Abundance and toxicity of *Planktothrix rubescens* in the pre-alpine Lake Ammersee, Germany

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ABSTRACT

Regular occurrences of the cyanobacterium *Planktothrix rubescens* have been observed in several lakes that have undergone recent re-oligotrophication, e.g. Lake Ammersee. *Planktothrix* species are known to produce microcystins, potent phosphatase inhibitors that have been associated with morbidities and mortalities in humans and animals. The aim of this study was to characterise the temporal and spatial abundance and toxicity of *P. rubescens* in Lake Ammersee.

P. rubescens cell densities and biovolumes were calculated via fluorescence image analyses. *P. rubescens* was present during the entire observation period from 1999 to 2004, albeit at different cell densities. Maximum biovolumes of $45 \text{ cm}^3 \text{ m}^{-2}$ were observed in May 2001. Filaments were regularly distributed over the entire water column during winter and stratified in distinct metalimnic layers during summer, reaching maximum cell densities of $\leq 15,000$ (winter) and $\leq 77,000$ cells ml^{-1} (summer). The results demonstrate that *P. rubescens* abundance is strongly influenced by water transparency, i.e. illumination in the metalimnion. Moreover, the *P. rubescens* abundance appears to result from regular phosphate depletion in the epilimnion, possibly additionally benefiting from high nitrogen loads.

Microcystin (MC) was detectable in 27 and 38 of 54 seston samples via HPLC and Adda-ELISA measurements, respectively. The main microcystin congeners in the seston samples were [Asp³]-MC-RR and [Asp³,Dhb⁷]-MC-RR. Microcystin concentrations correlated significantly with the respective phycoerythrin (PE)-concentrations. The variation in the MC/PE-ratios was low suggesting that the microcystin production of *P. rubescens* in Lake Ammersee is consistent and indicates that the appearance of *P. rubescens* coincides with measurable microcystin levels. Moreover, the observation of pronounced metalimnic oxygen depletions appears to be causally related to recurring high *P. rubescens* abundance.

In conclusion the results suggest that aquatic organisms such as indigenous fish populations (e.g. coregonids) are regularly confronted with potentially adverse *P. rubescens* densities, which might provide a possible explanation for the often observed impaired health and growth retardation of coregonid populations in *P. rubescens* containing pre-alpine lakes.

Keywords:
Planktothrix
Cyanobacteria
Re-oligotrophication
Phosphorous
Nitrogen
Transparency
Secchi depth
Microcystin
Coregonids
Fish

1. Introduction

Cyanobacteria are important constituents of phytoplankton communities and ubiquitous in lakes of different nutritional status. Approximately 50 of 2000 known cyanobacterial species are recognised to produce toxic molecules, e.g. alkaloids and peptides (Sivonen and Jones, 1999). Many of these toxins have

been associated with mortalities of wild and domestic animals as well as severe human intoxications. Among these toxins, microcystins¹ are most frequently found and have gained attention due to their potent inhibition of protein phosphatases and associated morbidities and mortalities in humans and animals (Falconer, 2001; Briand et al., 2003).

Microcystin-producing cyanobacteria are present in coastal and inland waters, primarily in naturally eutrophic waterbodies and waters that have experienced nutritional enrichment due to anthropogenic influences (eutrophication) (Bartram et al., 1999). However, in contrast to presently eutrophicated water bodies with *Anabaena* sp., *Aphanizomenon* sp. and *Microcystis aeruginosa* blooms, regular mass occurrences of the cyanobacterium *Planktothrix rubescens* have been observed in lakes that had undergone recent re-oligotrophication. This especially includes lakes in the

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¹ Cyclic heptapeptides, sharing the common structure cyclo(-Adda-D-Glu-Mdha-D-Ala-L-X-D-MeAsp-L-Z) where X and Z are variable L-amino acids, Adda is an uncommon amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid, D-MeAsp is 3-methylaspartic acid, and Mdha is N-methyl-dehydroalanine.

Table 1Size and nutritional status in (pre-) alpine lakes with documented *P. rubescens* abundance during the last decade

Country/lake	Size (km ²)	Trophic status	Abundance	References
Austria				
Wörthersee	19.4	Mesotrophic	2000–2006	Kärntner Seenberichte (2000–2007) www.kis.ktn.gv.at
Ossiacher See	10.8	Mesotrophic	2000, 2003–2006	Kärntner Seenberichte (2000–2007) www.kis.ktn.gv.at
Millstätter See	13.3	Oligo-mesotrophic	2000–2003, 2005–2006	Kärntner Seenberichte (2000–2007) www.kis.ktn.gv.at
Mondsee	16.6	Mesotrophic	1994–1997, 2001	Kurmayer et al. (2004), Teubner et al. (2004)
France				
Lac du Bourget	42	Mesotrophic	1996, 1999, 2001	Jacquet et al. (2005), Le Boulanger et al. (2002)
Germany (Bavaria)				
Ammersee	46.6	Mesotrophic	1996, 1998–2001	Teubner et al. (2004), Ernst et al. (2001)
Chiemsee	79.9	Mesotrophic	2004, 2006	Ernst (unpublished data)
Starnberger See	56.4	Mesotrophic	1997, 2005	Ernst (unpublished data), Nixdorf et al. (2004)
Italy				
Lago Maggiore	212.5	Oligo-mesotrophic	1995–1999	Morabito et al. (2002)
Lago di Garda	368.0	Oligo-mesotrophic	1995–2000	Salmaso (2000, 2002)
Lago d'Iseo	62	Mesotrophic	Regularly	Salmaso (2000)
Lago di Como	146.0	Mesotrophic	1997–1999	Buzzi (2002), Bettinetti et al. (2000)
Lago di Pusiano	5.3	Eutrophic	2001, 2002	Legnani et al. (2005)
Switzerland				
Lac de Neuchâtel	217.9	Oligo-mesotroph	1999–2004	www.die3seen.ch
Zürichsee	65.1	Mesotrophic	1993–2000	Hoeger et al. (2005), Walsby and Schanz (2002)
Lago di Lugano	48.8	Meso-eutroph	Regularly	Salmaso (2000)
Thunersee	47.8	Oligo-mesotroph	Regularly	Ochsenbein and Mattmann (2003)
Bielsee	37.8	Meso-eutroph	2000–2006	www.die3seen.ch
Murtensee	22.8	Mesotroph	2004–2006	www.die3seen.ch
Sempachersee	14.4	Eutroph	1989–1997	Bürgi and Stadelmann (2002), Mez (1998)

pre-mountainous areas of the Alps characterised by an ice-age modulated landscape of hills and valleys (pre-alpine regions) (Table 1).

The mass occurrence of *P. rubescens* is predominantly ascribed to two forms of specialisation providing for an ecological niche as well as a competitive advantage over green algae: i.e. an efficient regulation of buoyancy via semi-permeable gas vesicles enabling *P. rubescens* filaments to stratify effectively in the water column and the production of allophycocyanin, phycocyanin and phycoerythrin, photopigments enabling maximum utilisation of light energy and existence under low light conditions (Feuillade, 1994; Walsby and Schanz, 2002). Consequently, *P. rubescens* stratify in compact metalimnic layers overshadowed by the epilimnic community during summer stratification. Moreover, *P. rubescens* filaments can grow at low light conditions during circulation in the late autumn to early spring months or even below an ice cover during winter (Blikstad-Halstvedt et al., 2007).

