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ScienceDirect

Limnologica 38 (2008) 350–359

LIMNOLOGICA

www.elsevier.de/limno

Inter-annual stability of oligopeptide patterns of *Planktothrix rubescens* blooms and mass mortality of *Daphnia* in Lake Hallwilersee

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Received 8 May 2008; accepted 22 May 2008

Abstract

During mass developments of *Planktothrix rubescens*, the biomass of this cyanobacterium was collected over a period of four consecutive years (2002–2005) from Lake Hallwilersee, Switzerland. To avoid any shifts in analytical separation and sensitivity, the biomasses were extracted with 60% aqueous methanol at the end of the investigation period and were analysed within 1 week by LC-ESMS. A new mass spectrometric method to quantify oligopeptides was introduced. The sum of all major molecular species (quasi-molecular ion, double charged ion, adducts, dimers and molecular ions that had lost a water molecule) rather than just the signal of the quasi-molecular ion was used to determine the total abundance of oligopeptides. This procedure has become necessary because the variable presence of inorganic ions and the varying conditions of the mass spectrometric source strongly affect the formation of the different molecular species. Several anabaenopeptins, oscillapeptins and planktocyclins were found. [Asp^3, Dhb^7]microcystin-RR was the major microcystin. The oligopeptide patterns were relatively stable over the investigation period of 4 years. In June 2005, a mass mortality of *Daphnia* was observed. The dead *Daphnia*, which floated on the surface of the lake, were collected and analysed for oligopeptides. Planktocyclin and planktocyclin sulfoxide, which belong to the major cyclic peptides in *P. rubescens*, were found in the carcasses of *Daphnia*, but microcystins were missing. Live zooplankton of the epilimnion was represented by both *Daphnia* and copepods, while the patches of dead zooplankter on the lake surface were free of copepods and contained only *Daphnia*. Protease inhibitors rather than microcystins are discussed as the major bioactive compounds for grazer defence of *P. rubescens*.

Keywords: *Daphnia*; *Planktothrix rubescens*; Mass mortality; Oligopeptide; Inter-annual variation; Planktocyclin; Cyanobacterial toxin; Protease inhibitor; Quantification of mass spectrometric signals

Introduction

Mass developments of photoautotrophic microorganisms are frequently observed in lakes all over the world. In the temperate and Mediterranean climate zone, cyanobacteria of the genera *Microcystis* and *Planktothrix* are most widely observed as bloom formers in hypertrophic to eutrophic, and weakly eutrophic to mesotrophic lakes, respectively. Cyanobacteria possess physiological features to withstand physical, chemical and biological challenges in these lakes better than other photoautotrophic microorganisms (Dokulil and Teubner, 2000). Members of both genera, *Microcystis* and *Planktothrix*, are K-strategists and have developed efficient mechanisms to minimize top-down regulation (Hansson et al., 2007). Morphological features, such as filamentous growth and formation of big mucilaginous

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doi:10.1016/j.limno.2008.05.010

colonies, and chemical defence by secondary metabolites most likely essentially contribute to their superiority in nutrient-rich lakes.

Several compounds that belong to the chemical class of oligopeptides, and which are accumulated in extremely high concentrations (millimolar) in cyanobacterial cells (Long et al., 2001; Wiedner et al., 2003), seem to be involved in chemical defence. So far, all planktonic cyanobacteria that form mass developments and are K-strategists have been shown to contain oligopeptides. Based on the physiological impact of the oligopeptides on animals, two different groups can be distinguished: toxins and inhibitors of digestive proteases. The toxins are represented by a large number of microcystins (Chorus and Bartram, 1999), sulfated depsipeptides (Jakobi et al., 1996; Blom et al., 2003) and microviridin (Rohrlack et al., 2003). Targets of all microcystin congeners are the protein phosphatases 1 and 2A, which are active in all eukaryotes (Kennelly, 2001). Most of the prokaryotic protein phosphatases are, however, not affected by microcystins (Shi et al., 1999; Shi, 2004). The target site of cyanopeptoline SS (Jakobi et al., 1996), oscillapeptin J (Blom et al., 2006) and microviridin J (Rohrlack et al., 2003) was shown to be trypsin. While the molecular target sites of these oligopeptides have been elucidated, it is still unknown in which tissues of the invertebrates these enzymes are expressed, and in which metabolism they are involved that they can be affected to such an extent that death of the animals occurs.

