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Seasonal shifts in chemotype composition of *Microcystis* sp. communities in the pelagial and the sediment of a shallow reservoir

Martin Welker[1]
Technische Universität Berlin, Inst. Chemie-Biochem. und Mol. Biol., Franklinstr. 29, 10587 Berlin, Germany

Lenka Šejnohová
Czech Academy of Sciences, Inst. Botany, Květná 8, 6036 Brno, Czech Republic

Danka Némethová
Masaryk University, Research Centre for Environmental Chemistry and Ecotoxicology and Centre of Biostatistics and Analyses, Kamenice 126/3, 625 00 Brno, Czech Republic

Hans von Döhren
Technische Universität Berlin, Inst. Chemie-Biochem. und Mol. Biol., Franklinstr. 29, 10587 Berlin, Germany

Jiří Jarkovský
Masaryk University, Research Centre for Environmental Chemistry and Ecotoxicology and Centre of Biostatistics and Analyses, Kamenice 126/3, 625 00 Brno, Czech Republic

Blahoslav Maršálek
Czech Academy of Sciences, Inst. Botany; Květná 8, 6036 Brno, Czech Republic; Masaryk University, Research Centre for Environmental Chemistry and Ecotoxicology, Kamenice 126/3, 625 00 Brno, Czech Republic

Abstract

The various oligopeptides produced by individual *Microcystis* clones enable the classification of individual colonies of *Microcystis* in distinct peptide chemotypes. The dynamics and diversity of coexisting chemotypes are regarded as major factors influencing the microcystin-content of blooms of this potentially toxic cyanobacterial genus. We compared the chemotype composition in planktonic and benthic *Microcystis* communities in Brno reservoir (Czech Republic) from July to November 2004 by single-colony mass spectrometry ($n = 783$). Ninety-two peptides were selected to characterize 37 chemotypes as revealed by *K*-means clustering. In the course of the season the *Microcystis* community became significantly less diverse (linear regression of Shannon indices, $p < 0.001$) in the pelagic, and in November two chemotypes—both of which did not contain microcystins—accounted for nearly 80% of the colonies. In contrast, other chemotypes that were dominant in the pelagic in July were no longer encountered after August, whereas some chemotypes that never accounted for high relative abundances were encountered throughout the season. The shift to some few dominant chemotypes in the pelagic was also reflected by changes in the benthic community where the same chemotypes increased in relative abundances. Nonetheless, chemotypes were identified in the sediment in July and November that were never found in plankton samples. A principal component analysis revealed that communities in the pelagial and the benthal were very different in July but converged during the season because of the deposition of dominant planktonic chemotypes in the sediment. In accordance with the declining percentage of toxin-producing *Microcystis* colonies, the microcystin content of seston samples decreased significantly from 0.9 mg g^{-1} dry weight to levels below the detection limit (linear regression, $p < 0.001$).

[1] Corresponding author (martin.welker@chem.tu-berlin.de).

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Cyanobacterial blooms in waterbodies used for recreational purposes and as drinking water resources are of concern to public health because of the ability of some bloom-forming genera to produce hepatotoxic peptides, the microcystins. Whereas the modeling of *Microcystis* bloom events can be done with fair accuracy (Roelke and Buyukates 2002), no model has been developed yet to predict microcystin concentrations with sufficient accuracy with respect to the application of safety levels. Microcystins are produced by strains from diverse genera like *Microcystis, Planktothrix*, and *Anabaena*, but not by all strains of the respective genera (Rantala et al. 2004). The capability

of microcystin biosynthesis is not related to phylogenies based on housekeeping genes (Neilan et al. 1997), although evidence is given that the genes required for the biosynthetical pathway are of very ancient origin and repeated gene loss has led to the recent distribution of these genes (Rantala et al. 2004). Microcystin is synthesized by a nonribosomal peptide synthetase (NRPS) pathway (Tillett et al. 2000), a pathway by which many peptide antibiotics and other secondary metabolites are produced in heterotrophic bacteria, fungi, and cyanobacteria (von Döhren et al. 1997). Physiological experiments on microcystin production rates have shown that the microcystin content of a particular clonal strain can vary only within a narrow range, for about a factor of three (Orr and Jones 1998), whereas the microcystin content of field samples can vary for orders of magnitudes (Fastner et al. 1999). When comparing the microcystin content of toxigenic strains it became evident that these also can vary for orders of magnitude (Carrillo et al. 2003), thus suggesting that changes in the microcystin content of natural communities is the effect of the dynamics of individual clones rather than the result of physiological regulations. However, methods only recently have been developed that enable the tracking of the dynamics of distinct individual (cyano)bacterial clones or groups of clones in natural communities. With quantitative real-time polymerase chain reaction the number of microcystin synthetase gene (*mcy*) copies is estimated, and the relative share of toxigenic cells to total cell numbers of *Microcystis*, for example, can then be calculated (Vaitomaa et al. 2003). A distinction between individual toxigenic strains, however, cannot be made and only *mcy*$^+$ and *mcy*$^-$ genotypes are recorded. A higher resolution, i.e., more distinct groups of strains, is achieved by the application of denaturing gradient gel electrophoresis (Janse et al. 2004). Multiple genotypes based on genes like 16S-23S rRNA intergenic spacer sequences are distinguished and can be followed through the bloom season (Janse et al. 2004).

A third approach relies on the direct detection of secondary metabolites in individual *Microcystis* colonies by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Fastner et al. 2001; Welker et al. 2004). It makes use of the fact that clones of *Microcystis* produce a multitude of oligopeptides, most of which are likely synthesized by NRPSs in analogy to microcystins (Welker and von Döhren 2006), that give individual clones a distinct peptide fingerprint characterizing a peptide chemotype. The term ''chemotype'' applied in the present study refers thus to colonies having the same peptide pattern in mass spectra. There is good evidence that chemotypes are stable units representing clonal strains or very closely related clonal strains. Laboratory cultures of *Microcystis*, e.g., PCC 7806, have been observed for decades producing the same peptides (Tillett et al. 2000; Bister et al. 2004). Further, culture experiments showed that peptides other than microcystins, e.g., anabaenopeptin and cyanopeptolin variants, also seem to be produced constitutively (Repka et al. 2004; Ferreira Ferreira 2006), i.e., neither complete suppression of peptide production nor a switch to other congeners was observed, and relative changes in cell quota are of the same magnitude as observed for microcystins.