Metalimnic blooms of *Planktothrix* species are often observed to co-occur with marked oxygen deficiencies in the metalimnion (Lindholm and Meriluoto, 1991; Salmaso, 2000; Ernst et al., 2001; Buzzi, 2002; Krupa and Czernas, 2003). Indeed, the senescence of cyanobacterial blooms may generate an increased oxygen demand and consequently result in massive oxygen depletion. In addition, cyanobacterial senescence provides for the release of cyanobacterial toxins (Malbrouck and Kestemont, 2006). In comparison to other cyanobacterial species, *Planktothrix* sp. have been shown to contain the highest concentrations of microcystin per gram dry weight (Fastner et al., 1999b). Depending on the *P. rubescens* abundance, toxicity and distribution, both the release of cyanobacterial toxins as well as metalimnic oxygen deficiencies may result in adverse effects on aquatic organisms (Sivonen and Jones,

1999; Wiegand and Pflugmacher, 2005), especially on coregonids² (Ernst et al., 2006a, 2007).

The aims of this study were therefore

- to characterise the spatial and seasonal abundance of *P. rubescens* in Lake Ammersee,
- to characterise the variability of microcystin content in *P. rubescens* and
- to investigate the temporal co-occurrence of alterations in *P. rubescens* abundance and metalimnic oxygen depletion.

Exemplified by the indigenous coregonid (whitefish) population, the findings are finally discussed in the context of possible adverse effects on aquatic organisms.

2. Materials and methods

2.1. Chemicals

All reagents and solvents employed were of analytical or chromatographic grade and quality and purchased from Fluka (Switzerland), Merck (Germany), Riedel de Haen (Germany), Roth (Germany) or Sigma (Germany). Water was purified to 18.2 MΩ cm⁻¹.

2.2. Study location

Lake Ammersee is a typical pre-alpine lake, located in the south of Germany at an altitude of 553 m. The lake arose following the retreat of the glaciers at the end of the last ice age. Lake Ammersee is elongated in south-north orientation (16 km length and 2.9 km average width) and formed like a tube with steep shores on both west and east sides. Limnologically, Lake Ammersee is a dimictic lake (turning over twice a year) with a surface area of 46.6 km², a total volume of 1750 × 10⁶ m³ and a maximum and average depth of 81.1 and 37.5 m, respectively (Grimminger, 1982). Complete

² Coregonids (whitefish; *Coregonus* sp.) are among the dominant species of the ichthyofauna in most pre-alpine lakes, and, due to their high economic value, of great importance for the professional fishery.

Table 2
Time-points and intervals of sampling during the six-year survey

	J	F	M	A	M	J	J	A	S	O	N	D
1999											..	..
2000												
2001	.	.	..	..	..	..	..	..	..	..	..	..
2002	.	.	..	..	..	..	..	..	..	..	..	..
2003	.	.	..	.	..	..	..	..	..	..	.	.
2004	.		.	.	.		.	.	.			.

Sampling was shortened in 2000 (no Secchi measurements; sampling in August 2000 consisted of only seston-sampling).

winter circulation, including the lake bottom, is regularly achieved. The lakes principle water source is the river Ammer, with a mean flow rate of $16.6 \text{ m}^3 \text{ s}^{-1}$. Consequently, the water residence time is approximately 2.7 years. Due to the large catchment area of the river Ammer the lake collects water from an area of 993 km^2 , including widely natural and agricultural, but also urban and industrial influenced regions.

Until the late 1970s, the lake underwent a distinct phase of eutrophication, primarily as a result of increased urbanisation, detergent use and intensification of agriculture in the catchment area, reaching yearly mean total phosphorus concentrations of $60 \mu\text{g l}^{-1}$. Due to a reduction of anthropogenic influences, the continued eutrophication was halted and reversed, i.e. a re-oligotrophication process was initiated. As a consequence, the yearly mean total phosphorus concentrations decreased to approximately $10 \mu\text{g l}^{-1}$ (Kucklert et al., 2001).

2.3. Sample sites and sampling intervals

Samples and field measurements were generally taken in the middle of the lake ($47^\circ 58' (53.50) \text{N}$; $11^\circ 07' (35.70) \text{E}$). Sampling time-points and intervals are depicted in Table 2. Sampling consisted of water sampling for the determination of *P. rubescens* cell densities, measurement of water transparency, determination of temperature and oxygen profiles and of monthly seston sampling for the determination of the *P. rubescens* microcystin content. In addition, two seston samples collected previously (in August and November 1998) in an identical manner were included in the sample cohort.

2.4. *P. rubescens* abundance

For the determination of the *P. rubescens* cell densities, water samples (50 ml) were taken using a Ruttner flask sampler (Richter & Wiese, Germany). Samples were taken at 0, 3, 5, 25 and 40 m depth. Eight additional metalimnion-specific samples were taken, ranging between 6 and 15 m depth, always adjusted to the respective metalimnic temperature and thus depth stratification. Water samples were immediately fixed with Lugol's iodine solution and stored in darkness for at least 24 h until sample filtration. Defined sample volumes were filtered on nitrocellulose membranes (pore size $8 \mu\text{m}$ —diameter 25 mm, Schleicher & Schuell, Germany). Filters were dried in darkness at room temperature and subsequently analysed via fluorescence microscopy and image processing according to the protocol published in Ernst et al. (2006b).

P. rubescens biovolumes were estimated for each time-point and sample depth via multiplication of the cell density with the average cross section surface of $25.6 \mu\text{m}^2$ as given for *P. rubescens* (Walsby et al., 1998) and a mean cell length of $3 \mu\text{m}$ (Ernst et al., 2006b). Using GraphPad Prism 4[®] (USA), the biovolumes obtained were further integrated from the water surface to 40 m depth to provide for the *P. rubescens* biovolume per m^2 . Finally, the development of the *P. rubescens* biovolume was compared on a

ten-day scale throughout the investigated period (missing time-points were interpolated from data pre- and post-missing data-points).

2.5. Limnological parameters

Water transparency was determined via a Secchi disk (diameter 25 cm) with measurements carried out by the same person throughout the course of the study. Yearly mean values of the Secchi depth (Z_s) were determined via calculation of a time-weighted average, determined during the vegetation period, i.e. from the beginning of May until the end of October each year. No Secchi measurements were carried out in 2000.

The individual euphotic depths (Z_{eu}) were estimated from the respective Secchi depths (Z_s) as $Z_{eu} = 2.5 \times Z_s$ (Lemmin, 1978). Seasonal variations of metalimnic light conditions were estimated using the trends of the euphotic depth measurements throughout the vegetation period.

