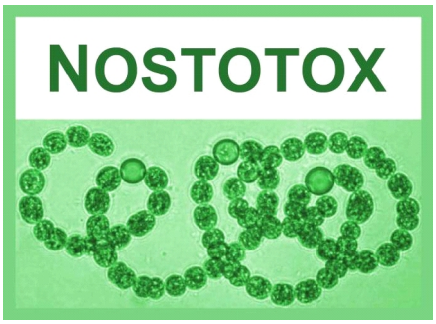
Development of Toxic Nostocales (Cyanobacteria) in the Course of Declining Trophic State and Global Warming

NOSTOTOX

Final Report



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The Kompetenzzentrum Wasser Berlin is an international centre for water research and knowledge transfer. The KWB links the capacities of the Berlin universities and research institutes to the Berlinwasser group of companies and Veolia.

The responsibilities for KWB within the project NOSTOTOX were the following:

- inform potential end-users (public authorities, water suppliers) about the project and relevant (intermediate) results,
- involve end-users in the discussion about possible countermeasures as well as in the development of a decision support system,
- organize technical discussions and conferences to present intermediate results,
- act as contact for the Berliner Wasserbetriebe and Veolia Water for topics relating the project progress, the contents of the project and its results,
- assure that the interests of the sponsors BWB and Veolia are considered during project progress, according to the aims of the project.

The research project was accompanied by a steering committee consisting of technical experts from the involved stakeholders and representatives of the funding organizations:

- R. Gnirss (BWB),
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- C. Pagotto (Veolia Eau),
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Summary

Background

Cyanobacteria of the order Nostocales – native species as well as alien species from tropical regions – were found to increase in many Brandenburg lakes while the formerly dominating microcystin (MC) producing cyanobacteria (*Microcystis* and *Planktothrix*) occurred less often and in lower amounts. As a consequence, lower MC concentrations were observed while the toxin cylindrospermopsin (CYN) that is produced by Nostocales was found to be widely distributed and to exceed sometimes the recommended guideline value for drinking-water of 1 µg L^{-1}. Recent data on the occurrence of further neurotoxins (paralytic shellfish poisoningtoxin, PSP and anatoxin, ATX) produced by cyanobacteria of the order Nostocales did not exist. Nostocales are superior competitors under conditions of high light intensity and nitrogen depletion because they can fix molecular nitrogen. Their germination is regulated by temperature and the temporal starting point of the pelagic population determines the population size (the earlier the larger). Therefore, the following working hypothesis has been put forward: Combined effects of declining trophic state and global warming favor the development of Nostocales and cause a shift in the species composition as well as in the occurrences of cyanobacterial toxins.

Objectives

The NOSTOTOX project aimed to determine the present occurrence and future development of Nostocales and their toxins in waterbodies. Special emphasis was paid to answer the question, which Nostocales species and which toxins can be expected under conditions of a proceeding decline in trophic state and increasing water temperature. The outcome of the project aims to contribute to developing recommendations and guidelines for the management of inland waters and drinking water supplies.

Concept

The occurrence and seasonal dynamics of cyanobacteria and toxins as well as of physical and chemical parameters were studied in 15 lakes in Berlin and Brandenburg. Data were integrated in an existing data base on long-term development of phytoplankton of 33 lakes. These data were analyzed with multivariate statistical approaches to identify main predictor variables of cyanobacteria performance and to evaluate threshold values for cyanobacterial dominance.

For the identification of toxin producers strains of different species from different lakes were isolated and analyzed morphologically (detection of species), genetically (detection of species and toxin encoding genes) and chemically (detection of toxins). Toxin produc-

tion under varying environmental conditions was studied for selected strains in laboratory culture experiments.

To gain further insights into the ecology of Nostocales, the performance and regulation of their whole life cycle were studied. Akinete production of the pelagic population, akinete loss during winter in the sediment and the regulation of the germination in spring was studied in selected lakes. Growth and competitiveness of native and invaded species were ascertained along light- and temperature gradients in culture experiments with isolated strains.

Based on data from field and laboratory experiments a mathematical model was developed for process analysis, simulation and prediction of the occurrence of Nostocales populations for case scenarios of lakes with declining trophic states and rising water temperatures. The model considers for the first time the whole life-cycle of Nostocales based on field observations and data form experimental field and laboratory experiments.

To estimate the risk of dissolved CYN and ATX in drinking-water obtained by bank filtration, the elimination of these toxins during the sediment passage was studied. Finally, results were/are implemented into concepts for lake management and risk assessment.