The targets of the digestion inhibitors are trypsin and chymotrypsin, which are the most important digestive proteases in the gut of *Daphnia* (von Elert et al., 2004) and most likely also in the gut of other invertebrates. When these digestive serine proteases are inhibited, the protein of the ingested cyanobacteria can no longer be hydrolyzed and hence amino acids and peptides are not available for uptake. Furthermore, the inhibitors when present in sufficient concentrations in the gut also prevent digestion of admixed suitable food organisms or particles. These inhibitors cause a general downgrading of food quality. This assumption is consistent with the negative effects that have been found when mixtures of a high-quality food (*Scenedesmus*) and *Microcystis* free of microcystins were offered to *Daphnia* (Lürling, 2003). Inhibition of the digestive serine proteases in the gut is easy to achieve, because the oligopeptides when liberated into the gut of the invertebrate can directly bind to the sensitive enzymes. As opposed to this, toxins need specific transporters to pass the cytoplasmic membrane to reach their targets and to become active. Inhibition of digestion is a common defence strategy of food organisms against grazers. It is interesting to note that this strategy has even been maintained in higher plants. But these organisms have no peptide synthetases that could produce suitable oligopeptides and therefore inhibitory proteins were developed (Ryan, 1990). The importance of digestion inhibition can be deduced from the fact that microcystin-deficient strains of *Microcystis* were frequently observed, whereas there are no reports on strains deficient of serine protease inhibitors. Glycosidase inhibitors, another class of digestion inhibitors, are much less frequently observed in cyanobacteria (Jüttner and Wessel, 2003) than protease inhibitors (Rohrlack et al., 2005). It can be expected that they are less effective in reducing the quality of food because the sugar C-sources can easily be replaced by anabolic syntheses starting from fatty acids, glycerol, amino acids and other compounds, while essential amino acids have to be taken up with the food and cannot be synthesised by animals.

An evolutionary consequence of the occurrence of potent grazer defence molecules is the development of deterrents and repellents as warning signals for grazers. The presence of deterrents has been demonstrated for *Planktothrix* (Kurmayer and Jüttner, 1999) and is the reason that copepods do not ingest this cyanobacterium. β-Cyclocitral that is rapidly formed upon attack on the integrity of *Microcystis* cells and can be perceived by *Daphnia* in the micromolar concentration range was shown to have the features of a repellent (Watson et al., 2007), but in *Planktothrix* repellents have not yet been detected.

A great number of oligopeptide chemotypes (organisms that differ in the composition of secondary metabolites) have been described for *Planktothrix rubescens* (Kurmayer et al., 2005) and in mass populations of this cyanobacterium (Fastner et al., 1999; Kurmayer and Gumpenberger, 2006). In our study over 4 years we wanted to investigate whether a population of *P. rubescens* exhibiting a certain pattern of oligopeptide chemotypes has been selected in a particular lake. We found a remarkable stability of the oligopeptide pattern and observed the rare event of mass mortality of *Daphnia.* Because copepods that avoid ingestion of *Planktothrix* were not affected, toxicity, malnutrition or at least weakening of the resistance of *Daphnia* against infection may have been caused by oligopeptides. Oligopeptides of *P. rubescens* could be detected in the dead bodies of the *Daphnia* neuston, and they may serve as indicators that ingestion has taken place.

Material and methods

Study site

The study site was Lake Hallwilersee, which is situated in the northern part of Switzerland (47°18′N, 08°12′E). It has a maximum depth of 47 m and a surface

area of 10 km^2. Intense agricultural land use around the lake caused massive eutrophication. Only recently the phosphorus load was reduced. The lake was aerated with air in the winter time, and during the summer dioxygen was introduced to prevent anoxic conditions (Bürgi and Jolidon, 1998). Every year mass developments of *P. rubescens* occur, and surface scums are regularly observed and concentrated by south-western winds in the littoral zone of the north-eastern bank.

Sample collection and analysis of oligopeptides

Samples of *P. rubescens* were collected with a 150-μm net from the scum in the shallow part of the littoral zone (near to the camping site Tennwil) or from the epilimnion by vertical tows (10 m depth to the surface) in four consecutive years between 2002 and 2005 (see Table 1). To remove the grazers, the suspension was successively passed through 400- and 250-μm screens. The filaments were concentrated from this suspension with 21-μm gauze (Sefar, Rüschlikon, Switzerland) and stored frozen at −20 °C. To avoid the differing separation capacities of the HPLC columns and sensitivities of the analytical instruments over such a long period, the filament samples were extracted and chromatographed only at the end of the investigation period within 1 week. Oligopeptides were isolated from the frozen biomass and analysed by LC-ESMS as outlined previously (Baumann et al., 2007). The weight of the wet biomass was converted into dry biomass using the equation given by Zotina et al. (2003). Chlorophyll *a* was determined in methanolic extracts using the equation of Ogawa and Vernon (1971). The amounts used for analysis are presented in Table 1. Peak areas were used to determine the relative abundance of the oligopeptides. The abundance of the quasi-molecular ion, double charged ion, major adducts, dimers and molecular ions that had lost a water molecule were summed up to give the total abundance of the ionised molecules. Signals of dimers were doubled for the calculation of total abundance.