Temperature, dissolved oxygen and oxygen saturation were determined using a portable oxy-meter (Oxi-197, WTW, Germany). Measurements were performed at intervals of 1 m starting from the surface to 20 m depth and in 5 m intervals starting from 20 to 40 m depth. The upper limit of the metalimnic layer (Z_{meta}) was defined as the depth with a decrease in temperature of $\geq 1^\circ \text{C m}^{-1}$ and is therefore approximately 1 m below the mixing zone (Z_{mix}); $Z_{meta} = Z_{mix} + 1$. The seasonal change of the upper metalimnic limit was estimated using the Z_{meta} determined from the beginning of May until the end of October each year.

In order to characterise the environmental conditions at the depth of *P. rubescens* stratification, the depths of maximum *P. rubescens* cell densities ($peak_{max}$) were compared to the Secchi depths (Z_s) and the upper limits of the metalimnic layer (Z_{meta}). For this, all measurements from the beginning of May until end of October obtained as of 1999 until 2004 were employed. In addition variations in light conditions and metalimnic stratification were compared within each year and among the years in order to illustrate seasonal and annual differences of metalimnic conditions.

2.6. Seston sample preparation

P. rubescens microcystin and phycoerythrin contents were analysed in monthly seston samples (see also Table 2). Samples were taken with a ballasted $40 \mu\text{m}$ gauze net hauled between 5 and 15 m depth horizontally through the water column. The taxonomical composition of the seston samples was determined via light microscopy using fresh sample aliquots. Classification of cyanobacterial genus was performed in accordance with Anagnostides and Komárek (1988), Suda et al. (2002) and Geitler (1932). The remaining sample volumes were immediately frozen (-20°C) and stored until lyophilisation. For analysis, frozen samples were thawed and lyophilised via speed-vac evaporation (Alpha RVC, Christ, Germany), weighed and portioned for methanolic microcystin and aqueous phycoerythrin extraction.

2.7. Seston sample microcystin contents

Seston sample microcystin (MC) contents were determined in order to characterise the variability of microcystin content in *P. rubescens* of Lake Ammersee. As the majority of *Planktothrix* cells lyse due to freezing and lyophilisation, *Planktothrix* cell counts were inappropriate as reference for toxin quantification. Thus, *P. rubescens* microcystin content was determined using the phycoerythrin (PE) concentration of the respective seston sample as reference and expressed as MC/PE. Use of phycoerythrin as reference has the advantage of minimizing the interference by zooplankton and algae often reported for chlorophyll and biomass measurements. The use of phycoerythrin as reference additionally minimises the interference due to variations within the seston sample composition since in Lake Ammersee seston samples, high intracellular phycoerythrin concentrations appear to be primarily associated with *Planktothrix* despite the presence of several other phycoerythrin containing cyanobacteria and of potentially phycoerythrin containing *Cryptophyta*. Indeed, in comparison to *Planktothrix* species the latter species contain much lower intracellular phycoerythrin concentrations (Bodemer, 2004), and their abundance in Lake Ammersee was noticeably lower than that of *Planktothrix rubescens*. Finally, Leboulanger et al. (2002) demonstrated that *Planktothrix rubescens* can be discriminated from eucaryotic algae and other cyanobacteria species on the basis of fluorescence properties. Therefore, interference in the Lake Ammersee seston phycoerythrin content due to phycoerythrin produced by other phytoplankton is probably negligible.

2.7.1. Quantification of phycoerythrin

Phycobilliprotein-concentrations in the lyophilised seston samples were determined via extraction of defined sample quantities (≤ 50 mg dw) in 1 ml phosphate buffered saline (pH 7.0) by three freeze-thaw cycles using liquid nitrogen. Each extract was centrifuged (45 min at $40,000 \times g$) and the absorption (A) of the resulting supernatants was determined at wavelengths of 562, 615 and 652 nm. Absorption was additionally determined at 750 nm for nullification (N). The optical density for the respective wavelength (OD_{xxx}) was calculated as $OD_{xxx} = A - N$. Phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) concentrations were calculated according to the description of Tandeau de Marsac (1977) using the following equations:

$$PC[\text{mg l}^{-1}] = \frac{(OD_{615} - 0.747 \times OD_{652})}{5.34}$$

$$APC[\text{mg l}^{-1}] = \frac{(OD_{652} - 0.208 \times OD_{615})}{5.09}$$

$$PE[\text{mg l}^{-1}] = \frac{(OD_{562} - 2.41 \times PC - 0.849 \times APC)}{12.7}$$

The extraction procedure was repeated at least three times and the mean phycoerythrin concentration for each seston sample calculated. The analytical protocol provided for a quantification limit of $\leq 0.1 \mu\text{g PE mg l}^{-1}$ dw.

2.7.2. Quantification of microcystin

Microcystin was extracted from a defined sample quantity (≤ 70 mg dw) via alternate shaking of the sample suspended in 10 ml of 75% methanol and ultrasonication at 35 kHz for 1 h. Subsequently the suspension was centrifuged (10 min at $10,000 \times g$) and the resulting supernatant stored at room temperature while the remaining pellet was re-extracted with 75% methanol. The extraction procedure was repeated three times for each seston sample and the supernatants were pooled to give one extract for each seston sample.

For further purification and microcystin concentration, sample extracts were reduced to their aqueous component (approximately 7.5 ml) under a gentle nitrogen stream, replenished to 30 ml with deionised water and applied to preconditioned C-18 end-capped solid phase extraction (SPE) cartridges (mass: 500 mg; Isolute[®] C₁₈(EC), Germany). Microcystins in the extracts were eluted from the cartridges with 12 ml methanol. Eluents were dried under a gentle nitrogen stream and re-dissolved in 3 ml 20% MeOH for HPLC- and anti-Adda ELISA-analyses (see below).

2.7.3. HPLC

HPLC was performed using a Shimadzu (Germany) HPLC equipment (including controller (SCL-10A_{VP}), autosampler (SIL 10AD_{VD}), two pumps (LC-10AT_{VD}), degasser (DGU-14A) and diode array detector (SPD-M10A_{VP})), with an analytical C₁₈ column (Grom Sil 120 ODS-4 HE, 5 μm , 250×4 mm, Stagroma, Germany). A gradient with water (0.05% TFA) and acetonitrile (0.05% TFA) was used as the mobile phase according to the method described by Lawton et al. (1994). Microcystin congeners were detected and identified via retention time and typical spectra in comparison with MC-LR, MC-RR, MC-YR, MC-LF, MC-LW standards (all purchased from Alexis, Switzerland), [D-Asp³]-MC-RR and [D-Asp³]-MC-LR (both kindly provided by Prof. Meriluoto, Åbo Akademi University, Turku, Finland) and [Asp³,Dhb⁷]-MC-RR (kindly provided by Dr. J. Blom, University of Zurich, Switzerland). Microcystin concentrations were calculated based on the peak area of the internal MC-LR standard employing a factor of 0.79 for quantification of [Asp³,Dhb⁷]-MC-RR (Hoeger et al., 2007). Based on a detection limit of 10 ng microcystin per injection and an injection volume of 20 μl , the limit of quantification was estimated at $0.05 \mu\text{g MC-LR}_{\text{equ}} \text{ mg}^{-1} \text{ dw}$.