Whereas the genetic and biochemical aspects of peptide production are known in principle, the role of the cyanobacterial peptides—including microcystins—has not yet been clearly revealed. Grazing protection (Ghadouani et al. 2004; Czarnecki et al. 2006) and allelopathic competition (Sukenik et al. 2002) in *Microcystis* have been demonstrated, but do not satisfyingly explain the metabolic diversity that has evolved despite high costs to the cells.

Besides clonal dynamics in the pelagic, the role of benthic inocula for the population dynamics is potentially of importance for the clonal composition of a *Microcystis* community. The overwintering of colonies in or on the sediment was recognized decades ago (Preston et al. 1980), but the inoculation from and to the sediment has been subject only recently to quantitative estimates (Brunberg and Blomqvist 2003; Ihle et al. 2005; Verspagen et al. 2005). Likewise, metabolic activity of benthic populations was estimated only recently, showing that in *Microcystis* colonies cell division continues during the winter in the sediment (Latour et al. 2004*a*).

For the present study the *Microcystis* community in the pelagial and benthal of a shallow, hypertrophic reservoir was sampled throughout a summer season. Isolated individual colonies were analyzed microscopically and by MALDI-TOF MS to determine chemotype composition and dynamics. The hypotheses we intended to test were as follows: (1) the pelagic *Microcystis* community at the onset of the bloom is influenced by inoculation from the sediment; (2) the pelagic *Microcystis* community shows dynamic changes in chemotype composition during the summer season; (3) the benthic *Microcystis* community at the end of the season reflects the sedimentation of pelagic colonies; and (4) the microcystin content of seston samples is correlated to the *Microcystis* community chemotype composition in the pelagic.

Methods and materials

Study site—The Brno reservoir (49°N, 16°30′E) is an artificial lake dammed up in 1940 at an altitude of 231 m. The reservoir stretches for 10 km in the valley of the Svratka River with a maximum width of 0.8 km in the main basin next to the dam. The maximum and average depths are 19 m and 7.7 m, respectively, and the total volume is some 15×10^6 m^3. The lake area is 2.59 km^2, and the annual average water discharge is 8.08 m^3 s^{-1}. The trophic state is considered as hypertrophic with annual averages of total phosphorus (TP) of 170 μg L^{-1} and maximum concentrations of chlorophyll *a* (Chl *a*) of a few hundred micrograms per liter. The reservoir is used mainly for recreational purposes but also serves as a backup drinking water reservoir.

Sampling—Phytoplankton samples were taken in the central part of Brno reservoir with a plankton net (42 μm) by repeated vertical tows in the uppermost 2 m of the water column at a central location. In the laboratory, colonies were selected randomly from rediluted samples under

Table 1. List of *Microcystis* samples taken at the Brno reservoir. *n* refers to the number of individual colonies isolated from a particular sample. "Failed" refers to the number of colonies that were lost during shipping or excluded from the further analysis because of the low quality of the mass spectra.

Sample	Date 2004	Origin	Location	Depth	*n*	Failed
P1	08 Jul	Pelagial	Central	0–2 m	100	—
P2	31 Aug	Pelagial	Central	0–2 m	100	—
P3	08 Oct	Pelagial	Central	0–2 m	100	7
P4	10 Nov	Pelagial	Central	0–2 m	100	—
S1a	08 Jul	Sediment	Upstream	0–5 cm	50	1
S1b	08 Jul	Sediment	Upstream	5–10 cm	100	1
S1c	08 Jul	Sediment	Central	0–5 cm	50	—
S4a	10 Nov	Sediment	Upstream	0–5 cm	50	3
S4b	10 Nov	Sediment	Upstream	5–10 cm	100	4
S4c	10 Nov	Sediment	Central	0–5 cm	50	—

a dissecting microscope and carefully washed thrice in droplets of sterile water on the day (and night) of sampling. Microscopical examination included micrography, cell and colony dimension measurement, description of colony morphology, and morphospecies determination. Colonies processed in this manner were then placed on a stainless steel template for MALDI-TOF MS analysis and allowed to dry at ambient temperature. Loaded templates were stored dry and cool (4°C) until further processing. An aliquot of the net sample was frozen to be lyophilized later.

Sediment samples were taken with a core sampler at two locations, central (12–14-m deep) and upstream (2–4-m deep), and the soft sediment was divided immediately after collection in two layers, 0–5-cm depth and 5–10-cm depth. A more refined separation of horizons was not possible because of the soft consistency of the sediments. In the laboratory, about 1 mL of sediment was suspended in sterile tap water, and colonies were isolated under a dissecting microscope and further processed like pelagic colonies. Isolation was continued until the envisaged number of colonies was isolated. For two sediment samples (central, 5–10 cm), the number of vital-looking colonies was very small, and respective samples thus were discarded. The number of colonies isolated from each sample is given in Table 1.

Mass spectrometry—Dried colonies were extracted directly on the template by placing a droplet of 0.05–0.2 μL of a matrix solution, dependent on the colony size, directly on the colony. The matrix solution was 20 mg of 2,5-dihydroxy benzoic acid dissolved in 1 mL of a mixture of water : acetonitril : ethanol (1 : 1 : 1) acidified with 0.1% v/v trifluoroacetic acid. MALDI-TOF MS analyses were carried out on a Voyager-DE PRO biospectrometry workstation as described previously (Welker et al. 2004). The mass spectra were qualitatively analyzed, and only those in which either peptides (verified by post-source-decay [PSD] fragmentation) or Chl *a* derivatives gave unambiguous mass signals (>1,000 counts) were used for further procedures.

Mass spectra were screened for known peptide masses (including associated peaks like $M + Na^+$ or $M\text{-}H_2O + H^+$) and mass signals of yet unknown metabolites. Metabolites that were detected with intense mass signals were further characterized by PSD fragmentation (Spengler et al. 1991). Peptide identification and elucidation are described in detail elsewhere (Welker et al. 2006). In colony mass spectra, peptides were identified by their mass to the nearest 0.05 Da, by characteristic isotopic distribution, and by characteristic patterns of associated peaks.