In June 2005, patchy mass accumulations of dead *Daphnia* sp. were observed that floated on the surface of Lake Hallwilersee. The neuston samples collected, consisting almost exclusively of dead *Daphnia*, were extracted with 60% aqueous MeOH and analysed by LC-ESMS for oligopeptides.

Results

Oligopeptide patterns

Chromatograms from samples of *P. rubescens* taken from the scum or from the epilimnion of Lake Hallwilersee are given in Fig. 1. The base peaks of the ESMS were extracted from the chromatograms and served with MS-MS spectra and retention times to determine the oligopeptides. For some of the compounds, analytical data and reference compounds were available in the lab. Four different chemical classes of the oligopeptides were observed: anabaenopeptins, microcystins, oscillapeptins and the new planktocyclins. The anabaenopeptins identified were anabaenopeptin B, anabaenopeptin A, anabaenopeptin E or F, which could not be differentiated, and oscillamide Y.

The microcystins were represented by [Asp^3, Dhb^7] microcystin-RR and two minor microcystins with the quasi-molecular ions m/z 1045 and m/z 981. The microcystins appeared primarily as doubly charged ions. Oscillapeptin J and several other oscillapeptins were also observed in the extracts. An unknown oscillapeptin with the monoisotopic mass of 1125.5 Da was a dominating compound of this compound class. Both planktocyclin as the major and planktocyclin sulfoxide as the minor compound were present in all samples. Interestingly the major peak of planktocyclin was caused by the sodium adduct of the dimeric form $[2M + Na]^+$.

The abundances of the oligopeptides (Table 2) were calculated from the sum of the peak areas of the quasi-molecular ions, doubly charged ions, adducts, molecular ions that had lost a water molecule and dimers of a particular compound (Table 3). In some cases, such as for planktocyclin and its sulfoxide, up to seven different

Table 1. Characteristics of the samples from Lake Hallwilersee used for the determination of oligopeptides by LC-MS

Sample	Sampling date	Biomass (g)	Chl *a* (μmol)	Biomass equivalent for LC-MS (mg)
A	18 April 2002	3.0	2.3	25
B	10 July 2002	6.5	4.9	54
C	9 October 2003	8.7	7.9	58
D	14 April 2004	9.3	ND	62
E	11 May 2005	3.2	ND	22

The biomass is given as wet weight (g), chlorophyll *a* in μmol and the amount injected for one LC-MS run in biomass equivalents (mg). ND—not determined.

