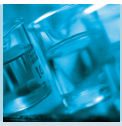
KOMPETENZZENTRUM
WasserBerlin





Volume 6

Cylindrospermopsis raciborskii and Cylindrospermopsin in Lakes of the Berlin Area

- Occurrence, Causes, Consequences -

Claudia Wiedner, Jacqueline Rücker, Bodo Weigert (eds.)

Project Funding VEOLIA WATER

Schriftenreihe Kompetenzzentrum Wasser Berlin

KOMPETENZZENTRUM Wasser Berlin

Kompetenzzentrum Wasser Berlin Publication Series

Volume 6

Cylindrospermopsis raciborskii and Cylindrospermopsin in Lakes of the Berlin Area

Occurrence, Causes and Consequences

- CYLIN -

Claudia Wiedner [a], Jacqueline Rücker [b], Anke Stüken [a],
Karina Preußel [c], Jutta Fastner [c], Ingrid Chorus [c] and Brigitte Nixdorf [b]

[a] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Limnology of Stratified Lakes, Alte Fischerhütte 2, D-16775 Stechlin, Germany

[b] Brandenburg University of Technology Cottbus, Department of Freshwater Conservation, Bad Saarow Research Station, Seestrasse 45, 15526 Bad Saarow, Germany

[c] Federal Environmental Agency , Corrensplatz 1, 14195 Berlin, Germany

Project Funding

VEOLIA WATER

Cylindrospermopsis raciborskii and Cylindrospermopsin in Lakes of the Berlin Area: Occurrence, Causes and Consequences

Claudia Wiedner [a], Jacqueline Rücker [b], Anke Stüken [a],
Karina Preußel [c], Jutta Fastner [c], Ingrid Chorus [c] and Brigitte Nixdorf [b]
Berlin 2007

Kompetenzzentrum Wasser Berlin Publication Series, 1.2004 ff., Volume 6

Editors:

Claudia Wiedner, Jacqueline Rücker, Bodo Weigert

[a] Leibniz-Institute of Freshwater Ecology and Inland Fisheries,
Department of Limnology of Stratified Lakes, Alte Fischerhütte 2, 16775 Stechlin, Germany

[b] Brandenburg University of Technology Cottbus, Department of Freshwater Conservation,
Bad Saarow Research Station, Seestraße 45, Bad Saarow, Germany

[c] Federal Environmental Agency,
Corrensplatz 1, 14195 Berlin, Germany

ISBN 978-3-00-021364-9

Cicerostrasse 24
10709 Berlin
www.kompetenz-wasser.de
Cover design: Votum GmbH, Berlin
Printer: Papyrus-Druck GmbH, Berlin
Translation: Suzyon O'Neal Wandrey, Berlin

Table of Contents

Summary

Project name:	***Cylindrospermopsis raciborskii* and *Cylindrospermopsin* in Lakes of the Berlin Area: Occurrence, Causes and Consequences**
Duration:	**2/2004 to 1/2007**
Project volume:	**EUR 470,000**
Project officers:	**Dr. Claudia Wiedner**, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Dept. of Limnology of Stratified Lakes; **Prof. Dr. Brigitte Nixdorf**, Brandenburg University of Technology Cottbus, Department of Freshwater Conservation; **Dr. Ingrid Chorus**, Umweltbundesamt (Federal Environmental Agency)
Sponsors:	**Kompetenzzentrum Wasser Berlin (KWB)** and **Veolia Water**
KWB contact:	**Dr. Bodo Weigert**

Cylindrospermopsis raciborskii, a cyanobacterium of tropical origin, can produce the toxin cylindrospermopsin (CYN). This originally tropical cyanobacterium (blue-green algae) has now spread to the distant waters of the Berlin area. Cylindrospermopsin has been detected in two lakes in the area, but none of the *C. raciborskii* strains isolated here so far were found to produce the toxin.

The main objectives of the CYLIN project were therefore to analyze the distribution and regulation of *C. raciborskii* and cylindrospermopsin and to determine which cyanobacteria are producing this toxin in order to establish a basis with which to predict the further course of development of this species and the related health hazards for humans.

The CYLIN project was implemented as a three-part program. A screening program was first conducted in 2004 to test regional water bodies for the presence of cylindrospermopsin and potential CYN-producing cyanobacteria in order to obtain an overview of their distribution in the study region. A total of 142 regional water bodies were sampled once each in this qualitative analysis of cylindrospermopsin and cyanobacteria. The screening program was followed by a monitoring program designed to generate quantitative data on the concentrations of dissolved CYN, particulate CYN, cyanobacteria and target environmental parameters at 20 selected lakes, which were sampled 3 times each. Last but not least, we investigated the seasonal dynamics of these parameters at two selected

lakes in 2004 and 2005. Apart from this we isolated different cyanobacterial strains and conducted chemical and molecular biological analyses of CYN and CYN-coding genes, in order to identify CYN-producing cyanobacteria.

The results show that *C. raciborskii* and CYN are much more widespread than was previously assumed. *C. raciborskii* was detected in 22 % of the investigated water bodies, and cylindrospermopsin in 52 %. Additionally, two other toxic cyanobacteria of tropical origin were found for the first time in the Berlin-Brandenburg region, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. The mean and maximum CYN concentrations were 1 µg L^{-1} and 12 µg L^{-1}, respectively. Since the particulate CYN fraction did not exceed 0.5 µg L^{-1}, the dissolved CYN fraction was found to be responsible for the high CYN concentrations. The proposed guideline safety value for cylindrospermopsin in drinking water (1 µg L^{-1}) was exceeded 18 times at 8 different lakes. Although *Aphanizomenon flos-aquae* (Nostocales) has been unequivocally identified as a producer of cylindrospermopsin, the observed cylindrospermopsin concentrations cannot be attributed to this cyanobacterial species alone. *Aphanizomenon gracile* was also identified as a potential CYN-producing cyanobacterium.

Based on the findings of the CYLIN project, we recommend that cylindrospermopsin be included as a risk factor in drinking and bathing water quality assessments. To identify hazard conditions associated with this cyanotoxin, further investigations are needed to identify all cyanobacteria that produce cylindrospermopsin and to elucidate the mechanisms regulating the occurrence of CYN-producing cyanobacteria, CYN synthesis by these organisms, and CYN decomposition in aquatic ecosystems.

Our analysis of *C. raciborskii* population dynamics showed that its germination is temperature-dependent and its population growth light-dependent. Population size was determined by the time of germination, that is, the earlier the time of germination, the bigger the population. Based on these findings, it appears highly likely that the climate-related early rise in water temperatures over the course of the years has promoted the spread of this species to temperate regions.

Our hypothesis for the future course of cyanobacterial and cyanotoxin development in German waters is as follows: The combination of trophic decline and global warming works to the general benefit of cyanobacteria of the order Nostocales and leads to a shift in cyanobacterial species and toxin composition. This may ultimately lead to an increase in the incidence of neurotoxins as well as cylindrospermopsin.

1. Introduction

Cylindrospermopsis raciborskii is a cyanobacterium that originally occurred in lakes and rivers of tropical and subtropical regions. Within the last few decades, it has spread to other parts of the world, out of the tropics into temperate climate zones. Taxonomically, *C. raciborskii* is a member of the order Nostocales. This cyanobacterium forms small filaments (trichomes) measuring an average 0.15 mm in length and 0.003 mm in diameter (Fig. 1). Like all Nostocales, *C. raciborskii* can produce vegetative cells as well as heterocysts for the fixation of molecular nitrogen and akinetes (resting cells) for survival in sediment during unfavorable growth conditions. *C. raciborskii* can produce potent toxins such as cylindrospermopsin (Fig. 1) and paralytic shellfish poisons. It is therefore important to carefully monitor the occurrence of this cyanobacterial species in water bodies used for drinking water or recreational purposes.

Fig. 1: *Cylindrospermopsis raciborskii* (left) and cylindrospermopsin (chemical formula, right).

Toxicity of cylindrospermopsin

The fact that *C. raciborskii* can be a health hazard for humans became acutely evident in the Palm Island incident in Australia in 1979. There, several cases of hepatoenteritis occurred in the local population due to contamination of drinking water with a cyanobacterium, which was later identified as *C. raciborskii* (Bourke et al. 1983). Epidemiological studies confirmed that a strain of *C. raciborskii* isolated from the drinking water reservoir produced serious hepatotoxic effects in a mouse bioassay and was therefore responsible for the occurrences of hepatoenteritis (Hawkins et al. 1985). Cylindrospermopsin, the causative alkaloid hepatotoxin named after *Cylindrospermopsis raciborskii*, was later isolated from the implicated *C. raciborskii* strain (Ohtani et al. 1992).

The toxicity mechanism of cylindrospermopsin (CYN) still is not fully understood. One decisive aspect demonstrated in laboratory experiments (in vitro) was that CYN blocks protein synthesis rapidly, completely and irreversibly (Froscio et al.

2003). Experiments with laboratory animals (in vitro) showed that CYN induces damage to the liver, kidneys, lung, heart, stomach, adrenals, circulatory system and lymphatic system (Hawkins et al. 1985). Liver damage is dose-dependent and can be very severe, especially after exposure to acute doses (inducing injury within 1 to 2 days). Although CYN is generally classified as a hepatotoxin, it has a much wider range of effects. Renal toxicity is a prominent effect, as was clearly demonstrated in animals with subchronic exposure (periods of several weeks) to daily doses of CYN (Humpage and Falconer 2003). Genotoxicity of cylindrospermopsin has also been demonstrated in a number of studies (e.g., Humpage et al. 2005). Concerning its potential for carcinogenicity in humans, the International Agency for Research on Cancer (IARC) concluded in 2006 that the available data was insufficient to resolve this question. So far, only one study has shown evidence of an increased tumor incidence in living cells exposed to very high doses of CYN (Falconer and Humpage 2001).

Toxic safety guideline values for CYN also have not been established to date due to insufficiency of the available data, and the World Health Organization (WHO) will presumably reassess the toxicity data in the near future because of this. In order to derive drinking water safety guideline values for a toxin, the WHO requires toxicity data from at least two independent studies or sufficient data from suborganismic tests. The one available animal study is a subchronic exposure trial (10 to 11 weeks) in mice (Humpage and Falconer 2003); similar to the WHO guideline value for microcystin-LR, the authors recommend 1 µg L^{-1} as the drinking water guideline value for CYN. Drinking water guideline values have been established in Brazil (15 µg L^{-1}) and New Zealand (2 µg L^{-1}).

Occurrence of cylindrospermopsin and CYN-producing cyanobacteria

CYN has been detected in waters in tropical regions such as Australia (McGregor and Fabbro 2000, etc.) and Brazil (Carmichael et al. 2001) as well as in water bodies in North America (Burns et al. 2002) and Europe (Fastner et al. 2003, Manti et al. 2005, Quesada et al. 2006). In the six available studies that provide quantitative assessments of CYN in natural waters, CYN concentrations reportedly ranged from 1 to 20 µg L^{-1} with maximum concentrations of up to 100 µg L^{-1} (Manti et al. 2005, Quesada et al. 2006, Chiswell et al. 1999, McGregor and Fabbro 2000, Hoeger et al. 2004, Burns et al. 2002). The total CYN concentration may have been underestimated in some of these studies because the dissolved CYN fraction was not determined. On the whole, quantitative CYN concentration data suitable for assessment of the hazard potential of this toxin is still very scanty.

Further investigation is also needed to determine the identity of the cyanobacteria responsible for the production of cylindrospermopsin. The first *C. raciborskii* and cylindrospermopsin distribution studies revealed that the toxin is produced by other cyanobacterial species besides *C. raciborskii*. Those identified by the start of

the CYLIN project were: *Umezakia natans* (Harada et al. 1994), *Aphanizomenon ovalisporum* (Banker et al. 1997), *Anabaena bergii* (Schembri et al. 2001), and *Raphidiopsis curvata* (Li et al. 2001a). Like *C. raciborskii*, these cyanobacterial species are also predominantly distributed in tropical and subtropical waters. Unlike *C. raciborskii*, however, they had not been detected in German waters before. *C. raciborskii* could not be implicated as the cause of the first occurrences of cylindrospermopsin at two German lakes (Melangsee and Langer See) because none of the *C. raciborskii* strains isolated from Melangsee to date produced the toxin (Fastner et al. 2003). Likewise, none of the *C. raciborskii* strains found in other European waters produce cylindrospermopsin (Bernard et al. 2003, Saker et al. 2003). These findings suggest that cyanobacterial species other than *C. raciborskii* produced the cylindrospermopsin detected in these waters or that *C. raciborskii* populations consist of both CYN-producing and non-producing genotypes. Another factor to consider is that the *C. raciborskii* strains found in Germany produce other toxic substances that have not yet been subjected to further analysis (Fastner et al. 2003). Therefore, further investigation of the spread and regulation of *C. raciborskii* is essential, even if this species is not the producer of cylindrospermopsin in regional waters.

Distribution and ecology of *C. raciborskii*

Cylindrospermopsis raciborskii (Woloszynká) Seenaya & Subba Raju was first discovered by Woloszynská (1912) in a lake in Java. This bloom-forming cyanobacterium has spread from the tropics to temperate climate zones during the last few decades, as was first extensively documented by Padisák (1997). *C. raciborskii* was first sighted in Europe in 1938 at a lake in Greece (Skuja 1938). It was later detected in other European lakes, including Balaton in Hungary in 1970 (Padisák 1977), Lieps in Germany in 1990 (Krienitz and Hegewald 1996), the Old Danube (Alte Donau) in Austria in 1993 (Dokulil and Mayer 1996), the Scharmützelsee region in Germany in 1994 (Rücker et al. 1997, Wiedner and Nixdorf 1997), and a small lake north of Paris, France in 1994 (Couté et al. 1997).

Such drastic spread of a phytoplankton species has never been observed before. Climate change and ecotype selection have been proposed as potential causes of the invasive spread of *C. raciborskii*. Some investigators who did not detect any ecophysiological differences between *C. raciborskii* strains from the tropics and temperate zones conclude that climate change is the cause of spread (Briand et al. 2004). Others found strains that can survive at low temperatures, which is more suggestive of adaptation and selection mechanisms (Chonudomkul et al. 2004). The cause of *C. raciborskii* invasion is therefore a subject of debate that still cannot be resolved definitively.

The highest biomass levels of *C. raciborskii* occur in deep stratified lakes in tropical regions and in polymictic shallow lakes in temperate regions (Padisák

1997). According to Padisák, the reason for this difference in habitat preference is that, in temperate climate zones, the mean water temperatures of shallow lakes are higher than those of deep lakes; shallow lakes are therefore more suitable to the high-temperature preferences of this tropical species. *C. raciborskii* populations can thrive all year round in the tropics (Fabbro and Duivenvoorden 1996, Bouvy et al. 1999). In temperate latitudes, however, pelagic growth of *C. raciborskii* is limited to the summer months. In order to survive the winter, the species must form akinetes, from which it germinates again in the spring.

Studies have shown that akinetes of tropical *C. raciborskii* populations germinate at temperatures above 22 °C. Peak biomass values for pelagic populations were found under the following conditions: water temperature range 27 to 30 °C, high pH, high water column stability, long water residence times, and high global radiation (Branco and Senna 1994, Fabbro and Duivenvoorden 1996, Souza et al. 1998, Bouvy et al. 1999, McGregor and Fabbro 2000). In temperate latitudes, germination also occurs at temperatures above 22 °C (Górzo 1987, in Padisák 1997), and population peaks coincide with annual temperature peaks, but the start of pelagic growth occurs at relatively low temperatures in the 15 to 17 °C range (Dokulil and Mayer 1996, Mischke 2003). Other investigators have postulated that *C. raciborskii* is a shade-tolerant species that benefits from low light intensity conditions, such as those occurring in eutrophic waters during the summer months (Dokulil and Mayer 1996, Padisák and Reynolds 1998), and from low concentrations of dissolved inorganic nitrogen and phosphorous (Mischke 2003, Briand et al. 2002a). Since most of the previous studies were only designed to characterize the conditions under which the populations occur, the actual mechanisms regulating *C. raciborskii* population dynamics and growth are still largely unclear. Consequently, it is not possible to predict the further course of development of *C. raciborskii* in our region at this time.

Based on the current data, the most northern occurrences of *C. raciborskii* are those in the lakes of the Scharmützelsee region (southeast of Berlin) and in Lake Lieps (north of Berlin). The extent of spread of the cyanobacterium in the region is, however, unclear. The Lieps sighting was based on an analysis of a single phytoplankton sample obtained in 1990. It is unclear whether *C. raciborskii* was there before or after this time or whether it occurs in neighboring lakes in the region. Further data from the lakes of the Scharmützelsee region show that the initial occurrence of *C. raciborskii* was not a transient event. Populations of this cyanobacterium have been found there regularly since 1994, and the *C. raciborskii* fraction sometimes comprises up to 20 % of the total phytoplankton biovolume (Wiedner and Nixdorf, 1997; Mischke 2003, Nixdorf et al. 2003). Although no "mass occurrence" of *C. raciborskii* has been observed in the region so far and although it has never occurred as the dominant phytoplankton species, its prevalence is remarkably high for recently invaded species.

In summary, we conclude that populations of the toxic cyanobacterial species *C. raciborskii* have become established in lakes in the Berlin area. Too little is known about the regional distribution of this new, toxic species to assess the potential health hazard it may pose in water bodies used for drinking water and recreational purposes. Further evidence clearly shows that CYN is present in lakes in the Berlin region, but the distribution and concentration of CYN and of the cyanobacteria that produce it are still unknown.

2. Objectives

The foremost objective of the CYLIN project was to assess current and future hazard potential of the toxic cyanobacterium *Cylindrospermopsis raciborskii* and of the cyanobacterial toxin cylindrospermopsin in water bodies in the Berlin-Brandenburg region.

Specifically, the three main work objectives of the CYLIN project were:

- To analyze the distribution of *C. raciborskii*, other potential CYN-producing cyanobacteria and CYN in regional water bodies with different trophic and morphological features and to characterize the lake types that provide suitable habitats for the CYN producers.

- To identify the mechanisms that regulate the seasonal dynamics of *C. raciborskii*, other potential CYN producers and CYN in order to predict the future course of development of CYN-producing cyanobacteria and CYN concentration in the study region.

- To determine which cyanobacteria produce CYN in the study region and to assess the variability of occurrence of the CYN producers.

3. Study design

3.1 Distribution of *C. raciborskii*, other potential cylindrospermopsin producers and cylindrospermopsin in the study region

Within in the scope of the CYLIN project, a three-part research program was used to investigate the spatial and temporal distribution of *C. raciborskii* in the study region (Fig. 2).

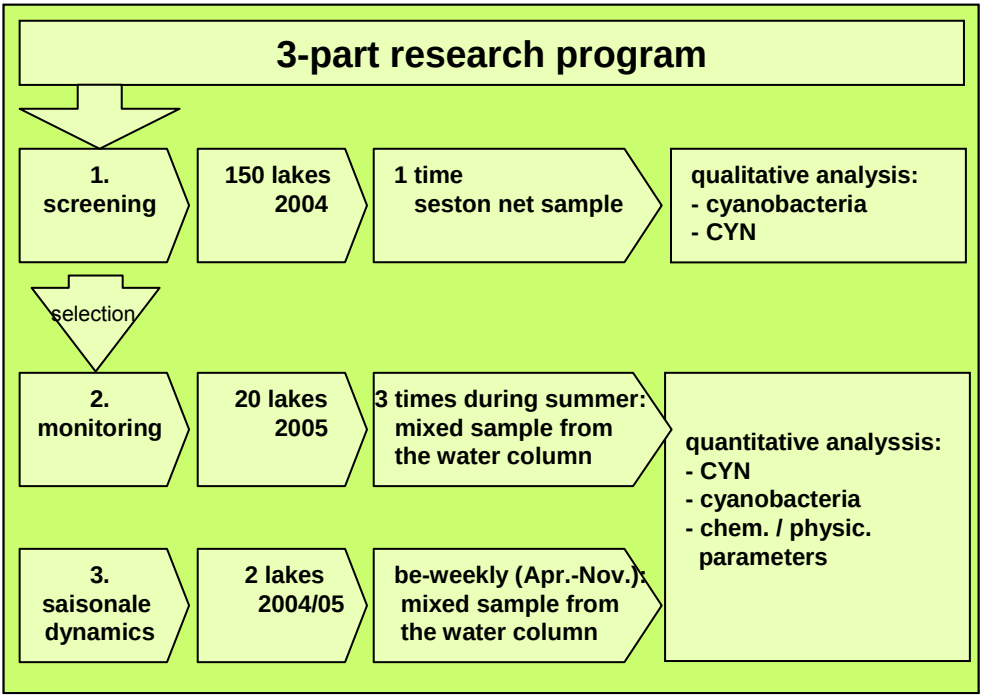


Fig. 2: Three-part research program of the CYLIN project.

Screening: The foremost objective of the screening program was to obtain an comprehensive overview of the spatial distribution of *C. raciborskii*, other potential CYN-producing cyanobacteria, and CYN. Water bodies throughout the entire study region and selected, representative lakes were included in the analysis. A large number of water bodies had to be sampled for this purpose: 60 was the

target number, and a total of 142 water bodies were actually sampled. Quantitative assessments of cyanobacterial species and CYN concentration parameters were made using plankton net samples taken once from each water body.

Monitoring: Twenty lakes in which potential CYN-producing cyanobacteria or CYN had been identified by screening were selected for inclusion in the monitoring program. Each of the lakes was sampled three times during the summer months. This generated the first quantitative data on dissolved and particulate CYN concentrations per volume water with which a risk assessment could be performed. Quantitative data on the biovolumes of the observed cyanobacterial species and relevant chemical and physical environmental parameters were also obtained. The overall data set was tested for correlations between CYN concentration and cyanobacterial biovolume and environmental parameters.

Seasonal dynamics: Quantitative measures of the variability of the seasonal dynamics of CYN concentration, potential CYN-producing cyanobacteria and environmental factors rounded off our comprehensive series of investigations. The quantitative seasonal data were analyzed to determine the mechanisms regulating CYN and cyanobacterial dynamics and to identify lake-specific correlations between CYN concentration and potential CYN-producing cyanobacteria.

3.2 Future course of *C. raciborskii* and cylindrospermopsin development

The results of the investigations of seasonal dynamics and regulatory mechanisms were used for forecasting purposes. In the case of *C. raciborskii* development, additional data sets from earlier study programs dating back to 1993 were also available for analysis. The serial data sets were analyzed for long-term trends and for factors that regulate *C. raciborskii* growth and population dynamics. Future development predictions were based on information derived from the analysis of regulatory mechanisms.

3.3 Identification of cylindrospermopsin-producing cyanobacteria

Strains of various cyanobacterial species of the order Nostocales (all of which are currently considered to be potential CYN producers) were studied using culture isolates obtained from samples collected from various lakes sampled in the scope of the screening program. The CYN content of all cultured strains was determined by chemical analysis, and the presence or absence of CYN-coding genes was determined by molecular biological analysis. A molecular biological method for detection of CYN-coding genes in individual filaments (trichomes) with which one

could estimate the fractions CYN-producing genotypes within a given population had to be developed. Representative quantities of individual filaments of potential CYN-producing cyanobacteria were isolated from selected monitoring program samples and stored for later analysis.

4. Water bodies studied and methods employed

4.1 Screening

A total of 142 water bodies were sampled from June to September 2004 in the scope of our screening program, which covered a wide range of lake types exhibiting differences in morphometry (depth, area, volume), water mixing (polymictic vs. dimictic) and trophic state (oligotrophic to hypereutrophic).

Fig. 3: Three of the sampled lakes: 1) Langer See (left), a polymictic, hypertrophic shallow lake; 2) Scharmützelsee (center), a mesotrophic, deep stratified lake; and 3) Große Kossenblatter See (right), a polymictic, eutrophic shallow lake.

Morphometric data on the lakes was extracted from a database on standing waters in Germany maintained by the Department of Freshwater Conservation of the Brandenburg University of Technology (BTU) Cottbus (Nixdorf et al. 2004, Hemm and Jöhnk 2004). All lakes investigated in the project are listed in the Appendix (page 84), and maps showing the distribution of the lakes within the study region are presented in the results sections (Chapters 5 and 7). If the point of maximum depth was known, each lake was sampled once at the deepest point; otherwise, samples were collected from the middle of the lake. Five lakes were not accessible by boat. In these cases, samples were collected from a pier or from the shore.

Plankton nets (mesh size: 25 µm) were used to collect suspended matter (seston) from the entire water column (Fig. 4). Each concentrated seston sample was divided into three aliquots. The first was examined under a microscope (200 to 400 X magnification) within one day of sampling, and cyanobacterial species detected in the sample were listed. After semi-quantitative assessment, the relative fraction of each individual species was classified as "not present", "rare", "common" or "dominant".

Fig. 4: Plankton net

Fig. 5: Secchi disk used for determination of transparency depth.

The second aliquot was fixed in formalin (final concentration: 4 % formalin) and saved as the reference sample for later control tests. The third aliquot was freeze-dried and used for later analysis of CYN concentration per unit seston dry weight (see Chapter 4.2.6).

Transparency depth (Secchi depth) was measured at each sampling date using a 25 cm Secchi disk (Fig. 5). These data permitted a rough estimate of vegetation-related water turbidity, and thus of the trophic state. Note that, according to the draft LAWA Guidance on Quality Assurance (LAWA, 1999) and the EU Water Framework Directive (EU, 2000), an exact estimate of the degree of eutrophication of a lake cannot be determined based on single sampling and secchi measurement. Additional parameters such as total phosphorous and chlorophyll *a* concentrations and phytoplankton biomass and composition must also be considered.

To determine which cyanobacterial species are producing cylindrospermopsin in German waters, strains of various cyanobacterial species in selected lakes were isolated from some of the unfixed seston samples. The isolation and culture methods are described in detail in Chapter 8, the chemical analysis of cylindrospermopsin in Chapter 4.2.6, and the molecular biological analysis in Chapter 9.

4.2 Monitoring and seasonal dynamics

A total of 20 lakes in which cylindrospermopsin and potential CYN-producing cyanobacteria had been detected during the 2004 screening program were selected for inclusion in the monitoring program, which was conducted during the second project year. The lakes studied in the monitoring program are listed in Table 4 (page 55). Samples were taken from the deepest point of the monitored lakes at two-week intervals on at least three occasions during the months of June to September 2005. Melangsee and Langer See were selected for study of cyanobacterial and cylindrospermopsin seasonal dynamics because cylindrospermopsin had already been detected there in 2000 (Fastner et al. 2003) and because earlier data from these lakes (from 1993 on) were available for inclusion in the analysis of *C. raciborskii* population dynamics (Rücker et al. 1997; Mischke

2003). Melangsee and Langer See were sampled every two weeks from April to November in the years 2004 and 2005.