Mass spectral data processing and analysis—For each colony a data set of presence/absence of individual peptides was generated, and all data for all colonies (including sampling date, morphospecies, etc.) were unified in a data matrix containing 92 peptides in 783 colonies. A number of colonies without peptides but otherwise positive mass spectral results (*see* above) were identified as *Microcystis wesenbergii*. The peptide containing colonies were subjected to a *K*-means clustering procedure, which is a nonhierarchical classification method. *K*-means clustering divides objects into a previously specified number of groups and is more appropriate than hierarchical clustering procedures in the case of a large data set such as for the present study. The general procedure is to find groups in multivariate space such that within-group similarities and between-group dissimilarities are maximized. The number of groups *K* must be specified in advance, and thus we repeatedly performed the clustering into 2, 3, 4, …., 50 groups (clusters) and calculated the ratio of intracluster versus intercluster variability (Ray and Turi 1999). The lowest value (the set of the most homogeneous clusters) for the range of 2–50 clusters was obtained for 36 groups of colonies; therefore we took this as the number of peptide chemotypes (CT). One additional group of colonies was formed by the 96 colonies of *M. wesenbergii*.

After assigning a chemotype (arbitrary number) to each of the colonies, the chemotype composition of each individual sample was expressed as the relative frequency of each chemotype. The chemotype diversity of a sample was expressed as the Shannon index of diversity.

In a principle component analysis (PCA; Jongman et al. 1997), communities of chemotypes were compared with each set of colonies originating from an individual sample representing one community. The PCA was applied to reduce dimensionality of the data set (37 chemotypes × 10 samples) and was performed on the correlation matrix.

The dependence of microcystin content on the percentage of microcystin-producing colonies was determined by means of linear regression analysis.

All statistical analyses were performed with the Statistica for Windows software package, version 7.1. (StatSoft).

High-performance liquid chromatography analysis—Some 5 mg (±0.1 mg) of lyophilized net-samples was extracted sequentially with 70% aqueous methanol (MeOH), water, and 5% acetic acid. Combined supernatants were dried in a SpeedVac to be stored at −20°C until analyzed. Dried extracts were redissolved in 50% MeOH in a volume resulting in an extract equivalent of 1 mg dry wt per 100 μL final volume. High-performance liquid chromatography (HPLC) analysis was carried out on a system and applying conditions as described previously (Welker et al. 2003). For the sample taken on 10 November 2004, not enough colonies were collected with the plankton net to be lyophilized after isolating the colonies. The remaining colonies were therefore concentrated on a glass-fiber filter, dried, and extracted without a determination of biomass.

Peak fractions of respective extracts were collected manually, dried, and further analyzed by MALDI-TOF MS as described earlier (Czarnecki et al. 2006).

Results

Microcystis *abundance*—The temporal pattern of *Microcystis* occurrence was in a good agreement with previous findings from eutrophied temperate lakes. In May, diatoms (*Asterionella formosa, Stephanodiscus* sp., and *Navicula* sp.), together with filamentous cyanobacteria (*Anabaena sigmoidea* and *Planktothrix agardhii*), dominated the phytoplankton community with total Chl *a* concentrations reaching 12 μg L^{-1}. After a pronounced clear-water phase in June, the first *Microcystis* colonies were encountered at the beginning of July with ever-increasing abundances peaking in August within the range of 130–450 μg L^{-1} Chl *a*, depending on the sampled location in the reservoir. During the period from July to October, *Microcystis* sp. was the dominant organism. In due course, *Microcystis* abundance declined to finally fall below detection levels in November. By this time, however, colonies could only be collected by repeated net tows, and in Lugol-fixed samples of the surface layer (0–2 m) no *Microcystis* cells were encountered. An increase in diatom abundance was observed together with the occurrence of (few) *Planktothrix* filaments in parallel to the disappearance of *Microcystis* from the water column.

The temperature and nutrient dynamics also followed typical trends, with T_{max} = 23°C reached in August coinciding with high TP (920 μg L^{-1}) and relatively low total nitrogen (1.1 mg L^{-1}) concentrations.

Peptides and chemotypes—The majority of 800 colonies originally isolated gave satisfying mass spectra, i.e., mass spectra in which at least one peptide could be identified or, in the case of *M. wesenbergii*, Chl *a* derivatives and repeatedly detected nonpeptidic metabolites (Fig. 1). Seven colonies from the pelagic sample collected on 08 October 2004 were lost during shipping. All other colonies that failed to give satisfying mass spectra originated from sediment samples collected at the upstream site and were most likely already decaying at the time of sampling. A total of 783 colonies gave mass spectra meeting the above-mentioned criteria and were subjected to the subsequent analytical procedures.

In 687 of the colonies at least one peptide was detected. The remaining 96 colonies were identified as *M. wesenbergii*, and in the respective mass spectra two compounds were detected that could not be further characterized (Fig. 1, CT 37, *see* below). Both compounds (M + H = 598.5 and 614.5, respectively) had isotopic patterns not consistent with peptidic compounds, and PSD fragmentation did not reveal amino acid fragments. Mass spectra with these characteristics were exclusively encountered for *M. wesenbergii* colonies. In Web Appendix 1 (http://www.aslo.org/lo/toc/vol_52/issue_2/0609a1.pdf) all peptides were listed that were included in the multivariate statistical analysis. The majority of the peptides listed in the table were detected before in a variety of different samples (cells, HPLC fractions, bloom extracts) of *Microcystis*. Nonetheless, for 14 mass signals we could only confirm the peptidic nature of the compounds, and for 15 peptides we could only make a classification with partial elucidation of the structure.

In mass spectral analyses, no qualitative difference was observed between colonies of pelagic and benthic origin (Fig. 1). Signal intensities were comparable and no habitat-specific background or noise was recorded. Colonies that were subsequently unified to a single chemotype gave very similar mass spectra, i.e., the same pattern of associated peaks and similar relative signal intensities, independent of the origin and the sampling date.