Results

In most (but not all) of the studied lakes the nutrient concentrations and the total cyanobacterial biomass declined during the last 10 years. This was accompanied by a shift in cyanobacterial dominance from species of the order Oscillatoriales towards species of the order Nostocales.

Statistical analysis revealed that the probability of dominance of Oscillatoriales increases with increasing phosphorus concentration while the probability of dominance of Nostocales increases with rising water temperatures. Moreover, the biovolume of Nostocales was significantly positively correlated with nitrogen concentrations.

Concerning cyanotoxins, the two most important results are that i) the concentrations of MCs followed the shift in cyanobacterial dominance and clearly declined in most of the lakes and ii) that CYN is more abundant than MCs in the lakes studied. Though the concentration of CYN only rarely exceeded 1 µg L^{-1} (max. 5 µg L^{-1}) higher concentrations may be expected if Nostocales further expand. Also other cyanotoxins predominantly produced by Nostocales such as ATX and PSP were found quite frequently, though at concentrations clearly below 1 µg L^{-1}.

For the first time, producers of PSP toxins (*Aphanizomenon gracile*) and of ATX (*Aphanizomenon issatschenkoi*) were identified in German lakes. From more than 200 isolated strains only 14 PSP toxin, one ATX, and no CYN producing strains were found. This indicates a very low portion of toxin-producing strains within natural populations.

Culture experiments showed that the temperature optima of Nostocales are above the mean water temperature of lakes in temperate regions. Moreover, it could be shown that invaded species are stronger competitors than native species at high temperature but weaker competitors at low temperatures. This difference in temperature dependent growth exerts a stronger influence on the successful establishment and size of a population after germination than the number of akinetes, i.e. the size of the inoculum which itself is determined by the size of the previous population. Furthermore resuspension of sediment proved to be an important mechanism controlling the recruitment of Nostocales.

Temperature was also found to have an influence on toxin production: For 2 strains tested, CYN production clearly decreased with increasing temperature up to an almost complete termination of the production at 30°C. For PSP-production the effect of temperature was less pronounced and strain-specific, showing either a decrease of PSP for some and an increase of PSP with increasing temperature.

Model simulations for rising water temperatures predict an increase of Nostocales combined with a shift in dominance towards invaded species. Simulations also demonstrate that further tropical species will be able to invade temperate waters and that they need less time to build up populations with increasing temperatures.

Drinking-water abstraction and underground passage: The high persistence of dissolved CYN eventually leading to year-round presence of this toxin in water bodies requires different monitoring schemes as for cellbound toxins to pick up risks where surface water is used as drinking-water source. For soil and sand filtration processes are used, an important result is that CYN is eliminated in sediments predominantly by microbial degradation. Sediment preconditioning and a low flow rate are essential for its efficient removal in sandy sediments. ATX is less stable in waters and sediments than CYN and is eliminated not only by microbial degradation but also by sorption.

Implications for lake assessment and management: The German lake assessment system for the implication of European Water Framework Directive uses cyanobacterial taxa as indicator organisms to determine the trophic status of lakes. New results from NOSTOTOX on the ecology of individual cyanobacterial taxa shall be used to modify the indicator values of certain taxa and to improve the lake assessment system.

For lake management it is important that the probability of Nostocales dominance increases with rising water temperatures. Also, NOSTOTOX found indication that not only phosphorus but also nitrogen is an important parameter in regulating Nostocales population dynamics. Therefore, it is now important to assess whether nitrogen reduction could be a successful measure to counteract increasing population size of Nostocales and to improve the ecological status of lakes.

Risk assessment: The low concentrations of total CYN found so far do not indicate a risk for recreational water use. However, the toxicological data available to date cannot exclude carcinogenic action, and for drinking water this implies a preliminary precautionary level of 0.1 µg L^{-1}. While most samples would meet this and the lakes studied are not

used for drinking-water production, the high frequency of – particularly dissolved – CYN and its persistence show that this cyanotoxin requires further attention in surface waters used as drinking-water source.

Conclusion

Many of the studied lakes were characterized by declining nutrient concentrations and as a consequence by decreasing cyanobacterial biomass and toxin concentration. Nevertheless, not all lakes are subject to nutrient limitation but all lakes are subject to global warming.

Overall, Nostocales were found to be the winners of increasing water temperatures and declining trophic status. In particular, we found that increasing water temperatures will chiefly promote Nostocales in the order of potentially invading species, those which have already invaded and native species. Since the probability of Nostocales dominance is also increasing with decreasing nutrient concentrations especially in the mesotrophic to low eutrophic range of trophic state, phosphorus and nitrogen reduction might be an appropriate measure to counteract increasing population sizes of Nostocales in the course of rising water temperatures.

