	N-NO2 mg.l^{-1}	P-PO4 mg.l^{-1}	Chlorophyll a µg.l^{-1}
Zámecký	1.19 ± 1.10	2.63 ± 0.82	0.012 ± 0.003	0.12 ± 0.06	125 ± 23
Hlohovecký	0.66 ± 0.44	3.17 ± 2.70	0.014 ± 0.003	0.41 ± 0.23	104 ± 58
Prostřední	1.57 ± 0.34	2.87 ± 0.95	0.050 ± 0.038	0.40 ± 0.09	377 ± 398
Nesyt	1.33 ± 0.77	2.93 ± 0.69	0.014 ± 0.002	0.29 ± 0.10	138 ± 43
Lužický	2.24 ± 0.27	2.43 ± 1.76	0.143 ± 0.080	0.69 ± 0.06	170 ± 89
Mlýnský	1.25 ± 0.61	2.97 ± 1.67	0.017 ± 0.003	0.24 ± 0.09	119 ± 31
Dvorský	0.55 ± 0.44	1.87 ± 1.87	0.011 ± 0.003	0.34 ± 0.25	224 ± 26
Vrkoč	0.96 ± 0.37	3.27 ± 0.90	0.028 ± 0.009	0.21 ± 0.09	196 ± 67
Novoveský	0.29 ± 0.01	3.00 ± 1.37	0.015 ± 0.007	0.62 ± 0.11	219 ± 31

DISCUSSION

In the 1950s and 1960s (average fish production was 350 kg.ha^{-1}) the dense bloom of *Aphanizomenon flos-aquae* was the most frequent type of plankton in Czech fish ponds during summer (Pechar and Fott, 1991). In the 1970s and 1980s (average fish production was 430 kg.ha^{-1}) *A. flos-aquae* was replaced by *Microcystis aeruginosa* and several species of *Anabaena* that form more than 60% of all recorded blooms together. Till the 1980s *Planktothrix agardhii* and *Limnothrix redekei* were rare in fish pond phytoplankton and no bloom of these species was recorded. During the last twenty years blooms of *P. agardhii* and *L. redekei* have become common in the fish ponds. (Pechar, 1995).

Aphanizomenon flos-aquae formed the dominant population (at least 50%) during summer if the fish stock was below production of 350 kg.ha^{-1}. With an increase of the fish stock, the number of large filter feeders of zooplankton decreased and small forms of algae became dominant. The course of the relationship after the summer fish stock reached 400 kg.ha^{-1} (Komárková, 1998).

During the last decades productivity of fish ponds in southern Moravia was markedly increased. High concentration of nutrients, intensive feeding by cereals caused eutrophication indicated by decreased transparency and increased chlorophyll a concentration. Komárková (1998) mentioned the relationship between the fish stock and chlorophyll a concentration in summer as significant, positive and linear. Our results did not certify these conclusions because all monitored ponds had high concentrations of chlorophyll a regardless fish stock density. Chlorophyll a fluctuated more in ponds with low fish stock as water transparency.

Composition of phytoplankton in ponds with fish production from 100 to 400 kg.ha^{-1} was formed mainly by chlorococcal green algae, diatoms and coccal cyanobacteria especially genus *Microcystis*. These ponds are characterised by high fluctuation of physical and chemical parameters and relatively fast phytoplankton and zooplankton succession. When fish stocks are low, nutrients can remain in water, despite being available for phytoplankton (Hrbáček et al. 1978). Such systems are very sensitive and, unfortunately, not stable for a longer time under natural conditions (Benndorf, 1987).

Genus *Microcystis* is noted as producer of liver toxins especially microcystins. Our results show high concentration of microcystins in water ponds with low fish production where cyanobacterium *Microcystis aeruginosa* was dominant. The value of microcystins in Prostřední pond (18.7 µg.l^{-1}) was the maximum concentration of 96 ponds and dams in the Czech Republic in the year 2005. Average values of microcystins in natural water were 0,88 µg.l^{-1} in the Czech Republic (Bláhová et al., 2007). Monitoring of 30 shallow eutrophic or hypertrophic lakes with dominating cyanobacteria *Microcystis* in China, showed average concentration of microcystins around 0.27 µg.l^{-1} and the maximum of 8.57 µg.l^{-1} (Wu et al., 2006). Similar values mentioned Kotak et al. (1996) from four lakes in Canada.

Composition of phytoplankton in ponds with fish production from 500 to 700 kg.ha^{-1} was dominated mainly by different kinds of pikoplanktonic cyanobacteria and diatoms. Basic physical and chemical parameters were relatively stable, mainly small species composition of zooplankton structure. Several kinds of pikoplanctonic cyanobacteria as *Aphanocapsa* have a potential to produce microcystins (Pearl et al., 2001). Our results did not show microcystins in water, total microcystins concentration in water was bellow detection limit (LOD 0.125 µg.l^{-1}) in all ponds with a dominance of pikoplanktonic cyanobacteria.