The most frequent peptide was an aeruginosin that could be identified as a chlorine-deficient variant of aeruginosin 89, aeruginosin 602 (603 Da; reference to individual peptides is made by giving the name and detected mass in Da as given in the Web Appendix 1) that was detected in 47% of the colonies. Other frequent peptides include three microcystin variants (Mcyst -LR, -YR, and -RR) that typically were produced by *Microcystis*. Mcyst-LR was most frequently detected (43% of colonies) whereas Mcyst-YR and Mcyst-RR were detected in about one-third of the colonies. One frequent cyanopeptolin congener (cyanopeptolin 1063, 1,046 Da) was detected in 28% of the colonies, but only in one chemotype that was encountered frequently itself.

Based on the peptide presence/absence data matrix and the clustering procedures, 37 CT were established with specific peptide patterns (Fig. 1). Chemotypes differed in the production of individual peptides as well as in the production of peptides in individual combinations. Aeruginosin 602, for example, was detected in colonies that were representatives of five chemotypes, three of which were among the most frequent ones (Fig. 1). In CT 7, the most frequent chemotype representing 28% of all colonies, aeruginosin 602 was the only aeruginosin detected and occurred together with cyanopeptolin 1063, whereas in CT 34, aeruginosin 602 was detected with two chlorinated

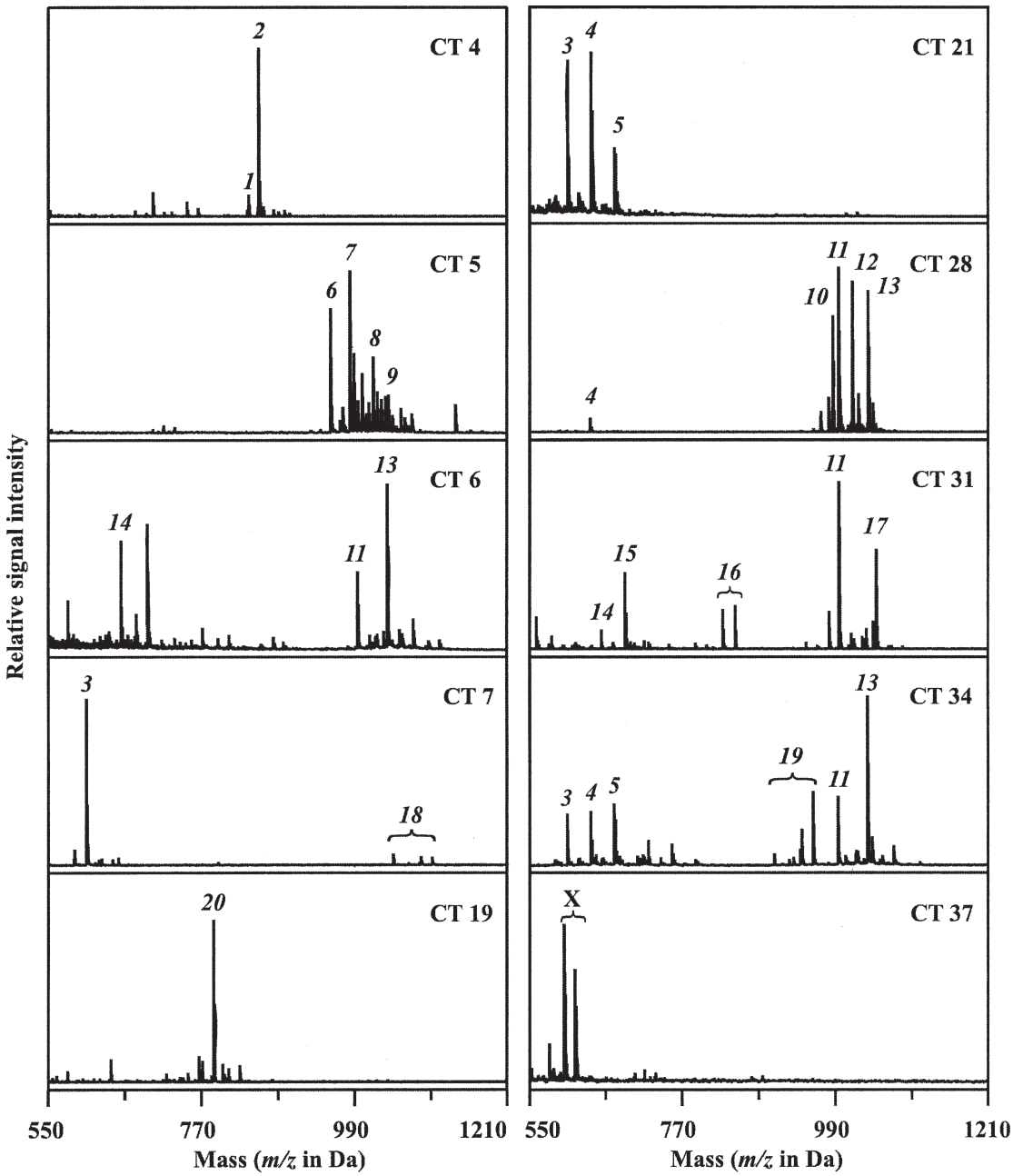

Fig. 1. Partial MALDI-TOF mass spectra of *Microcystis* sp. colonies representing the main peptide chemotypes encountered in the Brno reservoir in July–November 2004. Numbers on each panel with the prefix CT refer to the chemotype number. Numbers in italics above peaks refer to peptides: 1, anabaenopeptin B; 2, anabaenopeptin F; 3, aeruginosin 602; 4, aeruginosin 89; 5, aeruginosin 670; 6, cyanopeptolin 972C; 7, cyanopeptolin 1000A; 8, cyanopeptolin 1006D; 9, cyanopeptolin 1034A; 10, cyanopeptolin 986A; 11, microcystin-LR; 12, cyanopeptolin 1014A; 13, microcystin-RR; 14, aeruginosin 102; 15, aeruginosin 686; 16, cyanopeptolin S; 17, microcystin-H_4YR; 18, cyanopeptolin 1063; 19, cyanopeptolin 920; 20, kasumigamide; X, nonpeptidic compounds. Parentheses indicate multiple peaks of individual peptides, e.g., sodium adduct peaks. For a full list of detected peptides see Web Appendix 1.

variants (aeruginosins 89 and 670; 637 and 671 Da, respectively), cyanopeptolin 920 (921 Da), and microcystins LR and RR. In a further chemotype, CT 21, only the three aeruginosins (603, 637, and 671 Da, respectively) could be detected.