Thus, an important outcome of NOSTOTOX is the need for awareness of further changes in cyanobacterial composition in our lakes causing changes in the occurrence of toxins, with CYN as major toxin in the future as well as more frequent occurrence of ATX and STX.

However, we are still not able to predict toxin concentrations of Nostocales due to the following reasons: i) Nostocales are very variable in their abundance in time and space. ii) The percentage of toxin producers within a population remains unpredictable until we understand the factors driving the dominance of toxin-producing genotypes over nonproducing ones within a species.

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Introduction

Claudia Wiedner

Background

As a consequence of eutrophication cyanobacterial blooms often occur during the summer months in many European freshwaters. This causes serious impairments and expenses for a wide range uses of inland waters, including drinking-water treatment, because cyanobacteria can produce various toxic secondary metabolites.

A study conducted in 1995–97 showed that in German lakes microcystin producing cyanobacteria of the genus *Microcystis* (order: Chroococcales) and *Planktothrix* (order: Oscillatoriales) were widely distributed and occurred in high quantities. Microcystin occurred in 50 % of the studied lakes and concentrations above the provisional WHO Guideline Value for drinking-water of 1 µg L^{-1} for Microcystin-LR (WHO 1998), thus requiring effective removal by treatment if these resources were used for drinking-water production. Species of the order Nostocales that can fix molecular nitrogen and endure unfavorable growth conditions as akinetes (resting stages) occurred only subdominantly. Neurotoxins, as saxitoxin or anatoxin, which are produced by Nostocales, were only detected in a small number of lakes with low concentrations (results are summarized in Chorus (2001); the study was funded by the BMBF, grant no. 0339547).

Results of a study in 2004/05 show substantial alterations of the predominating cyanobacteria as well as in the occurrence of certain toxins, demonstrate, e.g during the summer months in 2004 the cyanobacteria community in 20 German lakes was mainly composed by Nostocales that contributed 50 % and more to the total cyanobacterial biovolume while the average share of *Planktothrix* was 30 % and that of *Microcystis* 10 %, which is significantly lower compared to earlier years (Rücker *et al.* 2007). In addition to the increase of native Nostocales species, Nostocales from tropical and subtropical regions invaded lakes in Germany and in other temperate regions. In 1990 *Cylindrospermopsis raciborskii* was detected for the first time in Germany (Krienitz and Hegewald 1996; Rücker *et al.* 1997). In 2004 this species occurred in 25 % of lakes in Brandenburg and contributes up to 30 % to the cyanobacterial biovolume and two other species with former tropic distribution, *Anabaena bergii* and *Aphanizomenon aphanizomenoides,* were detected for the first time in German lakes (Stüken *et al.* 2006).

The dominance shift from *Planktothrix* and *Microcystis* towards Nostocales was also reflected in alterations in the occurrences of certain toxins. As an example, the decreasing biovolume of *Planktothrix agardhii* in Lake Langer See resulted in declining microcystin concentrations from 31 µg L^{-1} in 1997 and 20 µg L^{-1} in 2001 to 6 µg L^{-1} in 2005 (Wiedner 1999, Wiedner 2002, Wiedner and Nixdorf 2006).

A precarious point is that another cyanotoxin, cylindrospermopsin (CYN), was detected in German lakes. CYN can be produced by Nostocales such as *C. raciborskii* (Hawkins et al 1995), *Anabaena bergii, Aphanizomenon ovalisporum* and *Raphidiopsis curvata* (Falconer 2005). It was first detected 2000 in two German lakes (Fastner *et al.* 2003) and in 2004 in 50 % of 142 German lakes studied (Fastner *et al.* 2007), and thus, it was as widely distributed as microcystin. CYN concentrations reached up to 12.1 µg L^{-1} (Rücker *et al.* 2007), which is clearly above the guideline value of 1 µg L^{-1} recommended by Humpage and Falconer (2003) for drinking-water, though probably not yet hazardously high for recreational water use. Other data on the occurrences of CYN exist from Australian waters, where values between 1.2-120 µg L^{-1} were measured and attributed to *C. raciborskii* (e.g. Chiswell *et al.* 1999; Shaw *et al.* 1999; McGreggor and Fabbro 2000). CYN-concentrations in this range were also reported from Florida, where they were attributed to *C. raciborskii* and *Aphanizomenon* sp. (Williams, Vortrag auf NALMS 2005) as well as from Spain, where they were attributed to *Aphanizomenon ovalisporum* (Quesada *et al.* 2006). The CYN concentrations in German lakes could not be attributed to *C. raciborskii*. The first CYN producer identified in German lakes is *Aphanizomenon flos-aquae* (Preußel *et al.* 2006), and strong evidence points towards *Aphanizomenon gracile* as a main CYN producer (Wiedner *et al.* 2008). However, CYN concentrations in German lakes could not be explained by the abundance of any of the known producers, suggesting the presence of further undetected producers. These were mainly suspected in the genus *Aphanizomenon* which is very diverse in respect of taxonomy and morphology. Therefore, it was not possible to break down this genus to a level that would have been needed to detect and quantify CYN producers by microscopy and thus, further molecular differentiation and detection methods had to be developed. In contrast to microcystin, a major concern with the occurrence of CYN is that it occurs with a high fraction dissolved in the water, often far more than 50 % of the total CYN (Rücker *et al.* 2007). During the annual cycle in two German lakes, the dissolved CYN fraction peaked after the particulate fraction, and reached concentrations higher than those observed for particulate CYN, indicating that CYN is poorly decomposed and accumulates in the water (Wiedner *et al.* 2008). Similar observations are also reported from Australia (McGregor and Fabbro 2000; Chiswell *et al.* 1999).