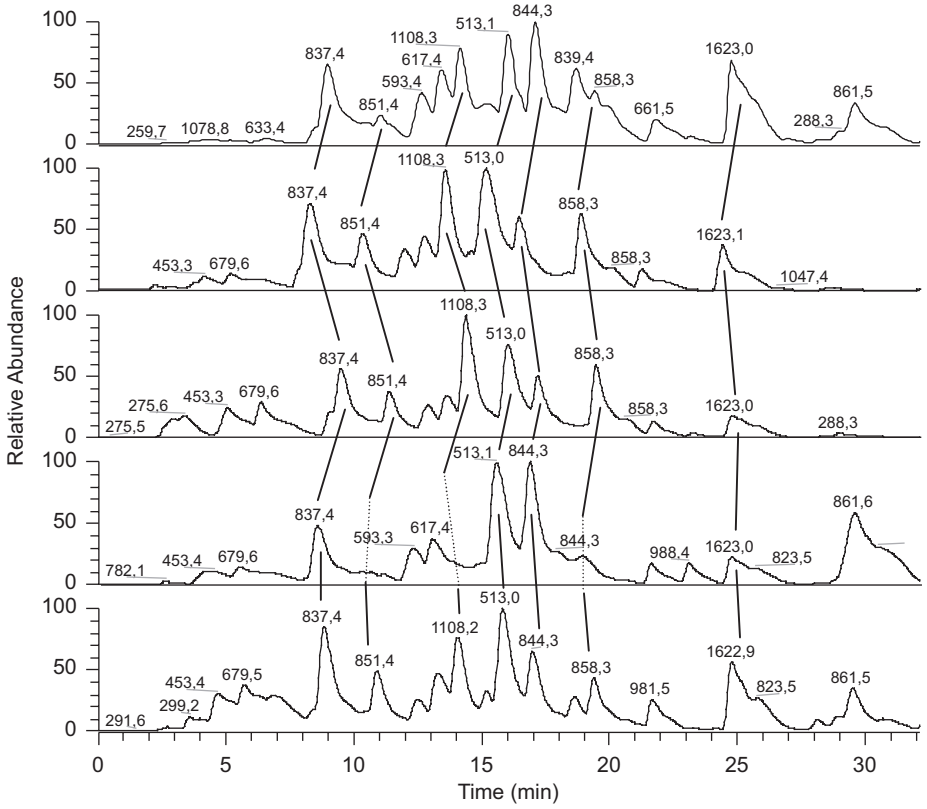

Fig. 1. LC-ESMS chromatograms of the methanolic extracts of *Planktothrix rubescens* scums collected on Lake Hallwilersee. (A) 18 April 2002, (B) 10 July 2002, (C) 9 October 2003, (D) 14 April 2004 and (E) 11 May 2005. The numbers above the peak maxima represent the base peaks observed.

molecule associated ions were observed and had to be considered because the ratio of the different ion species was strongly affected by the composition of the sample and the conditions of the ion source of the mass spectrometer.

The abundance ratios of the different oligopeptides varied only to a limited extend over the 4-year period (Table 4). It is remarkable that the trysin inhibitors planktocyclin and planktocyclin sulfoxide belonged in all samples to the prominent group of oligopeptides. High abundances were also observed for the unknown oscillapeptide 1125.5 Da. Oscillapeptide J was present only in low abundance.

Mass accumulation of dead *Daphnia*

Patches of neuston films consisting of dead *Daphnia* sp. as observed mid-lake of Lake Hallwilersee in June 2005 are displayed in Fig. 2A, while Fig. 2B was taken closer to the littoral zone. The microscopic examination of the collected neuston showed that it was composed exclusively of carcasses of *Daphnia* that were heavily populated by fungi (Figs. 2C and D). Only dead bodies of *Daphnia* were found while ephippia and copepods were missing. In vertical tows taken on the same occasion from the pelagic zone, live animals of both groups were present. The *Daphnia* in these samples were not populated by fungi (Figs. 2E and F).

Within the neuston samples collected, consisting almost exclusively of dead *Daphnia*, many different compounds were detected (Fig. 3). The majority of mass spectra could not be identified because these compounds were not normal constituents of *P. rubescens*. However, the more lipophilic oligopeptides planktocyclin and planktocyclin sulfoxide were found, exhibiting the signals of the sodium adducts m/z 823 $[M+Na]^+$ and m/z 839 $[M+Na]^+$, respectively (Fig. 3). Microcystins were not detected in these samples.

Discussion

The microcystins found during mass developments of *P. rubescens* were dominated by [Asp^3, Dhb^7] microcystin-RR. This is consistent with reports of *P. rubescens* in lakes of Northern Germany (Fastner et al., 1999), Central Italy (Messineo et al., 2006), Sicily (Naselli-Flores et al., 2007) and Spain (Barco et al., 2004), in which desmethyl-microcystin-RR was described as the major microcystin congener. The term desmethyl-microcystin-RR was used in these studies because the position of the lost methyl group was

Table 2. Composition and abundance of the quasi-molecular ion, adducts, dimers and molecules that had lost water of oligopeptides from *P. rubescens*