Many of the more frequent individual peptides (in >5% of colonies) were not restricted to a single chemotype but were found in combinations with other peptides in multiple chemotypes. This was the case for microcystins that were detected in 22 of the 37 chemotypes together with other peptides but never as the sole peptides. Other peptides were restricted to a single chemotype and were only found in one particular combination, such as cyanopeptolin 1063 in CT 7 or a series of cyanopeptolins in CT 5 (cyanopeptolins 972C, 1000A, 1006D, and 1034A with 955, 983, 1,011, and 1,039 Da, respectively; Fig. 1).

About half of the peptides were encountered only in <1% of the colonies. These were either peptides that produced only low-intensity mass signals and were thus close to the detection limit (e.g., oscillamide Y [858 Da] and [Asp^3,Dha^7]Mcyst-LR [1,067 Da]) or were produced exclusively by rare chemotypes but then detected with high-signal intensity (e.g., microginin FR9 [751 Da] and microginin 764 [765 Da]).

Morphospecies and chemotypes—Regarding the relationship between the morphospecies of the colonies and the chemotyping based on peptide pattern, a dilemma is evident: some 20 morphospecies are described for temperate lakes (Komárek and Anagnostidis 1999), eight of which have been reported for the Czech Republic, whereas in our samples, 37 distinct chemotypes could be detected. Thus, a particular morphospecies can be represented by several chemotypes. In the present study, only two clear relationships were found: first, no *M. wesenbergii* colony produced any peptide, and second, all colonies representing CT 28 were identified as *Microcystis viridis*. These two morphospecies generally have distinct morphologies, allowing an unambiguous morphospecies determination, whereas for all other morphospecies (*aeruginosa, novaceckii, botrys, flos-aquae, smithii, ichtyoblabe*) numerous colonies had hybrid morphologies, e.g., *aeruginosa/novaceckii* or *aeruginosa/ichtyoblabe*). For the following we therefore focused on the chemotype classification of the colonies and refer to morphospecies only in unambiguous cases.

Chemotype dynamics—The phenology of individual chemotypes in the pelagic was very variable (Fig. 2). About half of the chemotypes (17 out of 37) never reached relative abundances in an individual sample exceeding 5%, and some 10 chemotypes made up 60–90% of colonies in all individual samples. Of these dominant chemotypes, some had their maximum at the onset of the bloom, such as CT 34 that accounted for 19% of colonies in the pelagic sample of July (P1) and declined to a mere percent at the end of the season in November. A similar phenology was observed for CT 31, the relative abundance of which declined steadily. Another chemotype, CT 28, was abundant in July (16%) but vanished completely from the pelagic thereafter and was encountered only in a sediment sample in November. Other chemotypes showed a contrasting phenology with steadily increasing relative abundance. CT 7 started with 1% relative abundance in July (sample P1) and soon reached some 30%, increasing to a final 41% in November (P4). A similar, though less dramatic, increase was observed for CT 37 (*M. wesenbergii*), which increased from 10% to 37% relative abundance (in P1 and P4). A third type of phenology was observed for CT 8 that was encountered in only one sample (10% in P2).

As a result of the contrasting phenologies, the pelagic *Microcystis* community was dominated by two chemotypes (CT 7 and CT 37) at the end of the season, accounting together for nearly 80% of the colonies in November, whereas the relative abundance of all other individual chemotypes was 4% at highest. In contrast, the sample of July (P1) was more diverse, with eight chemotypes with relative abundances of 5% or more. This is also reflected by the Shannon diversity index that declined steadily and significantly ($p < 0.001$) from 2.63 in July to 1.58 in November.

In the benthic samples, the *Microcystis* communities showed also a shift in chemotype composition in the course of the season (Fig. 2). Some chemotypes that were dominant in July declined in relative abundance to the end of the year, e.g., CT 5 and CT 6. Others were not or were scarcely present in the sediment in July but dominated the community in November. Most decidedly this was the case for CT 7, which was found in relative abundances of >60% in sediment surface samples (S4a, S4c). Chemotypes encountered exclusively or nearly exclusively in benthic samples were CT 4, CT 5, or CT 21, for example, all of which occurred in high relative abundances in particular samples. The most widely distributed chemotype was CT 34 that was found in all samples and, with two exceptions, in abundances of at least 10%, both in pelagic and benthic samples.

The comparison of all samples is shown as a factorial PCA plot in Fig. 3. The first principal component (factor 1) was significantly correlated negatively with chemotypes CT 9, CT 13, CT 28, and CT 31 (among others) whereas no positive correlation was significant. The second principal component (factor 2) was significantly correlated positively with chemotypes CT 7 and CT 37 and negatively with chemotypes CT 5, CT 20, CT 29, and CT 34. The first two factors accounted for 54.5% of the variance in the data set. The position of samples in the PCA ordination space indicates their similarity in chemotype composition, i.e., the closer the respective data points were placed to each other, the more resembling was the chemotype composition in respective samples.

The dynamic of the pelagic *Microcystis* community is expressed by a shift of the data points representing samples P1 to P4 across a wide range. The shift is noncircular, and no tendency of a return to the origin could be noticed: The community at the end of the season differed markedly from the one at the beginning.

The data points representing the benthic samples showed a less scattered distribution. This was found for sets of samples taken on the same date as well as for the two sets of benthic samples in comparison. The samples from July (S1a–c) were placed close to each other, indicating that the benthic community is rather homogeneous, and only a weak tendency of an influence of the location was expressed in a contiguous ordination of the two upstream samples (S1a–b). A similar result could be stated for the benthic samples taken in November, except that the highest similarity was found between the two samples from the sediment surface (S4a and S4c), with a more diverging sample from the deeper layer (S4b). The shift of data points representing benthic samples was much less pronounced as compared to the pelagic samples.