4.2.1 Underwater light conditions

Fig. 6: Light sensor

Sunlight is the primary source of energy used by cyanobacteria and other eukaryotic phytoplankton algae to accomplish photosynthesis. The intensity of underwater light intensity is therefore an important growth-limiting factor for cyanobacteria. Here, light intensity was measured as the intensity of photosynthetically active radiation (PAR) using two spherical quantum sensors (Li Cor SA 193) fixed at 0.5 m interval and submerged - to variable depth along the vertical profile of the water column (Fig. 6).

The attenuation coefficient (K_d), or fraction of solar energy lost due to depth-related attenuation and scattering, was calculated as the difference between the upper and lower sensor readings using Kirk's equation (Kirk 1994):

$$K_d = \frac{\ln I_1 - \ln I_2}{z_1 - z_2}$$

where I_1 = PAR at depth z_1 and I_2 = PAR at depth z_2. Mean PAR in the mixed water column (I_{mix}) was calculated as the product of underwater light attenuation and total incident radiation (I_0) using Riley's equation (Riley 1957):

$$I_{mix} = 0.45 \cdot Io \cdot \left(\frac{1 - e^{-K_d \cdot z_{mix}}}{K_d \cdot z_{mix}} \right)$$

where z_{mix} equals the depth of the mixed water column. For shallow polymictic lakes, z_{mix} was assumed to be equivalent to the mean water depth (ratio of lake volume to lake area); for dimictic lakes, it was derived from the vertical profiles for water temperature. I_0 was defined as the 14-day mean of total incident radiation before the sampling date. Total incident radiation data was kindly provided by the meteorological observatory of the German Weather Service in Lindenberg. The ratio of photosynthetically active radiation to total incident radiation and losses due to reflection on the water surface were considered using a factor of 0.45 according to Behrendt and Nixdorf (1993)

4.2.2 Water temperature, oxygen, pH and mixing

Fig. 7: Multi-parameter probe (H20)

Vertical depth profiles for temperature, pH, oxygen concentration and oxygen saturation were obtained using a multi-parameter probe (H20, Hydrolab, Fig. 7) or multiple WTW probes positioned at 0.5-meter intervals. Based on these depth profiles, the depth of the mixed water column from which the mixed sample was to be collected was determined on-site. Unlike deep and thermally stable stratified lakes (dimictic lakes), which form epi-, meta- and hypolimnion layers in summer, shallow lakes tend to be mixed from top to bottom (polymictic).

4.2.3 Sampling

Fig. 8: Water sampler

Mixed water samples were derived from the entire water column of shallow polymictic lakes and from the epilimnion of stratified lakes. Samples were collected at 0.5 meter intervals using a LIMNOS 2.3 L water sampler (Fig. 8) and stored in a single vat to yield the mixed water sample. The mixed water sample, which was the source of the aliquots used for chemical and microscopic testing, was preserved by appropriate means until the time of analysis.

4.2.4 Nutrients and chlorophyll *a*

Total phosphorous (TP) and total nitrogen (TN) concentrations were determined in a laboratory equipped with the FIA-LAB II system (MLE GmbH, Radebeul) using 100 ml aliquots of mixed water sample acidified with sulfuric acid (Lippert et al. 2004). The acidified samples were kept frozen until the time of analysis. To obtain samples for dissolved inorganic phosphorous (DIP), ammonium (NH_4-N) and nitrite plus nitrate (NO_{total}-N) concentration analyses, part of the mixed sample was filtered through a membrane filter (0.45 µm pore size) and kept frozen until the time of analysis.

Fig. 9: Multichannel segmented flow analyzer

Dissolved nutrient concentration analyses were performed using an automated multichannel segmented flow analyzer (SFA; Perstorp, Fig. 9) according to German standard procedures (DEV, 1976 - 1998).The limits of detection were 1.7 µg L^{-1} for DIP, 64 µg L^{-1} for ammonium and 3.2 µg L^{-1} for nitrite plus nitrate. If the concentration of dissolved nutrients was below the detection limit on a given date, which was frequently the case in the summer months, one-half the detection limit was inserted as the value for that sampling date.

For chlorophyll *a* determinations, a defined volume of the mixed sample (according to Secchi disk transparency depth) was passed through a glass fiber filter (Whatman GF/C), which was kept frozen in aluminum foil until the time of analysis. The analyses were performed within 10 days maximum. Chlorophyll was extracted using 96 % ethanol and was determined according to German standard DIN 38412-L16 (Nixdorf et al. 1995).

4.2.5 Phytoplankton analysis

A concentrated net sample (25 µm) was used for the qualitative analysis of phytoplankton, especially cyanobacterial species (generation of a species list), as described above. Taxonomic classification of the cyanobacteria was done mainly according to the recommendations of Geitler (1932), Huber-Pestalozzi (1938), Komárek and Ettl (1958), Horecká and Komárek (1979), and Hindák (1992, 2000). Quantitative analyses of the individual species biovolumes were performed using 100 ml aliquots of mixed sample fixed in Lugol's solution. The samples were first filled into plankton counting chambers (Hydrobios) and allowed to settle. Cyanobacteria at the bottom of the chamber were then counted and measured using an inverted microscope according to the methods of Utermöhl (1958) and Rott (1981) (see also Rott et al. 2007).

4.2.6 Cylindrospermopsin analysis

Cylindrospermopsin analyses were accomplished by LC-MS/MS, a method that combines analytical HPLC (high-performance liquid chromatography) with tandem mass spectrometry (MS/MS) for reliable detection of even small traces of toxin in a complex matrix. LC-MS/MS systems use two serially linked mass spectrometers (MS/MS), which drastically increases the selectivity. In the selected reaction mode

monitoring (SRM), the first mass spectrometer selects an analyte from of a variably complex mixture of substances. The target analyte is then fragmented in the collision cell, and selected substance-specific fragments are analyzed by the second mass spectrometer.

Particulate CYN concentrations were measured in either freeze-dried or filtered and frozen seston samples that were extracted twice using purified water (Welker et al. 2002). During each extraction step, the samples were treated for 10 min in an ultrasonic bath and shaken for 1 hour. The samples were then centrifuged and the supernatant removed. The supernatants were combined, dried in a vacuum centrifuge and stored at -20°C until the time of analysis. Before analysis, each sample was redissolved in 1 ml of water, centrifuged and filtered (0.45 µm pore size). For dissolved CYN, the samples were filtered (0.45 µm) before measurement and directly injected without further processing.

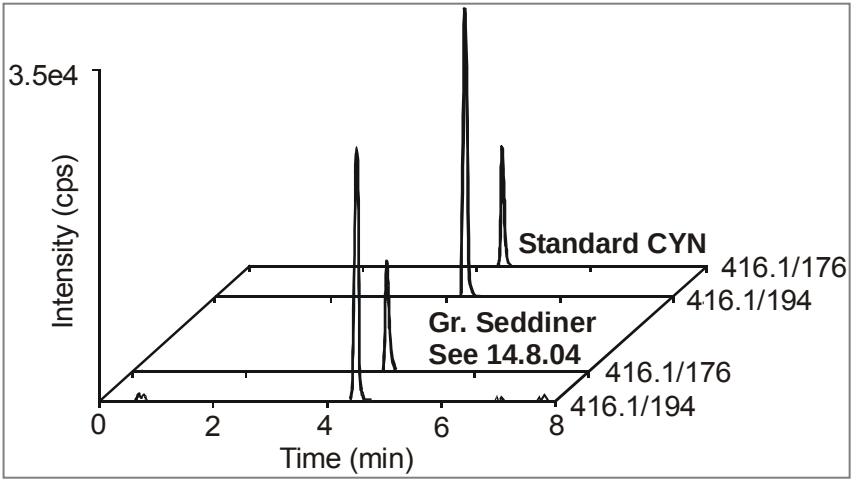


Fig. 10: Selected reaction monitoring (SRM) chromatogram for cylindrospermopsin standard and sample taken from Grosser Seddiner See on 14 Aug 2004 showing the characteristic transitions for cylindrospermopsin (*m/z* 416.1/194 and 416.1/176).

Analysis of CYN was accomplished by LC-MS/MS using an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 4000 triple quadrupole mass spectrometer system (Applied Biosystems/MDS Sciex, Framingham, MA) with a TurboIonSpray Interface. The extract was separated on a NovaPak C18 column (150 x 4.6 mm, 5 µm particle size; Waters, USA) under the following conditions: temperature: 30 °C; flow: 0.8 ml min^{-1}; solvent gradient: 1 min of 100 % A, to 100% B in 5 min 100 % B, held for 3 min, and back to 100% A in 1 min (A = 1 % methanol/water, B = 60 % methanol/water, and solvent A and B = 5 mM ammonium acetate). The equilibration time was 7 minutes.

The mass spectrometer was operated in the positive SRM mode with a collision energy of 48 eV. For the determination of CYN (*m/z* 416.1 [M+H+]) the characteristic transitions *m/z* 416.1/194 and 416.1/176 were monitored with a dwell time of 0.2 sec (Fig. 10). Quantification of CYN was accomplished using the *m/z* 416.1/194 transition. The detection limit was ca. 10 pg on column, corresponding to 0.002 µg L^{-1} for particulate CYN and 0.05 µg L^{-1} for dissolved CYN (after accounting for the employed filtered water volume, solvent volume, and injection volume).

5. Distribution of *C. raciborskii* and other neo-cyanobacteria

Anke Stüken

We use the term neo-cyanobacteria to describe non-indigenous cyanobacteria that have spread to Germany and are now distributed in German waters. Other authors have referred to *C. raciborskii* as a neophyte (Mischke 2003). This term suggests that *C. raciborskii* belongs to the phylum Phyta or Viridiplantae and to the kingdom Eukaryota—which is not correct. Like all other cyanobacteria, *C. raciborskii* belongs to the phylum Cyanobacteria and the kingdom Eubacteria. We therefore feel that the term "neo-cyanobacterium" is more appropriate.

The objective of the 2004 screening program was to obtain an overview of the spatial distribution and relative frequency of occurrence of *C. raciborskii* and other potential CYN-producing neo-cyanobacteria. A total of 142 water bodies in the Berlin-Brandenburg region were sampled for this purpose (see Chapter 3.1). The results show that C. raciborskii (Fig. 11) is much more widespread in German waters than was previously assumed. The regional distribution of *C. raciborskii* in Berlin-Brandenburg is shown in Figure 12. In the summer of 2004, we found this cyanobacterium in 39 of the 142 water bodies sampled. In terms of frequency of occurrence, *C. raciborskii* occurrence was classified as "rare" in the majority of the cases, but as "common" in nine lakes (Fig. 13). Regional occurrence of the species did not display a geographical pattern. Statistical analysis of environmental parameters showed that *C. raciborskii* occurred in a wide variety of lake types. It was present in deep and shallow lakes and in lakes with high and lower Secchi depths as well as in small and large lakes (surface area: 0.06 to 12.07 km^2, volume: 0.20 to 108.23 million m^3; Fig. 14). However, lakes in which *C. raciborskii* occurred were significantly different from those in which it did not occur: those where it was detected were more shallow and had low Secchi depths (Mann-Whitney U test: maximum lake depth, $n = 132$, $Z = -2.871$, $P = 0.004$; Secchi depth, $n = 136$, $Z = -3.861$, $P < 0.001$). Since there was a strong correlation between lake depth and Secchi depth (Spearman's rank order correlation: $n = 127$, $r_S = 0.727$, $P < 0.001$), it was not possible to analyze the individual effects of depth and turbidity on *C. raciborskii* occurrence in this study. Surface area and volume of the water body did not have a direct effect on *C. raciborskii* occurrence.

Our extensive search for unpublished and non-refereed publications and communications (gray literature) on *C. raciborskii* yielded 24 written reports on the occurrence of this species in German waters dating between 1994 and 2003 (Zippel 1996; Weithoff 1998; Mischke 2001; Täuscher 2003a, b, 2004a, b; Teubner et al. 2004). We also received 9 other reports from the same time period by oral communication (U. Mischke, L. Täuscher). Some of these reports included lakes surveyed in the scope of our 2004 screening program. All in all, *C. raciborskii* has been detected in 62 lakes in Germany to date.

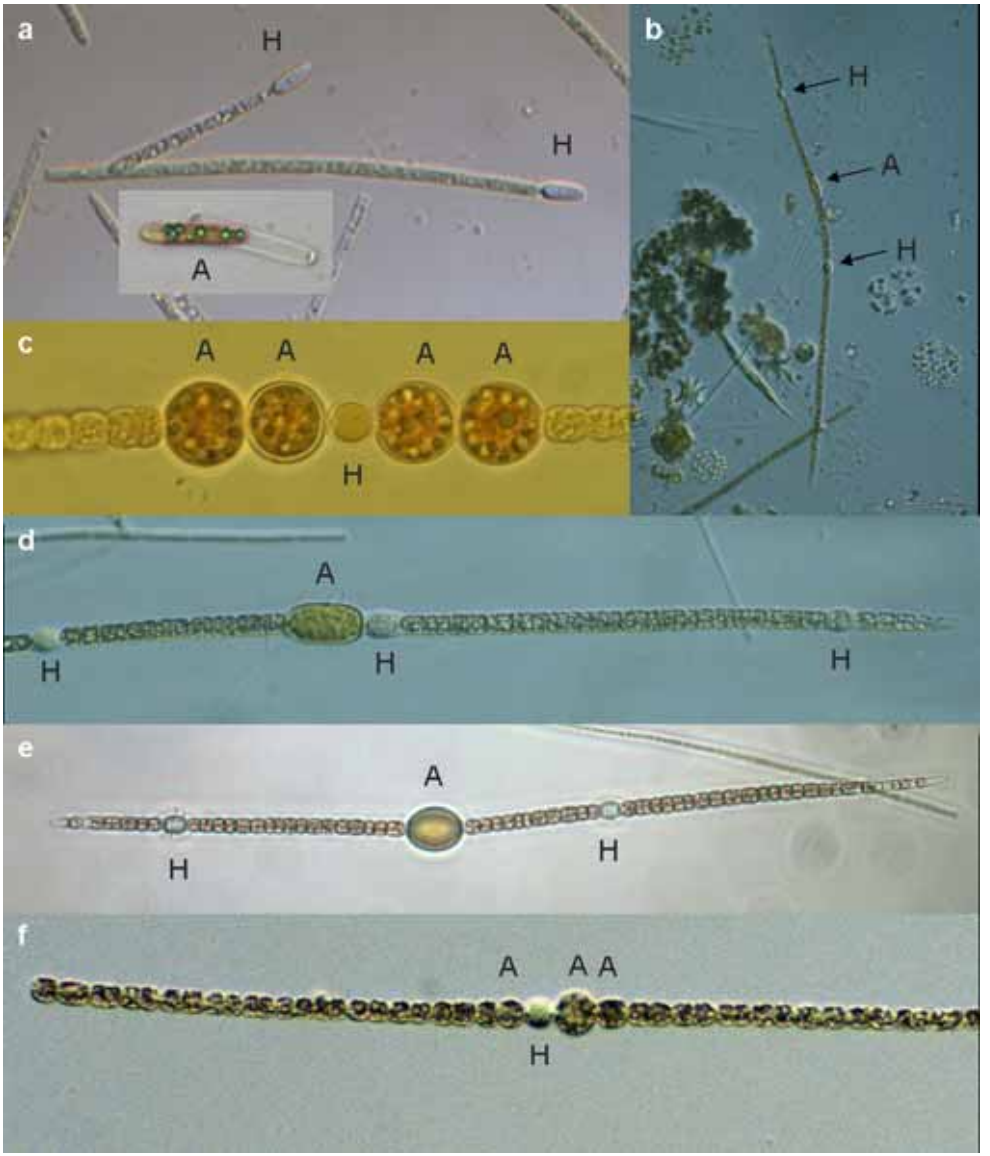


Fig. 11: Photographs of the three originally tropical cyanobacterial species now found in lakes in northeast Germany. **(a)** *C. raciborskii* (inlet: germinating akinetes); **(b)** *Anabaena bergii* in a field sample; **(d/e)** *Anabaena bergii*; **(c/f)** *Aphanizomenon aphanizomenoides.* A: Akinete. H: Heterocyst. Photo (c) was kindly provided by H. Täuscher.

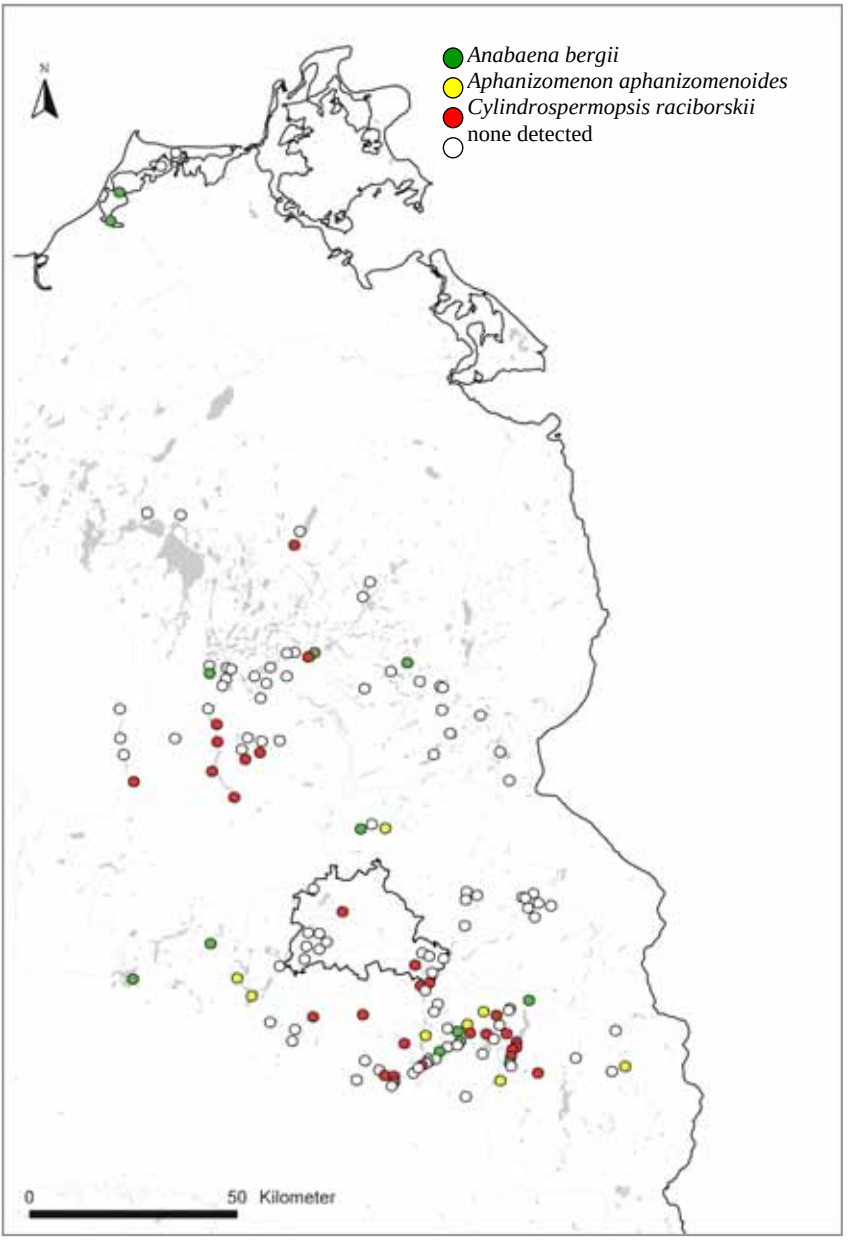


Fig. 12: Occurrence of *Cylindrospermopsis raciborskii, Anabaena bergii* and *Aphanizomenon aphanizomenoides* during the 2004 screening program.

Two other neo-cyanobacterial species besides *C. raciborskii* were also found in the Berlin-Brandenburg region: *Anabaena bergii* Ostenfeld 1908 (Figs. 11b, d, e) and *Aphanizomenon aphanizomenoides* (Forti) Horécka and Komárek 1979 (Figs. 11 c and f). These are the first reported sightings of these species in Germany. Both species have previously been detected in warmer climate zones such as the tropics, subtropics, and Southeast Europe (Komárek and Ettl 1958; Horecká and Komárek 1979; Cirkaltindag et al. 1992). Only recently have these species been reported in temperate latitudes: *A. aphanizomenoides* was reported in the Neuse River Estuary in the USA (Moisander et al. 2002) and *A. bergii* in a pond in Bratislava, Slovakia (Hindák and Hindákova 2003).

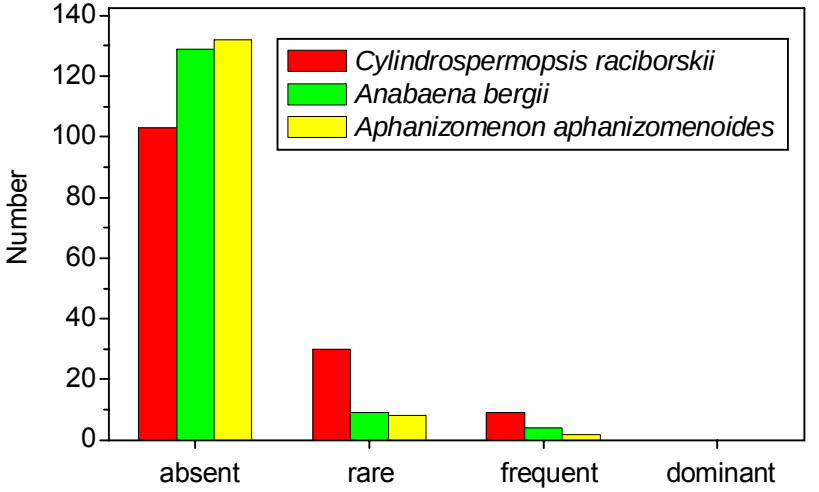


Fig. 13: Frequency of occurrence of *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides* in the surveyed water bodies (semi-quantitative assessment).

The distribution of *A. bergii* and *A. aphanizomenoides* in the Berlin-Brandenburg region is shown together with that of *C. raciborskii* in Figure 12. Like *C. raciborskii*, *A. bergii* and *A. aphanizomenoides* also did not exhibit a geographic distribution pattern. *A. bergii* was present in 13 of the 142 water bodies surveyed, including two slightly saline water bodies in the Darss-Zingst chain of lagoons ("*Bodden*"). As in the case of *C. raciborskii*, the waters in which *A. bergii* occurred were significantly more shallow and turbid than those in which it did not occur (Mann-Whitney U test: maximum lake depth, n = 132, Z = -2.590, P = 0.010; Secchi depth, n = 136, Z = -2.281, P = 0.023). In addition, neither lake surface area nor lake volume had an effect on *A. bergii* occurrence. *A. aphanizomenoides* was detected in 10 samples (Fig. 13), but none of the analyzed parameters had a significant effect on its occurrence.

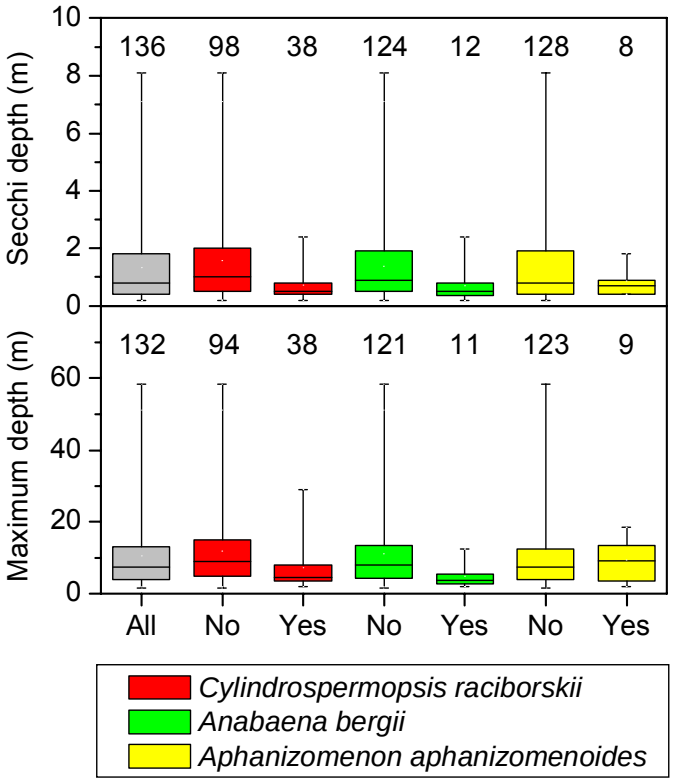


Fig. 14: Comparison of maximum depth and Secchi depth of the lakes in which *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides* were detected (YES) or not detected (NO). The bars on the vertical 'whiskers' mark the minimum and maximum data values, and the numbers above the bars indicate the number of samples studied. The boxes show the 95th percentile of the statistical distribution. The horizontal line dividing the box indicates the median value.

Like *C. raciborskii*, *A. bergii* and *A. aphanizomenoides* are cyanobacteria of the order Nostocales and, as such, they can produce heterocysts and akinetes. Photographs of filaments (trichomes) of all three, including heterocysts and akinetes, are presented in Figure 11. The filaments produced by *A. bergii are* shown in Figures 11 b, d and e. The position of the akinetes in Figure 11 d is unusual for *A. bergii* because its akinetes and heterocysts normally do not lie directly adjacent to one another. However, the shape of the akinetes, heterocysts, vegetative cells and terminal cells in particular are clearly characteristic of *A. bergii*. Figures 11 b and f show *A. aphanizomenoides* filaments with typically spherical akinetes lying directly adjacent to heterocysts. Table 1 provides a morphological overview comparing the filament types and sizes of these species.