Oligopeptide	Ion composition	Mass (m/z)	Abundance $\times 10^6$ (arbitrary units)	Total of ionised molecules
Anabaenopeptin B	$[M+H]^+$	837	3112	4074
	$[M+2H]^{2+}$	419	698	
	$[2M+H]^+$	1674	132	
Anabaenopeptin E or F	$[M+H]^+$	851	1029	1317
	$[M+2H]^{2+}$	426	240	
	$[2M+H]^+$	1701	24	
Oscillapeptin (1125.5)	$[M+H]^+$	1126	1150	6208
	$[M+Na]^+$	1148	1852	
	$[M\text{-}H_2O+H]^+$	1108	3206	
[Asp^3, Dhb^7]microcystin-RR	$[M+H]^+$	1024	1186	4980
	$[M+2H]^{2+}$	513	3794	
Anabaenopeptin A	$[M+H]^+$	844	4487	4569
	$[2M+H]^+$	1687	41	
Oscillamide Y	$[M+H]^+$	858	1777	1811
	$[2M+H]^+$	1715	17	
Planktocyclin	$[M+H]^+$	801	1619	8425
	$[M+Na]^+$	823	3054	
	$[M+K]^+$	839	180	
	$[2M+H]^+$	1601	10	
	$[2M+H_2O]^+$	1618	21	
	$[2M+Na]^+$	1623	1704	
	$[2M+K]^+$	1639	51	
Planktocyclin sulfoxide	$[M+H]^+$	817	1684	6387
	$[M+Na]^+$	839	3459	
	$[M+K]^+$	855	156	
	$[2M+H]^+$	1633	5	
	$[2M+H_2O]^+$	1650	6	
	$[2M+Na]^+$	1655	510	
	$[2M+K]^+$	1671	23	

The total of ionised molecules of a compound was used as a measure of the abundance of an oligopeptide. The structure of the oscillapeptin-type oligopeptide with the mass of 1125.5 Da was not determined. Sample: 18 April 2002.

Table 3. Total abundance (arbitrary units) of eight oligopeptides of different samples from Lake Hallwilersee (A–E) and *P. rubescens* (H9)

Oligopeptide	Abundance ($\times 10^6$)				
	A	B	C	D	E
Anabaenopeptin B	4074	12,022	8150	7192	372
Anabaenopeptin E or F	1317	6962	5067	1365	2077
Oscillapeptin (1125.5)	6208	18,735	16,336	3226	4916
[Asp^3, Dhb^7]microcystin-RR	4980	19,678	11,514	14,514	4539
Anabaenopeptin A	4569	7381	5255	11,214	2155
Oscillamide Y	1811	6930	5572	1470	1266
Planktocyclin	8425	12,167	5493	7347	5328
Planktocyclin sulfoxide	6387	1498	974	1254	1453

The structure of the oscillapeptin-type oligopeptide with the mass 1125.5 Da was not determined. Sampling dates: A (18.04.2002), B (10.07.2002), C (09.10.2003), D (14.04.2004) and E (11.05.2005).

Table 4. Percentage of eight oligopeptides of different samples from Lake Hallwilersee (A–E) and *P. rubescens* (H9)

Oligopeptide	Percentage					Mean %
	A	B	C	D	E	
Anabaenopeptin B	10.8	14.1	14.0	15.1	14.6	14.5
Anabaenopeptin E or F	4.5	8.2	8.7	2.9	8.2	7.0
Oscillapeptin (1125.5)	16.4	21.9	28.0	6.8	19.3	19.0
[Asp^3, Dhb^7]microcystin-RR	13.2	23.0	19.7	30.5	17.8	22.8
Anabaenopeptin A	12.1	8.6	9.0	23.6	8.5	12.4
Oscillamide Y	4.8	8.1	9.5	3.1	5.0	6.4
Planktocyclin	22.3	14.3	9.4	15.4	20.5	14.9
Planktocyclin sulfoxide	16.9	1.8	1.7	2.6	5.7	3.0

The structure of the oscillapeptin-type oligopeptide with the mass 1125.5 Da was not determined. Sampling dates: A (18.04.2002), B (10.07.2002), C (09.10.2003), D (14.04.2004) and E (11.05.2005). Mean percentage is given for the samples B–E (A was disregarded to avoid overrepresentation of the year 2002).