The initial benthic and pelagic communities (P1 and S1a–c) were placed wide apart, indicating a rather dissimilar composition. Pelagic and benthic samples placed closest to each other were the benthic samples from November (S4a–c) and the pelagic sample taken during

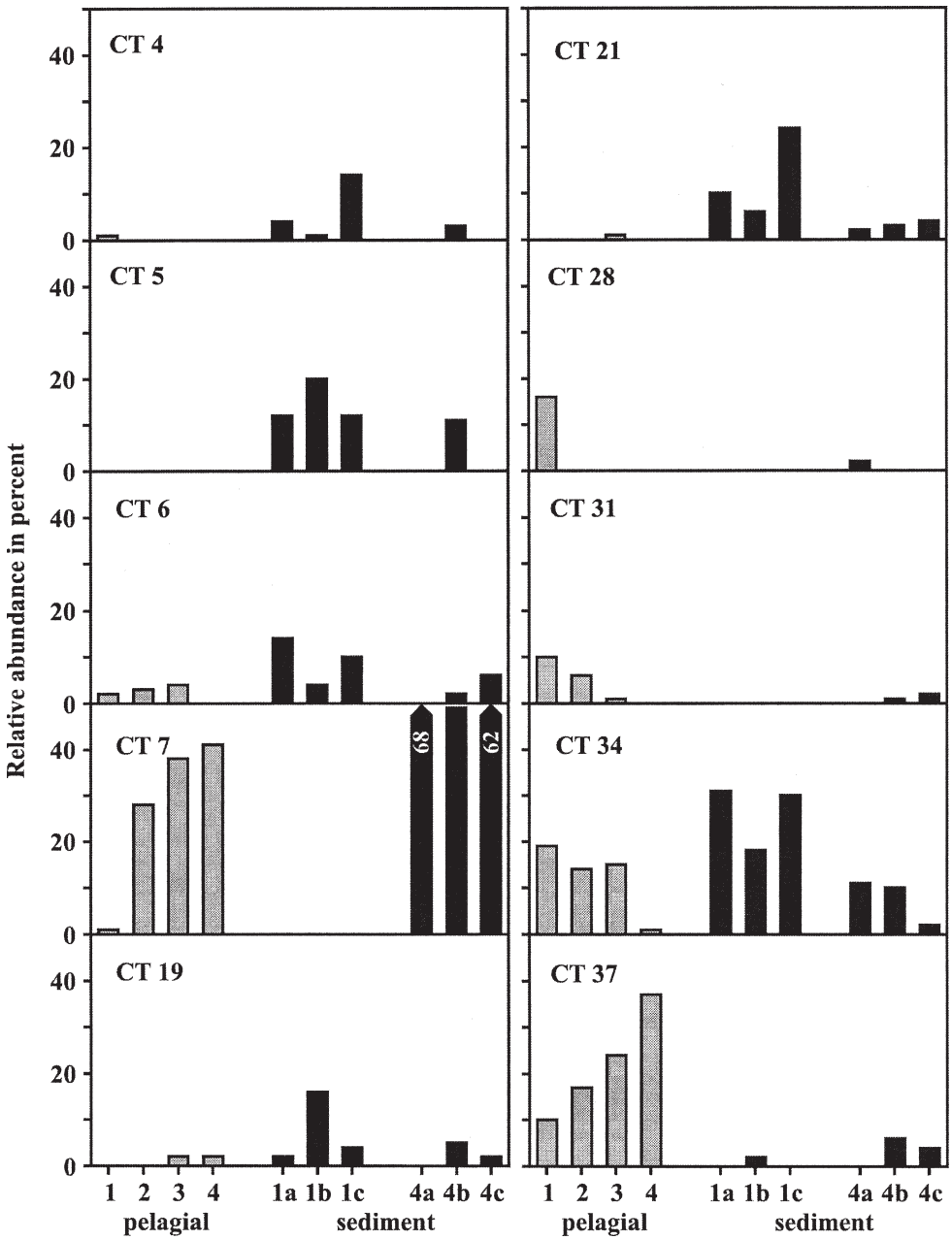

Fig. 2. The relative abundance of selected peptide chemotypes (CT) of *Microcystis* colonies in samples collected in the Brno reservoir in 2004. Gray columns represent pelagic samples and black columns benthic samples. Only those that occurred in a relative abundance of at least 10% in at least one of the samples out of 37 chemotypes as in Fig. 1 are shown. For sampling dates and locations *see* Table 1.

the summer bloom (P2). The dynamics of the benthic community thus could be interpreted as a rapprochement to the pelagic community from an initially larger distance. The pelagic community, in turn, developed further from the point of highest similarity leading again to a higher dissimilarity at the end of the season.

Microcystins and chemotype dynamics—The shift in peptide chemotype composition of the *Microcystis* community was also reflected in HPLC chromatograms of seston extracts (Fig. 4). In July, the highest peaks were anabaenopeptins B and F and microcystins RR and LR. In later bloom samples, anabaenopeptins were no longer detected and microcystin peaks diminished steadily. On the other hand, peaks of cyanopeptolin 1063 and the non-peptidic compound of *M. wesenbergii* (X) occurred with increasing peak heights. Both compounds produced the two major peaks in the chromatogram of the October

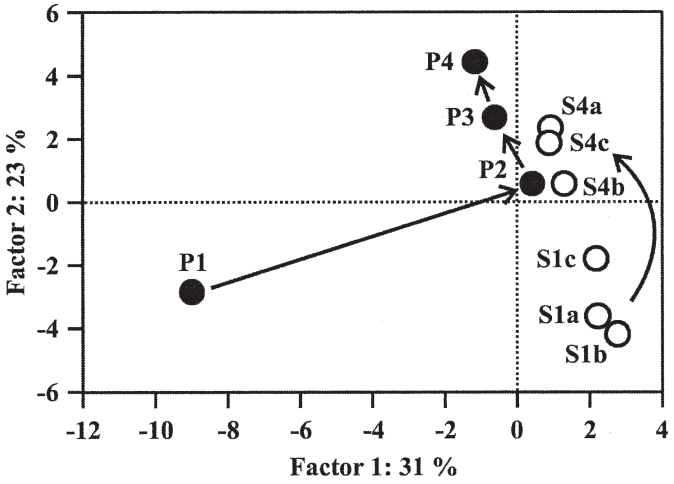

Fig. 3. An ordination plot (factor 1 × factor 2) of a PCA of *Microcystis* chemotype communities sampled in the Brno reservoir during July–November 2004. Closed circles represent pelagic samples, and open circles represent benthic samples. Next to each data point the sample number is given (*see* Table 1). Arrows indicate the temporal suite of the samples.

bloom sample. In Fig. 4 only those peaks were marked that could be clearly identified by mass spectrometry of peak fractions. Besides the major peaks, a high number of smaller peaks were found in the chromatograms, likely representing other peptides.