Taxonomic classification of the two 'new' neo-cyanobacteria is problematic. *Aphanizomenon sphaericum* Kiselev and *Anabaena aphanizomenoides* Forti (Horéka & Komárek) are synonyms for *A. aphanizomenoides,* and the name *Anabaena aphanizomenoides* is still commonly used in the literature (Moisander et al. 2002, etc.). It is unclear which genus applies to this species: *Anabaena* or *Aphanizomenon*. In the case of *A. bergii*, taxonomic classification is even more unclear. Morphologically, *Anabaena minderi* Huber-Pestalozzi [= *A. bergii* var. *limnetica* Couté & Preising (Hindák 2000)] and *Aphanizomenon ovalisporum* Forti are very similar to *A. bergii*. *Anabaena minderi* and *A. bergii* differ only in terms of shape and akinete size (Hindák 2000), and it has even been suggested that *A. bergii* and *A. ovalisporum* may be different morphotypes of the same species (Komarék and Ettl 1958, Shaw et al. 1999, Fergusson and Saint 2000). The difficulties associated with identifying species that are the source of filaments derived from lake samples are well documented (Bazzichelli and Abdelahad 1994). In light of these difficulties, the possibility that the species found in this study might be classified elsewhere as *A. minderi* or *A. ovalisporum* cannot be excluded.

Mass development of *A. ovalisporum* has been reported in Israel (Banker et al. 1997) and Australia (Shaw et al. 1999). It was found that *A. ovalisporum* and *A. bergii* strains can synthesize the toxin cylindrospermopsin (Banker et al. 1997, Schembri et al. 2001, Fergusson and Saint 2003). *A. aphanizomenoides* strains are also known to be toxic, but the toxin they produce has not yet been identified (Hiripi et al. 1998).

Our screening program showed a) that *C. raciborskii* and at least two other neo-cyanobacterial species occur in Germany and b) that all three of these species are widespread and potentially toxic. Especially when dealing with these 'new' neo-cyanobacteria, more data is needed in order to estimate the hazard potential they may pose. [1]

[1] Project-related follow-up publications on this subject: Stüken et al. (2006), Stüken et al. (submitted).

Table 1: Morphology of *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides* filaments occurring in German waters.

Species	*C. raciborskii*	*A. bergii*	*A. aphanizomenoides*
Photos	Fig. 11a	Fig. 11 b, d, e	Fig. 11 c, f
Filaments	Solitary; do not form colonies; straight, curled or coiled; only the straight form has been reported in Europe	Solitary; do not form colonies; straight or slightly curved and usually tapered; at least one terminal cell is clearly pointed and wedge-shaped	Solitary; do not form colonies; straight or slightly curved and tapered
Vegetative cells	Cylindrical; length > width: length: 4 to 12 µm, width: 2 to 4 µm	Stout and barrel-shaped; length < width: length: 3 to 5 µm, width: 4 to 6 µm	Extremely variable in size and shape, but usually rounded & barrel-shaped; length < width: length: 3 to 6 µm; width: 4 to 6 µm; rounded terminal cells
Heterocysts	Solitary, always terminal, almost always distal to akinetes; flame-shaped; length: 3.5 to 6 µm, width: 1.5 to 2.5 µm	Solitary; 1 to 3 per filament; intercalary; usually distal to akinetes; roundish; diameter: 5 to 7 µm	Solitary; 0 to 3 per filament; spherical to ellipsoid; length: 5 to 6.5 µm, width: 5 to 6 µm
Akinetes	Usually solitary; long and cylindrical to slightly oval; length: 8 to 15 µm, width: 2.5 to 4 µm	Solitary and usually distal to heterocysts; oval to cylindrical; length: 11 to 20 µm, width: 10 to 16 µm	Solitary or multiple, in rows; directly adjacent to heterocysts, on one or both sides; spherical; diameter: 8 to 14 µm

6. Population dynamics of *C. raciborskii* in temperate regions and causes of its spread

Claudia Wiedner

As mentioned in the introduction, there are still many unanswered questions concerning the reasons for the spread of *C. raciborskii* to temperate regions and the mechanisms regulating its population dynamics and long-term development in those latitudes. To shed light on these important issues, we analyzed long-term data from Melangsee and Langer See, both of which are eutrophic, polymictic, shallow lakes. Detailed background information on these lakes can be found in our previous publications: Rücker et al. (1997), Mischke (2003), and Nixdorf et al. (2003).

Long-term development of *C. raciborskii*

Melangsee and Langer See have been sampled regularly since 1993, and *C raciborskii* has been detected at both lakes every year since 1995 (Fig. 15). In peak years, *C. raciborskii* contributed up to 23 % of the total cyanobacteria biovolume and 19 % of the total phytoplankton biovolume. The size of the fraction is substantial considering that *C. raciborskii* is a neo-cyanobacterium, so the spread of this species can justifiably be classified as invasive.

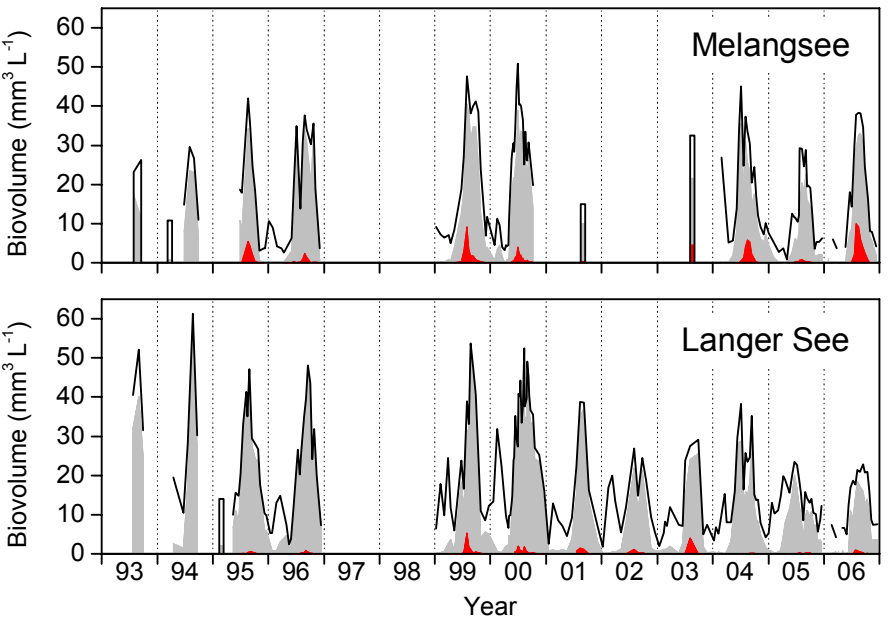


Fig. 15: *C. raciborskii* biovolume (red area), total cyanobacteria biovolume (gray area), and total phytoplankton biovolume (black line).

In view of the invasive nature of spread, it is important to address the following questions: When did *C. raciborskii* initially invade the region? How long did it take the species to establish its populations in German waters? Has the maximum population size in regional waters already been reached, or will the populations continue to grow? Can mass development of *C. raciborskii* be expected in the future?

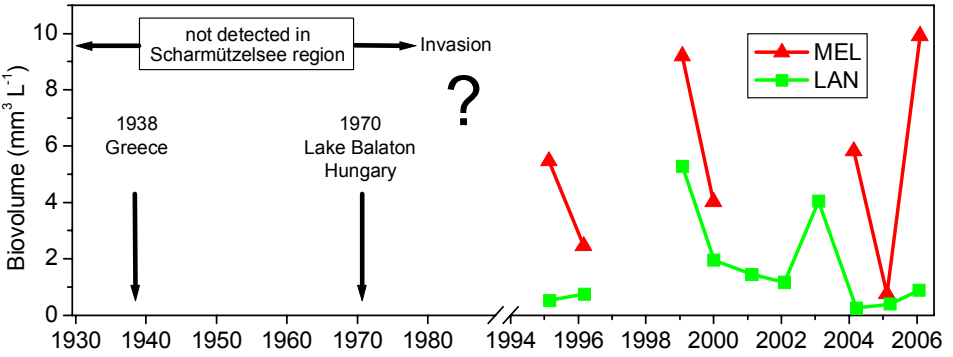


Fig. 16: Invasion of *C. raciborskii* to Europe and annual population peaks measured at lakes Melangsee (MEL) and Langer See (LAN).

The current data only provides partial answers to certain questions, as illustrated in the case of the two regional lakes. Figure 16 shows the most important European sites of *C. raciborskii* invasion over time and annual its peak biovolumes at Melangsee and Langer See. The time window for initial *C. raciborskii* invasion can be narrowed down to the years 1940 to 1990. *C. raciborskii* did not occur in the region prior to this period, as is supported by evidence from early limnological studies from the 1930s and 1940s (Czensny 1938, Wundsch 1940, Schäperclaus 1941). Since the *C. raciborskii* biovolumes measured at Lake Lieps in 1990 (Krienitz and Hegewald 1996) and at Melangsee and Langer See from 1995 on were quite considerable, one can safely assume that initial invasion occurred at an earlier time. Great inter-annual variation in maximum *C. raciborskii* population size was observed at both lakes. The study period was too short for a trend analysis, and the available data does not allow us to predict whether the species has already reached its maximum population size.

Regulation of *C. raciborskii* population dynamics

Well-founded knowledge of the mechanisms regulating *C. raciborskii* population dynamics is essential for forecasting the further course of species development.

To identify these mechanisms, we tested the data for correlations between the specific population growth rate (μ) and relevant growth-related parameters. The specific growth rate was calculated based on biovolume data using the following equation:

$$\mu = \frac{\ln Cbiov_2 - \ln Cbiov_1}{t_2 - t_1} \quad (1)$$

where $Cbiov_1$ is the *C. raciborskii* biovolume at sampling time t_1 and $Cbiov_2$ is the biovolume at sampling time t_2. Accordingly, μ is a net population growth rate that accounts for population losses due to feeding losses or decline, etc. We tested for correlations between the growth rate and environmental parameters using Pearson's correlation analysis, which was run using a statistical software package (SPSS 12.0 for Windows, SPSS Inc.); the calculated μ values were assigned to time t_2 on the data matrix. In addition, the mean annual course of growth rate over time was compared with the mean annual course of environmental parameters. This was done by extracting the mean annual courses for light intensity (I_{mix}), water temperature, DIN, DIP and *C. raciborskii* biovolume from the pooled data for the entire observation period (1993 to 2005) for both lakes individually using the "Polynomial Fit" tool of the Origin Pro 6.1 graphics program (OriginLab Corporation, Northampton, USA). The order of polynomial fit was adjusted to ensure an optimal statistical result and good curve fit. Since nonlinear curve fits (exponential and quadratic) did not improve the statistical results for specific growth rate $\mu_{trend}(t)$, we opted for linear regression using the pooled data (Fig. 17).

$$\mu_{trend} = a \cdot t + b \quad (2)$$

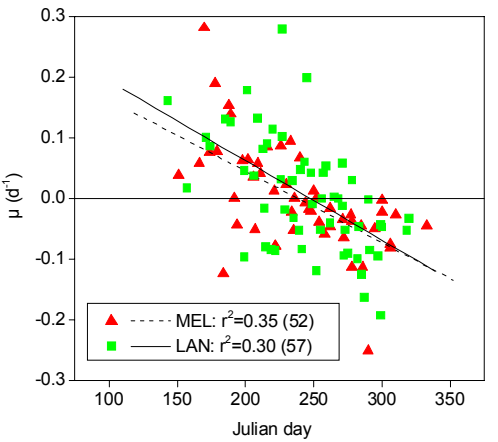


Fig. 17: *C. raciborskii* growth rate (μ) over time (Julian days) at Melangsee (MEL) and Langer See (LAN) and results of linear regression (r^2) with number of samples (n) in parentheses.

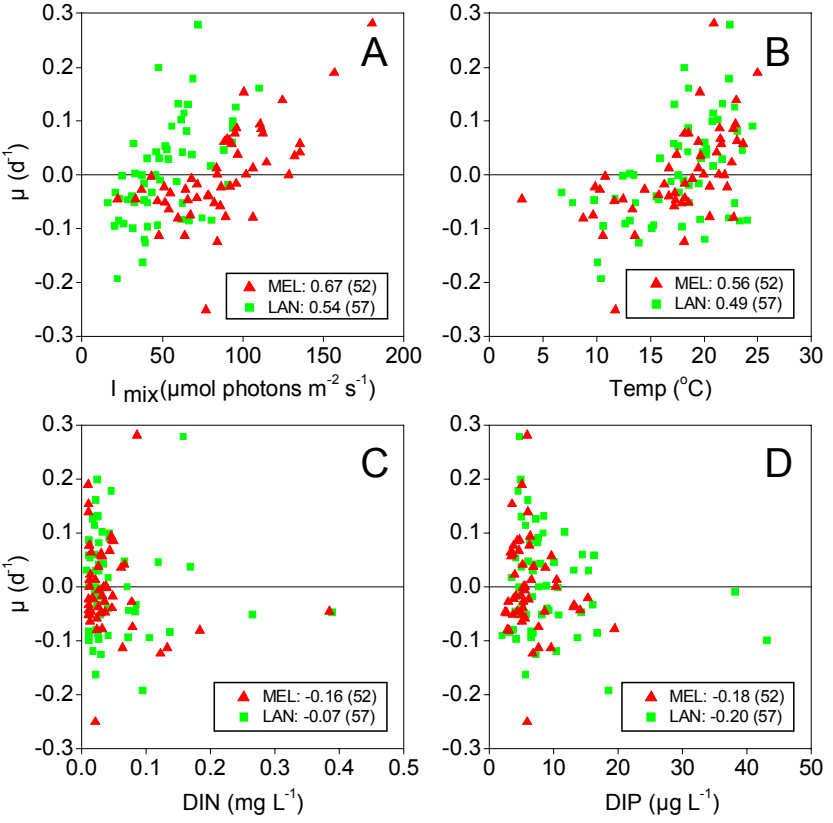


Fig. 18: (A) *C. raciborskii* growth rate (μ) as a function of mean light intensity in the mixed layer (I_{mix}), (B) temperature and concentration of (C) dissolved inorganic nitrogen (DIN) and (D) phosphorous at Melangsee (MEL) and Langer See (LAN), including the results of the correlation analysis, i.e., correlation coefficients and number of samples (n).

The correlation analysis revealed significantly positive correlations between growth rate and mean light intensity (I_{mix}) and temperature, but no significant correlations between growth rate and dissolved inorganic phosphorous (DIP) or nitrogen (DIN) (Fig. 18). This suggests that *C. raciborskii* population growth should increase with increasing light intensity (I_{mix}) and temperature. However, the plot of population growth (μ) versus temperature clearly shows that temperature can be ruled out as a growth-limiting factor until the time of maximum population size (Fig. 19). Unlike the growth rate, which decreases linearly over the course of the year, temperature rises during the first half of the growth phase, so it cannot have a positive effect on growth during this time. Light intensity, on the other hand, peaked before the beginning of the growth phase and decreased parallel to the growth rate. We therefore consider light intensity (I_{mix}) to be the most important determinant of *C. raciborskii* growth.

Temperature does, however, play an important role in controlling the start of the annual pelagic populations. The first *C. raciborskii* filaments were observed at temperatures between 15 and 17°C. These findings suggest that the germination of *C. raciborskii* akinetes is temperature-dependent.

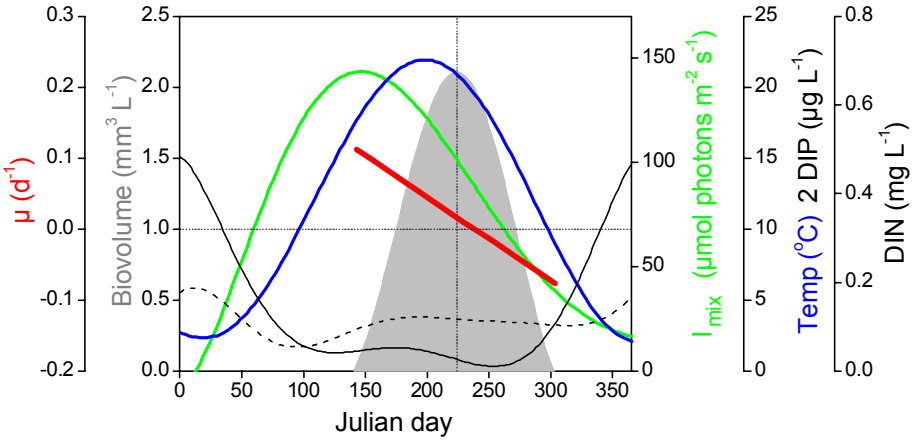


Fig. 19: Mean annual courses of biovolume (gray area) and specific growth rate (μ, red) of *C. raciborskii* plus mean intensity of photosynthetically active radiation in the mixed water column (I_{mix}, green), water temperature (Temp, blue), and concentrations of dissolved inorganic phosphorous (DIP, black dotted line) and nitrogen (DIN, black solid line) as determined from the pooled data for Melangsee from 1993 to 1995.

The overall results of the data analyses permit us to draw the following summary conclusions concerning the regulation of *C. raciborskii* population dynamics:

- Akinete germination is temperature-dependent and takes place at temperatures below 15 °C.
- Pelagic growth is regulated by light intensity, which acts as a growth-limiting factor right from the beginning (Fig. 20).
- The low levels of dissolved inorganic nitrogen and phosphorous (Fig. 19) suggest that phytoplankters compete with *C. raciborskii* for these nutrients during the summer months. In cases of nitrogen deficiency, *C. raciborskii* would have a competitive advantage because of its ability to fix gaseous nitrogen.

These findings shed new light on the regulation of this tropical cyanobacterial species in temperate latitudes. Until now, *C. raciborskii* growth in temperate climate zones was assumed to be limited by temperature — which is completely plausible considering that this is a tropical cyanobacterium. This assumption seemed to be supported by evidence like the coincidence of population and temperature peaks (Hamilton et al. 2005, etc.). Our study also revealed a temporal coincidence of population and temperature peaks. However, this coincidence appears to be

purely accidental — our results clearly demonstrate that *C. raciborskii* growth is not limited by temperature. Furthermore, it was previously assumed that *C. raciborskii* is a shade-adapted species that benefits from low light conditions (Dokulil and Mayer 1996, Padisák and Reynolds 1998, Bouvy et al. 1999, Briand et al. 2002a, Mischke 2003, etc.). Our findings clearly refute this assumption: We found that *C. raciborskii* growth is light-limited from the beginning. Previous studies failed to detect this because the parameters used for assessment of light intensity either measured only light attenuation (attenuation coefficient, transparency depth, etc.) without taking incident radiation into consideration or they measured only incident radiation without taking the attenuation of light in water into account. Also, there were few data sets available in the past that had a temporal resolution suitable for growth rate analysis. Consequently, most researchers analyzed correlations between environmental parameters and biovolume. This method yields an assessment of the current state of conditions under which a species occurs but does not address the issue of growth regulation processes.

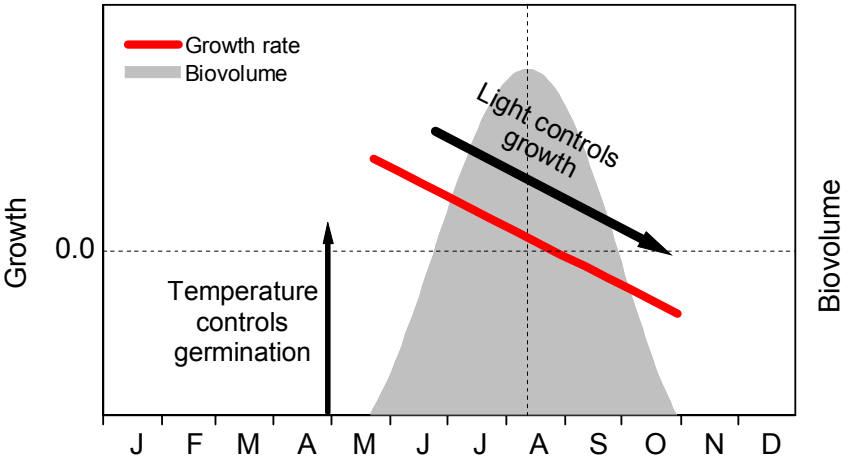


Fig. 20: Schematic representation of *C. raciborskii* population dynamics over the course of a year, showing the effects of the growth-limiting factors "light" and "temperature".

Two of the most important findings of the CYLIN project were that *C. raciborskii* germination is temperature-dependent and that light intensity acts as a growth-limiting factor. Since light intensity is highest before the start of the pelagic phase, any shift in the life cycle to an earlier period improves the light conditions and, thus, the growth conditions for this species.

It is important to remember that climatic conditions frequently affect the phenology of a species, that is, the annual course of its life cycle. An earlier onset of spring activities has generally been observed since the 1960s (Walther et al. 2002). Especially in aquatic ecosystems in northern temperate regions, scientists have observed changes in the phenology of plankton communities, which have been attributed to higher winter and spring temperatures. At the German lakes Constance and Müggelsee, *Daphnia* populations now tend to appear two weeks earlier than in previous years (Straile 2000, Gerten and Adrian 2000). Typical spring phytoplankters appeared 20 days earlier at Lake Washington, USA (Winder and Schindler 2004), 30 days earlier at Lake Erken, Sweden (Weyhenmeyer et al. 1999), and 30 days earlier at Lake Müggelsee, Germany (Gerten and Adrian 2000). Investigators studying the regulatory mechanisms of North Sea plankton did not detect a seasonal shift in light-dependent life cycles, whereas temperature-dependent life cycles were shifted to an earlier season (Edwards and Richardson 2004).

Considering that the start of the pelagic phase in *C. raciborskii*'s life cycle is temperature-dependent, one must assume that *C. raciborskii* populations would tend to benefit from global warming. We therefore tested for effects of life cycle shift on *C. raciborskii* population size. The following mathematical model was developed and used to simulate population size for different points in the germination phase:

$$\mathrm{d}\ Cbiov/\mathrm{dt} \sim \mu(\mathrm{t}) \qquad (3)$$

where d $Cbiov$ is the change in *C. raciborskii* biovolume and *dt* is the change in time. This model uses the specific growth rate (μ) as a function of time (t), as was determined based on the pooled μ_{trend} data for Melangsee (equation 2). The computed slope of the linear regression line (*a*) was -1.177 10^{-3}, and the absolute constant (*b*) 0.28. Both values were highly significant ($p<0.001$). Introduction of the proportionality factor *skal* (= 0.15) yielded the following model:

$$\frac{d\,Cbiov}{dt} = \begin{cases} skal \cdot \mu_{trend}(t) \ \ if \ \ t \geq t_{begin} \\ 0 \ \ else \end{cases} \qquad (4)$$

The simulations were run using Model Maker 3.0 software. Population size was simulated using three different times for the start of germination (t_{begin}) over the course of the year: Julian day 90, 120 and 150. Two different scenarios were simulated. In the first, equal numbers of akinetes (biovolume: 1 $mm^3\ L^{-1}$) were assumed to be present on all three Julian days. The basic assumption of the second scenario was that germination takes place earlier each year, leading to a corresponding increase in *C. raciborskii* population size and akinetes number each

year. Akinete number i.e. biovolume, which was assumed to increase linearly, was defined as: 1.5 mm^3 L^{-1} at t_{begin} = day 90, 1.0 mm^3 L^{-1} at t_{begin} = day 120, and 0.5 mm^3 L^{-1} at t_{begin} = day 150.

According to the simulation results, the population size doubles when germination takes place 60 days earlier while the number of akinetes remains constant. If the annual increase in akinete number is also simulated, doubling of population size occurs when germination takes place 30 days earlier (Fig. 21).

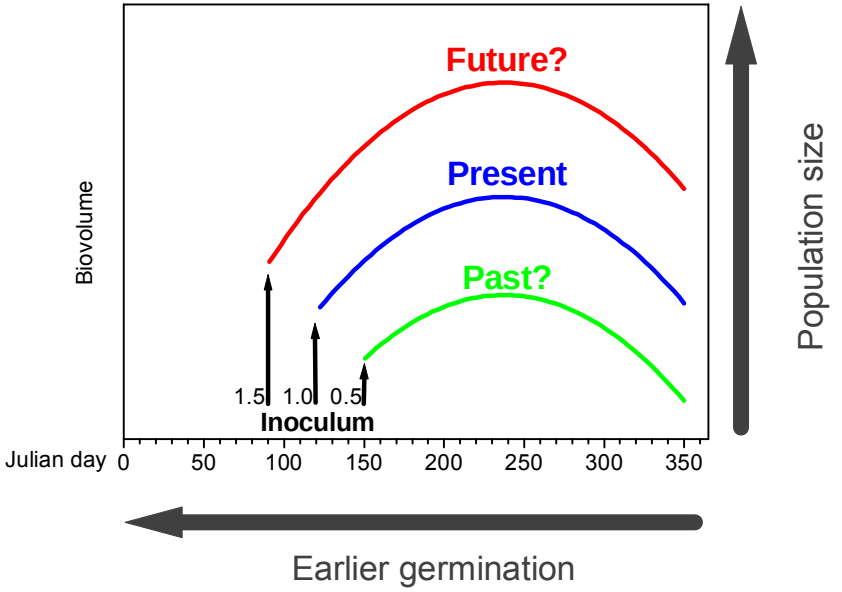


Fig. 21: Simulation of *C. raciborskii* population size assuming that akinete germination occurs on Julian day 120 and that the number of akinetes (inoculum size) equals 1.0, corresponding to the situation today. Past and future population development was also simulated assuming earlier and later times of germination and lower and higher inoculum sizes, respectively.

Based on these findings, there is a high probability that the climate-related earlier rise in water temperatures over the course of the years has promoted the spread of *C. raciborskii*. We predict that, if this trend continues, the size of *C. raciborskii* populations will continue to grow.

It is highly probable that the identified regulatory mechanisms for *C. raciborskii* population dynamics also apply to the other two neo-cyanobacteria, *Aphanizomenon aphanizomenoides* and *Anabaena bergii*, as well as to indigenous Nostocales. This hypothesis should be tested when analyzing the development of phytoplankton in our waters. Current gaps in our knowledge about the life cycle of Nostocales must also be closed. Last but not least, the extent to which adaptation and selection mechanisms play a role in the spread of tropical species must also be determined. [2]

[2] Project-related follow-up publications on this subject: Wiedner et al. (2007), Tingwey et al. (2007), Rücker et al. (2006), and Haande et al. (submitted).

7. Distribution of cylindrospermopsin

Jutta Fastner

The distribution of cylindrospermopsin (CYN) in Germany was studied based on samples from 127 Berlin-Brandenburg water bodies with different trophic and morphological characteristics. All water bodies studied are listed in the Appendix. Methods of sampling, phytoplankton analysis and CYN analysis are described in sections 4.2.3, 4.2.5, and 4.2.6, respectively.