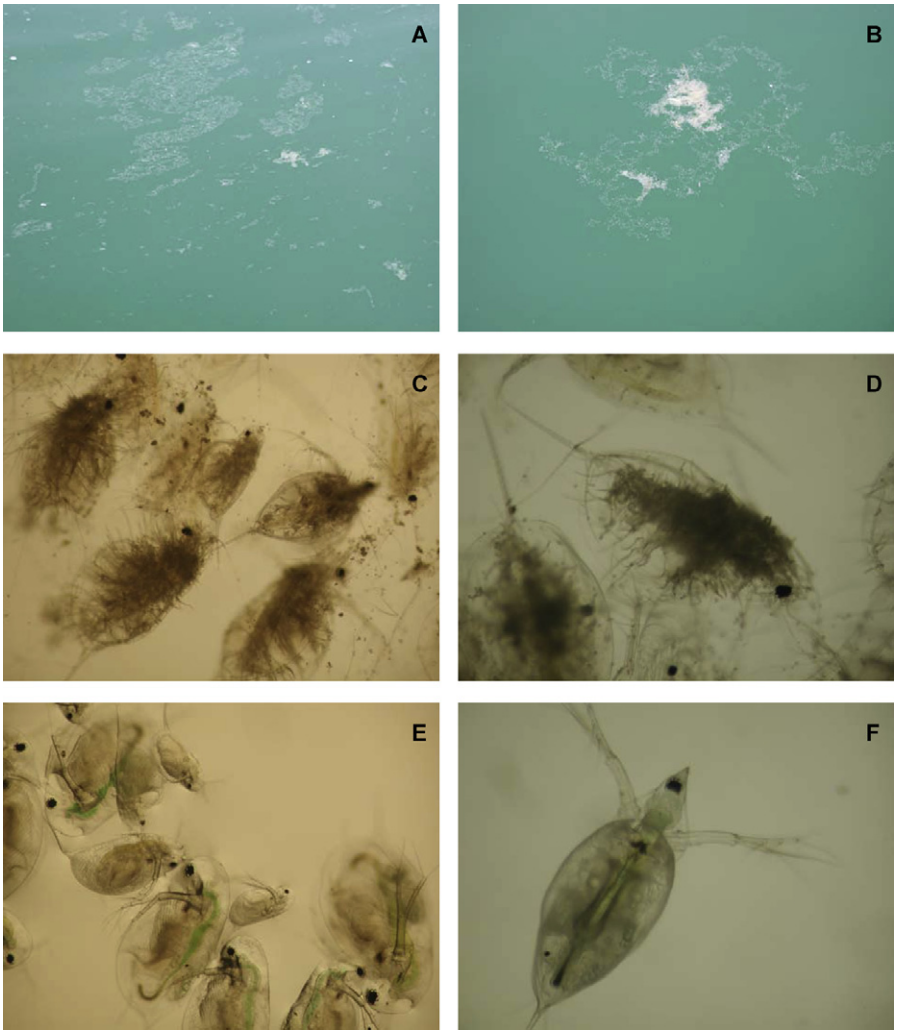

Fig. 2. Neuston patches of dead *Daphnia* on Lake Hallwilersee (June 2005). (A) mid-lake, (B) close to the littoral zone, (C) and (D) dead *Daphnia* populated by fungi, (E) and (F) *Daphnia* from 10 m depth mid-lake.

unknown. Therefore, it is not ruled out that the microcystin congener was [Asp^3, Dhb^7]microcystin-RR. Anabaenopeptin B, anabaenopeptin E/F and oscillamide Y have been described for strains of *P. rubescens* (Welker et al., 2004) and natural mass developments of *P. rubescens* (Barco et al., 2004). We