An additional consequence of the increase of Nostocales in German waters could probably be a more frequent occurrence of the neurotoxins, anatoxin (ATX) and saxitoxin (STX) – i.e. in a greater number of lakes as well as in higher concentration than determined in the survey of 1995-1997 (Chorus 2001). However, no more recent data on the occurrence of theses neurotoxins in German inland waters existed. The study in 1995-97 addressed them only in a smaller fraction of samples, and subsequent studies were not designed to gain information on their occurrence. Therefore it remained important to clarify whether CYN, ATX and STX bear a risk for drinking-water supplies; they are all very potent toxins, occur mainly dissolved in the water and cannot be eliminated by filtration.

In comparison to microcystin producing *Planktothrix* and *Microcystis* species that formerly predominated German lakes, only little was known about the diversity, distribution and regulation of native and invasive Nostocales species.

The invasion of *C. raciborskii* could be related to climate change: The earlier rise of water temperature in spring enables earlier germination and shifts the pelagic population into a phase of higher light intensity, which benefits *C. raciborskii*'s growth and allowed to build up stable reoccurring populations (Wiedner *et al.* 2007). Whether this trend will proceed and lead to bloom formation of the species in the future cannot be foreseen hitherto. Generally, it can be assumed that the described mechanism (forward shift of the life cycle into a more favorable growth period) also functions in regulating other Nostocales' populations and is one major cause for increasing species diversity and abundance. At this point it is essential to close gaps in knowledge of the life cycle of Nostocales that is poorly understood and the little information existing is concerning the pelagic phase of the populations.

An additional reason for increasing Nostocales populations is most probably the decline in the trophic state observed in many German lakes. In this process, both, increasing light intensity in the water column and declining nutrient concentrations, enhance the competitive success of Nostocales. The fact that Nostocales do not become dominant in highly eutrophic and turbid polymictic shallow lakes has mostly been explained by light limitation (Zevenboom and Mur 1980). This is supported by results from studies on the seasonal dynamics of cyanobacteria in shallow lakes: population growth of *C. raciborskii* was found to be limited by light (Wiedner *et al.* 2007) and it could be shown that Nostocales in general were outcompeted by *Planktothrix agardhii* with declining light intensity (Wiedner *et al.* 2002). *Vice versa*, it can be assumed that increasing light intensities will advance the competitive ability of Nostocales. The competitiveness of Nostocales will further increase with decreasing nitrogen concentrations because Nostocales can fix molecular nitrogen in their heterocysts. Moreover, indication exists that *Planktothrix agardhii*, the previously dominating species of eutrophic shallow lakes, is outcompeted by Nostocales species under decreasing phosphorous concentrations (Wiedner and Nixdorf 2006).

Per se, nutrient reduction in natural waters is a desirable process that is achieved by substantial investment, aiming at long-term improvement of water quality in terms of reduced abundance of cyanobacteria in particular and phytoplankton in general (EU WRRL 2000, Nixdorf *et al.* 2005). However, in a short and intermediate term it has to be taken into consideration that the diversity and abundance of Nostocales as well as the concentrations of their toxic secondary metabolites will increase. It also has to be taken into account that effects of declining trophic state and climate change can probably reinforce each other.