2.7.4. ELISA

The anti-Adda ELISA Kit (Abraxis LLC, USA) is based on an antiserum raised against the unique C₂₀ amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda), which is common to the majority of known microcystin variants (Fischer et al., 2001). Therefore, Adda-ELISA analyses represent a good approach for the determination of the overall microcystin concentration in the seston samples. The ELISA was performed according to the manufacturer's instructions. Each sample extract was analysed twice using duplicate measurements yielding a mean microcystin content per sample. Due to unspecific matrix-interfering compounds in the highly concentrated seston sample extracts, the limit of quantification (LOQ) was estimated at $0.05 \mu\text{g mg}^{-1} \text{ dw}$.

2.8. Statistical analyses

Statistical analyses were carried out using GraphPad Prism 4[®] (USA) Software. Values are given as mean $\pm$ S.E.M., unless indicated otherwise. The yearly mean values of the Secchi depth ($^{\circ}Z_s$) were analysed using a two-tailed one-way ANOVA followed correction of multiple analyses using a Bonferroni's post-test.

The depth of maximum *P. rubescens* cell densities (peak_{max}) was analysed for statistical correlation versus the Secchi depth (Z_s) and the upper limit of the metalimnic layer (Z_{meta}) via Pearson's correlation test. Statistical significance of the correlation was determined at the $p < 0.05$ level and indicated as *** $p < 0.001$ and * $p < 0.05$. Seasonal tendencies in light conditions were estimated via polynomial trends of the euphotic depth (Z_{eu}) from May until October ($n = 26$ in 1999, $n = 17$ in 2001, $n = 18$ in 2002 and 2003). No trends were given for 2000 and 2004 due to missing samples or limited sampling intervals ($n = 4$), respectively. The seasonal change in lake stratification was estimated via determination of

polynomial trends of the upper limit of the metalimnic layer (Z_{meta}) determined from May to October ($n = 25$ in 1999, $n = 18$ in 2001, $n = 17$ in 2002 and $n = 15$ in 2003). No trends were given for 2000 and 2004 due to limited sampling intervals ($n \leq 4$).

The microcystin congener composition of microcystin-positive Lake Ammersee seston samples as determined via HPLC analyses is given as mean percentage of total microcystin $\pm$ S.E.M. Correlation

of the determined MC- and PE-contents were performed using the Pearson's correlation test. Correlation analyses included all microcystin-positive samples acquired from August 1998 to September 2004 and were performed for HPLC- and ELISA-determinations independently. Statistical significance of the correlation was determined at the $p < 0.05$ level and indicated as $***p < 0.001$. MC/PE ratios were analysed for outliers using the Tukey box plot rule.

Fig. 1. The spatial and seasonal abundance of *P. rubescens* in the upper 40 m of Lake Ammersee from 1999 to 2004.

Table 3

Summary of periods displaying *P. rubescens* cell densities which have experimentally been shown to affect exposed coregonids (Ernst et al., 2007), and the time-weighted, yearly mean values of the Secchi depths (0Z_s) determined during the vegetation period, i.e. from the beginning of May until end of October for each year

	<i>P. rubescens</i> (cells/ml)			0Z_s (m)
	$\geq 55,000$	$\geq 15,000$	≥ 1500	
1999 ^b	–	July	April–October	3.3 ± 0.19
2001 ^b	July–August	August–September, October–December ^a	August–December ^a	
2001	–	January, March–October ^a	January–October ^a	3.3 ± 0.34
2002	–	–	May–September	2.7 ± 0.21
2003	–	–	August–September	2.9 ± 0.26
2004 ^b	–	July	June–September	3.6 ± 0.15

0Z_s values are given as mean ± S.E.M. ($n \geq 15$). No Secchi measurements were carried out in 2000.

^a Distributed over the entire water column.

^b Measurements were not performed throughout the whole year.

3. Results

3.1. *P. rubescens* abundance

During the 261-week observation period, starting in April 1999 and ending September 2004, *P. rubescens* was always present, albeit at varying cell densities. The distribution patterns observed included phases with a distribution over the entire 40 m water column investigated, as well as phases with distinct metalimnic layers (Fig. 1). Maximum cell densities during winter circulation, whereby *P. rubescens* was mostly distributed throughout the entire water column, reached 15,000 cells ml⁻¹. Maximum *P. rubescens* cell densities in the metalimnic layer reached 77,000 cells ml⁻¹ and 45,000 cells ml⁻¹ as observed in 8–10 m depth in the end of August 2001 and 2000, respectively. *P. rubescens* cell densities of $\geq 55,000$ cells ml⁻¹ occurred during 6 weeks, densities of $\geq 15,000$ cells ml⁻¹ during 53 weeks, and densities of ≥ 1500 cells ml⁻¹ during 123 weeks, corresponding 2%, 22% and 47% of the 261-week observation period, respectively (Table 3). Integrated *P. rubescens* biovolumes are summarised in Fig. 2. Maximum biovolumes were observed at the onset of lake stratification in May 2001, reaching 45 cm³ m⁻².

Yearly onset of lake thermal stratification was recurrent between 20th of April and the 10th of May. Layers of *P. rubescens* filaments were regularly observed as of the beginning of thermal lake stratification (Fig. 1). During stratification, the depth of maximum *P. rubescens* cell densities (peak_{max}) ranged between 7 and 13 m depth and significantly correlated with the Secchi depth and the upper limit of the metalimnic layer (Fig. 3).

The time-weighted, yearly mean values of the Secchi depths (0Z_s) are depicted in Table 3. Although differences in 0Z_s were not statistically significant, metalimnic conditions differed from May to October as well as from year to year. There were vegetation periods with a high water transparency and sustained euphotic depths reaching the upper limit of the metalimnic layer (i.e. 1999, 2001 and 2004), as shown in Fig. 4. However, there were also vegetation periods, where the euphotic depths did not or only temporarily reach the upper limit of the metalimnic layer (i.e. 2002 and 2003). With the exception of 1999, the seasonal development of Lake Ammersee thermal stratification was roughly identical throughout the observation period. However, there were marked differences regarding water transparency and respective extent of the euphotic depth (Fig. 5).

3.2. Seston sample phycoerythrin and microcystin contents

Phycoerythrin (PE) was detectable in all Lake Ammersee seston samples. The overall mean seston PE-content was 3.4 ± 0.7 µg PE mg⁻¹ dw ($n = 54$). Microcystins (MC) were detectable

in 27 and 38 of 54 seston samples via HPLC and Adda-ELISA measurements, respectively.

In all of the 27 Lake Ammersee seston samples that were microcystin-positive, as determined via HPLC analyses, the main microcystin congener (79 ± 4% of the total microcystin) had a retention time and spectrum consistent with [Asp³]-MC-RR. Seventeen samples contained a microcystin variant with a retention time and spectrum consistent with [Asp³Dhb⁷]-MC-RR (14 ± 1% of the total microcystin). Twenty two samples contained a microcystin variant with a retention time and spectrum consistent with [Asp³]-MC-LR (9 ± 2% of the total microcystin), and 18 samples contained a further MC-RR-variant with a retention time which was not comparable to the employed internal standards, however presenting with a spectrum characteristic for microcystin congeners (8 ± 2% of the total microcystin) (Fig. 6).