Until now, there were only sporadic reports of cylindrospermopsin in Europe (and Germany) and comprehensive data on the distribution of cylindrospermopsin were not available. The present data set is therefore unique, not only for Germany, but also for the rest of Europe.

Cylindrospermopsin was frequently detected in the study region, i.e., in roughly half of the investigated water bodies and samples (Fig. 22). The frequency of CYN occurrence in NE Germany is therefore similar to that in Australia and North America (McGregor and Fabbro 2000, Burns et al. 2000) and equal to that of microcystin, which was previously considered be the most common cyanotoxin in Germany (Fastner et al. 1999).

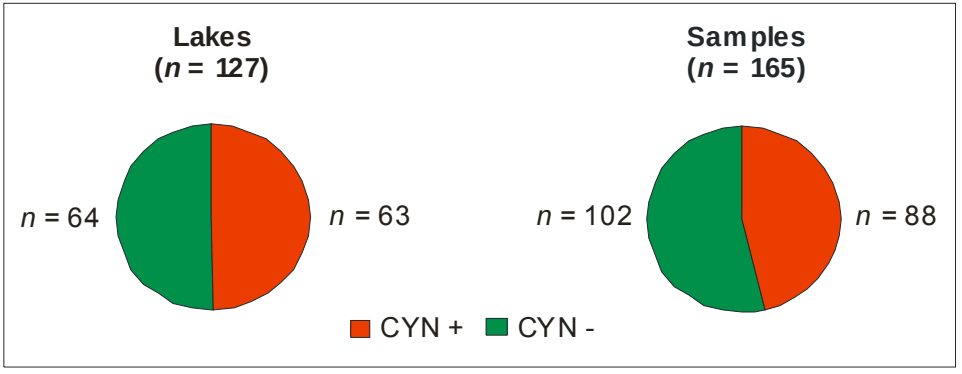


Fig. 22: Frequency of occurrence of cylindrospermopsin (CYN) in 127 water bodies (n = 165 samples) in the Berlin-Brandenburg region.

Cylindrospermopsin was distributed throughout the entire study region and was not restricted to any specific local areas (Fig. 23). This also applied to the measured CYN concentrations; no local area exhibited only high or only low cylindrospermopsin concentrations (Fig. 23).

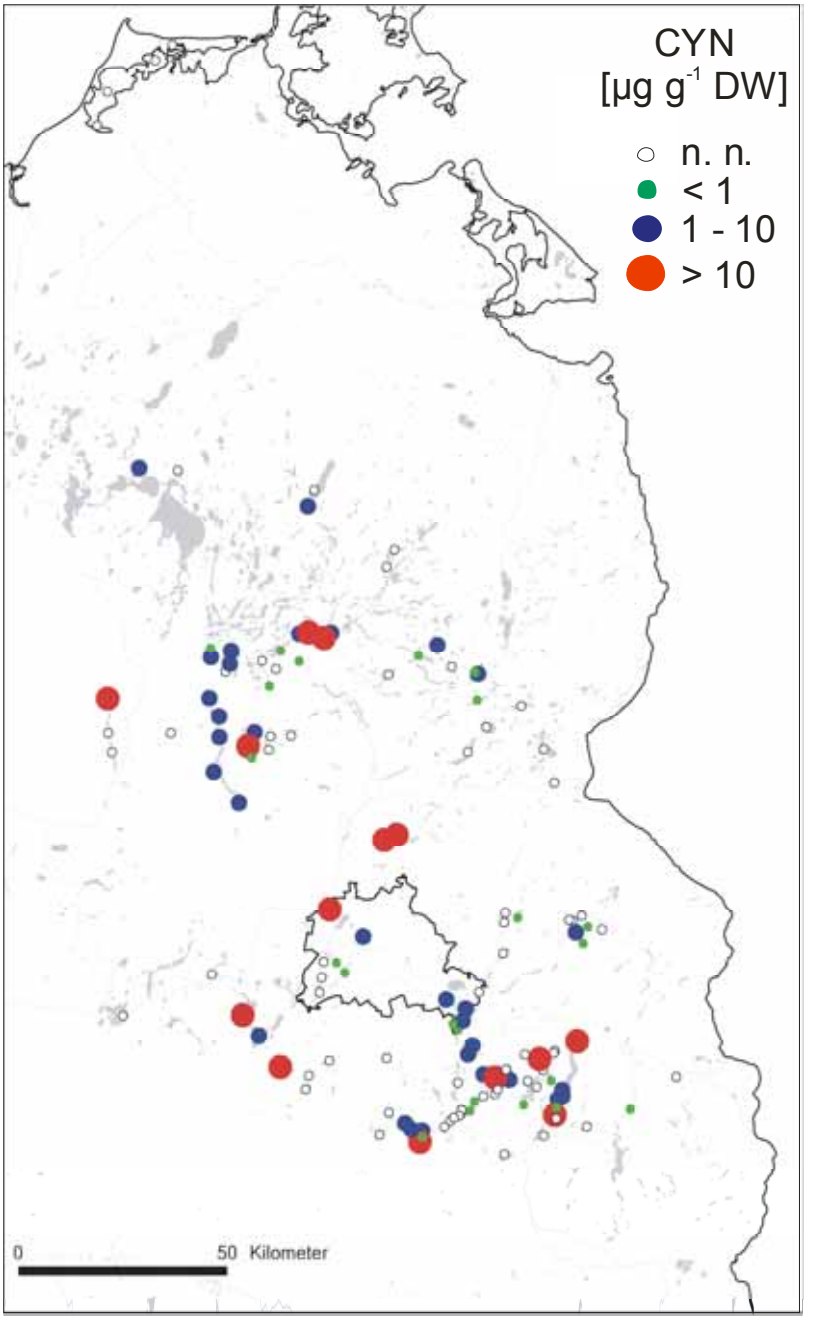


Fig. 23: Distribution of cylindrospermopsin (CYN) in the study region.

Cylindrospermopsin concentrations in the study region ranged from 0.1 to 73.2 µg g^{-1} dry weight (site: Grosser Plessower See), with a median of 2.3 µg g^{-1} dry weight (Fig. 24). CYN concentrations were lower than 10 µg g^{-1} dry weight in over 80 % of samples and higher in only 18 %. These values are in the range of the cylindrospermopsin concentrations determined for the first time in Langer See and Melangsee in 2000 (Fastner et al. 2003). Isolated CYN-producing strains of various species, including the *Aphanizomenon flos-aquae* strains found in the study region (Chapter 8), generally produce significantly higher CYN concentrations (ca. 0.5 to 6 mg g^{-1} dry weight) (Banker et al. 1997, Hawkins et al. 1997, Preußel et al. 2006). The lower concentrations in the field can be attributed to either a low abundance of CYN-producing species or genotypes in the water bodies (toxin-producing and non-producing genotypes of the same species generally occur simultaneously) and/or to the production of lower cylindrospermopsin concentrations by the toxin-producing genotypes compared to the isolated strains. Last but not least, it is highly probable that not the entire CYN concentration in the water was determined. This study (Chapter 10) and others have shown that over 90 % of cylindrospermopsin may be present in the dissolved fraction. Dissolved CYN passes through typical meshes and is not included in samples harvested using plankton nets.

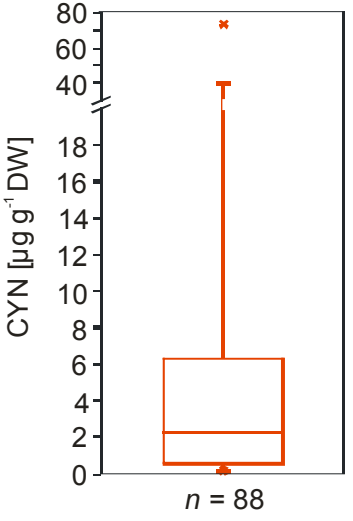


Fig. 24: Box whiskers plot of cylindrospermopsin (CYN) concentration (µg g^{-1} DW) in 88 seston samples.

Box plot: The box represents 50% of values between the 25th and 75th percentile, and the vertical lines ('whiskers') represent the minimum and maximum data values. The horizontal line dividing the box indicates the median value. Extreme outliers are represented by an asterisk.

In order to identify potential CYN producers in the water bodies representatives of the order Nostocales were determined in the samples since most CYN-producing cyanobacteria identified to date belong to this order. In addition to *Cylindrospermopsis raciborskii, v*arious *Anabaena*, *Aphanizomenon*, and *Raphidiopsis* species were also found in the water bodies. Most samples contained more than one genus and, in the case of *Anabaena* and *Aphanizomenon*, more than one species. Since unequivocal species classification was not possible in all cases, the detected species were grouped at the genus level.

Most of the samples (n = 61) contained a combination of different *Aphanizomenon* and *Anabaena* species; 26 contained *Aphanizomenon* and *Anabaena* species plus *C. raciborskii*, and 14 samples contained *Aphanizomenon* species plus *C. raciborskii* (Fig. 25). *Raphidiopsis* occurred in combination with *Aphanizomenon, Anabaena* and/or *C. raciborskii* in a total of 17 samples. However, since some of these combinations occurred only once, they were not included in Figure 25 for reasons of clarity. Cylindrospermopsin was detected in 50 to 90 % of samples from all of these groups, and there were no signs of especially high or low CYN concentrations in the individual groups.

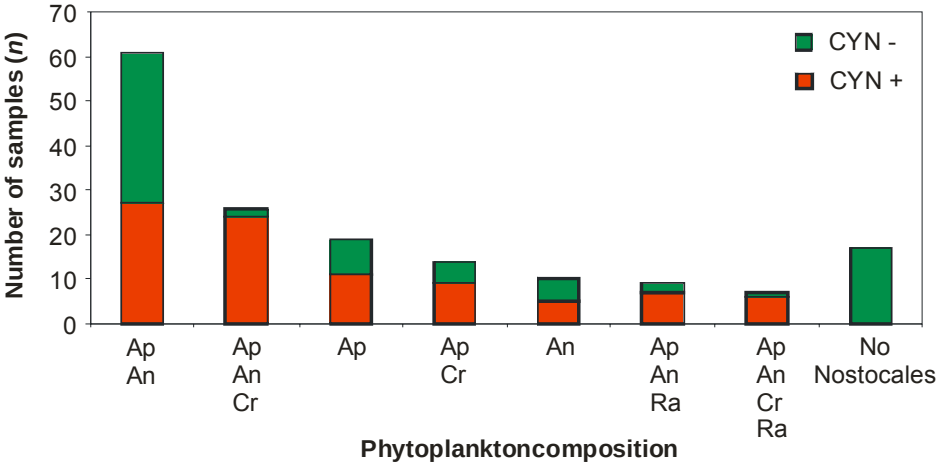


Fig. 25: Frequency of samples with different combinations of Nostocales. The total number of samples per group and the number of cylindrospermopsin-positive (CYN +) and cylindrospermopsin-negative (CYN –) samples are indicated by the red and green column segments. Ap = *Aphanizomenon*, An = *Anabaena*, C r = *C. raciborskii*, Ra = *Raphidiopsis*.

Raphidiopsis was the sole representative of the order Nostocales in only one sample, which did not contain any cylindrospermopsin (data not shown). Consequently, it is still unclear whether the native *Raphidiopsis* species produce CYN, as has been detected in a strain of *Raphidiopsis curvata* in Thailand (Li et al. 2001a).

Exclusively *Aphanizomenon* (*n* = 19) or *Anabaena* species (*n* = 10) were found in the remaining samples, roughly half of which contained cylindrospermopsin (Fig. 25). These findings clearly identify *Aphanizomenon* and *Anabaena* species as potential CYN producers in Germany, which was confirmed by the identification of *Aphanizomenon flos-aquae* as a CYN-producing organism in the lakes studied here (Preußel et al. 2006). *Aphanizomenon ovalisporum* (Banker et al. 1997, Shaw et al. 1999), *Anabaena bergii* (Schembri et al. 2001), and *Anabaena lapponica* (Spoof et al. 2006) are also known to produce cylindrospermopsin. It is very likely that even more species of these genera will be identified as CYN-producing cyanobacteria in the future.

Apart from Nostocales, *Umezakia natans,* a member of the order Stigonematales, and most probably *Lyngbya wollei* (Oscillatoriales) also produce cylindrospermopsin (Harada et al. 1994, Seifert et al. 2007). Currently, it still cannot be definitively determined whether cylindrospermopsin has as similar taxonomic distribution as other cyanotoxins (such as anatoxin-a and microcystin) and is also produced by cyanobacteria of other orders, such as Chroococcales and Oscillatoriales. In this study, cylindrospermopsin was not present in any of the 17 samples not containing Nostocales, but most of them also did not contain other cyanobacteria.

Likewise, the role of *C. raciborskii* as a CYN producer in Germany is still unclear and could not be determined based on the screening results because none of the field samples contained *C. raciborskii* alone. As before in 2000, no CYN-producing strain of *C. raciborskii* could be isolated from the water bodies studied in the CYLIN project (Fastner et al. 2003, Chapter 8). This also has not been possible in other European countries (Saker et al. 2003; Bernard et al. 2003). Although the cylindrospermopsin detected in two Italian lakes has been ascribed to the occurrence of *C. raciborskii*, this has not been confirmed by isolates (Manti et al. 2005). The continued spread of *C. raciborskii* necessitates further study of CYN production of this species in Europe.

Considering the frequency of occurrence of cylindrospermopsin in this study and evidence that *Aphanizomenon* and *Anabaena* produce this cyanotoxin, more attention should be devoted to study the occurrence of this toxin in Germany and Europe. *Aphanizomenon* and *Anabaena* are commonly part of the summer phytoplankton in our latitudes, and the quantitative data (Chapter 10) show that, as in Spain and Italy, the CYN concentrations in Germany are clearly above the proposed WHO drinking water guideline value of 1 µg l^{-1} (Humpage and Falconer

2003, Manti et al. 2005, Quesada et al. 2006). Also much higher CYN concentrations of 100 µg l^{-1} (or multiples thereof) as observed during blooms of *C. raciborskii* or *A. ovalisporum* in Australia and North America cannot be excluded for Germany as *Aphanizomenon flos-aquae* can form persistent blooms in temperate regions (Kann and Welch 2005). [3]

[3] Project-related follow-up publications on this subject: Fastner et al. 2007.

8. Identification of cylindrospermopsin-producing cyanobacteria

Karina Preußel

Cylindrospermopsin (CYN) was first discovered in Brandenburg in 1999 in plankton samples from two regional lakes, Melangsee and Langer See (Fastner et al. 2003). This was also the first report of the finding of CYN in Europe. Since *Cylindrospermopsis raciborskii*, which is also known to produce cylindrospermopsin (Ohtani et al. 1992), had also been detected at both lakes, the objective of the first strain isolation studies was to confirm that the CYN was being produced by this cyanobacterium. However, CYN was not found in any of the *C. raciborskii* strains isolated from Melangsee. Furthermore, none of the gene sequences presumed to be involved in CYN synthesis in tropical *C. raciborskii* strains have been detected (Schembri et al. 2001). The fact that none of the *C. raciborskii* strains isolated from European waters to date produce the toxin (Bernard et al. 2003, Saker et al. 2003) supports our assumption that other species must be responsible for the occurrence of cylindrospermopsin in our waters.

Other cyanobacterial species that produce CYN in subtropical and tropical waters have already been identified (see Chapter 1). Since most of the known CYN producers are cyanobacteria of the order Nostocales, our isolation studies targeted this group as the most likely producers of cylindrospermopsin.

Isolation of potential CYN producers

Different isolation strategies were used. First, individual filaments were isolated directly from fresh plankton samples obtained from water bodies with high abundances of cyanobacteria of the order Nostocales. Plankton samples with low densities of candidate species were cultivated in nitrogen-free culture medium. Since cyanobacteria of the order Nostocales are able to fix atmospheric N_2, cultivation in nitrogen-free medium allows them to grow preferentially. The specimens were enriched in this manner before isolation. The same type of enrichment culture was used for a sediment sample from Langer See. The enrichment cultures were incubated for approximately 2 weeks at 20 °C to 25 °C with a light intensity of 65 µE m^{-2} s^{-1} and a 12/12-hour hour light-dark cycle.

The individual strains were isolated using sterile equipment only. Phytoplankton material (fresh or enrichment cultures) was transferred to Petri dishes and then washed and diluted with Z8 medium until it was possible to transfer individual filaments to a microtiter plate using an ultra-thin Pasteur pipette under stereomicroscopic control. After addition of 300 µL Z8 medium to each well of the microtiter plate, the samples were incubated under the same conditions as the enrichment cultures. Successfully cultured isolates were first transferred to 5-ml reaction vessels and later to a 50-ml Erlenmeyer flask (Fig. 26).

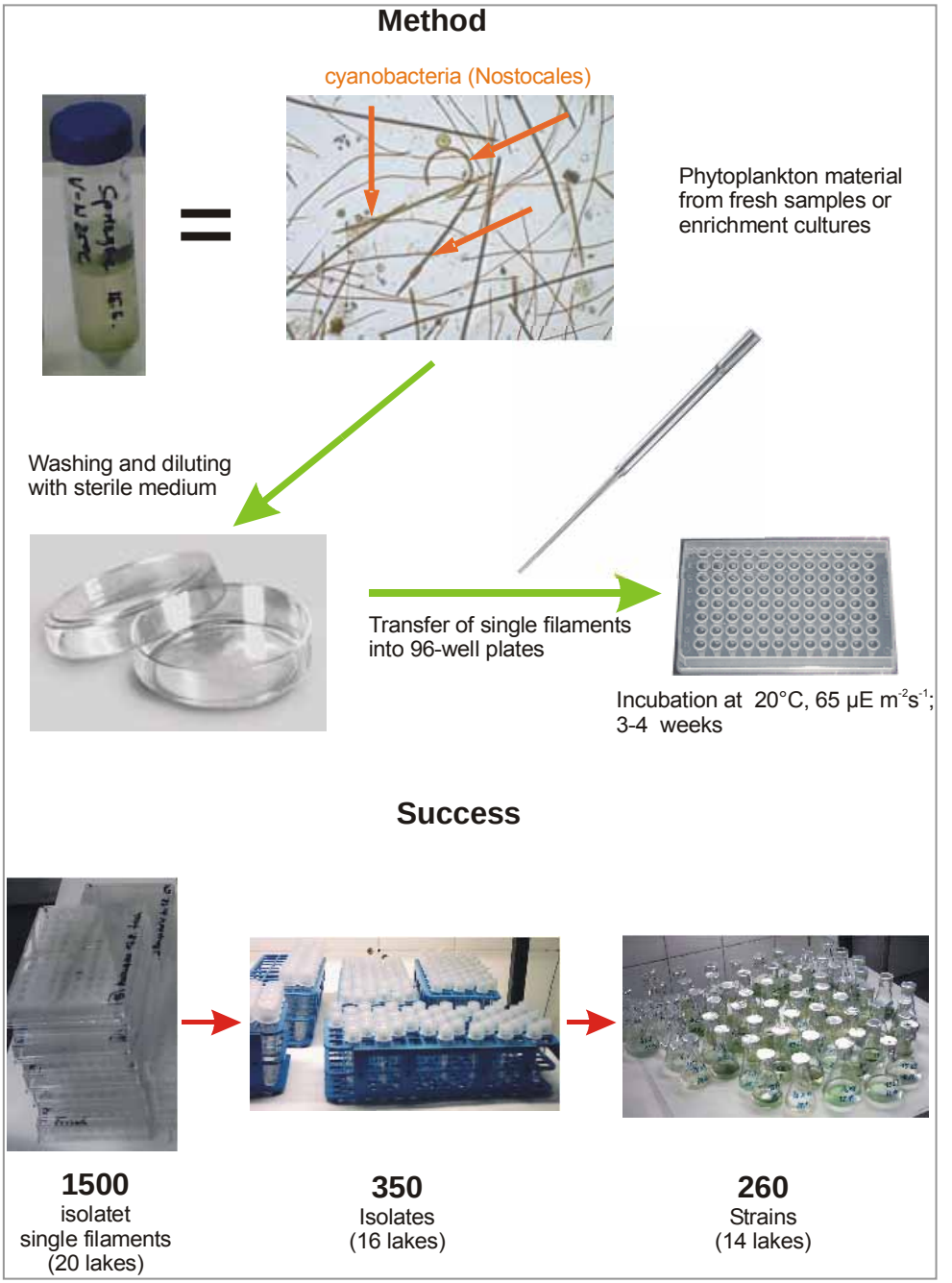


Fig. 26: Method used for isolation of cyanobacteria of the order Nostocales.

This method enabled us to isolate a total of roughly 1500 individual filaments from 24 plankton samples collected at 20 different lakes. It was later discovered that only half of the plankton samples used for the isolation studies actually contained cylindrospermopsin, which reduced our chances of identifying CYN-producing cyanobacteria in the remaining samples to virtually nil. After 3 to 4 weeks of incubation, roughly 350 isolates from 16 lakes were found to be suitable for further cultivation. Ultimately, 240 strains were successfully isolated for sustainable harvesting of cell material for toxin analysis. Results of taxonomic classification analysis and the variability of isolate origin are shown in Table 2.

Table 2: Number of isolates of the various taxa and variability of origin, i.e., the number of lakes in which the isolates were detected.

Number of Strains	Taxon	Number of Lakes
32	*Cylindrospermopsis raciborskii*	9
18	*Anabaena* sp. / *Anabaenopsis* sp.	5
	Unequivocally identified:	
4	*Anabaena crassa*	1
2	*Anabaena viguieri*	1
2	*Anabaena affinis*	1
1	*Anabaenopsis elenkinii*	1
200	*Aphanizomenon* sp.	16
	Unequivocally identified:	
19	*A. gracile*	6
2	*A. aphanizomenoides*	1
57	*A. flos-aquae*	11

Initial isolation of individual filaments under the stereomicroscope permitted classification of the cyanobacterial taxa to the genus level. The cyanobacterial isolates were then grown and transferred to a large cultivation vessel before being microscopically examined for taxonomic classification. Some of the species, such as *C. raciborskii* and *A. aphanizomenoides*, were easy to identify because of their distinctive features. A number of other strains, however, could not be identified unequivocally due to the absence of features necessary for reliable classification. For example, akinetes were absent in many *Anabaena* isolates, and so-called transition forms were observed exhibiting the characteristics of different species

simultaneously. This was frequently the case in the genus *Aphanizomenon*. Since experts are calling for taxonomic revision of the cyanobacterial species and genera, we decided to forgo species classification in equivocal cases. In other cases, we observed that isolates that were originally identified unequivocally had changed so much during cultivation that unequivocal confirmation of the classification was no longer possible. This phenomenon is a well-known problem associated with classifying cyanobacterial isolates.

Identification of CYN producers and quantitative toxin assay (LC-MS/MS)

The harvested cell material was lyophilized (freeze-dried), and 5 mg of each strain was placed in extraction vessels and extracted and analyzed as described in section 4.2.6. Identification of CYN in the isolates was accomplished by comparing them with a CYN references standard.

Cylindrospermopsin was detected in 4 of the 240 isolates studied, corresponding to a fraction of 1.7 %. All CYN-producing isolates were *Aphanizomenon* species originating from three lakes (Table 3, Fig. 27). As in our earlier studies (Fastner et al. 2003), the currently isolated *C. raciborskii* strains also did not produce cylindrospermopsin. Neither did the *Anabaena* strains. Although it turned out that half of the starting material for the isolation studies did not contain any CYN, we were still surprised to find such a low proportion of CYN-producing strains overall. It would therefore seem that CYN-producing cyanobacteria make up only a small fraction of phytoplankton in the sampled lakes. However, it cannot be excluded that the selected cultivation conditions might have promoted CYN non-producer growth more than CYN producer growth, which would result in the suppression or exclusion of CYN-producing cyanobacteria.

Table 3: Identified cylindrospermopsin (CYN) producers.

Strain	CYN content (mg g^{-1} dry weight)	Isolated as:	Lake of origin
10 E 6	2.3	*A. flos-aquae*	Melangsee
10 E 9	3.2	*A. flos-aquae*	Melangsee
22 D11	6.6	*A. flos-aquae*	Heiliger See, Potsdam
30 D 11	1.6	*Aphanizomenon* sp.	Petersdorfer See

The cylindrospermopsin content ranged from 0.16 to 0.66 % of the dry weight of the strains. Compared to the previously data for other CYN-producing

cyanobacteria (for overview, see Spoof et al. 2006), these concentrations are relatively high and are comparable to the highest toxin concentrations (0.55 %) measured for *C. raciborskii* (Hawkins et al. 1997). However, the variability of toxin content for the different *C. raciborskii* strains is much greater that that of the CYN-producing cyanobacteria identified in the CYLIN project. In various Australian isolates, for example, the authors reportedly measured CYN contents of 0.00012 to 0.46 % under identical growth conditions (Saker and Neilan 2001). If, based on geographic origin, it is assumed that our strains consist of at least 3 different genotypes, then the interspecific variability of CYN content would appear to be smaller than that of *C. raciborskii*. In addition, environmental factors are reported to have a strong impact on cylindrospermopsin production by *C. raciborskii*. According to Saker and Griffith (2000), a drastic decrease to complete cessation of CYN synthesis occurs in the 20 to 35 °C temperature range. In the case of *A. flos-aquae*, further studies should therefore be performed to determine whether various environmental factors such as light, temperature and nutrients have an effect on toxin production.

Based on the large number of CYN-negative isolates and the relatively high cylindrospermopsin content of CYN producers, it can be concluded that the cylindrospermopsin found in the water bodies studied most likely originated from only a few toxin-producing genotypes. Since these strains are able to produce relatively large quantities of the toxin, high cylindrospermopsin concentrations can be expected if they become the dominant strains in the waters.