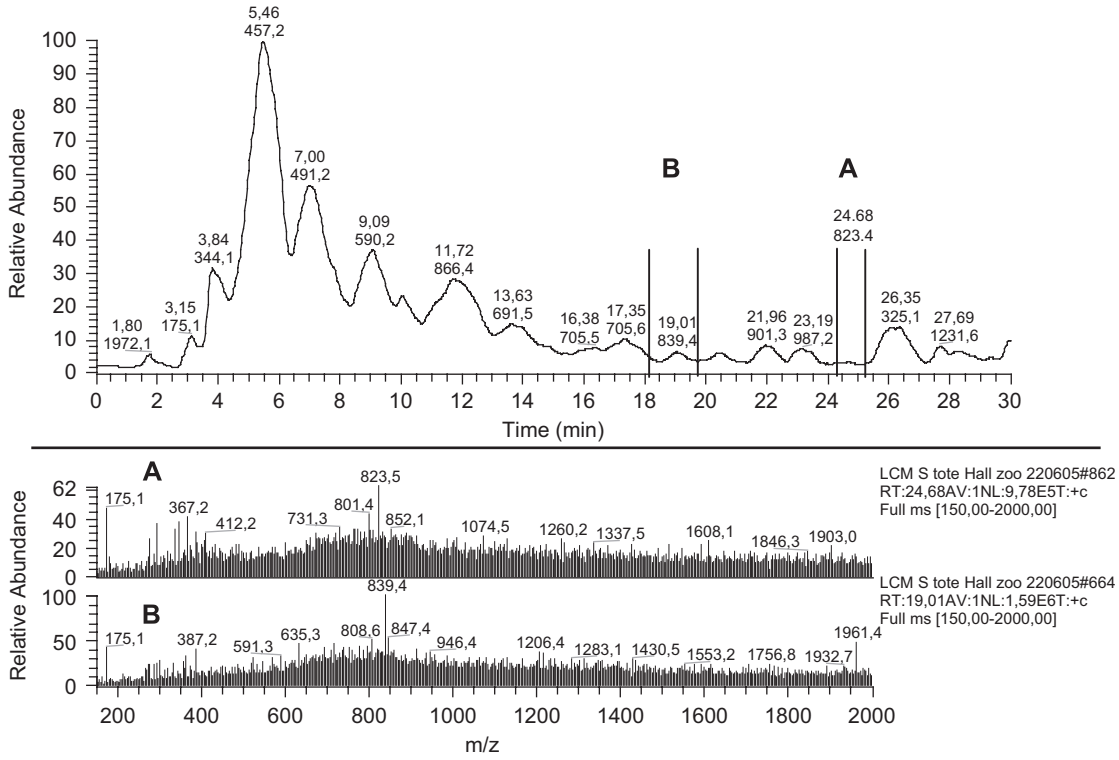

Fig. 3. LC-MS chromatogram of the neuston primary composed of dead *Daphnia* collected in June 2005. The vertical lines in the chromatogram limit the peaks of planktocyclin (A) and planktocyclin sulfoxide (B). The ES-MS of (A) shows the sodium adduct m/z 823 $[M + Na]^+$ of planktocyclin at R_t 24.68 min and (B) the sodium adduct m/z 839 $[M + Na]^+$ of planktocyclin sulfoxide at R_t 19.01 min.

found several congeners of the oscillapeptins in the lake samples of *P. rubescens*, but only oscillapeptin J, which has been previously described for *P. rubescens* A7 (Blom et al., 2003), could be unequivocally determined. Oscillapeptin J was present only in low concentrations. The serine protease inhibitors planktocyclin and planktocyclin sulfoxide have only recently been found and their structures determined (Baumann et al., 2007). Since dissolved planktocyclin is easily oxidized by air to its sulfoxide, the finding of the latter compound can be an artefact, and in this case the amount observed for planktocyclin sulfoxide has to be added to planktocyclin. But planktocyclin sulfoxide may have originated also partially or totally from the samples.

The patterns of the oligopeptides over the period of 4 years were rather stable. In all samples, the anabaenopeptins A, B and E or F, oscillamide Y, planktocyclin and [Asp^3, Dhb^7]microcystin-RR were found to be dominant oligopeptides. Recent studies have confirmed earlier findings (Long et al., 2001) that the synthesis of oligopeptides is dependant on the growth rate of cyanobacteria rather than on environmental factors. This is true for microcystins (Jähnichen et al., 2001; Briand et al., 2005), and anabaenopeptins and microviridin (Rohrlack and Utkilen, 2007), but may also apply for other oligopeptides. Variations of the intracellular concentrations on changing environmental factors frequently reported are in a relatively small range (Wiedner et al., 2003). Therefore, according to this observation it is rather likely that any new appearing or vanishing oligopeptide in the lake community of *Planktothrix* must be caused by the emergence and disappearance of oligopeptide chemotypes.

The bioactive oligopeptides observed belong either to the group of toxins or digestion inhibitors. [Asp^3, Dhb^7]microcystin-RR is a strong toxin to non-adapted crustaceans (Blom et al., 2006) and the planktocyclins are efficient inhibitors of trypsin (Baumann et al., 2007). Several oscillapeptins that were not determined in their structures were also present in high abundances. This type of oligopeptides has been shown to be potent inhibitors of either trypsin or chymotrypsin. A biological activity of the anabaenopeptins, which were also permanently present in high concentrations, has not yet been found. Previously reported inhibitory activities of these oligopeptides on various enzymes needed unrealistically high concentrations (Sano et al., 2001), and other inhibitory activities could not be confirmed and may have been caused by contaminating compounds (Fujii et al., 2000). In general, digestion inhibitors become active at much lower concentrations than toxins because after release from the ingested cyanobacterium

they remain in the same compartment and can easily bind to the target enzymes while toxins have to pass cytoplasmic membranes by suitable transporters to become active. The observation that micocystin-deficient chemotypes can be found, but chemotypes that are free of oligopeptide inhibitors against digestive enzymes have not been reported, supports the idea that digestive protease inhibitors are particularly important components of cyanobacterial defence.