Irrespective of the analytical method, the detected microcystin amounts significantly correlated with the PE-contents determined (Fig. 7). MC/PE ratios are summarised in Table 4. The variation in MC/PE ratios throughout the six-year observation period was low, with statistical outliers only in May and December 1999 (for both HPLC and ELISA analyses), and in June and August 1999 and August 2000 (for ELISA analyses only). The mean of the determined seston microcystin contents was 0.21 ± 0.03 µg MC-LR_{equ} µg⁻¹ PE ($n = 27$) via HPLC- and 0.43 ± 0.06 µg MC-LR_{equ} µg⁻¹ PE ($n = 38$) via ELISA determination, corresponding 0.85 ± 0.11 µg MC-LR_{equ} mg⁻¹ dw ($n = 27$) and 1.50 ± 0.22 µg MC-LR_{equ} mg⁻¹ dw ($n = 38$), respectively.

3.3. Oxygen

The water column was saturated with oxygen as of the beginning of circulation usually starting at the end of November (±2–3 weeks) and attaining full circulation by the end of December (Fig. 8). With the beginning of lake stratification in May, the epilimnion (corresp. approximately 0–8 m depth) was often super-saturated (e.g. 120% saturation; data not shown), occasionally reaching maximum levels of 150% saturation (August 2001). Subsequently and simultaneous with prolonged lake stratification and a sustained shift of the thermocline into deeper layers, oxygen measurements suggested decreasing oxygen saturation in the metalimnion (as of June in 2002 and July in 1999, 2001, 2003 and 2004). Metalimnic oxygen values regularly were minimal at the end of September and in October, i.e. 3.7 mg l⁻¹ in 1999, 4.0 mg l⁻¹ in 2001, 5.1 mg l⁻¹ in 2002, and 4.6 mg l⁻¹ in 2003.

4. Discussion

4.1. *P. rubescens* distribution and succession

Comparable to *P. rubescens* occurrences observed in deep pre-alpine lakes in Italy (Salmaso, 2000; Buzzi, 2002; Morabito et al.,

Fig. 2. Integrals of the *P. rubescens* biovolume in Lake Ammersee, as determined from the surface to 40 m depth.

2002; Legnani et al., 2005), Switzerland (Walsby et al., 1998) and France (Jacquet et al., 2005) the *P. rubescens* assemblage in Lake Ammersee appears to proceed in a uniform annual pattern. As demonstrated by Walsby et al. (1998), *P. rubescens* cells can remain viable during winter mixing down to 80 m depth. Considering the maximum depth of 82 m, the *P. rubescens* population in Lake Ammersee thus obviously can endure winter circulation. This is confirmed by the constantly high integrated *P. rubescens* bio-

volume during mixing in winter 2000/2001. The winter endurance of the *P. rubescens* population may provide *P. rubescens* with an early competitive advantage in exploiting the resources available at the beginning of the vegetation period (Walsby et al., 1998; Legnani et al., 2005). Despite this, a regular short-term decline in *P. rubescens* abundance is observed during early spring in Lake Ammersee, as is also observed in other lakes (Walsby et al., 1998; Bettinetti et al., 2000; Salmaso, 2000; Walsby and Schanz, 2002). The latter is assumed to stem from the change in hydrostatic conditions. As shown by Walsby et al. (1998), the beginning of lake stratification provides a natural selection for *P. rubescens* cells with strong gas vesicles, enabling filaments to remain buoyant after winter mixing and consequently remain able to stratify in the water column. Filaments that float to the surface mixed layer or remain within the hypolimnion disappear at the beginning of lake stratification, most likely due to high UV irradiance and nutritional competition in the surface layer and cold temperature as well as light limitation in the hypolimnion (Mur et al., 1999; Walsby and Schanz, 2002).

Maximum *P. rubescens* cell densities are observed in Lake Ammersee, as in other lakes, within compact stratified layers as of late spring (Walsby and Schanz, 2002; Jacquet et al., 2005; Blikstad-Halstvedt et al., 2007). As indicated by the correlation of peak_{max} and Z_{meta} ($Z_{\text{meta}} = 0.45 \times \text{peak}_{\text{max}} + 3.3$), the *P. rubescens* peaks – regularly located at 7–13 m depth – are situated approximately 0.5–4 m below the upper limit of the metalimnion, thus closely related to the strong thermal gradient stabilizing the stratified water column. Moreover, the depth of *P. rubescens* stratification broadly corresponds (for peaks in 7–13 m) with the triple of the Secchi depths ($\text{peak}_{\text{max}} \approx 3 \times Z_s$), as demonstrated by the correlation of peak_{max} and Z_s ($Z_s = 0.11 \times \text{peak}_{\text{max}} + 2.1$). Thus the depth of *P. rubescens* stratification is slightly below the standard estimation for the euphotic depth ($Z_{\text{eu}} = 2.5 \times Z_s$, Lemmin, 1978), albeit in accordance with the approximation given by Dokulil and Teubner (2000) and euphotic depths recently estimated in other meso- and oligotrophic lakes (Salmaso, 2000; Buzzi, 2002; Morabito et al., 2002).

The light intensity at the observed *P. rubescens* stratification depth probably corresponds to $\geq 1\%$ of the surface irradiance, which is suggested to be sufficient for the low light requirements of *P. rubescens* (Mur et al., 1999). The latter data thus confirm that the border between epilimnion and metalimnion represents an ecological niche that prevents *P. rubescens* filaments from mixing within the surface layer while simultaneously providing sufficient light for *P. rubescens* growth (Micheletti et al., 1998; Walsby et al., 1998; Morabito et al., 2002). The decrease of the thermal gradient – due to cooling of the surface following lower daytime temperatures and convective cooling during night in autumn – provides for an increased mixing of the near-surface layers, which may include near metalimnic layers (Walsby et al., 1998; Salmaso, 2000; Buzzi, 2002; Teubner et al., 2004; Blikstad-Halstvedt et al., 2007). Consequently, *P. rubescens* filaments may be mixed within the surface layer, where they can accumulate and provide for the surface blooms occasionally observed in stratified lakes (Walsby et al., 1998; Walsby and Schanz, 2002; Blikstad-Halstvedt et al., 2007).

4.2. *P. rubescens* cell densities and biovolumes

P. rubescens occurred in Lake Ammersee continuously, throughout the observation period of six years, as shown via *P. rubescens* cell counts and presence of detectable phycoerythrin. Cell densities and integrated biovolumes were in the range of those previously observed in other pre-alpine lakes, irrespective of whether they were determined during winter circulation or summer stratifica-

Fig. 3. Correlation ($\pm 95\%$ confidence interval of the mean) of the depth of maximum *P. rubescens* densities ($peak_{max}$) to (A) the upper metalimnic limit (Z_{meta}) and (B) the Secchi depth (Z_s). Correlation was statistically significant (Pearson's correlation test) for Z_{meta} ($n = 72$, $r = 0.40$, $p < 0.001$) as well as for Z_s ($n = 71$, $r = 0.25$, $p < 0.05$).