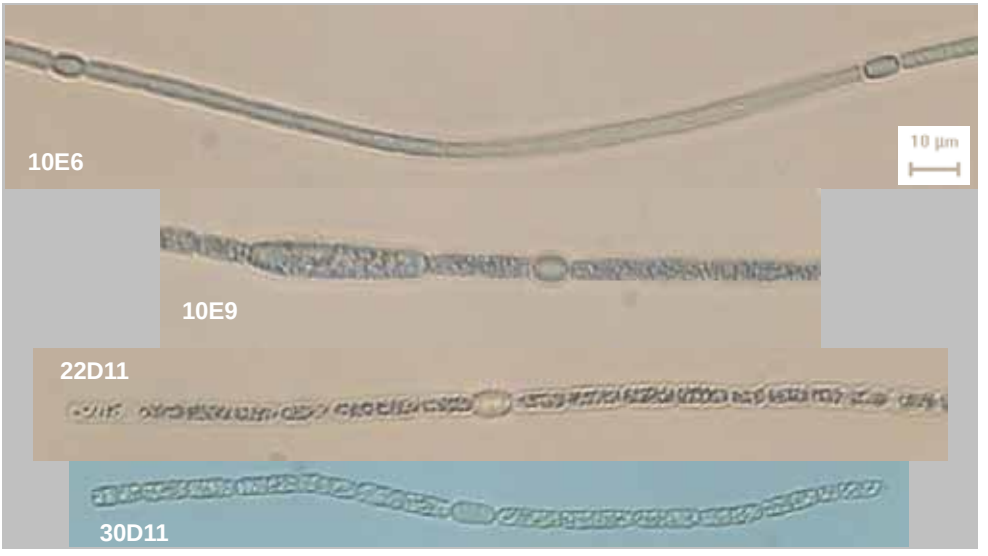


Fig. 27: Typical filaments of CYN-producing strains (microscopic images magnified ca. 400 times). Strain 10E6 does not form gas vesicles.

Molecular biological identification of CYN-coding genes

Although CYN biosynthesis is not fully understood, it is known to be an integrated system involving polyketide synthetase (PKS) (Burgyone et al. 2000) and non-ribosomal peptide synthetase (NRPS) (Shalev-Alon et al. 2002). Schembri et al. (2001) and Fergusson and Saint (2003) showed that the presence of certain PKS and NRPS genes is directly associated with the presence of cylindrospermopsin in *C. raciborskii* and *A. ovalisporum* strains. It can therefore be assumed that these genes are involved in CYN biosynthesis. In the literature, the primer pairs M13/M14 (NRPS) and M4/K18 (PKS) have been used for identification of biosynthesis genes involved in the multi-enzyme complex. We also used these primer pairs to test the identified CYN producers 10 E 6, 10 E 9 and 22 D 11.

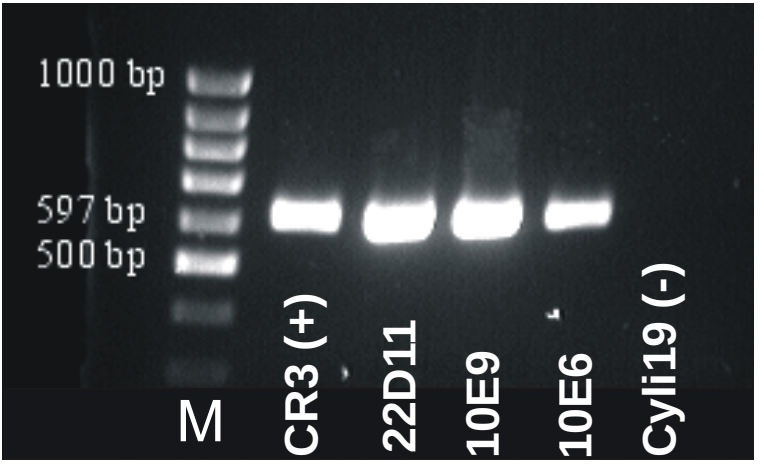


Fig. 28: Appearance of peptide synthetase-PCR fragments in CR3, the Australian *Cylindrospermopsis raciborskii* strain used as the positive control in the present study, and in 22D11, 10E9 and10E6, the *Aphanizomenon flos-aquae* strains isolated by us. Cyli19, a German *C. raciborskii* strain that does not produce CYN, was used as the negative control. Since one peptide synthetase band is absent, i.e., since this fragment is not present in the Cyli19 genome, Cyli19 is unable to synthesize CYN.

For a detailed description of the PCR method, see Chapter 9. PCR using the primer pair M13/M14 results in the appearance of a 597 base pair (bp) fragment in CYN-positive strains and in CR3, the control strain known to produce CYN, but not in Cyli19, the negative control (Fig. 28). Unequivocal identification is therefore possible in the case of the peptide synthetase component, but not for the PKS domain. The absence of a DNA fragment when a suitable primer pair such as

M4/K18 is used for detection of the PKS component suggests that the structural organization of the CYN biosynthesis system may differ from one CYN-producing species to another. Such inter-specific differences in the modular structure and sequences of synthesis systems have been reported for the cyanobacterial toxin microcystin. In the case of the genera *Microcystis, Planktothrix* and *Anabaena*, for example, multi-enzyme complexes of very different structure formed identical molecules (microcystins) (Tillet et al. 2000, Christiansen et al. 2003, Rouhiainen et al. 2004, Mbedi et al. 2005).

Such variations in the genetic code of the CYN synthetase of different cyanobacterial species or strains should be targeted when investigating the global pattern of spreading of CYN-producing cyanobacteria and their phylogenetic relatives. The CYN-producing organisms identified by us should be further investigated by further molecular biological analysis and compared with tropical and subtropical cylindrospermopsin producers. This could help to elucidate the pathways by which this cyanobacterial toxin has spread to temperate regions and to predict the further course of expansion in the coming years. [4]

[4] Project-related follow-up publications on this subject: Preußel et al. (2006).

9. Detection of cylindrospermopsin-producing cyanobacteria by PCR

Anke Stüken

Polymerase chain reaction (PCR) is a gene technology method used to replicate specific DNA fragments. The working principle of PCR is described below:

The first step is to identify the DNA segments located directly before and after the DNA fragment to be replicated. The second step is to synthesize two short DNA sequences (length: ~ 15 to 30 base pairs), which are complementary in base sequence to the identified flanking sequences and serve as the primers. The next step is to extract DNA, i.e. isolate the DNA from the rest of the cell material. The primers, an enzyme (*Taq* polymerase in this case), the four nucleotides (DNA building blocks), and a few reagents required for PCR are added to the extracted DNA and heated to 94 to 96 °C. This heat treatment separates the strands of the DNA double helix, producing two single-stranded DNA fragments (Fig. 29). The mixture is then cooled to ~ 45 to 60 °C, which allows the primers to dock onto the complementary DNA fragments. The required temperature for this step varies in accordance with the length and base composition of the primers. Once annealing of the primer on the DNA is completed, the temperature is increased to 72 °C. This is the optimal temperature for *Taq* polymerase. *Taq* extends the primers in the direction of the target DNA fragment by "adding on" nucleotides according to the rules of base pairing. This yields two copies of the original DNA fragment. The heating and cooling cycles are repeated roughly 30 times, yielding an exponential number of identical copies of the target DNA fragment.

After PCR, the PCR product is applied to an agarose gel containing ethidium bromide and stimulated with electrical current. In response to the current, the PCR product migrates through the gel from the negative terminal to the positive terminal of the gel electrophoresis system. The PCR product is applied together with a so-called ladder. A "ladder" is a special marker containing a mixture of PCR products of different but defined length. Since small DNA fragments travel through the gel more quickly than large fragments, DNA fragments in the gel separate according to their size. Once migration is completed, the gel is photographed under ultraviolet light. The ethidium bromide in the gel attaches to the DNA fragments and fluoresces under ultraviolet light. This permits visualization of the DNA fragments, i.e., the PCR products and the ladder. The ladder is used to estimate the length of the amplified PCR product. A photograph of a gel electrophoresis system is shown in Figure 30.

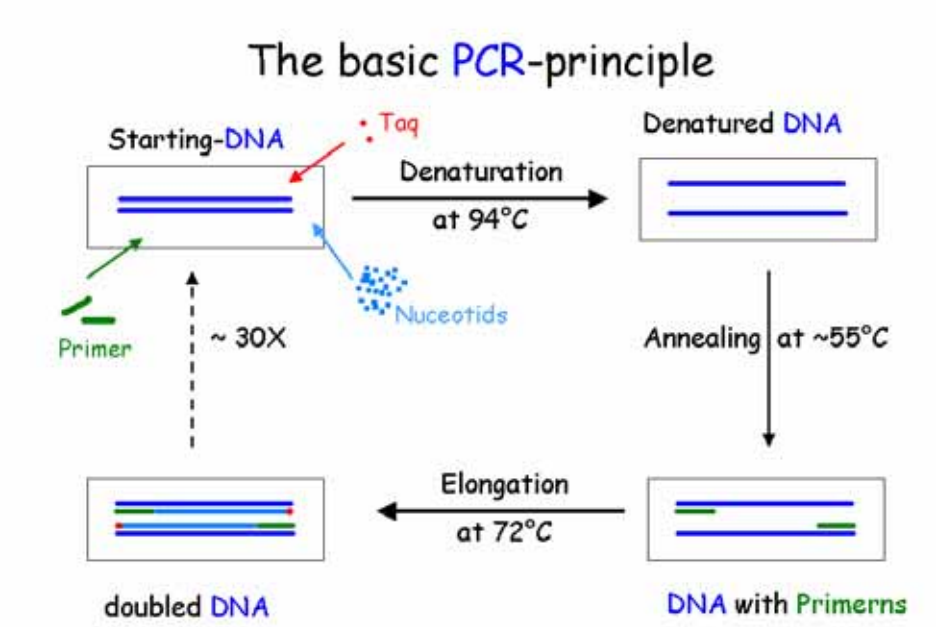


Fig. 29: The PCR procedure. See the text section for a detailed description.

In the present research project, PCR was performed to identify CYN-producing cyanobacteria. First, all isolated cyanobacterial strains were to be tested for genes involved in CYN synthesis. Second, a method that permitted the detection of these genes in individual cyanobacterial filaments was to be developed.

Before the start of the CYLIN project, other investigators had developed and published data on primers and a PCR method for replicating DNA segments that coded for a polyketide synthetase (PKS) and a non-ribosomal peptide synthetase (NRPS), both of which are presumed to be essential for CYN synthesis (Schembri et al. 2001, Fergusson and Saint 2003). For further details, see Chapter 8. Furthermore, a multiplex PCR assay specific for *Cylindrospermopsis raciborskii* and capable of distinguishing between CYN-producing and non-producing *C. raciborskii* strains had been described (Fergusson and Saint 2003). Multiplex PCR assays amplify multiple gene fragments simultaneously. These fragments vary in length and can therefore be separated like a ladder in a gel system. Three fragments were amplified in this case: a PKS fragment and a NRPS fragment thought to be involved in CYN-synthesis, and a *C. raciborskii*-specific fragment. Absence of the PKS and NRPS fragments in the gel means that the tested

cyanobacterium does not produce cylindrospermopsin. Absence of the *C. raciborskii*-specific fragment means that the tested cyanobacterium is not *C. raciborskii*.

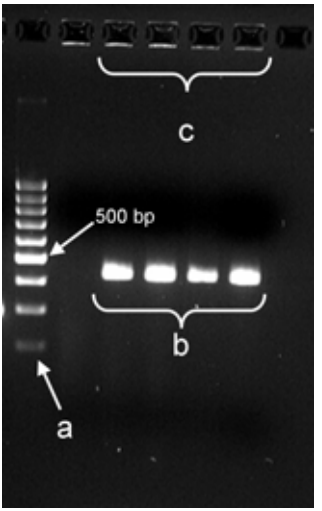


Fig. 30: Photograph of ethidium bromide-stained agarose gel. **a**) Ladder: the thick band in the middle is 500 base pairs (bp) in length, and the top band is 1000 bp long. **b**) Four PRC products with a length of ca. 450 bp. **c**) "Pockets" (small depressions) in the gel marking the initial sites of application of the PCR products and the ladder.

Both methods were developed on tropical strains at a time when no CYN-producing strains had been isolated from temperate latitudes. In our laboratory, the first method worked for the CYN-producing strains CR3 and ILC-146. CR3 is an Australian *C. raciborskii* strain kindly provided by Martin Saker, and ILC-146 is an Israeli *A. ovalisporum* strain kindly provided by Aaron Kaplan so that we could establish our own methods. Unfortunately, 100 % transfer of this method to our CYN-producing *A. flos-aquae* strains was not possible. In our four CYN-producing A. *flos-aquae* strains, only the NRPS fragment could be reliably identified. The tests for the PKS fragments produced either no bands or multiple bands. The formation of multiple bands means that the primers do not bind specifically enough, leading to the replication of multiple fragments. The second method did not work reliably in any of the strains tested at our laboratory. We therefore concluded that the primers for the *C. raciborskii*-specific fragment are not suitable for all *C. raciborskii* strains isolated from temperate latitudes. The corresponding bands were absent in many cases. Furthermore, the *C. raciborskii*-specific primers also amplified fragments from non-*C. raciborskii* strains.

The most likely reason for these problems is that the investigated DNA sequences vary between the strains from tropical and temperate regions as well as between the different species. As a result, the primers did not work or worked poorly with our strains, i.e., they amplified no bands or multiple bands.

Because of these problems, it was necessary to design new primers for this project. We therefore designed primers for amplification of a segment of the PKS fragment and primers for amplification of a segment of a cyanobacteria-specific gene. The two primer pairs selected had different segment lengths yet similar temperature properties, that is, they were suitable for use in a multiplex PCR system. This method worked reliably in all cultures tested. Both bands appeared on the gel in the presence of CYN-producing cultures, and only the cyanobacteria-specific band appeared in the case of non-CYN-producing cultures (Fig. 31).

When testing cyanobacterial strains, detection of the cyanobacteria-specific band is not necessary for identification of the CYN-coding gene. Theoretically, identification of PKS and NRPS alone is sufficient proof of potential CYN production. Actually, identification of only one of these segments is sufficient because the two segments seem to always occur either together or not at all (Schembri et al. 2001; Fergusson and Saint, 2003). However, detection of the cyanobacteria-specific band is imperative when performing single-filament PCR (SF-PCR).

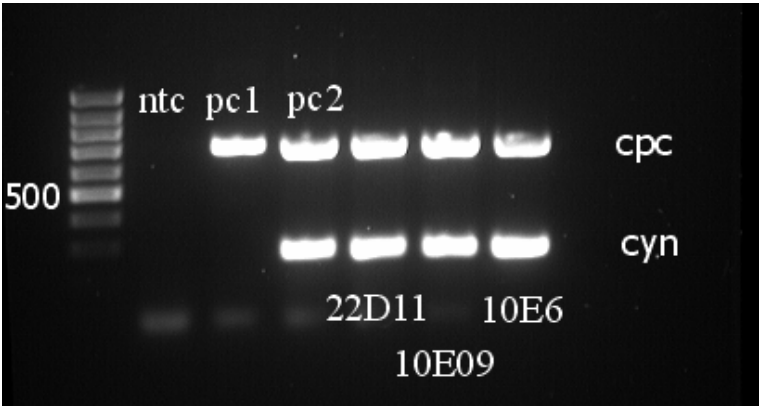


Fig. 31: Multiplex PCR. The ladder is shown on the far left. The negative control (ntc), in which only water was used as the starting material, appears in the next lane to the right. This is followed by the first positive control (ptc1), for which DNA material from Cyli19, a non-CYN-producing *C. raciborskii* strain was used. Only the cyanobacteria-specific fragment (cpc) was amplified (upper band). CR3, a CYN-producing *C. raciborskii* strain, was used as the second positive control (ptc2). This led to amplification of 2 bands: the cyanobacteria-specific band (top) and the CYN-specific band (bottom). *A. flos-aquae* strains 22D11, 10E9 and 10E6 were used in the last three lanes. Double bands for all three strains signify that they all have the cyanobacteria-specific gene fragment as well as the fragment needed for CYN synthesis.

Individual cyanobacterial filaments are used as the starting material for SF-PCR. Consequently, the initial quantity of DNA is very small. To ensure that the absence of PKS bands signifies the absence of the PKS fragment and not the failure of the PCR, the cyanobacteria-specific fragment must also be amplified as a control.

Absence of both bands means that PCR failed or that the isolated filament did not land in the PCR tube. This can easily occur because the filaments are so small—the isolated filaments in the tube can neither be seen with the naked eye nor under a microscope. To break up the cells, the tubes with the filaments are frozen and subsequently heated to boiling.

Unfortunately, the method that we designed works well on filaments isolated from cyanobacterial cultures, but not on filaments isolated directly from field samples. We presume that the field samples contain substances that inhibit PCR. In "normal" PCR with large quantities of starting materials, the DNA is extracted, that is, separated from all inhibitory substances. When individual filaments are used, it is not possible to extract the DNA, so the whole sample, including the cell debris, goes into the PCR. Work on a method to neutralize the PCR inhibitors is currently in progress. We are confident that this method will soon be available. We will then be able to analyze the more than 1000 filaments that we have isolated during the course of this project by SF-PCR.

10. Dissolved and particulate cylindrospermopsin concentration and seasonal course in target lakes

Jacqueline Rücker

A widespread occurrence of cylindrospermopsin (CYN) can be observed in water bodies in the Berlin-Brandenburg region, as was confirmed by the qualitative data from our initial screening program in 2004 (cf. Chapter 7). Quantitative data on the CYN content per unit volume of lake water is, however, required for assessment of the human hazard potential of this toxic cyanobacterium. In all of Europe, the only published quantitative data suitable for this purpose was derived from three Italian lakes (Manti et al. 2005) and a Spanish reservoir (Quesada et al. 2006). Filling this information gap was therefore an important goal of the CYLIN project.

Our monitoring program conducted in 2005 was specifically designed for this purpose. A total of 20 lakes in which cylindrospermopsin and potential CYN-producing cyanobacteria (*Cylindrospermopsis raciborskii* and *Anabaena bergii*) had been identified in the 2004 screening program were thereby monitored. We additionally studied the seasonal dynamics of cylindrospermopsin at two selected lakes, Langer See and Melangsee, in 2004 and 2005. For quantitative CYN analysis, a defined volume of water sample was passed through a membrane filter; the cylindrospermopsin concentration on the filter was then measured as the particulate CYN fraction (CYN_{part}), and that in the filtrate was measured as the dissolved fraction (CYN_{gel}). Biovolumes of cyanobacteria and relevant environmental parameters were also measured. The monitored lakes were sampled at least three times at 14-day intervals between June and September. If there were more than 3 data sets per lake, monthly means were calculated to ensure equal weighting of the lakes. In these cases, 3 to 4 data sets per lake were used in the statistical analysis. In the end, the statistical analysis was performing using 69 data sets generated using data obtained on 93 sampling dates. Langer See and Melangsee were sampled every 14 days in 2004 and 2005. Table 4 provides an overview of the investigated lakes, the mean Secchi depths, and the total phosphorous and total CYN concentrations.

Cylindrospermopsin concentrations

Cylindrospermopsin was detected in 18 out of 20 monitored lakes or in 80 of 93 samples from the lakes. The CYLIN project provided the first quantitative data on cylindrospermopsin for a large region of Europe. The highest total CYN concentration measured by us was 12.1 $\mu g\, L^{-1}$. Sixteen samples from 7 lakes exceeded 1 $\mu g\, L^{-1}$, the proposed guideline value for drinking water safety (Humpage and Falconer 2003). A total of 41 samples contained only particulate CYN, and 42 samples contained particulate and dissolved CYN, with individual fractions ranging from 24.3 to 99.8 % of the total CYN concentration (Fig. 32).

Table 4: Mean concentrations of total phosphorous (TP), total CYN (CYN_{tot}), and Secchi depth (SD) at the 20 lakes investigated from June to September 2005 in the scope of our monitoring program. The lakes are grouped according to water mixing type and total phosphorous. CYN_{tot} concentrations exceeding the proposed guideline safety value of 1 µg L^{-1} are highlighted in bold print.

Lake Name	Code	TP (µg L^{-1})	SD (m)	CYN_{tot} (µg L^{-1})
Polymictic lakes:				
Kleiner Zeschsee	KZ	32.9	1.05	0.14
Moderfitzsee	MF	34.2	0.63	0.01
Petersdorfer See	PE	37.7	0.50	0.02
Petznicksee	PZ	41.6	0.83	0.01
Bützsee	BU	42.3	0.70	**2.39**
Rahmer See	RA	42.9	0.82	0.19
Melangsee	ME	51.1	0.53	0.09
Zermützelsee	ZE	70.2	0.77	**8.40**
Langer See	LA	75.8	0.57	0.13
Braminsee	BR	78.6	0.38	0.79
Kutzingsee	KU	87.3	0.33	0.52
Vielitzsee	VI	98.3	0.43	0.00
Lieps	LI	107.0	0.25	**5.07**
Dimictic lakes:				
Großer Glubigsee	GL	17.2	1.02	0.00
Springsee	SP	19.9	0.96	0.01
Pätzer Vordersee	PA	25.4	1.40	0.00
Großer Plessower See	GP	33.0	2.03	**1.10**
Ruppiner See	RU	36.2	0.97	**5.29**
Stolpsee	ST	41.3	0.98	**4.31**
Motzener See	MZ	47.9	1.73	0.00

Dissolved CYN concentrations ranged from 0.077 to 11.75 µg L^{-1}, whereas those of particulate CYN were only 0.002 to 0.048 µg L^{-1}. Hence, the dissolved CYN fraction was clearly responsible for the high concentrations exceeding the safety limit. The absolute concentrations and fractions of dissolved and particulate CYN relative to total CYN are shown in Figures 33 C and 33 D. Seasonal variation of cylindrospermopsin at Langer See and Melangsee is shown in Figures 34 and 35. The seasonal variation data show that dissolved CYN follows the course of

particulate CYN with a time lag. This finding suggests that there is enrichment of dissolved CYN in the water column.

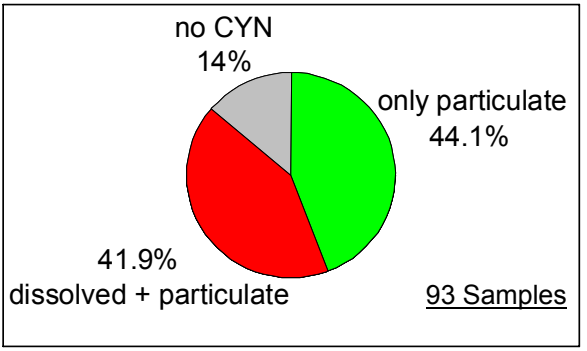


Fig. 32: Percentage fractions of cylindrospermopsin (CYN) in the 93 lake samples collected in 2005.

Compared to the worldwide values, the CYN concentrations measured by us are in the medium range, which was defined by Falconer and Humpage (2006) as 1 to 10 µg L^{-1}. The highest concentration detected by us (12.1 µg L^{-1} CYN_{tot} at Zermützelsee) is below the peak values measured in Australia and Florida. However, the latter peak values were measured in artificial water bodies that are not used for drinking water or recreation purposes, e.g., fish ponds (Saker and Eaglesham 1999), agricultural reservoirs (Shaw et al. 2000), and in waters with cyanobacterial surface scum (Shaw et al. 1999). After eliminating these data, only 6 international publications remain that provide relevant volume-related data on cylindrospermopsin concentrations in natural waters. All publications are summarized in Table 5.

Analysis of the data revealed that cylindrospermopsin is just as widespread as microcystin and that the concentrations of the two toxins are comparable, as shown by the data from various European water lakes. The maximum particulate microcystin concentration was 0.6 to 2.6 µg L^{-1} at two lakes in the Netherlands (Janse et al. 2004), 1 to 3 µg L^{-1} in Müggelsee (Welker 2003), 5.2 µg L^{-1} in a shallow French lake (Briand et al. 2002b), and 6 µg L^{-1} in Langer See in 2005 (Wiedner in preparation). Microcystin occurs in predominantly cell-bound form. The dissolved fraction ranges from 0.2 to 2.5 % (Wiedner et al. 2003), and it does not accumulate in the water (Wiedner et al. 2002). In the case of CYN, however, the dissolved fraction can exceed the particulate fraction many times over, and it can accumulate in water over time, as our findings clearly demonstrate.

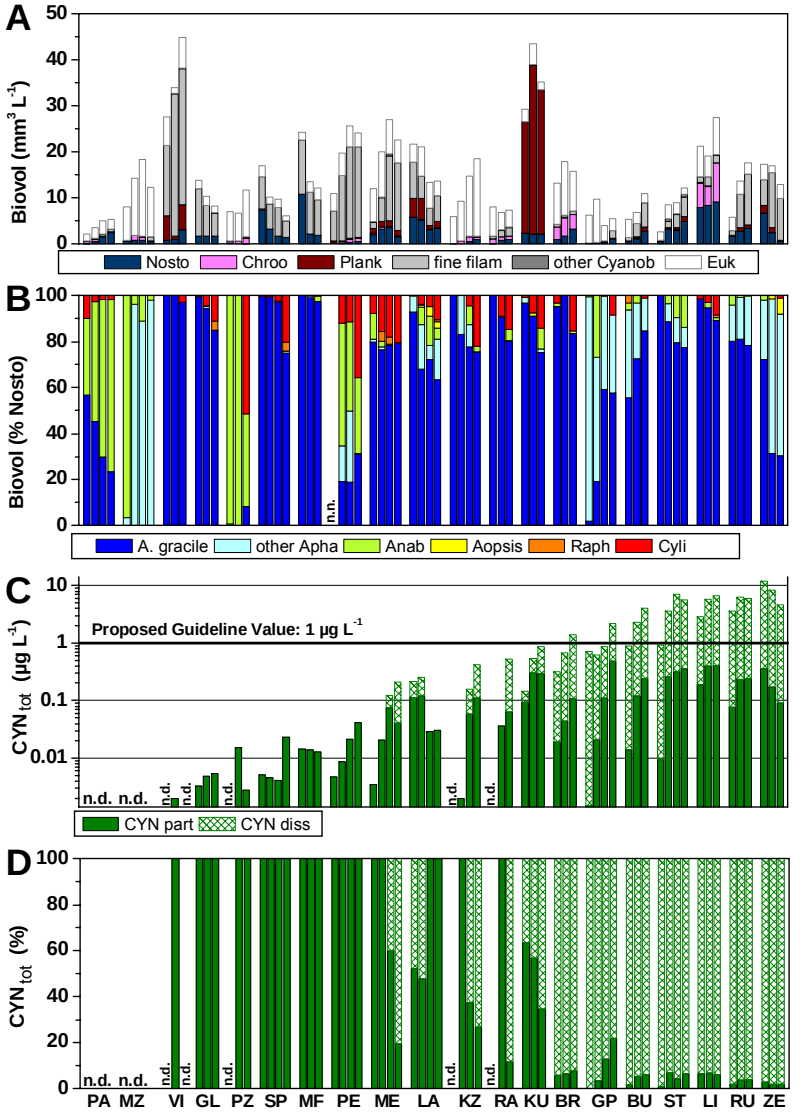


Fig. 33: (A) Phytoplankton composition; (B) Fractions of individual genera relative to total biovolume (Biovol) of Nostocales; (C) particulate CYN (CYN_{part}) and dissolved CYN (CYN_{diss}) fractions calculated logarithmically and (D) as a percentage of total CYN (CYN_{tot}) at the 20 lakes (sorted according to CYN_{tot}) studied from June to September 2005 (data sets used for statistical analysis). Nosto – Nostocales; Chroo – Chroococcales; Plank – *Planktothrix agardhii*; fine filam – fine-filamentous cyanobacteria (*Limnothrix* spp., *Pseudanabaena* spp., *Leptolyngbya* spp.); other Cyanob – other cyanobacteria; Euk – eukaryotic algae; A. gracile – *Aphanizomenon gracile*; other Apha – other *Aphanizomenon* species; Anab – *Anabaena* spp.; Aopsis – *Anabaenopsis* spp.; Raph – *Raphidiopsis mediterranea*; Cyli – *Cylindrospermopsis raciborskii*. See Table 4 for key to abbreviations for lake names.