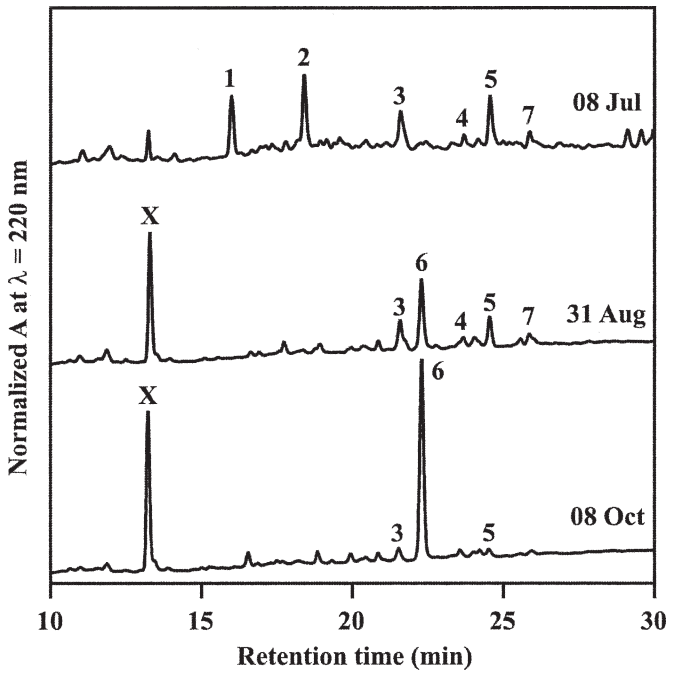

Fig. 4. HPLC chromatograms of seston sample (> 42 μm) extracts corresponding to three sampling dates in 2004 (Table 1). Numbers above the peaks indicate peptides identified by fraction collection followed by mass spectral analysis: 1, anabaenopeptin B; 2, anabaenopeptin F; 3, microcystin-RR; 4, microcystin-YR; 5, microcystin-LR; 6, cyanopeptolin 1063; 7, cyanopeptolin 1014A; X, nonpeptidic compound from *M. wesenbergii*. Chromatograms are normalized and correspond to an extract equivalent of one milligram seston per injection.

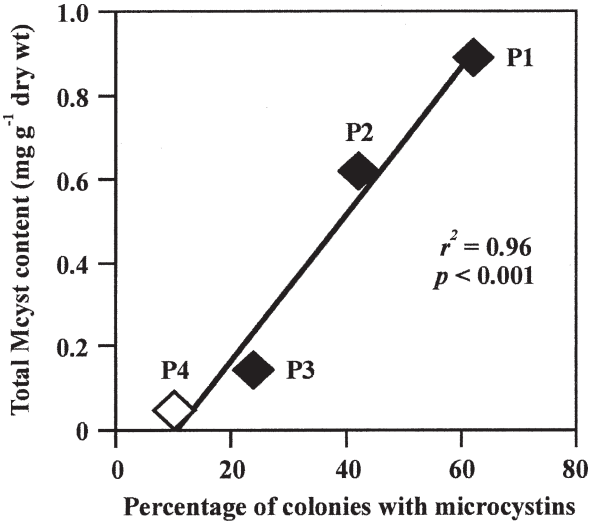

Fig. 5. Dependence of microcystin content of seston (> 42 μm) on the percentage of microcystin-producing *Microcystis* colonies in samples taken in the Brno reservoir during July–November 2004. Microcystin content was determined by HPLC and summarized for three major structural variants (RR, YR, and LR). Closed diamonds, analysis of lyophilized seston samples; open diamond, analysis and estimate from filter sample. Data points are marked as given in Table 1.

According to the decrease in peak heights of microcystin variants, the microcystin content of seston samples declined gradually from 890 mg g^{-1} dry wt in July to 140 mg g^{-1} dry wt in October. For the last sampling date no seston sample was available (*see* above), and only a qualitative analysis was possible that produced a negative result. Considering the detection limit of about 5 ng on column, it is safe to assume a microcystin content <50 mg g^{-1} dry wt.

In parallel to the decline in microcystin content, the percentage of toxin-producing colonies declined from 62% in July to 24% in October (10% in November).

Plotting the microcystin content versus the percentage of microcystin-producing colonies in individual samples revealed a linear relationship (Fig. 5). A linear regression was highly significant ($p < 0.001$) also when only the first three samples are considered for which true gravimetric microcystin contents were available ($r^2 = 0.96$, $p = 0.005$).

Discussion

MALDI-TOF MS and the subsequent statistical analyses proved to be efficient tools to recognize distinct peptide chemotypes in a *Microcystis* community. The similar detectability of peptides in benthic and pelagic colonies supports the integrity of *Microcystis* cells during the benthic stage (Ihle et al. 2005). The low dropout rate that could be attributed to lysis showed that colonies with an apparently functional photosynthetic apparatus, i.e., green color of the cells, also retain peptides intracellularly. The diversity of peptides that can be potentially detected in sediment samples thus depends on the chemotype composition as in pelagic samples.

The number of chemotypes was in a range estimated previously, as was the number of individual peptides

detected and identified in the samples (Fastner et al. 2001; Welker et al. 2004). Both numbers, however, have to be considered as minimum numbers because not all peptides are detected with equal response by MALDI-TOF MS and because of the limited number of colonies that could be analyzed for practical reasons. Individual strains of *Microcystis* often produce dozens of individual oligopeptides, only a few of which are accessible to fast and easy detection, generally the ones that are produced in high cell quota, whereas other peptides in low cell quota are only detected with repeated fractionation (Czarnecki et al. 2006; Ishida pers. comm.; Welker et al. unpubl. data) of mass-cultured cell material.