* Trends were not estimated due to deficient sample intervals

Fig. 4. Spatial and seasonal development of the euphotic depth Z_{eu} (black) and the upper metalimnic limit Z_{meta} (grey) in Lake Ammersee (depicted as polynomial trends) from the beginning of May until end of October (periods of vegetation) in 1999, 2001, 2002, 2003 and 2004.

tion (Walsby and Schanz, 2002; Hoeger et al., 2005; Jacquet et al., 2005).

Spatial differences in *P. rubescens* abundance within Lake Ammersee appear to be minimal and to occur only temporarily as suggested by sporadic sampling at a second sampling point in the north of the lake (Ernst et al., unpublished data). In contrast, regarding both maximum cell densities and the integrated *P. rubescens* biovolume, considerable differences in *P. rubescens* abundances were observed between different years. Based on the cell densities and integrated biovolumes, the highest *P. rubescens* abundance was observed in 2001 followed by 2000 > 1999 > 2004 > 2002, 2003. This raises the question which factors influence these apparent differences in annual *P. rubescens* abundance.

Obviously, *P. rubescens* growth in deep pre-alpine lakes is likely to be affected by multiple factors and/or processes (Dokulil and Teubner, 2000; Walsby and Schanz, 2002; Jacquet et al., 2005; Blikstad-Halstvedt et al., 2007, etc.) including nutrient conditions. As nutrient concentrations were not part of the current investigation, the discussion on the influence of the nutritional conditions remains largely hypothetical. However, *P. rubescens* dominates the phytoplankton biomass in stratified, re-oligotrophicated lakes frequently at total phosphorus-concentrations of approximately $10 \mu\text{g l}^{-1}$ (Dokulil and Teubner, 2000; Salmaso, 2000; Morabito et al., 2002). This applies to Lake Ammersee, where the mean annual total phosphorus concentration never exceeded $15 \mu\text{g l}^{-1}$ during the past decade (Teubner et al., 2004). In re-oligotrophicated lakes with $< 15 \mu\text{g}$ total phosphorus-concentration per litre, phosphate depletion strongly influences seasonal phytoplankton succession in the epilimnion in the course of a year (Anneville et al., 2002). Indeed, Jacquet et al. (2005) observed *P. rubescens* mass occurrence to arise when phosphate had severely been depleted in Lake Bourget, France. This appears possible due to the ability of *P. rubescens* to grow at the border of the euphotic zone allowing it to benefit from the nutrient rich metalimnion (Anneville et al., 2002; Walsby and Schanz, 2002). Moreover, *P. rubescens*, which is capable of excreting alkaline phosphatases into the ambient water, has the physiological advantage of utilizing organic phosphorus when inorganic phosphates are growth limiting (Feuillade, 1994). Indeed growth limitation for phytoplankton species other than *P. rubescens* will also reduce the degree of shading in the surface mixed layer, therefore providing for sufficient illumination in deeper zones, as discussed below (Bürge and Stadelmann, 2002; Walsby and Schanz, 2002; Legnani et al., 2005).

Blikstad-Halstvedt et al. (2007) observed that the nutritional conditions determined in Lake Steinsfjorden, Norway, were often below the minimum N and P levels which allowed maximum *P. rubescens* growth rate in culture studies. This applied to both, the epi- and metalimnion, particularly with regard to nitrogen levels. Thus, *P. rubescens* populations may thrive under high nitrogen

Fig. 5. Comparison of the spatial and seasonal development of the euphotic depth Z_{eu} (A) and the upper metalimnic limit Z_{meta} (B) in Lake Ammersee (depicted as polynomial trends) during the periods of vegetation (beginning of May until end of October) in 1999, 2001, 2002, 2003 and 2004.

concentrations. This interpretation is supported by the observation that *P. rubescens* mass occurrence primarily arises in lakes with low phosphate and high nitrogen loads as observed in many oligo- and mesotrophic pre-alpine lakes (Salmaso, 2000; Zotina et al., 2003; Teubner et al., 2004; Jacquet et al., 2005), including Lake Ammersee, where mean nitrogen concentrations regularly exceed 1 mg l^{-1} (Nixdorf et al., 2004).

Another important factor influencing *P. rubescens* growth appears to concern the temperature regime (Blikstad-Halstvedt et al., 2007). Jacquet et al. (2005) suggested that a possible reason for the increasing success of *P. rubescens* may be an earlier onset of lake stratification and thus longer water column stratification in lakes due to global warming. However, it is unlikely that the annual variation in *P. rubescens* abundance observed in Lake Ammersee was caused by differences in the thermal stratification, especially as the onset of lake thermal stratification was comparable throughout the investigation period. In addition, the latitude of the mixed surface layer and the temporal development of the

thermal stratification were roughly identical throughout the study (Z_{meta} exceeded the long-standing average only in 1999, which can without doubt be ascribed to an extreme flood, which raised the water level by approximately 2 m; www.hnd.bayern.de). However, the suggestion by Jacquet et al. (2005) cannot be totally dismissed as decisively longer observation periods would be required to definitively assign global warming induced small changes in onset of lake temperature stratification to *P. rubescens* abundance.

The observation that time-weighted yearly mean values of the Secchi depths ($\bar{Z}_s$) were $>3 \text{ m}$ in 1999, 2001 and 2004 and contrastingly $<3 \text{ m}$ in 2002 and 2003 however, suggests that *P. rubescens* growth might primarily be related to differences in water transparency and therefore to light conditions. Indeed, the comparison of the annual *P. rubescens* abundances with the respective seasonal trend of the upper metalimnic limit (Z_{meta}) and the latitude of the euphotic depth demonstrated that *P. rubescens* abundance was high, when the vegetation periods were characterised by high water transparency and sustained euphotic

Fig. 6. HPLC-chromatogram of a Lake Ammersee seston sample (November 2000) with four MC-variants and respective retention times and spectra consistent with $[\text{Asp}^3]$ -MC-RR (A), $[\text{Asp}^3\text{Dhb}^7]$ -MC-RR (B) and $[\text{Asp}^3]$ -MC-LR (D). The fourth spectrum (C) being comparable to those characterised for MC-congeners is proposed to represent another MC-RR variant.