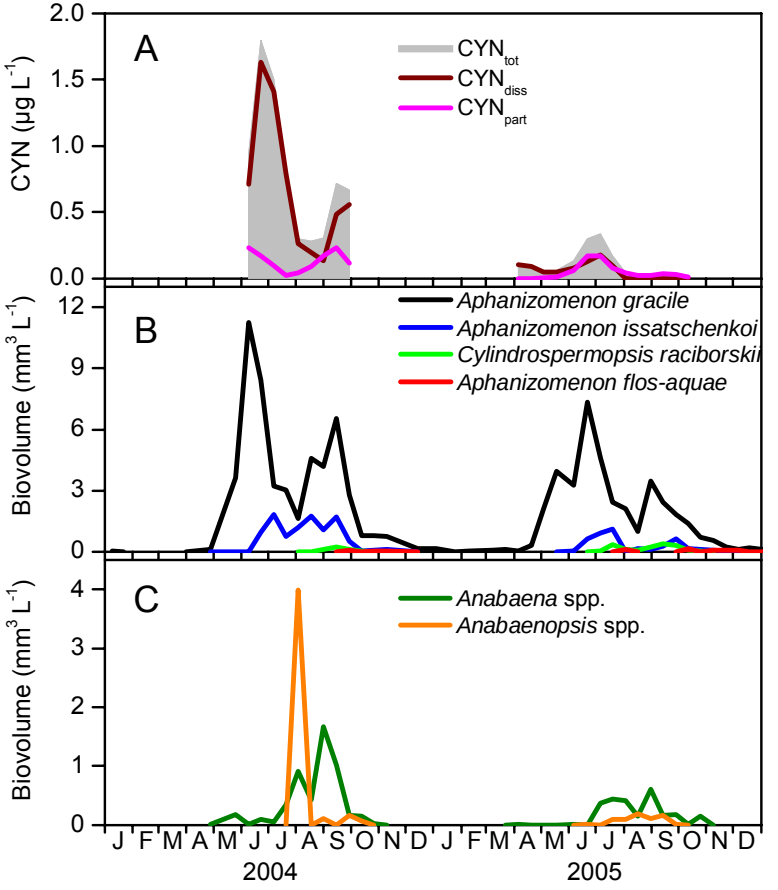


Fig. 34: Seasonal variation **(A)** of dissolved CYN (CYN_{dis}), particulate CYN (CYN_{part}) and total CYN (CYN_{tot}) and **(B/C)** of the cyanobacterial species found at Langer See in 2004 and 2005.

The release of dissolved CYN can occur due to active or passive mechanisms (e.g., the death of cyanobacterial cells). The seasonal variation data show that the dissolved fraction was much higher than the cell-bound fraction. It can therefore be assumed that active release by CYN-producing cyanobacteria is the main source of dissolved CYN. In addition, the temporal shift in dissolved CYN peaks is suggestive of poor decomposition of CYN in the waters, which results in accumulation of the toxin. Chiswell et al. (1999) have made similar observations. These features distinguish cylindrospermopsin from microcystin, which decomposes quickly in the water column. The high levels and persistence of dissolved CYN pose a health hazard for humans because the toxin is not adequately removed from raw water by conventional drinking water treatment methods such as precipitation, sedimentation and filtration.

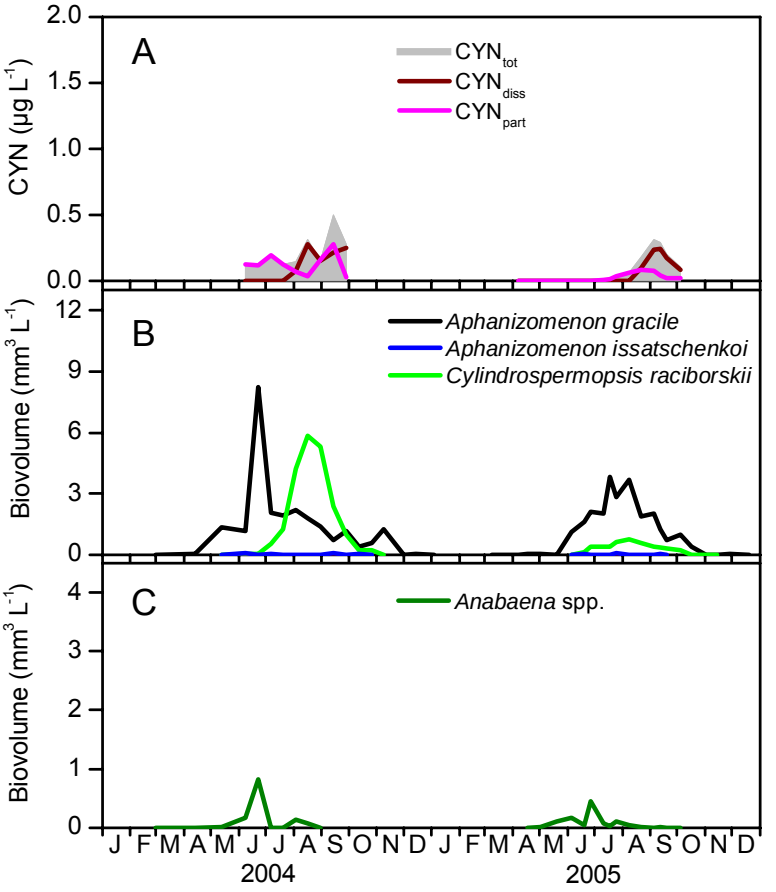


Fig. 35: Seasonal variation **(A)** of dissolved CYN (CYN_{dis}), particulate CYN (CYN_{part}) and total CYN (CYN_{tot}) and **(B/C)** of the different cyanobacterial species found at Melangsee in 2004 and 2005. The axes are on the same scale as in Fig. 34 to ensure direct comparability of the two lakes.

Under which conditions does cylindrospermopsin occur?

We did not detect any significant correlations between cylindrospermopsin occurrence and morphometric variables, such as lake surface area, volume or maximum depth of the monitored lakes (data not shown). Consequently, one cannot define a certain lake type predestined for cylindrospermopsin occurrence but must expect to find this cyanobacterial toxin in water bodies of various types. Particulate CYN exhibited significantly positive correlations with total phosphorous, chlorophyll a, and plankton biovolume, and significantly negative correlations with Secchi depth and mean photosynthetically active radiation in the mixed water

column, respectively (Table 6). These findings suggest that the probability of cylindrospermopsin occurrence is somewhat higher in lakes with higher trophic level than in those with lower trophic level. However, it is not possible to predict cylindrospermopsin concentrations based on abiotic or trophic lake data because of the low correlation coefficients. Although correlation coefficients between cylindrospermopsin and the aforementioned variables were slightly higher at Langer See and Melangsee (data not shown), it still is not possible to predict the occurrence of cylindrospermopsin there based on abiotic variables.

Which cyanobacteria produce cylindrospermopsin?

Cyanobacteria were the dominant members of the phytoplankton community in the monitored lakes (Fig. 33 A). The cyanobacterial community in most lakes was very diverse, as illustrated in Figure 36. We identified a total of 43 cyanobacterial species from a total of 20 genera. Cyanobacteria of the order Nostocales contributed significantly to the overall cyanobacterial biovolume (Fig. 33 A). Species of the genus *Aphanizomenon,* especially *A. gracile* dominated the order Nostocales (Fig. 33 B). *A. gracile* was the dominant Nostocales in 47 % of all cases and was absent in only 8 samples from 3 lakes. All other species or genera of the order Nostocales were found in far fewer samples and, in most cases, in lower numbers (Fig. 33 B, Table 6).

Figures 34 B/C and 35 B/C illustrate the seasonal dynamics of cyanobacteria of the order Nostocales at Langer See and Melangsee. *A. gracile* was the species with the highest biovolume at both lakes. In addition, high biovolumes of *Pseudanabaena limnetica* (Oscillatoriales) also occurred at both lakes, and high *Planktothrix agardhii* biovolumes were observed at Langer See (data not shown).

Anabaena bergii, the neo-cyanobacterium first discovered in the study region in 2004, also deserves special mention (cf. Chapter 5). This species was identified in quantitative assessments of data obtained from 12 of the lakes studied in our Monitoring program. The maximum biovolume of *Anabaena bergii* at the monitored lakes was 0.24 $mm^3 L^{-1}$, and it made up 55 and 64 % of the total biovolume of Nostocales at the lakes Petersdorfer See and Petznicksee, respectively. These findings clearly demonstrate that *Anabaena bergii*, another non-indigenous species originally from the tropics and subtropics, has established itself in North German waters.

Table 5: Summary of global quantitative data on total (CYN_{tot}), particulate (CYN_{part}) and dissolved cylindrospermopsin (CYN_{dis}).

Study region	CYN_{tot} (µg L^{-1})	CYN_{part} (µg L^{-1})	CYN_{dis} (µg L^{-1})	Organisms	Source
Europe					
93 samples from 20 lakes in the lowlands of northeastern Germany, June-October 2005.	0-12.1	0-0.1	0-11.8	*Aphanizomenon gracile, A. flos-aquae C. raciborskii, Anabaena bergii*	CYLIN project
Samples from 3 Italian lakes, July-October 2004.	0.5-15			*C. raciborskii*	Manti et al. 2005
Arcos Reservoir, southern Spain, Aug.-Sept. 2004.	n.d.	1.5-9.4	n.d.	*Aphanizomenon ovalisporum*	Quesada et al. 2006
Australia					
Hervey Bay, Queensland, Nov. 1997-January 1998.	10-92	0.7-29	7-63	*C. raciborskii*	Chiswell et al. 1999
183 samples from 15 reservoirs in Queensland. Oct. 1997- June 1999.	0-80	n.d.	n.d.	*C. raciborskii*	McGregor and Fabbro 2000
Raw water and treated water from a water treatment plant, Sept. 2001.	1.17	n.d.	20.5%		Hoeger et al. 2004
Surface scum samples from two small shallow lakes near Hervey Bay, Queensland, Feb., April, Oct. & Nov. 1997.	4-120	0-4	4-120	*Aphanizomenon ovalisporum* blooms, no *C. raciborskii*	Shaw et al. 1999
Aquaculture pond in Queensland, August 1997.	589 max.	93%	7%	*C. raciborskii*	Saker and Eaglesham, 1999
Subtropical farm dams, Australia.	800 max.	?	?	*C. raciborskii*	Shaw et al. 2000
USA					
Surface waters in Florida, 1999-2000.	8.1-97.1	n.d.	n.d.	*C. raciborskii*	Burns et al. 2002

n.d. = not detected

Fig. 36: Plankton community in a plankton net sample collected at Langer See in August 2004, microscopic appearance under 200-times magnification.

Compared to earlier studies, the current findings indicate interesting increases in cyanobacteria of the order Nostocales in the study region over time. In 2005, the *A. gracile* population at Langer See was larger than that of *P. agardhii* for the first time since 1993 (cf. Wiedner et al. 2002, Mischke 2003). Trophic decline is the main reason for the decrease in microcystin-producing *P. agardhii* populations, which is associated with a significant decrease in microcystin levels. However, declining trophic levels do not lead to a decrease in *Aphanizomenon* populations (Wiedner et al., in preparation). Compared to the values measured in 1993 to 1996 (Rücker et al. 1997, Wiedner 2002), there has also been a relative increase in the fraction of Nostocales at some of the lakes monitored over time. As explained in Chapter 6, synergistic effects of climate change and trophic decline could play a role in this process. Future studies should be performed to test this hypothesis.

Fig. 37: Microscopic appearance of *Aphanizomenon gracile* at 400-times magnification.

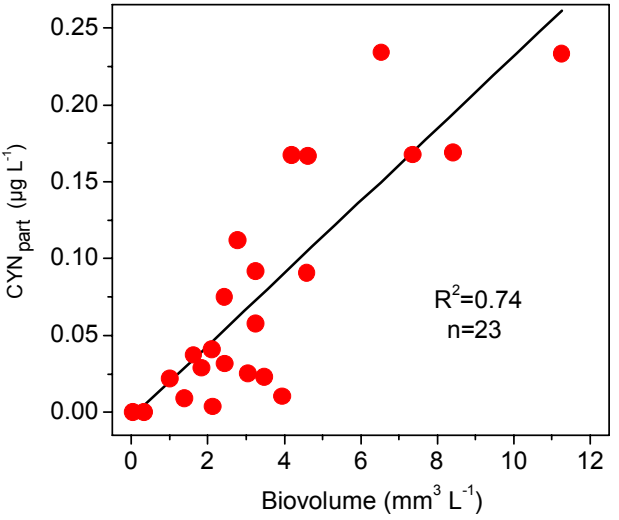


Fig. 38: Relationship between particulate cylindrospermopsin concentration (CYN_{part}) and *Aphanizomenon gracile* biovolume at Langer See in 2004 and 2005.

Statistical tests for correlations between cylindrospermopsin concentration and composition of the cyanobacterial communities in the monitored lakes (Table 6) revealed a relatively high correlation coefficient between the concentration of particulate CYN (CYN_{part}) and the biovolume of cyanobacteria of the order Nostocales. These findings suggest that Nostocales are the cyanobacteria that produce cylindrospermopsin. The highest correlation coefficients were obtained for *Aphanizomenon* at the genus level, and for *Aphanizomenon gracile* at the species level. *Planktothrix agardhii* and *Cylindrospermopsis raciborskii* also had high correlation coefficients. In the case of *Aphanizomenon flos-aquae* and *Anabaena bergii*, no positive correlation with particulate CYN was observed. Analysis of the seasonal data revealed significantly higher correlation coefficients between CYN_{part} and *A. gracile* (0.8) and *P. agardhii* (0.7) than for other cyanobacteria at Langer See. At Melangsee, on the other hand, there was no significant correlation between particulate CYN and any of the above-mentioned taxa. Data from Langer See also showed a linear correlation between the particulate CYN concentration and *A. gracile* biovolume (Figures 37 and 38); no linear relationship with CYN_{part} was found for any of the other species or lakes studied.

Table 6: Spearman's rank correlation coefficients for particulate (CYN_{part}), dissolved (CYN_{dis}) and total cylindrospermopsin (CYN_{tot}) and for selected abiotic and biotic parameters for the lakes monitored in 2005 (the data sets for statistical analysis were sometimes formed using monthly mean values). Double asterisks (**) indicate correlations with a significance level of $p<0.01$; n represents the number of samples included in the analysis.

Parameter	n	CYN_{part} (µg L^{-1})	CYN_{dis} (µg L^{-1})	CYN_{tot} (µg L^{-1})
Total phosphorous (µg L^{-1})	69	0.389**	0.338**	0.319**
Total nitrogen (µg L^{-1})	69	0.214	0.226	0.230
DIP (µg L^{-1})	69	0.032	-0.064	-0.075
NH_4-N (µg L^{-1})	69	-0.123	-0.179	-0.185
NO_{tot}-N (µg L^{-1})	69	0.154	0.149	0.143
Temperature (°C)	69	-0.205	-0.020	-0.140
Secchi depth (m)	68	-0.440**	-0.176	-0.287**
I_{mix} (µE m^{-2} s^{-1})	69	-0.304**	-0.166	-0.165
Chlorophyll-*a* (µg L^{-1})	69	0.333**	0.059	0.130
Biovolume (mm^3 L^{-1}):				
Phytoplankton, total	69	0.292**	0.061	0.127
Cyanobacteria, total	69	0.399**	0.114	0.234
Nostocales	68	0.537**	0.344**	0.417**
Aphanizomenon spp.	67	0.585**	0.388**	0.479**
A. gracile	63	0.501**	0.278	0.353**
A. flos-aquae	21	-0.252	0.108	0.035
A. issatschenkoi	23	0.362	0.283	0.324
A. aphanizomenoides	7	-0.536	-0.204	-0.536
Aphanizomenon sp.	22	0.117	0.309	0.283
Anabaena spp.	47	0.080	0.036	-0.018
Anabaena bergii	20	-0.064	-0.146	-0.100
Anabaenopsis spp.	7	-0.607	-0.523	-0.536
Cylindrospermopsis raciborskii	40	0.428**	0.223	0.348
Raphidiopsis mediterranea	14	0.591	0.079	0.266
Planktothrix agardhii	58	0.486**	0.329**	0.365**
Fine-filamentous Oscillatoriales	67	0.103	-0.080	0.039
Chroococcales	69	-0.061	-0.130	-0.132
Other cyanobacteria	28	0.191	0.149	0.155

DIP – dissolved inorganic phosphorous; NH_4-N – ammonia; NO_{tot}-N – sum of nitrate plus nitrite; I_{mix} – mean photosynthetically active radiation in the mixed water column

The coefficients of correlation between cyanobacterial biovolume and dissolved CYN were invariably lower than those between biovolume and particulate CYN. The reasons for this must be a decoupling of population growth, CYN production, and CYN decomposition, resulting in enrichment of dissolved CYN in the water body. Theoretically, the cyanobacteria that produced dissolved CYN may not have been present in the phytoplankton samples because they died before sampling.

Based on the overall body of evidence, it can be concluded with high probability that *Aphanizomenon gracile* produces cylindrospermopsin and is mainly responsible for the occurrence of cylindrospermopsin in the sampled waters. This conclusion is based on evidence from our statistical analyses and on the fact that *Aphanizomenon gracile* was detected as the sole representative of the order Nostocales in 5 CYN-positive samples. This has far-reaching implications because *A. gracile* is a widespread indigenous species in North German waters. It would also explain the widespread distribution of cylindrospermopsin (cf. Chapter 7). Isolates of *A. gracile* strains are needed for definitive confirmation of CYN production by this cyanobacterium.

Aphanizomenon flos-aquae has already been identified as a potential producer of cylindrospermopsin (cf. Chapter 8). However, considering its relatively low frequency (19 of 80 CYN-positive samples) and very low biovolume, it cannot be the main source of cylindrospermopsin. Even if both *Aphanizomenon* species are analyzed as a single group, they still could not account for the widespread occurrence of cylindrospermopsin. Therefore, other CYN producers must be considered. One potential producer detected in several CYN-positive samples is *C. raciborskii*, the biovolume of which correlated significantly with CYN concentration. However, cylindrospermopsin has not been detected in any of the *C. raciborskii* strains isolated from water bodies in the study region (Chapter 8; Fastner et al. 2003) or in other parts of Europe (Bernard et al. 2003, Saker et al. 2003). CYN-producing strains of *C. raciborskii* have only been detected in Australia (Hawkins et al. 1997, Saker and Griffiths 2000), New Zealand (Wood and Stirling 2003), Thailand (Li et al. 2001b), and Japan (Chonudomkul et al. 2004).

Planktothrix agardhii was also present in many CYN-positive samples, and its biovolume correlated significantly with CYN_{part}. No CYN-producing strains of *Planktothrix agardhii* (Oscillatoriales) have been detected so far, but their existence cannot be ruled out altogether. The most likely explanation, however, is that the correlation between *Planktothrix agardhii* biovolume and CYN concentration was merely coincidental due to joint occurrence of the species with *A. gracile*: the coefficient of correlation between the biovolumes of the two species was 0.6.

The neo-cyanobacterium *Anabaena bergii* and other species of the genus *Anabaena* must also be considered as potential cylindrospermopsin producers.

CYN-producing strains of *A. bergii* have been found in Australia (Schembri et al. 2001). Various species of the genus *Anabaena*, including *A. bergii,* were the only representatives of the order Nostocales detected in one CYN-positive sample. This confirms that *Anabaena* is a CYN-producing genus in Europe. Analysis of further isolates is required to determine which *Anabaena* species is responsible for the toxin production. Spoof et al. (2006) detected cylindrospermopsin in *Anabaena lapponica* isolated from Finnish waters, but this species does not occur in our study region.

In summary, we conclude that it is not possible to reliably predict cylindrospermopsin concentration based on cyanobacterial composition with the present data. Even the recommendation to avoid waters dominated by blue-green algae is not always a successful strategy of cylindrospermopsin avoidance, as is shown in the following examples: The phytoplankton community at Vielitzsee was dominated by cyanobacteria, but very low concentrations of cylindrospermopsin were detected in the lake (Fig. 33). In contrast, Großer Plessower See exhibited cylindrospermopsin concentrations close to the guideline value, but cyanobacteria made a minor contribution to its phytoplankton community and the Secchi depth (> 2 m) was the highest of all regional lakes studied (Table 4).

Further investigation is required to explain this puzzling variability of cylindrospermopsin concentration. Several possible causes are briefly discussed below.

- The cylindrospermopsin detected in a given lake could theoretically be produced by cyanobacteria of more than one species. As we already explained, not all CYN-producing species have been identified yet. It is therefore essential to isolate and analyze more strains from the pool of potential CYN producers described above.

- The fact that microscopic differentiation based on morphological features is not possible for all strains is a serious impediment to taxonomic classification of the strains and to differentiation between strains in the scope of biovolume analyses (see Chapters 5 and 8). Differentiation between *A. flos-aquae* and *A. gracile*, for example, is not always possible, as Figures 27 and 37 clearly illustrate. Moreover, the morphology of cultured strains tends to change slightly (cf. Chapter 8). Molecular biological studies are therefore needed for further taxonomic classification of these strains, and new methods for quantitative analysis of the individual species must be developed.

- A population of a given species can theoretically consist of CYN-producing and non-producing genotypes and chemotypes. This has proved to be the case with other species and toxins. *P. agardhii* populations consisting of microcystin-

producing and non-producing genotypes have been found (e.g., Mbedi et al. 2005, Kurmayer and Gumpenberger 2006). Little is known about the variability of toxin-producing genotypes. Our findings indicate that the proportion of CYN producers in the cyanobacterial populations is very low: only 4 out of 240 isolated strains were found to produce cylindrospermopsin (see Chapter 8). Since it is not possible to distinguish between the genotypes by microscopic methods, further development of molecular biological detection methods is imperative.

- Last but not least, the regulatory mechanisms controlling cylindrospermopsin production are not adequately understood, and those for *Aphanizomenon* have not been investigated yet. Consequently, it currently is not possible to assess the variability of cylindrospermopsin concentration as a function of regulatory parameters. [5]

[5] Project-related follow-up publications on this subject: Rücker et al. (submitted), Wiedner et al. (submitted).

11. Hazard potential, guidelines and perspectives

Based on the findings of the CYLIN project, our preliminary assessment of the hazard potential of cylindrospermopsin in waters in the study region and issues requiring further investigation are summarized below.

Hazard potential of cylindrospermopsin

Because cylindrospermopsin is produced by at least one widespread indigenous species (see below), it is highly likely that the toxin first appeared in the study region decades ago. It can therefore be presumed that cylindrospermopsin might be the cause of several yet unexplained cases of cyanobacterial poisoning.

The guideline safety value 1 µg L^{-1} for cylindrospermopsin in drinking water, which was proposed by Humpage and Falconer (2003), can be used for a preliminary toxicological assessment of current CYN concentrations.

Basic information concerning guideline safety values:

Extrapolation and uncertainty factors with which guideline safety values are calculated must be taken into account when assessing toxin levels measuring up to 10 times the guideline safety value. An uncertainty factor[6] of 100 to the highest "no observed adverse effect level" (NOAEL) determined in laboratory animals during subchronic exposure trials is generally used as the safety margin. Similar to Humpage and Falconer's proposal for cylindrospermopsin, the uncertainty factor for microcystin also includes uncertainty factors of 10 to account for extrapolation from laboratory animals to humans and for extrapolation of data from part of the life cycle to lifetime exposure. The overall assessments of health hazards associated with toxin levels exceeding the guideline safety value by a factor of 10 must therefore be weighed against a background of extrapolation and uncertainty factors that, when multiplied, yield a total uncertainty factor of 1000.

These considerations play an increasingly important role in defining action levels for toxins in drinking water management. Since drinking water guideline safety values for tolerable lifetime exposure are extrapolated values, they may be up to 10 times lower than subchronic exposure values, as was explained above. Consequently, action levels cannot be higher. Action levels are needed to determine whether drinking water contaminated with a toxin poses an acute health hazard and whether countermeasures must be enacted immediately (interruption of the water supply in extreme cases). As long as a temporary, transient violation of the safety limit remains below the action level and the affected population has been informed, the situation can be tolerated under certain conditions if a remedial plan is submitted and put into effect. This helps to combine

[6] Product of a factor of 10 for extrapolation from animals to humans times a factor of 10 for susceptibility differences between individuals in a given population.

forces to eliminate the root cause of the problem (e.g., reduction of eutrophication instead of more rigorous drinking water treatment). Action levels are derived specifically for each individual substance in consideration of its mechanism of action. An action level for cylindrospermopsin does not yet exist.

The proposed guideline safety value for CYN in drinking water (1 µg L^{-1}) was exceeded 18 times at lakes studied by us. In the study region, cylindrospermopsin now occurs just as frequently as the well-studied cyanobacterial toxin microcystin and at comparable concentration ranges. As explained below, an increase in CYN distribution and concentration can be expected in the future. These findings indicate that cylindrospermopsin may pose a health risk in certain situations.