Composition of phytoplankton in ponds with fish production from 900 to 1500 kg.ha^{-1} consisted mainly of filamentous cyanobacteria and green algae. Ponds with high fish stock density were characterised by very low transparency and high fluctuations of dissolved oxygen. Filamentous cyanobacteria, mainly species *Planktothrix agardhii*, *Limnothrix redekei* and *Pseudanabaena limnetica* were dominant in turbid ponds and lakes. The basic mechanism is that cyanobacteria are the superior competitors under conditions of low light (Scheffer et al., 1997).

Cyanobacterial species *Planktothrix agardhii* has a potential to produce microcystins (Yepremian et al., 2007). Our results show the maximum concentration of microcystins 5.6 µg.l^{-1}. The concentrations of microcystins are well comparable with levels from other localities in the Czech Republic (Maršálek et al., 2001).

CONCLUSIONS

In recent years, higher fish stock densities combined with high level of manuring has caused a permanent dense phytoplankton bloom. Low grazing pressure of zooplankton, low light conditions and high nutrient concentrations are suitable conditions for mass development of cyanobacteria. The dominance of cyanobacteria species shifts from coccal (especially genus *Microcystis*) to small-colonial pikoplanctonic (genus *Aphanocapsa*, *Aphanothece*) and to single-filamentous (genus *Planktothrix*, *Limnothrix*, *Pseudanabaena*) cyanobacteria related to increasing fish stock density.

Toxicity concentrations of cyanobacteria were different among monitored ponds. The concentrations of microcystin in water were the highest in ponds with low fish production, lower in ponds with high fish production, and below the detection limit in ponds with average fish production.

SOUHRN

Složení a toxicita sinic rybníků jižní Moravy v závislosti na intenzitě rybářského hospodaření

Během vegetačního období roku 2005 (červen–říjen) probíhal monitoring devíti rybníků jižní Moravy. Rybníky byly vybrány v závislosti na intenzitě hospodaření s rybí produkcí od 100 do 1500 kg.ha^{-1}. Sledovali jsme základní fyzikálně-chemické parametry vody (teplota vody, pH, rozpuštěný kyslík, vo-

divost, průhlednost, N-NH_4^+, N-NO_2^-, N-NO_3^-, P-PO_4^{3-}), chlorofyl a, koncentraci microcystinů a složení fytoplanktonu.
Všechny rybníky byly charakterizovány nízkou průhledností, vysokou hodnotou pH, amoniakálního dusíku, fosforečnanů a vysokou koncentrací hodnot chlorofylu a. Koncentrace rozpuštěného kyslíku výrazně kolísala především u rybníků s nízkou hustotou rybí obsádky, ale nejnižší hodnoty nasycení byly zjišťovány u rybníků s nejvyšší hustotou obsádky ryb. Ostatní sledované parametry nevykazovaly výraznější rozdíly mezi jednotlivými rybníky, mimo hodnot vodivosti, které závisely především na obsahu solí v sedimentech dna a přítokové vodě.
Složení fytoplanktonu jednotlivých rybníků bylo typické pro mělké hypertrofní nádrže s vysokou produktivitou. Převažovali zástupci sinic, chlorokokálních zelených řas a rozsivek. Intenzitě rybářského hospodaření odpovídalo i druhové složení planktonních sinic. Sinice rybníků s nízkou hustotou rybí obsádky byly zastoupeny převážně kokálními druhy sinic rodu *Microcystis*, u kterých byly zjišťovány i nejvyšší hodnoty microcystinů. Maximální zjištěná koncentrace rozpuštěného microcystinu ve vodě byla 18,7 μg.l^{-1} (Prostřední rybník). Pikoplanktonní sinice (*Aphanothece*, *Aphanocapsa*) byly hlavní dominantou rybníků se střední hustotou rybí obsádky, u těchto populací sinic nebyla zjištěna toxicita, koncentrace microcystinů byla pod detekčním limitem (LOD < 0,125 μg.l^{-1}). V rybnících s nejvyšší hustotou rybí obsádky převažovaly vláknité sinice (*Planktothrix*, *Limnothrix*, *Pseudanabaena*), které jsou adaptovány na život v prostředí s nízkou světelnou intenzitou. V těchto populacích sinic jsme detekovali různé úrovně koncentrace microcystinů, avšak dosahovaly nižších hodnot než u kokálních sinic rodu *Microcystis*. Maximální zjištěná koncentrace rozpuštěného microcystinu ve vodě byla 5,6 μg.l^{-1} (Novoveský rybník).

sinice, rybníky, microcystin

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