The ecologically relevant effect of these oligopeptides is the formation of mass developments that indicate a severe unbalance of primary production and grazing efficiency. The increase of inedible phytoplankton observed on enhanced grazing pressure by herbivorous zooplankton (Benndorf, 1995) may partially be caused by defence chemicals. Negative correlations of *Daphnia* abundances to cell bound microcystin concentrations were observed in several eutrophic lakes (Hansson et al., 2007). However, since planktonic cyanobacteria also contain oligopeptides that are directed against digestion enzymes, the contribution of these two classes of compounds (toxins and digestion inhibitors) cannot be separated.

The stability of oligopeptide patterns and the moderate variation of their abundances indicate the stability of the community of *P. rubescens* chemotypes over quite a long time in Lake Hallwilersee. The chemotypes seem to represent an optimised mixture for the defence of the grazers, which are or would become active in this lake. There are some difficulties in understanding the selection of suitable chemotypes of *P. rubescens*. Chemicals for selection may be toxins (microcystins) and the oligopeptide inhibitors of digestive proteases. Communities of *P. rubescens* are composed of a large number of chemotypes that synthesise different oligopeptides and differ in their intracellular concentrations of the oligopeptides. It is not clear how chemotypes with insufficient defence can be eliminated. Experiments have shown that the oligopeptides are not responsible for differences of the ingestion rates (Kurmayer and Jüttner, 1999). A differentiation of so many chemotypes by different deterrents, however, is rather unlikely. One can hypothesize that a high number of spatially separated communities of *P. rubescens* exist in lakes. Only those communities will prosper that have a sufficient high number of chemotypes with an optimised composition and intracellular concentration of oligopeptides. Communities that have a too high number of chemotypes with weak defence properties will particularly suffer from grazing. They will be replaced by *Planktothrix* communities from a habitat that has maintained a chemotype composition for optimal defence. Differences of the environmental requirements for the different chemotypes have so far not been found, but more experiments are necessary to show this definitely.

It is rather likely that a cyanobacterial community of a lake shows adaptation to the grazers, but also a counteradaptation can be expected. For aquatic organisms an impressive case of adaptation has been reported for saxitoxin with clams, which are frequently exposed to mass developments of toxic algae. Clams have gained resistance to this toxin by a sodium channel mutation of a single amino acid (Bricelj et al., 2005). While the adaptation on the molecular level has not been reported for crustaceans, the acclimation of cladocerans to *Microcystis* (Gustafsson and Hansson, 2004; Guo and Xie, 2006) has been reported. This can also be expected for *P. rubescens*.

P. rubescens is ingested by *Daphnia*, but is rejected by copepods (Kurmayer and Jüttner, 1999). The rejection is caused by a so far unknown deterrent of lipophilic structure. Although copepods are much more sensitive to microcystins (Blom et al., 2006) and possess digestive proteases that are more sensitive to serine protease inhibitors, they can live in lakes with intense developments of *P. rubescens* because they avoid the uptake of *Planktothrix* filaments. *Daphnia*, however, are more resistant, but have to withstand the effects of these oligopeptides because only an inefficient selection of food particles takes place. On one occasion we observed mass mortality of *Daphnia* and formation of a neuston film from their dead bodies. This is a rare event, and declines of *Daphnia* during summer normally do not lead to such a phenomenon (Wagner et al., 2004). Interestingly, this event happened only 1 month after our sampling date during a bloom of *Planktothrix* with production of oligopeptides (Tables 3 and 4). The carcasses of daphnids were intensively populated by fungi. It was not clear whether the mortality of *Daphnia* was caused by cyanobacterial toxins or the digestion inhibitors of *Planktothrix* had weakened the population by starvation to the extent that they became susceptible to fungal infections. Also infection by other parasites (e.g. as reported by Decaestecker et al., 2005) cannot be ruled out, with fungi only appearing post-mortem. Mass spectrometric analysis of the dead *Daphnia* did not show the presence of any microcystin, but both planktocyclins were found. Planktocyclin is a signature oligopeptide for *P. rubescens* and has so far not been found in any other cyanobacterium. Since planktocyclin is more lipophilic than [D-Asp^3, Dhb^7]microcystin-RR, a better retention of this compound in dead *Daphnia* can be expected than that of the more hydrophilic microcystin. The presence of planktocyclin in the dead *Daphnia* is an indication that ingestion of *P. rubescens* had taken place, which had possibly killed or weakened a considerable part of the population. Further experimental work is needed to confirm or reject the hypothesis of a causal link between the occurrence of dead daphnids containing planktocyclin and blooms of *P. rubescens* producing these oligopeptides.

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