Based on the current evidence, we recommend that cylindrospermopsin be included as a risk factor in drinking and bathing water safety risk assessments. Data on the monitoring, risk assessment and toxin elimination of microcystin is available but cannot be transferred directly to cylindrospermopsin because of the need for further scientific investigation of the following major issues:

1. Unlike microcystin, cylindrospermopsin often occurs in dissolved form. This poses a risk of penetration of cylindrospermopsin into the water supply because simple particle removal-based methods of drinking water treatment (flocculation and filtration) eliminate the cell-bound, particulate CYN fraction but not the dissolved CYN fraction. Microbial decomposition of cylindrospermopsin may be slower than that of microcystin, which often decomposes within a few days. Data on the persistence and biological degradation of cylindrospermopsin is therefore required, especially for assessment of the risk of cylindrospermopsin penetration into the drinking water.

2. The main producers of cylindrospermopsin and the conditions for their development have not been unequivocally determined. *Aphanizomenon flos-aquae* is a confirmed producer of cylindrospermopsin, but this cyanobacterial species is not the main producer of cylindrospermopsin in water bodies. *A. gracile*, also an indigenous cyanobacterial species, probably is one of the main producer, but unequivocal proof of this has not been demonstrated. Further cyanobacterial species must be targeted as potential producers of the cyanotoxin. Once they have been identified, suitable methods for detection of these organisms must be developed. Further investigation of the mechanisms that regulate the occurrence of the CYN-producing cyanobacteria is also essential. This will be discussed in the following section in conjunction with the population dynamics of *Cylindrospermopsis raciborskii*.

Hazard potential of *Cylindrospermopsis raciborskii*

C. raciborskii is a widespread cyanobacterium that occurs at relevant biomass levels. Although *C. raciborskii* has been ruled out as the main source of cylindrospermopsin occurrence, no "all-clear signal" can be given for this species for three reasons:

1. *Hazard potential:* In a predecessor project, *C. raciborskii* isolates were shown to induce significant toxicity that was not related to cylindrospermopsin. The resulting risk for humans still has not been determined. Further studies for structural determination of the toxin and extensive toxicity tests are therefore required.

2. *Relevant ecological issues: C. raciborskii* invasion can be expected to induce changes in our aquatic ecosystems. This will mainly affect biodiversity in that indigenous species will be supplanted. Relevant changes in biotic interactions and other processes cannot be excluded.

3. *Water management:* Water management measures are based on knowledge of (biotic) colonization patterns and processes in aquatic ecosystems (see above). Any changes in these parameters must be managed by adapting existing water purification and management measures accordingly. However, there is much less research data on predicting the effects of trophic decline than on eutrophication, e.g., "What Vollenweider couldn't tell us“ (Reynolds 1992).

Prognosis of further cyanobacteria development and toxin occurrence

The analyses of *C. raciborskii* population dynamics conducted in the present study provide fundamental new insights into the regulation of tropical species in temperate regions, the causes for its spread, and its further course of development.

Climate change (earlier rise in water temperatures over the course of the year) has promoted the establishment of *C. raciborskii* populations because it shifts the life cycle of this cyanobacterium to a period of improved growth conditions. If this trend in climate change continues, an increase in the size of *C. raciborskii* populations can be expected. Contrary to previous reports, we found that light, not temperature, is the growth-limiting factor for *C. raciborskii* growth during the vegetative phase. This finding should be considered in water management, because decreases in trophic level are always associated with an increase in water transparency and, thus, in light intensity. Trophic decline not only leads to a reduction of phosphorous concentrations but also, in many cases, to a reduction of

nitrogen concentrations. As a nitrogen-fixing species, *C. raciborskii* would then have a competitive advantage over other phytoplankters.

If global warming continues, one can expect to see an increase in *C. raciborskii* populations and, in all probability, the further spread of this species within our region and further north. Two other neo-cyanobacteria (also of the order Nostocales) were also detected in our waters; their life cycle begins with the germination of akinetes, and they are also able to fix nitrogen. Consequently, a northern shift in the expansion of these species can generally be expected. Further studies should be conducted to determine whether the causes of spread of these species are the same as for *C. raciborskii*.

It must be assumed that the mechanisms that regulate *C. raciborskii* population dynamics, as determined in the present study, also apply to indigenous species of the order Nostocales. If these changes in climate continue, especially the trophic decline of water bodies, then a general increase in species of the order Nostocales can be expected. The first signs of this have already been detected. This also applies to the *Aphanizomenon* species that produce cylindrospermopsin. An increase in cylindrospermopsin concentrations in our waters can therefore be expected.

A change in the species composition of cyanobacteria will ultimately lead to a change in toxin occurrence. When assessing the related risk potential, it is important to remember that some species of the order Nostocales can produce neurotoxins such as anatoxin and saxitoxin in addition to cylindrospermopsin.

Last but not least, we would like to emphasize that the CYLIN project data on the current status and future course of toxic cyanobacterial development in the Berlin-Brandenburg region can be extended to comparable water body types throughout the entire temperate climate zone.

12. Acknowledgements

We are sincerely grateful to all of the many national and international external cooperation partners who contributed to the success of the CYLIN project. We would expressly like to thank:

- Prof. Jiří Komárek (Institute of Botany, Czech Academy of Sciences, Třeboň, Czech Republic) and Dr. Larelle Fabbro (Centre for Environmental Management, Central Queensland University, Rockhampton, Australia) for their helpful comments concerning the taxonomic classification of *Anabaena bergii*.

- Prof. Aaron Kaplan and Gali Shalev-Alon (Dept. of Plant and Environmental Sciences, The Hebrew University of Jerusalem, Israel), Dr. Martin Saker (Ecotoxicology Laboratory, Faculty of Sciences, University of Porto, Portugal), Dr. Julianne Dyble (Institute of Marine Sciences, University of North Carolina, USA) and Dr. Andrew Chapman (GreenWater Laboratories, Palatka, Florida, USA) for kindly providing cultured strains of various CYN-producing and non-producing cyanobacteria needed for establishment of our molecular biological method.

- Dr. Chris Saints and Dr. Paul Rasmussen (CRC for Water Quality and Treatment, Australian Water Quality Centre, Bolivar, Australia) and Prof. Antonio Quesada (Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Spain) for kindly supplying DNA extracts of CYN-producing cyanobacteria.

- Prof. Ulf Karsten, Dr. Rhena Schumann, Dr. Henning Baudler and Ms. Jana Woelfel (Institute of Biosciences, Dept. of Applied Ecology, University of Rostock, Germany) for kindly enabling and conducting the sampling of "Bodden" (lagoon) waters at Baltic coast.

- Dr. Antje Köhler (ILAT - Institute of Food, Drug and Animal Disease Research, Berlin, Germany) for kindly providing samples from various Berlin lakes.

- Dr. Lothar Täuscher (Institute of Applied Water Ecology, Seddiner See, Germany) for kindly providing previously unpublished data on the distribution of *C. raciborskii* in 16 water bodies in the years 2002-2004.

We would also like to thank a number of colleagues from our home institutions (IGB, BTU, and UBA) for their assistance:

- It would not have been possible to sample such a large number of water bodies without the active assistance of Ingo Henschke, Wolfgang Terlinden, Michael Sachtleben and Roman Degebrodt.

- Gudrun Lippert, Ute Abel, Tina Hanke, Johanna Dalchow and Uta Mallok analyzed the nutrient and chlorophyll *a* concentrations of countless numbers of samples.

- The cyanobacterial qualitative analysis was carried out with support from Beate Müller, Andrea Launhardt and Tina Endrulat.

- Martin Beck isolated a portion of the cyanobacterial strains.

- Monika Degebrodt was a big help in cyanobacterial strain cultivation and molecular biological analysis.

- We are sincerely grateful to Christa Woodgett and Gabriele Gericke for assisting in the cylindrospermopsin analysis.

- Helgard Täuscher and Ute Mischke kindly provided unpublished data on the occurrence of *C. raciborskii*.

- Dr. Jan Köhler organized the sampling of several Berlin lakes.

- Mike Hemm generated the distribution maps.

Last but not least, we would like to thank our sponsors, Kompetenzzentrum Wasser Berlin (KWB) and Veolia Water, for providing full financial support for the CYLIN project.

13. References

List of publications originating from the CYLIN project

Fastner, J., Rücker, J., Stüken, A., Preußel, K., Nixdorf, B., Chorus, I., Köhler, A., Wiedner, C. 2007. Occurence of the Cyanobacterial Toxin Cylindrospermopsin in Germany. Environmental Toxicology 22 (1): 26-32.

Haande, S., Rohrlack, T., Ballot, A., Wiedner, C. Genetic characterisation of *Cylindrospermopsis raciborskii* isolates (Nostocales, Cyanobacteria) from Africa and Europe (submitted to: Harmful Algae).

Preußel, K., Stüken, A., Wiedner, C., Chorus, I., Fastner, J. 2006. First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon 47: 156-162.

Rücker, J., Stüken, A., Nixdorf, B., Fastner, J., Chorus, I., Wiedner, C. Concentrations of particulate and dissolved cylindrospermopsin (CYN) in 21 *Aphanizomenon* dominated lakes of North East Germany (submitted to: Toxicon.).

Rücker, J., Stüken, A., Nixdorf, B., Wiedner, C. 2006. Distribution and regulation of the originally tropical cyanobacterium *Cylindrospermopsis raciborskii* at its northern limits. In: Rabitsch, W., Klingenstein, F., Essl, F. (Eds.): BfN-Skripten 184, Bundesamt für Naturschutz, Bonn: p. 229.

Stüken, A., Preußel, K., Beck, M., Quesada, A., Sukenik, A., Dadheech, P., Wiedner, C. Phylogenetic position of the three cyanobacterial species *Anabaena bergii*, *Aphanizomenon ovalisporum* and *Aphanizomenon aphanizomenoides* (order Nostocales) (submitted to: Int. J. Syst. Evol. Microbiol.).

Stüken, A., Rücker, J., Endrulat, T., Preussel, K., Hemm, M., Nixdorf, B., Karsten, U., Wiedner, C. 2006. Distribution of three alien cyanobacterial species (Nostocales) in Northeast Germany: *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. Phycologia 45: 696–703.

Tingwey, E.I., Rücker, J., Launhardt, A., Wiedner, C. Nixdorf, B. 2007. Germination of *Cylindrospermopsis raciborskii* and *Aphanizomenon* species under natural and experimental conditions. Deutsche Gesellschaft für Limnologie. Tagungsbericht 2006 (in press).

Wiedner, C., Rücker, J., Brüggemann, R., Nixdorf, B. 2007. Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia. Online first: DOI 10.1007/s00442-007-0683-5.

Wiedner, C., Rücker, J., Stüken, A., Fastner, J., Chorus, I., Nixdorf, B. Seasonal dynamics of cylindrospermopsin and cyanobacteria in two German lakes (submitted to: FEMS Microbiol.).

Cited references

Banker, R., Carmeli, S., Hadas, O., Teltsch, B., Porat, R., Sukenik, A. 1997. Identification of cylindrospermopsin in the cyanobacterium *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from Lake Kinneret. Israel J. Phycol. 33: 613-616.

Bazzichelli, G.B., Abdelahad, N. 1994. Charctérisation morphométrique et statistique de deux populations d' *Aphanizomenon* du groupe *Aphanizomenon ovalisporum* FORTI des lacs de Nemi et Albano (Italie). Algological Studies 73: 1-21.

Behrendt, H., Nixdorf, B. 1993. The carbon balance of phytoplankton production and loss processes based on in situ measurements in a shallow lake. Int. Rev. Ges. Hydrobiol. 78: 439-458.

Bernard, C., Harvey, M., Briand, J.F., Biré, R., Krys, S., Fontaine, J.J. 2003. Toxicological comparison of diverse *Cylindrospermopsis raciborskii* strains: evidence of liver damage caused by an French *C. raciborskii* strain. Environ. Toxicol. 18: 176-186.

Bourke, A.T.C., Hawes R.B., Neilson A., Stallman, N.D. 1983. An outbreak of the hepatoenteritis (the Palm island mystery disease) possibly caused by algal intoxication. Toxicon Suppl. 3: 45-48.

Bouvy, M., Molica, R., De Oliveira, S., Marinho, M., Beker, B. 1999. Dynamics of a toxic cyanobacterial bloom (*Cylindrospermopsis raciborskii*) in a shallow reservoir in the semi-arid region of northern Brazil. Aqua Micobial. Ecol. 20: 285-297.

Branco, C.W., Senna, P.A.C. 1994. Factors influencing the development of *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* in Paranoá Reservoir, Brasíl. Algological Studies 75: 85-96.

Briand, J.F., Leboulanger, C., Humbert, J.F., Bernard, C., Dufour, P. 2004. *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, or global warming? Journal of Phycology 40: 231-238.

Briand, J.F., Robillot, C., Quiblier-Lloberas, C., Humbert, J.F., Couté, A., Bernard, C. 2002a. Environmental context of *Cylindrospermopsis raciborskii* (cyanobacteria) blooms in a shallow pond in France. Water Research 36: 3183-3192.

Briand, J.F., Robillot, C., Quiblier-Llobéras, C. Bernard, C. 2002b. A perennial bloom of *Planktothrix agardhii* (Cyanobacterial) in a shallow eutrophic French lake: Limnological and microcystin production studies. Arch. Hydrobiol. 153: 605-622.

Burgoyne, D.L., Hemscheidt, T.K., Moore, R.E. , Runnegar, M.T.C. 2000. Biosynthesis of cylindrospermopsin. J. Org. Chem. 65: 152-156.

Burns, J., Williams, C., Chapman, A. 2002. Cyanobacteria and their toxins in Florida surface waters. In: Johnson, D., Harbison, R.D. (Eds.) Proceedings of Health Effects of Exposure to Cyanobacteria Toxins – State of Science, Saratoga, Florida, August 13-14, 200: 16-21.

Carmichael, W.W., Azevedo, S.M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Persp. 109: 663-668.

Chiswell, R.K, Shaw, G.R, Eaglesham, G.K, Smith, M.J, Norris, R.L, Seawright, A.A, Moore, M.R., 1999. Stability of cylindrospermopsin, the toxin from the cyanobacterium *Cylindrospermopsis raciborskii*: effect of pH, temperature, and sunlight on decomposition. Environ. Toxicol. 14: 155-165.

Chonudomkul, D., Yongmanitchai, W., Theeragool, G., Kawachi, M., Kasai, F., Kaya, K., Watanabe, M.M. 2004. Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) strains from Thailand and Japan. FEMS Microbiology Ecology 48: 345-355.

Christiansen, G., Fastner, J., Erhard, M., Börner, T., Dittmann, E. 2003. Microcystin biosynthesis in *Planktothrix*: genes, evolution and manipulation. J. Bacteriol. 185: 564-572.

Couté, A., Leitao, M., Martin, C. 1997. Premièr observation du genre Cylindrospermopsis (Cyanophyceae, Nostocales) en France. Cyptogaie, Algol 18: 57-70.

Czensny R. 1938. Die Oscillatorienerkrankung unserer Seen, Biologie und Chemismus einiger märkischer Seen. Vom Wasser 8: 36-57.

DEV (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). 1976-1998. Schlammuntersuchung. Verlag Chemie, Weinheim, D11, E5, D9, C9, E1, H7

Dokulil, M.T., Mayer, J. 1996. Population dynamics and photosynthetic rates of a *Cylindrospermopsis - Limnothrix* association in a highly eutrophic urban lake, Alte Donau, Vienna, Austria. Algological Studies 83: 179-195.

Edwards, M., Richardson, A.J. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 40: 881-884.

EU (Europäische Union) 2000. Richtlinie 2000/60/EG des Europäischen Parlaments und des Rates vom 23. Oktober 2000 zur Schaffung eines Ordnungsrahmens für Maßnahmen der Gemeinschaft im Bereich der Wasserpolitik. Amtsblatt der EU vom 22.12.2000 (L327/1).

Fabbro, L.D., Duivenvoorden, L.J. 1996. Profile of a bloom of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Ruju in Fitzroy river in tropical central Queensland. Mar. Freshwater Res. 47: 685-694.

Falconer, I.R., Humpage, A.R. 2001. Preliminary evidence for in vivo tumour initiation by oral administration of extracts of the blue-green alga *Cylindrospermopsis raciborskii* containing the toxin cylindrospermopsin. Environmental Toxicology 16(2): 192-195.

Falconer, I.R., Humpage, A.R., 2006. Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsin. Environ. Toxicol. 21: 299-304.

Fastner, J., Heinze, R., Humpage, A.R., Mischke, U., Eaglesham, G.K., Chorus, I. 2003. Cylindrospermopsin occurrence in two German lakes and preliminary

assessment of toxicity and toxin production of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolates. Toxicon 42: 313-321.

Fastner, J., Neumann, U., Wirsing, B., Weckesser, J., Wiedner, C., Nixdorf, B., Chorus, I. 1999. Microcystins (hepatotoxic heptapeptides) in German fresh waterbodies. Environ. Tox. 14:13-22.

Fergusson, K.M., Saint, C.P. 2000. Molecular phylogeny of *Anabaena circinalis* and its identification in environmental samples by PCR. Applied and Environmental Microbiology 66: 4145-4148.

Fergusson, K.M., Saint, C.P. 2003. Multiplex PCR assay for *Cylindrospermopsis raciborskii* and cylindrospermopsin-producing cyanobacteria. Environ. Toxicol. 18: 120-125.

Froscio, S.M., Humpage, A.R., Burcham, P.C., Falconer, I.R. 2003. Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. Environ. Toxicol. 18(4): 243-251.

Geitler, L. 1932. Cyanophyceae. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz, pp. 1-1196. Akademische Verlagsgesellschaft, Leipzig, Germany.

Gerten, D., Adrian, R. 2000. Climate-driven changes in spring plankton dynamics and the sensitivity of shallow polymictic lakes to the North Atlantic Oscillation. Limnol Oceanogr 45: 1058-1066.

Hamilton, P.B., Ley, L.M., Dean, S., Pick, F.R. 2005. The occurrence of the cyanobacterium *Cylindrospermopsis raciborskii* in Constance Lake: An exotic cyanoprokaryote new to Canada. Phycologia 44: 17-25.

Haney, J.F. 1987. Field studies on zooplankton-cyanobacteria interactions. New Zeeland J. Mar. Freshw. Res. 21: 467-475.

Harada, K.I., Ohtani, I., Iwamoto, K., Suzuki, M., Watanabe, M.F., Watanabe, M., Terao, K. 1994. Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. Toxicon 32: 73-84.

Hawkins, P.R., Chandrasena, N.R., Jones, G.J., Humpage, A.R., Falconer, I.R. 1997. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. Toxicon 35: 341-346.

Hawkins, P.R., Runnegar, M.T.C., Jackson, A.R.B., Falconer, I.R. 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju isolated from a domestic water supply reservoir. Appl. Env. Microbiol. 50: 1292-1295.

Hemm, M., Jöhnk, K.D. 2004: Datenbank stehender Gewässer in Deutschland - Beschreibung und deren Anwendungen. In: J. Rücker & B. Nixdorf (Eds.) Gewässerreport Nr. 8. BTUC-AR 3/2004 - Eigenverlag der BTU Cottbus: 145-159.

Hindák, F. 2000. Morphological variation of four planktic nostocalean cyanophytes - members of the genus *Aphanizomenon* or *Anabaena*? Hydrobiologia 438: 107-116.

Hindák, F., 1992. Several interesting planktic cyanophytes. Algological Studies 66: 1-15.

Hindák, F., Hindáková, A. 2003. Diversity of cyanobacteria and algae of urban gravel pit lakes in Bratislava, Slovakia: a survey. Hydrobiologia 506 (1-3): 155-162.

Hiripi, L., Nagy, L., Kalmár, T., Kovács, A., Vörös, L. 1998. Insect (*Locusta migratoria migratorioides*) test monitoring the toxicity of cyanobacteria. Neurotoxicology 19: 605-608.

Hoeger, S.J., Shaw, G., Hitzfeld, B.C., Dietrich, D.R., 2004. Occurrence and elimination of cyanobacterial toxins in two Australian dinking water treatment plants. Toxicon 43: 639-649.

Hoffmann, L. 1996. Geographic distribution of freshwater blue-green algae. Hydrobiologia 336: 33-40.

Horecká, M., Komárek, J. 1979. Taxonomic position of three planctonic blue-green algae from the genera *Aphanizomenon* and *Cylindrospermopsis*. Presilia 51: 289-312.

Huber-Pestalozzi, G. 1938. Das Phytoplankton des Süßwassers. Systematik und Biologie. 1. Teil: Blaualgen, Bakterien, Pilze. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart: 342 pp.

Humpage, A.R., Falconer, I.R. 2003. Oral toxicity of the cyanobacterial toxin cylindrospermopsin male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. Environ. Toxicol. 18: 94-103.

Humpage, A.R., Fontaine, F., Froscio, S., Burcham, P., Falconer, I.R. 2005. Cylindrospermopsin genotoxicity and cytotoxicity: Role of cytochrome P-450 and oxidative stress. Journal of Toxicology and Environmental Health-Part a-Current Issues 68(9): 739-753.

Janse, I., Kardinal, W.E.A., Meima, M., Fastner, J., Visser, P.M., Zwart, G. 2004. Toxic and nontoxic Microcystis colonies in natural populations can be differentiated on the basis of rRNA gene internal transcribed spacer diversity. Appl. Environ. Microbiol. 70: 3979-3987.

Kann, J., Welch, E.B. 2005. Wind control on water quality in shallow, hypereutrophic Upper Klamath Lake, Oregon. Lake and Reservoir Management 21 (2): 149-158.

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press: 509 pp.

Komárek, J., Ettl, H. 1958. Algologische Studien: Die Taxonomische Revision der Planktischen Blaualgen der Tschechoslowakei. Ceskoslovenska Akademie Ved, Prague. 358 pp.

Krienitz, L., Hegewald, E. 1996. Über das Vorkommen von wärmeliebenden Blaualgenarten in einem norddeutschen See. Lauterbornia 26: 55-63.

Kurmayer, R., Gumpenberger, M., 2006. Diversity of microcystin genotypes among populations of the filamentous cyanobacteria *Planktothrix rubescens* and *Planktothrix agardhii*. Molecular Ecology 15 (12): 3849-3861.

LAWA (Länderarbeitsgemeinschaft Wasser) (Ed.) 1999. Gewässerbewertung – stehende Gewässer - Vorläufige Richtlinie für eine Erstbewertung von natür-

lichen entstandenen Seen nach trophischen Kriterien. - Kulturbuch-Verlag, Berlin: 74 pp.

Li, R., Carmichael, W.W., Brittain, S., Eaglesham, G.K., Shaw, G.R., Liu, Y.D., Watanabe, M.M. 2001a. First report of the cyanotoxins cylindrospermopsin and deoxy-cylindrospermopsin from *Raphidiopsis curvata* (Cyanobacteria). Journal of Phycology 37: 1121-1126.

Li, R., Carmichael, W.W., Brittain, S., Eaglesham, G.K., Shaw, G.R., Mahakhant, A., Noparatnaraporn, N., Yongmanitchai, W., Kaya, K., Watanabe, M.M. 2001b. Isolation and identification of the cyanotoxin cylindrospermopsin and deoxy-cylindrospermopsin from a Thailand strain of *Cylindrospermopsis raciborskii* (Cyanobacteria). Toxicon 39: 973-980.

Lippert, G., Herold, I., Heckemann, H.J., Stadler, B. 2004. Erfahrungen und Ergebnisse zur Bestimmung von Gesamtphosphor mit Festphasenanreicherung im Bereich von 10 bis 200 µg l-1 in Gewässern mit der FIA-LAB II (MLE GmbH Radebeul). In: J. Rücker & B. Nixdorf (Eds.) Gewässerreport Nr. 8. BTUC-AR 3/2004 - Eigenverlag der BTU Cottbus: 133-144.

Manti, G., Mattei, D., Messineo, V., Melchiorre, S., Bogialli, S., Sechi, N., Casiddu, P., Luglio, L., Di Brizio, M., Bruno, M. 2005. First report of *Cylindrospermopsis raciborskii* in Italy. Harmful Algal News 28: 8-9.

Mbedi, S., Welker, M., Fastner, J., Wiedner, C. 2005. Variability in the microcystin synthetase gene cluster in the genus *Planktothrix* (Oscillatoriales, Cyanobacteria). FEMS Microbiol. Lett. 245: 299-306.

McGregor, G.B., Fabbro, L.D. 2000. Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: Implications for monitoring and management. Lakes & Reservoirs: Research and Management 5: 195-205.

Mischke, U. 2003. Cyanobacteria associations in shallow polytrophic lakes: influence of environmental factors. Acta Oecologica 24: 11-23.

Mischke, U., 2001. Der Neophyt Cylindrospermopsis raciborskii: Eine Blaualge aus tropischen Regionen in Gewässern des Spree-Dahme-Einzugsgebietes. In Krumbeck, H., Mischke, U. (Eds.), Gewässerreport (Nr. 6). BTUC-AR: 39-62.

Moisander, P.H., McClinton III, E., Pearl, H.W. 2002. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecology 43: 432-442.

Nixdorf, B., Hemm, M., Hoffmann, A., Richter, P., 2004: Dokumentation von Zustand und Entwicklung der wichtigsten Seen Deutschlands. Umweltbundesamt. UBA-Texte Forschungsbericht 299 24 274, UBA-FB 000511: 1-1056.

Nixdorf, B., Mischke, U., Rücker, J. 2003. Phytoplankton assemblages and steady state in deep and shallow eutrophic lakes - an approach to differentiate the habitat properties of Oscillatoriales. Hydrobiologia 502: 111-121.

Nixdorf, B., Rücker, J., Deneke, R., Zippel, P. 1995. Limnologische Zustandsanalyse von Standgewässern im Scharmützelseegebiet, Teil I. BTUC-AR 1/95, Eigenverlag der BTU Cottbus: 52 pp.

Ohtani, I., Moore, R.E., Runnegar, M.T.C. 1992. Cylindrospermopsin: a potent hepatotoxin from blue-green alga *Cylindrospermopsis raciborskii*. J. Am. Chem. Soc. 114: 7941-7942.

Padisák, J. 1997. *Cylindrospermopsis raciborskii (*Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. Archiv für Hydrobiologie, Supplement 107: 563-593.

Padisak, J., Reynolds, C.S. 1998. Selection of phytoplankton associations in Lake Balaton, Hungary, in response to eutrophication and restoration measures, with special reference to the cyanoprokaryotes. Hydrobiologia 384: 41-53.