Fig. 7. Correlation ($\pm 95\%$ confidence interval of the mean) of the microcystin (MC)-content with the phycoerythrin (PE)-content in Lake Ammersee seston samples. MC-analyses were performed using (A) HPLC and (B) ELISA analyses. Correlation was statistically significant (Person's correlation test) for both HPLC ($n = 27$, $r = 0.70$, $p < 0.001$) and ELISA analyses ($n = 38$, $r = 0.74$, $p < 0.001$).

depths reaching the upper limit of the metalimnic layer (i.e. 1999, 2001 and 2004). In contrast, *P. rubescens* abundance was low, when the euphotic depths did not or only temporarily reached the upper limit of the metalimnic layer (i.e. 2002 and 2003). Moreover, the development of the thermal stratification was comparable throughout the observation period, further corroborating the hypothesis that *P. rubescens* abundance in Lake Ammersee is primarily affected by water transparency and Z_{eu}/Z_{mix} -ratios ≥ 1 (whereby Z_{mix} is the latitude of the mixed surface layer ($Z_{mix} = Z_{meta} - 1$). Indeed, Mur and Schreurs (1995) noted that stratifying *Planktothrix* species usually grow in water columns with Z_{eu}/Z_{mix} ratios close to 0.7–1.2. An increased average Z_{eu}/Z_{mix} ratio to 0.9–1 is also discussed as a key to the success of *P. rubescens* in Lake Bourget, France (Jacquet et al., 2005). The importance of light conditions for *P. rubescens* growth is also supported by findings of Dokulil and Teubner (2000), showing that dominance of stratifying *P. rubescens* in Lake Mondsee, Austria, is associated with light climate correlates (Z_{mix}/Z_s) of approximately 4 (Dokulil and Teubner, 2000), which corresponds Z_{eu}/Z_{mix} ratios of ≥ 0.7 , largely depending on the Z_{eu} -approximation used.

4.3. *P. rubescens* microcystin contents

The average microcystin content in microcystin-positive seston samples in this study was $0.85 \pm 0.11 \mu\text{g MC-LR}_{\text{equ}} \text{mg}^{-1} \text{dw}$ via HPLC and $1.50 \pm 0.22 \mu\text{g MC-LR}_{\text{equ}} \text{mg}^{-1} \text{dw}$ via ELISA determination. This is within the range of microcystin contents previously determined in *P. rubescens* extracts and seston sample extracts of *P. rubescens* dominated lakes (Fastner et al., 1999a; Jann-Para et al., 2004; Kurmayer et al., 2005). The results obtained in Lake Ammersee thus confirm that *Planktothrix* species contain comparably high amounts of microcystin (Fastner et al., 1999a; Akcaalan et al., 2006).

The HPLC-analyses demonstrated that *P. rubescens* in Lake Ammersee primarily produce one main microcystin congener, i.e. [Asp³]-MC-RR, far lower concentrations of two additional microcystin congeners, i.e. [Asp³,Dhb⁷]-MC-RR and [Asp³]-MC-LR, and one putative uncharacterised microcystin variant. This observation concurs with previous studies, describing demethylated variants of MC-RR to be the predominant microcystin congeners of *P. rubescens* (Luukkainen et al., 1993; Fastner et al., 1999a; Blom et al., 2001; Kurmayer et al., 2005), accompanied by a varying number of characterised microcystin variants such as [Asp³]-MC-LR, [Asp³]-MC-HtyR and [Asp³]-MC-YR, and as yet uncharacterised congeners (Fastner et al., 1999a; Grach-Pogrebinsky et al., 2003; Kurmayer et al., 2005). It is thus not surprising that differences were observed between microcystin determination via ELISA and HPLC. Indeed, HPLC analysis of microcystins is entirely dependent on the availability of standards, whereas the Adda-ELISA recog-

Table 4

Microcystin contents and potentially toxic cyanobacterial species present in Lake Ammersee seston samples collected from August 1998 until September 2004

	($\mu\text{g MC}_{\text{equ}}/\mu\text{g PE}$)		Abundant species
	HPLC	ELISA	
1998			
August	0.2	0.2	Ptx
November	0.2	0.7	Ptx
1999			
April	0.1	0.2	Ptx
May	0.8	1.2	Ptx
June	0.3	1.2	Ptx
July	0.3	0.5	Ptx
August	0.3	1.1	Ptx
September	0.1	0.4	Ptx
October	0.3	0.7	Ptx
November	0.2	0.6	Ptx
December	0.7	1.9	Ptx
2000			
August	0.4	1.1	Ptx
October	0.2	0.5	Ptx
November	0.3	0.5	Ptx
December	0.1	0.2	Ptx
2001			
January	0.1	0.3	Ptx
February	0.2	0.4	Ptx
March	0.1	0.1	Ptx
April	0.1	0.3	Ptx
May	0.1	0.2	Ptx
June	0.1	0.3	Ptx
July	0.1	0.4	Ptx
August	0.1	0.2	Ptx
September	0.1	0.3	Ptx
October	n.d.	0.4	Ptx
November	*	*	Ptx
December	*	*	Ptx
2002			
January	*	*	Ptx
February	n.d.	n.d.	Ptx
March	n.d.	n.d.	Ptx
April	n.d.	n.d.	Ptx
May	n.d.	0.3	Ptx
June	0.1	0.3	Ptx
July	0.1	0.2	Ptx
August	n.d.	0.3	Ptx, Mic, Ana
September	n.d.	n.d.	Ptx, Mic, Ana
October	n.d.	n.d.	Ptx, Mic, Ana
November	n.d.	n.d.	Ptx
December	n.d.	n.d.	Ptx
2003			
January	n.d.	n.d.	Ptx
February	n.d.	n.d.	Ptx
March	n.d.	n.d.	Ptx
April	n.d.	n.d.	Ptx

Table 4 (Continued)

	($\mu\text{g MC}_{\text{equiv}}/\mu\text{g PE}$)		Abundant species
	HPLC	ELISA	
May	n.d.	0.2	Ptx
June	n.d.	n.d.	Ptx
July	n.d.	n.d.	Ptx
August	n.d.	0.1	Ptx, Mic, Ana
September	n.d.	0.1	Ptx, Mic, Ana
October	n.d.	0.1	Ptx, Mic, Ana
November	n.d.	n.d.	Ptx
December	n.d.	0.2	Ptx
2004			
January	n.d.	0.1	Ptx
March	n.d.	n.d.	Ptx
April	n.d.	n.d.	Ptx
May	*	*	Ptx
July	0.1	0.3	Ptx
August	n.d.	0.1	Ptx, Mic, Ana
September	n.d.	0.1	Ptx, Mic, Ana

Microcystin-amounts were analysed via HPLC and ELISA and expressed as Microcystin-LR equivalents ($\text{MC-LR}_{\text{equiv}}$) per μg phycoerythrin (PE). Abundant cyanobacterial species were *Planktothrix rubescens* (Ptx), *Microcystis* sp. (Mic) and *Anabaena* sp. (Ana). Cyanobacteria marked in bold represented a main component of the phytoplankton community. n.d.: not detectable ($<\text{quant. limit}$). *Deficient sample volumes.

nises nearly all microcystin congeners with comparable sensitivity. Nevertheless, microcystins were detectable in 50% and 70% of the investigated seston samples via HPLC and Adda-ELISA measurements, respectively. The results also demonstrated frequent presence and high microcystin concentrations in samples taken during periods of high *P. rubescens* abundance, whereas samples taken during periods of low *P. rubescens* abundance were less frequently microcystin-positive and, when microcystin was present, the quantities detected were low. The latter data therefore suggest that the presence and quantity of microcystin detected in Lake Ammersee seston is strongly if not exclusively correlated with the abundance of *P. rubescens* and moreover that a nearly constant MC: *P. rubescens* biomass ratio predominates in Lake Ammersee. Consequently, variations in microcystin content per unit biomass of seston samples appear strongly dependent on the species composition of the seston sample itself (*P. rubescens* representation within the sample) rather than a result of variations in *P. rubescens* microcystin content.