The diversity of peptides within an individual strain is considered first as a result of the presence of specific biosynthetic gene clusters. Second, multiple structural variants originate from (sometimes nonstringent) substrate specificities of NRPS enzymes such as, for example, adenylation domains (Mikalsen et al. 2003). In natural populations, variants of particular gene clusters can be found determining the structural peptide variants produced by respective strains (Mikalsen et al. 2003). Like the *mcy*-cluster, other cyanobacterial NRPSs presumably also have an erratic distribution among *Microcystis* clones (Kehr et al. unpubl. data) leading to a practically endless number of peptide fingerprints and chemotypes. The results of the present study showed, on the other hand, that in a given system the number of clones is not endless as could be shown by genetic approaches (Janse et al. 2004).

Changes in community peptide content thus are arguably the result of a change in clonal composition. In the present study, a steady decline in the microcystin content of seston samples could be construed as the consequence of a changing chemotype composition. Coincidentally, other peptides typical for particular clones that steadily increased in relative abundance showed increases in respective seston contents as expressed in chromatogram peak heights. Although the driving forces of chemotype (genotype) dynamics are not known, a trend of higher microcystin content at the bloom onset has been observed previously (Welker et al. 2003; Janse et al. 2005; Kardinaal and Visser 2005). This observation was interpreted as a potentially better grazing protection through more toxic *Microcystis* cells on average but has not been supported by experiments. Indeed, although intoxication upon ingestion dependent on actual toxin uptake has been demonstrated (Rohrlack et al. 1999), it does not explain what the advantage of such a postmortem grazing protection could be.

Peptide chemotypes can be considered as evolutionary units, and the interaction between chemotypes expectedly resembles more the competitive interactions between bacterial species (although not well defined, Cohan 2002) than co-operative interactions between clonal cells (Taga and Bassler 2003). Therefore, the term ''population'' did not seemed justified to us to summarize all *Microcystis* colonies in a sample, and instead the term ''community'' was chosen. Only very few, recent studies deal with the dynamics of clones or subspecific units of prokaryotes in environmental samples, making it difficult to judge whether our findings represent general trends (Janse et al. 2005). On the other hand, the sheer number of different clonal strains of *Microcystis* that have been isolated worldwide and the varying physiological capacities indicate that mutual influences between clonal strains are likely to happen in every *Microcystis* bloom.

The seasonal success of particular chemotypes, i.e., increasing relative abundance, can be the result of either bottom-up or top-down regulation (Carpenter and Kitchell 1993). For bottom-up regulation, the nutrient and light availability or the ability to exploit resources efficiently, respectively, is crucial, and ecophysiological studies have shown that individual *Microcystis* strains differ considerably in their functional responses to variable growth factors (Hesse and Kohl 2001; Schatz et al. 2005). As a top-down factor, the susceptibility of individual clones to grazing by herbivores—or cyanophage infection (Tucker and Pollard 2005)—is important. Several peptides are discussed as grazing protecting agents such as microcystins (Ghadouani et al. 2004), microviridins (Rohrlack et al. 2004), and cyanopeptolins (Czarnecki et al. 2006) acting either as toxins or inhibitors of digestive enzymes. The peptide composition of a clone could thus directly influence the rate by which it is consumed or contribute to a peptide pool that makes it difficult for grazers to adapt to the changing biochemical composition of food particles (Hairston et al. 2001; Sarnelle and Wilson 2005).

A third way of interaction is allelopathy. Several studies have shown that compounds released from cyanobacterial strains can negatively influence the growth of other cyanobacteria and phototrophs in general (Sukenik et al. 2002; Schatz et al. 2005). With regard to the structural diversity of potentially bioactive oligopeptides, it is evident that a very complex network of chemical and biochemical interactions could influence the dynamics of metabolically diverse strains (Engelke et al. 2003). We are, however, only beginning to recognize patterns of metabolic diversity, clonal dynamics, and competition.

Surprisingly, benthic inocula apparently did not influence the pelagic chemotype composition at the onset of the bloom, and only a few chemotypes were found in both compartments, e.g., CT 6, CT 29, and CT 34, whereas others were confined to one habitat at this time. Other chemotypes, in turn, were confined to one habitat throughout the season, like CT 5 that was found exclusively in sediment samples. From our data we cannot conclude whether this chemotype never entered the water column or whether it was not successful after inoculating the water column because of inferiority in competition with other chemotypes. The re-invasion of the water column from the sediment seems to be critical for the success of particular *Microcystis* clones: a respective estimation for the Quitzdorf reservoir was a meager 3% of benthic *Microcystis* cells exported during a re-invasion phase forming the pelagic inoculum whereas the majority of the cells was assumed to lyse shortly after entering the water column (Ihle et al. 2005). In contrast, studies by Brunberg and Blomqvist (2003) and Verspagen et al. (2005) came to the conclusion that pelagic inoculation from shallow areas accounted up to half of the original benthic populations. The successful

re-invasion of colonies likely depends on many factors determining the growth capacity in the pelagic whereas the initial inoculation apparently is driven mainly by physical forces, i.e., resuspension by wind-induced mixing (Verspagen et al. 2005). This apparent contradiction can be partly explained by the different approaches, yet we rather would expect that successful re-invasions and losses occur not at average ratios but are strain specifically dependent on actual physicochemical factors, grazing rates, etc,. and are therefore highly dynamic and highly unpredictable.

Because most studies quantifying the exchange between benthic and pelagic populations did not consider the strain composition, no conclusions could be drawn on the success of particular clones. The clonal composition of *Microcystis* populations studied as 16S-23S rRNA ITS clone libraries revealed that the same clones were present in sediment and plankton samples in the course of a season (Humbert et al. 2005). In the Brno reservoir, particular chemotypes persisted in the sediment for an entire season without successfully entering the pelagic. We could not test whether respective chemotypes would successfully re-invade the water column or whether colonies would simply gradually disappear from the sediment. Chemotypes that were successful in the pelagic, on the other hand, clearly dominated the benthic population at the end of the season. The survival rate of respective chemotypes likely is one crucial step for the determination of the chemotype composition in the following seasons (Latour et al. 2004*b*). In conclusion, we suppose that benthic and pelagic *Microcystis* communities interact on a multiseasonal timescale, hampering the prediction of chemotype composition and, hence, toxin contents of eventual blooms.

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