Preußel, K., Stüken, A., Wiedner, C., Chorus, I., Fastner, J. 2006. First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon. 47: 156-162.

Quesada, A., Moreno, E., Carrasco, D., Paniagua, T., Wormer, L., Hoyos, C., Sukenik, A. 2006. Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. European Journal of Phycology 41: 39-45.

Reynolds, C.S. 1992. Eutrophication and the management of planktonic algae: What Vollenweider couldn't tell us. In Sutcliffe, D.W., Jones J.G. (Ed.), Research and application to water supply. Freshwater Biol. Ass.

Riley, G.A. 1957. Phytoplankton in the north central Sargasso Sea 1950-1952. Limnol. Oceanogr. 2: 252-272.

Rott, E. 1981. Some results from phytoplankton counting intercalibration. Schweiz. Z. Hydrol. 43: 34-62.

Rott, E., Salmaso, N., Hoehn, E., 2007: Quality control of Utermöhl-based phytoplankton counting and biovolume estimates – An easy task or a Gordian knot? Hydrobiologia 578:141-146.

Rouhiainen, L., Vakkilainen, T., Siemer, B.L., Buikema, W., Haselkorn, R., Sivonen, K. 2004. Genes coding for hepatotoxic heptapeptides (microcystins) in the cyanobacterium *Anabaena* strain 90. Appl. Environ. Microb. 70: 686-692.

Rücker, J., Wiedner, C., Zippel, P. 1997. Factors controlling the dominance of *Planktothrix agardhii* and *Limnothrix redekei* in eutrophic shallow lakes. Hydrobiologia 342/343: 107-115.

Saker, M.L., Eaglesham, G.K., 1999. The accumulation of cylindrospermopsin from the cyanobacterium *Cylindrospermopsis raciborskii* in tissues of the red-claw crayfish *Cherax quadricarinatus*. Toxicon 37: 1065-1077.

Saker, M.L., Griffith, D.J. 2000. Effects of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. Phycologia 39: 349-354.

Saker, M.L., Neilan, B.A. 2001. Varied diazotrophies, morphologies and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from Northern Australia. Appl. Env. Microbiol. 67: 1839-1845.

Saker, M.L., Nogueira, I.C.G., Vasconcelos, C.M., Neilan, B.A., Eaglesham, G.K., Pereira, P. 2003. First report and toxicological assessment of the cyanobacte-

rium *Cylindrospermopsis raciborskii* from Portuguese freshwaters. Ecotoxicol Environ Safety 55: 243-250.

Schäperclaus, W. 1941. Seenverschlechterung. Z. Fischerei XXXVIII: 345-375.

Schembri, M.A., Neilan, B.A., Saint, C.P. 2001. Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. Environ. Toxicol. 16: 413-421.

Seifert, M., McGregor, G., Eaglesham, G., Wickramasinghe, W., Shaw, G. 2007: First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck. Harmful Algae 6: 73-80.

Shalev-Alon, G., Sukenik, A., Livnah, O., Schwarz, R., Kaplan, A. 2002. A novel gene encoding amidinotransferase in the cylindrospermopsin producing cyanobacterium *Aphanizomenon ovalisporum*. FEMS Microbiol. Lett. 209: 83-87.

Shaw, G.R., Seawright, A.A., Moore, M.A., Lam, P.K.S. 2000. Cylindrospermopsin, a cyanobacterial alkaloid: Evaluation of its toxicological activity. Therapeutic Drug Monitoring 22: 89-92.

Shaw, G.R., Sukenik, A., Livne, A., Chiswell, R.K., Smith, M.J., Seawright, A.A., Norris, R.L., Eaglesham, G.K., Moore M.R. 1999. Blooms of cylindrospermopsin containing cyanobacterium *Aphanizomenon ovalisporum* (Forti), in newly constructed lakes, Queensland, Australia. Environ. Toxicol. 14: 167-177.

Skuja, H. 1938. Süsswasseralgen aus Griechenland und Kleinasien. Hedwigia 77: 15-70.

Souza, R.C.R., Carvalho, M.C., Truzzi, A.C. 1998. *Cylindrospermopsis raciborskii* (Wolosz.) Seenaya and Subba Raju (Cyanophyceae) dominance and a contribution to the knowledge of Rio Pequeno Arm, Billings reservoir, Brazil. Environ. Toxicol. Water Qual. 13: 73-81.

Spoof, L., Berg, K.A., Rapala, J., Lahti, K., Lepistö, L., Metcalf, J.S., Codd, G.A., Meriluoto, J. 2006. First observation of cylindrospermopsin in *Anabaena lapponica* isolated from boreal environment (Finland). Environ. Toxicol. 21: 552-560.

Straile, D. 2000. Meteorological forcing of plankton dynamics in a large and deep continental European lake. Oecologia 122: 44-50.

Täuscher, L. 2003a. Qualitative und quantitative Untersuchungen des Phytoplanktons in Seen des Dahmegebietes im Land Brandenburg als Beitrag zu Grundlagen der Umsetzung der Wasserrahmenrichtlinie der Europäischen Union. Projektbericht des Seenprojektes Brandenburg e.V., Seddin, Germany.

Täuscher, L. 2003b. Phytoplankton. In: Möller, D., Mietz, O., Täuscher, L., Henker, H. (Eds.). Güterfelder Haussee. Limnologischer Abschlussbericht für das Jahr 2002. Institut für angewandte Gewässerökologie, Seddin, Germany.

Täuscher, L. 2004a. Phytoplankton. In: Meisel, J., Täuscher, L., Kabus, T., Henker, H., Vedder, F. (Eds.). Ökologische und limnologische Untersuchungen zur wissenschaftlichen Vorbereitung der Umsetzung der EU-WRRL an Seen des Landes Brandenburg im Jahr 2003. Projektbericht des Seenprojektes Brandenburg e.V., Seddin, Germany.

Täuscher, L. 2004b. Gutachten für das Gesundheitsamt Oberhavel, Oranienburg, Germany.

Teubner, K., Morscheid, H., Tolotti, M., Greisberger, S., Morscheid, H., Kucklentz, V. 2004. Bedingungen für Auftreten toxinbildender Blaualgen (Cyanobakterien) in bayrischen Seen und anderen stehenden Gewässern. In: Materialien Nr. 113. Bayerisches Landesamt für Wasserwirtschaft (Ed.), München, Germany.

Tillet, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T., Neilan, B.A. 2000. Structural organisation of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system. Chem. Biol. 7: 753-764.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Internat. Verein. Limnol. 9: 1-38.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoeg-Guldenberg, O., Bairlein, F. 2002. Ecological responses to recent climate change. Nature 416: 389-395.

Weithoff, G. 1998. Steuermechanismen der Phytoplanktondynamik im flachen eutrophen Flakensee. Dissertation, 51 pp. Humboldt-Universität, Berlin.

Welker, M., Bickel, H., Fastner, J. 2002. HPLC-PDA detection of cylindrospermopsin - opportunities and limits. Water Res. 36: 4659-4663.

Welker, M., von Döhren, H., Täuscher, H., Steinberg, C.E.W., Erhard, M. 2003. Toxic microcystis in shallow lake Müggelsee (Germany) - Dynamics, distribution, diversity. Arch. Hydrobiol. 157: 227-248.

Weyhenmeyer, G.A., Blenckner, T., Petterson, K. 1999. Changes of the plankton spring outburst related to the North Atlantic Oscillation. Limnol. Oceanogr. 44: 1788-1792.

Wiedner, C., Nixdorf, B., 1997. Verbreitung und Steuerung der Entwicklung von toxischen und nicht-toxischen Cyanobakterien in ostbrandenburgischen Gewässern unterschiedlicher Trophie und Hydrographie im Zuge der reduzierten Belastung. WaBoLu Hefte 4/97: 17-26.

Wiedner, C., Nixdorf, B., Heinze, R., Wirsing, B., Neumann, U., Weckesser, J. 2002. Regulation of cyanobacteria and microcystin dynamics in polymictic shallow lakes. Arch. Hydrobiol. 155: 383-400.

Wiedner, C., Visser, P.M., Fastner, J., Metcalf, J.S., Codd, G.A., Mur, L.R. 2003. Effects of light on the microcystin content of Microcystis strain PCC 7806. Appl. Environ. Microbiol. 69: 1475-1481.

Winder, M., Schindler, D.E. 2004. Climate change uncouples trophic interactions in an aquatic ecosystem. Ecology 85: 2100-2106.

Woloszynska, J. 1912. Das Phytoplankton einiger Javanian Seen mit Berücksichtigung des Sawa-Planktons. Bull. Int Aad. Sci. Cracoviae Ser. B. 6: 649-709.

Wood, S.A., Stirling, D.J. 2003. First identification of the cylindrospermopsin-producing cyanobacterium *Cylindrospermopsis raciborskii* in New Zealand. New Zealand Journal of Marine and Freshwater Research 37: 821-828.

Wundsch, H.H. 1940. Beiträge zur Fischereibiologie märkischer Seen, VI. die Entwicklung eines besonderen Seentypus (H_2S-Oscillatorien-Seen) im Flußgebiet

der Spree und Havel, und seine Bedeutung für die Fischereibiologischen Bedingungen in dieser Region. Z Fischerei XXXVIII: 443-648.

Zippel, P. 1996. Phytoplanktonsukzession und -dynamik in den Gewässern des Scharmützelseegebiets. In: Nixdorf, B., Kleeberg, A. (Eds.) Gewässerreport (Teil II), BTUC-AR 2/1996 - Eigenverlag der BTU Cottbus: 44-60.

Appendix

List of water bodies studied in the 2004 Screening Program, including the name of each lake (German: "*See*") or water body and its geographic coordinates, volume, maximum depth (max. depth), Secchi depth (SD) and seston dry weight (wt) concentration of cylindrospermopsin (CYN). n.d. – not detected.

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Alter Wochowsee 52°13'15" N; 13°55'43" E	0.32	0.45	2.5	0.2	n.d.
Bikowsee near Zechlinerhütte 53°08'50" N; 12°53'27" E	0.31		11.0	0.6	0.532
Blankensee 52°13'35" N; 13°07'06" E	2.90	3.42	3.9	0.4	n.d.
Bodtstedter Bodden 54°23'37" N; 12°37'19" E				0.4	n.d.
Bötzsee 52°34'01" N; 13°49'44" E	0.86	5.46	12.0	1.6	n.d.
Braminsee 53°08'18" N; 12°48'09" E	0.69		2.2	0.3	5.770
Breiter Luzin 53°21'25" N; 13°28'03" E	3.45	77.04	58.3	3.4	n.d.
Breitlingsee (Havel River) 52°23'03" N; 12°28'45" E	5.13		4.5	0.6	n.d.
Bruchsee, near Templin 53°08'07" N; 13°32'44" E	0.20		2.2	0.7	0.444
Buckowsee, Schermützelsee 52°34'00" N; 14°04'13" E	0.13		11.0	2.4	n.d.
Bückwitzer See 52°52'19" N; 12°29'18" E	0.49		8.0	0.4	
Bützsee 52°49'50" N; 12°53'51" E	2.23		4.0	0.7	4.925
Dagowsee 53°09'03" N; 13°03'10" E	0.22	1.20	9.5	0.9	0.147
Dämeritzsee 52°25'22" N; 13°44'03" E	1.03	2.74	5.7	0.9	n.d.
Dobrasee 52°11'05" N; 13°52'52" E	0.24	0.86	10.0	4.2	0.151
Dolgensee near Dolgenbrodt 52°14'59" N; 13°44'35" E	1.52	3.30	2.5	0.4	1.993
Dollgower See 53°04'32" N; 13°00'38" E	0.18		1.7	0.4	0.584
Düstersee 53°05'32" N; 13°45'28" E	0.43		9.0	2.6	2.004
Fängersee 52°35'13" N; 13°50'02" E	0.46	1.31	5.0	1.5	n.d.
Globsowsee (Altglobsower See) 53°07'41" N; 13°07'07" E	0.14		4.0	1.2	0.247

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Grimnitzsee 52°58'45" N; 13°46'59" E	7.77	29.50	9.0	2.0	n.d.
Groß Schauener See 52°14'01" N; 13°53'54" E	1.46		4.5	0.4	n.d.
Große Steinlanke (Unterhavel) 52°27'03" N; 13°11'10" E	3.44	20.51	9.3		n.d.
Großer Däbersee near Waldsieversdorf 52°32'32" N; 14°04'50" E	0.19		16.0	1.9	3.220
Großer Glubigsee 52°11'38" N; 14°00'02" E	0.58	2.66	13.0	0.8	4.333
Großer Klobichsee 52°33'14" N; 14°07'24" E	0.47		9.5	1.4	0.362
Großer Kolpiner See 52°17'43" N; 13°59'45" E	0.22	0.45	4.6	0.6	n.d.
Großer Kossenblatter See 52°08'04" N; 14°06'02" E	1.68		3.2	0.2	n.d.
Großer Linowsee 53°06'24" N; 12°51'17" E	0.34		18.0	3.9	n.d.
Großer Moddersee near Groß Köris 52°10'08" N; 13°39'45" E	0.29	0.26	2.0	0.4	n.d.
Großer Möggelinsee 52°08'04" N; 13°31'29" E	0.37	0.48	2.6	0.3	6.294
Großer Müggelsee 52°26'15" N; 13°38'55" E	7.67	36.52	8.9	4.1	n.d.
Großer Müllroser See 52°13'57" N; 14°25'06" E	1.27	4.34	7.0	1.5	n.d.
Großer Plessower See 52°23'01" N; 12°54'00" E	3.22	20.80	13.4	1.8	73.213
Großer Präßnicksee 53°02'13" N; 13°45'09" E	1.14		12.0	1.6	0.125
Großer Schlagenthinsee 52°31'10" N; 14°06'20" E	0.16	0.41	7.0	1.9	0.103
Großer Seddiner See 52°16'24" N; 13°01'49" E	2.18	6.62	7.2	0.6	39.565
Großer Storkower See, Nordbecken 52°15'17" N; 13°57'10" E	1.39	6.59	11.4	2.0	n.d.
Großer Storkower See, Südbecken 52°14'01" N; 13°58'47" E	2.33	8.79	8.0	1.5	0.288
Großer Tornowsee near Buckow 52°34'38" N; 14°06'12" E	0.09		9.6	2.3	n.d.
Großer Treppelsee 52°08'37" N; 14°27'04" E	0.71	1.60	5.6	0.4	
Großer Wannsee 52°25'43" N; 13°10'22" E	2.82	15.42	9.8	1.1	n.d.
Großer Wünsdorfer See 52°09'02" N; 13°27'55" E	1.65			0.5	1.625

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Großer Zechliner See 53°09'26" N; 12°48'06" E	1.84	20.81	36.8	3.7	0.210
Großer Zeschsee 52°06'39" N; 13°30'52" E	0.38	1.70	15.0	4.6	13.559
Großer Zug 52°20'43" N; 13°39'24" E	0.88		2.5	0.4	0.734
Grössinsee 52°15'18" N; 13°07'49" E	0.93	1.34	2.6	0.4	n.d.
Grunewaldsee 52°28'11" N; 13°15'47" E	0.17	0.50	6.5	0.7	0.310
Gudelacksee 52°58'38" N; 12°57'21" E	4.25		25.5	2.5	2.260
Heiligensee 52°36'14" N; 13°12'55" E	0.32	1.92	9.5	1.6	28.311
Heiliger See near Potsdam 52°24'44" N; 13°04'18" E	0.35	2.28	13.0		
Hölzener See 52°11'37" N; 13°42'33" E	1.15	9.10	12.5	1.0	0.211
Huwenowsee 52°58'10" N; 13°05'11" E	0.39		6.0	1.6	n.d.
Jabeler See 53°32'09" N; 12°33'07" E	2.44	12.90	22.6	1.1	3.913
Jürgenlanke (Unterhavel) 52°29'44" N; 13°11'51" E	2.82	10.37	6.8	0.4	n.d.
Kalksee near Binenwalde 53°03'02" N; 12°47'43" E	0.54		21.0	1.2	9.843
Katerbower See 52°58'41" N; 12°39'27" E	0.54		2.0	2.0	n.d.
Klarer See near Alt Temmen 53°05'48" N; 13°44'50" E	0.45		8.5	1.5	0.599
Klein Köriser See 52°10'31" N; 13°41'33" E	1.57		10.0	0.6	0.142
Kleiner Glubigsee 52°11'59" N; 14°01'03" E	0.06		4.5	0.9	3.624
Kleiner Kolpiner See 52°17'26" N; 13°59'28" E	0.09	0.11	2.0	0.3	n.d.
Kleiner Moddersee near Groß Köris 52°10'34" N; 13°39'52" E	0.20	0.39	4.0	0.4	n.d.
Kleiner Müggelsee 52°25'47" N; 13°40'36" E	0.16		7.5	1.2	
Kleiner Zeschsee 52°07'17" N; 13°31'26" E	0.23	0.61	5.6	0.8	0.733
Kölpinsee near Milmersdorf 53°06'32" N; 13°39'50" E	1.65		9.5	2.0	n.d.
Königsberger See 53°03'06" N; 12°25'56" E	0.46		9.0	0.4	48.908

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Köthener See 52°04'50" N; 13°48'36" E	1.48		2.0	0.5	n.d.
Krossinsee 52°21'48" N; 13°40'27" E	1.61		5.5	0.4	1.349
Krumme Lanke 52°27'08" N; 13°13'59" E	0.14	0.54	6.8	2.1	
Krüpelsee (Dahme) 52°17'33" N; 13°41'26" E	1.69		4.5	0.4	3.516
Kutzingsee 52°14'14" N; 13°49'59" E	0.32	0.78	6.0	0.3	7.664
Langer See 52°14'32" N; 13°47'03" E	1.38	3.27	3.5	0.4	6.897
Langer See (Dahme) 52°24'32" N; 13°37'05" E	2.51	10.59	8.5	0.4	1.567
Lebbiner See 52°16'45" N; 13°56'26" E	0.28	0.55	4.0	0.5	7.405
Liepnitzsee 52°44'54" N; 13°30'33" E	1.15		16.5		
Lieps 53°27'07" N; 13°09'30" E	4.31	9.70	3.8	0.3	2.773
Melangsee 52°09'40" N; 13°59'18" E	0.12	0.20	2.8	0.4	5.743
Mellensee 52°10'29" N; 13°24'32" E	2.15	7.20	10.0	0.6	n.d.
Moderfitzsee near Himmelpfort 53°11'09" N; 13°14'08" E	0.59		5.5		2.514
Molchowsee 52°58'05" N; 12°49'49" E	0.47			0.9	2.389
Motzener See 52°12'54" N; 13°34'06" E	2.06	12.70	13.5	2.4	
Nehmitzsee 53°07'46" N; 12°59'12" E	1.61	9.30	18.6	0.6	n.d.
Neuendorfer See near Sperenberg 52°07'40" N; 13°22'28" E	0.62		7.5	1.0	n.d.
Obersee 52°58'46" N; 12°26'02" E	1.63	11.80		0.4	n.d.
Oderberger See 52°51'32" N; 14°01'10" E	1.03		2.5	0.5	n.d.
Oelsener See 52°07'58" N; 14°23'53" E	1.07		2.9	0.6	
Parsteiner See 52°55'47" N; 13°59'06" E	10.03	77.00	31.0	4.3	n.d.
Pätzer Vordersee 52°14'02" N; 13°39'12" E	1.65		18.5	1.8	n.d.
Petersdorfer See 52°18'52" N; 14°04'27" E	0.24	0.45	3.8	0.4	11.803

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Petznicksee 53°09'20" N; 13°36'54" E	0.72		2.0	0.4	2.424
Plötzensee 52°32'38" N; 13°19'49" E	0.08	0.23	6.2	1.0	9.023
Rahmer See, w Wandlitz 52°44'53" N; 13°24'36" E	0.80		4.0	1.1	19.983
Rangsdorfer See 52°17'19" N; 13°24'14" E	2.45	3.75	2.0	0.2	n.d.
Rheinsberger See 53°07'26" N; 12°52'15" E	2.62		30.0	1.0	5.154
Ribnitzer See 54°15'30" N; 12°24'24" E				0.2	n.d.
Röblinsee 53°11'06" N; 13°07'14" E	0.90		6.0	1.9	2.229
Röddelinsee 53°05'41" N; 13°26'15" E	1.83		38.5	1.4	n.d.
Roofensee 53°06'41" N; 13°02'06" E	0.57	5.12	19.1	2.5	n.d.
Ruppiner See 52°53'45" N; 12°48'31" E	8.08	66.00	23.0	0.7	4.981
Saaler Bodden 54°19'45" N; 12°26'40" E				0.3	n.d.
Scharmützelsee 52°12'45" N; 14°01'04" E	12.07	108.23	29.0	2.8	0.245
Schermützelsee 52°34'09" N; 14°03'31" E	1.35	20.98	38.0	1.9	n.d.
Schlabornsee (Hüttensee) 53°09'02" N; 12°52'31" E	0.69		8.0	0.7	6.634
Schmaler Luzin 53°19'17" N; 13°26'17" E	1.45	20.98	33.5	3.0	n.d.
Schmöldesee 52°12'14" N; 13°44'25" E	1.05		5.5	1.0	n.d.
Schulzensee near Groß Köris 52°09'53" N; 13°39'16" E	0.12	0.18	2.5	0.3	n.d.
Schwedtsee 53°11'13" N; 13°09'13" E	0.55		4.0	1.0	52.531
Schweriner See (part of Teupitzer See) 52°09'14" N; 13°37'24" E				0.5	n.d.
Schwielowsee (Havel River) 52°20'22" N; 12°57'27" E	7.86	22.17	9.1	0.7	1.968
Seddinsee 52°23'19" N; 13°41'12" E	2.81	11.04	7.5	0.7	0.804
Siethener See 52°17'08" N; 13°12'14" E	0.71	1.66	4.3	0.4	n.d.
Spree River near Alt Schadow (outlet: Neuendorfer See) 52°07'05" N; 13°56'59" E					n.d.

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Spree River near Beeskow (outlet: Schwielochsee) 52°22'02" N; 13°59'34" E					0.171
Springsee 52°10'37" N; 13°59'41" E	0.59	5.86	18.0	1.0	0.351
Stahnsdorfer See 52°17'23" N; 13°53'24" E	0.50	0.43	2.0	0.4	n.d.
Stienitzsee 2°30'10" N; 13°49'21" E	2.12	14.05	14.5	2.3	n.d.
Stolpsee 53°10'31" N; 13°12'28" E	3.81		12.0	0.7	42.403
Straussee 52°34'38" N; 13°52'34" E	1.36	13.48	20.0	5.5	0.165
Streganzer See 52°12'28" N; 13°46'55" E	0.34		2.6	0.8	n.d.
Teufelssee 2°29'28" N; 13°14'01" E	0.02	0.07	5.9	0.6	0.285
Teupitzer See 52°08'30" N; 13°36'07" E	4.76	19.00	7.5	0.6	n.d.
Tholmannsee 52°56'56" N; 12°55'51" E	0.36		7.5	1.6	24.694
Tiefer See near Prieros 52°13'03" N; 13°47'32" E	0.29	2.06	11.5	2.4	n.d.
Tiefer See oder Grubensee 52°09'11" N; 13°59'39" E	0.61	7.02	23.4	6.1	n.d.
Tiefwarensee 53°31'48" N; 12°41'25" E	1.41	13.58	23.6	5.5	n.d.
Tollensesee 53°29'09" N; 13°10'58" E	17.90	315.89	31.3	8.1	n.d.
Trebelsee (Havel River) 52°28'11" N; 12°47'38" E	2.47		2.3	0.8	n.d.
Untersee 52°56'20" N; 12°26'50" E	2.16		8.1	0.6	n.d.
Vielitzsee 52°56'27" N; 13°00'19" E	1.11		3.0	0.8	n.d.
Vordersee near Obersdorf 52°32'47" N; 14°10'25" E	0.60		7.5	1.5	n.d.
Wandlitzer See 52°45'30" N; 13°27'18" E	2.05		24.5	2.9	19.073
Werbellinsee near Altenhof 52°55'41" N; 13°42'52" E	7.82	173.00	51.0	2.7	n.d.
Werbellinsee near Schönberg 52°55'27" N; 12°56'37" E	0.36		3.5	0.5	0.257
Wolletzsee 53°01'19" N; 13°54'34" E	3.10	26.86	16.0	2.5	n.d.
Wolziger See near Storkow 52°15'30" N; 13°49'20" E	5.55	32.02	13.2	1.8	n.d.

Name of Water Body	Area (km^2)	Volume (10^6 m^3)	Max. Depth (m)	Secchi Depth (m)	CYN (µg/g dry wt)
Wolziger See near Wünsdorf 52°08'15" N; 13°29'13" E	0.52	0.74	2.8	0.4	2.817
Wutzsee near Lindow 52°58'07" N; 13°00'44" E	1.12		17.0	2.5	n.d.
Zemminsee 52°09'38" N; 13°38'11" E	0.45	0.61	2.3	0.4	n.d.
Zermützelsee 53°00'43" N; 12°49'47" E	1.25		7.0	0.7	1.018
Zernsdorfer Lankensee 52°18'39" N; 13°42'25" E	0.41	0.90	4.0	0.5	1.619
Zeuthener See 52°21'34" N; 13°38'28" E	2.33	5.84	4.8	0.3	0.721
Zingster Stream 54°25'32" N; 12°41'07" E				0.4	n.d.

Cylindrospermopsis raciborskii, a toxic blue-green algae (cyanobacterium) of tropical origin, has spread in recent years to water bodies in the Berlin-Brandenburg region. Its typical toxin - cylindrospermopsin (CYN) – has also been detected in local waters, but it is unclear whether local *C. raciborskii* strains actually produce the toxin.

The aim of the CYLIN project was therefore to analyze the distribution and regulation of *C. raciborskii* and cylindrospermopsin and to identify which organisms are producing the toxin in order to predict their further development and associated risk potential.
The results of the CYLIN project show that *C. raciborskii* is widely distributed in the Berlin-Brandenburg region. Two other cyanobacteria of tropical origin were also detected. We observed that springtime water temperature regulates the time of germination of *C. raciborskii*, whereas water transparency influences its biomass production. Considering the effects of climate change, these findings suggest that the neo-cyanobacteria will have an increasing competitive advantage over our endemic cyanobacterial species.

ISBN 978-3-00-021364-9 Berlin, 2007