P. rubescens phycoerythrin content has been shown to be relatively constant; especially as *P. rubescens* shows no photopigment adaptation subsequent to environmental alterations (Skulberg and Skulberg, 1985). Moreover, high intracellular phycoerythrin concentrations appear to be specifically a *Planktothrix* inherent trait (Ernst et al., 2006b). Therefore, the ratio of MC: phycoerythrin (PE) would appear a better indicator for detection of *P. rubescens* microcystin content than seston biomass in seston samples. Mez (1998) determined PE-concentrations of up to $4 \mu\text{g l}^{-1}$ during a survey on the abundance of toxic cyanobacteria in Swiss lakes, which would correspond to approximately $4 \mu\text{g mg}^{-1}$ dw seston. This compares well with the overall mean PE-content of $3.4 \pm 0.7 \mu\text{g PE mg}^{-1}$ dw detected in the Lake Ammersee seston samples.

The average MC/PE ratio as analysed in the Lake Ammersee seston samples was $0.21 \pm 0.03 \mu\text{g MC-LR}_{\text{equ}} \mu\text{g}^{-1}$ PE and $0.43 \pm 0.06 \mu\text{g MC-LR}_{\text{equ}} \mu\text{g}^{-1}$ PE when analysed via HPLC and Adda-ELISA, respectively. MC/PE ratios varied only little, suggesting that the variation in the *P. rubescens* microcystin content was limited. This concurs with observations in other German freshwaters where temporal variability of microcystin variants as well as the variability of relative microcystin amounts within persisting *P. rubescens* blooms was rather low (Fastner et al., 1999a).

4.4. Oxygen

Concurrent to prolonged lake stratification and a sustained shift of the thermocline into deeper layers, the oxygen measurements suggested decreasing oxygen-saturation in the metalimnion regularly leading to minimal metalimnic oxygen values in September and October. The observed decrease in available oxygen is assumed to result from a shift of the metalimnion below the euphotic depth, limiting metalimnic photosynthetic activity and thus metalimnic oxygen release, while oxygen consumption due to decomposition of organic matter remained constant (Kucklentz et al., 2001).

In this study, a ranking of the metalimnic oxygen minima according to the lowest values of individual years yielded the following result: $1999 < 2001 < 2003 < 2002$. This ranking compared very well with the ranking obtained with ordering yearly *P. rubescens* abundance from highest to lowest values. The results thus demonstrated that metalimnic oxygen depletion is more pronounced in years of high *P. rubescens* abundance while lower metalimnic depletion could be observable in years with lower *P. rubescens* abundance. Repeated observable metalimnic oxygen depletions therefore appear to be causally related to recurring high *P. rubescens* abundance. This apparent coherence of observations may be assumed to result from oxygen consuming decomposition of senescent *P. rubescens* cells in the metalimnion, e.g. a decline of oxygen saturation was observed subsequent to a decrease in *P. rubescens* abundance coincident with increasing microcystin concentrations in the metalimnic water of Lake Ammersee in August 1999 (Ernst et al., 2001). It may also be caused by a shift of the stratified *P. rubescens* population into deeper layers below the photosynthetic compensations point thus enforcing a change from oxygen producing photosynthesis to respiration of oxygen (Walsby et al., 2001). Similarly, marked metalimnic oxygen depletions have been observed in several other *P. rubescens*-containing lakes (Lindholm and Meriluoto, 1991; Salmaso, 2000; Buzzi, 2002; Krupa and Czernas, 2003), lending more weight to above interpretation.

4.5. Possible consequences

Microcystin-containing cyanobacteria have been demonstrated to detrimentally affect various aquatic organisms (Sivonen and Jones, 1999; Wiegand and Pflugmacher, 2005) including fish (Carbis et al., 1996; Fischer and Dietrich, 2000; Tencalla and Dietrich, 1997). This is very interesting as in many pre-alpine *P. rubescens*-containing lakes, coregonids have recently suffered massively reduced growth and changed population demographics resulting in recurrent slumps in yearly yields in coregonid fishery (Wißmath, 2000; Gammeter and Forster, 2002; Müller, 2003; Kirchhofer, 2004).

Laboratory experiments clearly demonstrated adverse effects, including dose- and time-dependent physiological stress and progressive organ damage with subsequent elevated susceptibility to ectoparasitic infestations and increased mortality, following subchronic exposure of coregonids to *P. rubescens* (Ernst et al., 2006a, 2007). This has been shown to apply to *P. rubescens* densities greater than $1500 \text{ cells ml}^{-1}$, which occurred in Lake Ammersee during 47% of the 261-week observation period. The results of this study thus suggest that Lake Ammersee coregonids are indeed regularly confronted with potentially adverse *P. rubescens* densities. As the microcystin content of *P. rubescens* appeared to be constant, detrimental effects on the coregonid population resulting from exposure to prolonged *P. rubescens* abundance thus cannot be excluded. This pertains specifically to periods with *P. rubescens* filaments distributed over the entire water column – as for example continuously observable from

Fig. 8. Oxygen conditions in the upper 40 m of Lake Ammersee from July until December (1999–2004).

December 2000 to May 2001 – and thus making avoidance of *P. rubescens* impossible.

As in addition, pronounced metalimnic oxygen depletion has regularly been observed to result in oxygen concentrations at the limit of the coregonid tolerance (Kischnik, 1992; Müller and Stadelmann, 2004), pronounced oxygen depletion may provide a further stress factor in the metalimnic layer, in addition to stratified microcystin-containing *P. rubescens*.

Consequently, it is plausible that the observed challenge of coregonids in *P. rubescens* containing lakes is a result of adverse environmental conditions that are causally linked to the appearance of microcystin-containing *P. rubescens*.

5. Conclusion

In summary *P. rubescens* abundance appears to be strongly influenced by water transparency, i.e. illumination in the

metalimnion. In addition, the current data support the hypothesis that *P. rubescens* abundance in the re-oligotrophicated Lake Ammersee results from regular phosphate depletion in the epilimnion and possibly benefits additionally from high nitrogen loads. The results as presented thus confirm that the increasing *P. rubescens* abundance in pre-alpine lakes is a paradoxical outcome of lake restoration efforts (Morabito et al., 2002; Jacquet et al., 2005). In addition, the variation in the MC/PE-ratios was low suggesting that the microcystin production of *P. rubescens* in Lake Ammersee is consistent thus indicating that the appearance of *P. rubescens* coincides with measurable microcystin levels. Aquatic organisms such as coregonids thus appear to be regularly confronted with potentially adverse *P. rubescens* densities which might provide a possible explanation for the often observed challenge to coregonid populations in *P. rubescens* containing pre-alpine lakes.

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