A reliable answer to the question, which toxic Nostocales species benefit most during the processes of declining trophic state and rising water temperature, cannot be given, since profound data on the ecophysiology of the most frequent species are missing. Effects of temperature and light were mainly studied for *Aphanizomenon flos-aquae* (e.g. Uehlinger

1981 a, b). Equivalent data do not exist for species that are distributed more widely and occur in higher abundances, for example *Aphanizomenon gracile*, which is one of the main CYN producers in German lakes or for the neo-cyanobacteria *Anabaena bergii* (which is a potential CYN producer) and *Aphanizomenon aphanizomenoides*. Ecophysiological data on the growth of the most frequent Nostocales are essential for determining the overlap in their ecological niche, the competitive ability between different Nostocales species as well as between Nostocales and *Microcystis* and *Planktothrix*. This is of major importance in estimating which species and which toxins can be expected when threshold values of certain environmental parameters are crossed in the processes of declining trophic state and climate change as well as for simulating the occurrence of Nostocales and their toxins for different scenarios with mathematical models.

The occurrence and variability of certain toxins is also determined by the cellular production, release and decomposition of the toxins. Only little is known about the production of CYN and ATX (determined on the basis of particulate and dissolved concentrations per cell). Concentrations of anatoxin-s were found to vary by a factor of 2-7 depending on light, temperature and nutrient conditions (Rapala *et al.* 1993, Rapala and Sivonen 1998). Only one study is published on the effect of temperature on CYN production of two strains of *C. raciborskii*, showing an increase of cellular CYN content with decreasing temperature and CYN production of almost zero when strains reach their maximum growth rate at high temperatures (Saker 2002). Only little information is available on extra-cellular concentrations of CYN and ATX from field surveys. This is mainly due to technical detection problems: the sample has to be concentrated, or very sensitive detection methods, as LC-MS-MS as well as quantitative standards are needed (Eaglesham *et al.* 1999). Both the sensitive detection methods and the standards became available only recently. In contrast to microcystin, high concentrations of dissolved CYN and ATX were found in culture experiments (Preussel *et al.* 2005; Bumke-Vogt *et al.* 1999). In case of CYN this holds also true for field studies where high and variable concentrations of CYN were detected as outlined above. So far it is unclear whether the high and variable dissolved concentrations of CYN result from strain (genotype) specific production and release rates or if certain environmental conditions affect the production, release and decomposition of CYN in different ways.

Finally, it is of great importance to gain further insight into the decomposition of CYN, ATX and STX. While a fair body of evidence is available for their elimination in technical drinking-water treatment, very little information was available on their persistence versus degradation in waterbodies as well as on their retention or breakthrough in natural and close-to-natural processes of underground passage and slow sand filtration.

Objectives

The NOSTOTOX project aimed to determine the present occurrence and future development of Nostocales and their toxins in waterbodies. Special emphasis was paid to answer the question, which Nostocales species and which toxins can be expected under conditions of a proceeding decline in trophic state and increasing water temperature. The outcome of the project aims to contribute to developing recommendations and guidelines for the management of inland waters and drinking water supplies.

Concept

The occurrence and seasonal dynamics of cyanobacteria and toxins as well as of physical and chemical parameters were studied in 15 lakes in Berlin and Brandenburg. Data were integrated in an existing data base on long-term development of phytoplankton of 33 lakes. These data were analyzed with multivariate statistical approaches to identify main predictor variables of cyanobacteria performance and to evaluate threshold values for cyanobacterial dominance.

For the identification of toxin producers strains of different species from different lakes were isolated and analyzed morphologically (detection of species), genetically (detection of species and toxin encoding genes) and chemically (detection of toxins). Toxin production under varying environmental conditions was studied for selected strains in laboratory culture experiments.

To gain further insights into the ecology of Nostocales, the performance and regulation of their whole life cycle were studied. Akinete production of the pelagic population, akinete loss during winter in the sediment and the regulation of the germination in spring was studied in selected lakes. Growth and competitiveness of native and invaded species were ascertained along light- and temperature gradients in culture experiments with isolated strains.

Based on data from field and laboratory experiments a mathematical model was developed for process analysis, simulation and prediction of the occurrence of Nostocales populations for case scenarios of lakes with declining trophic states and rising water temperatures. The model considers for the first time the whole life-cycle of Nostocales based on field observations and data form experimental field and laboratory experiments.

To estimate the risk of dissolved CYN and ATX in drinking-water obtained by bank filtration, the elimination of these toxins during the sediment passage was studied. Finally, results were/are implemented into concepts for lake management and risk assessment.

1. Occurrence and regulation of Nostocales and cyanotoxins in lakes of the Berlin Brandenburg area

The abundance and diversity of cyanobacteria, the concentration of cyanotoxins as well as physical and chemical parameters were studied in various types of lakes in the Berlin Brandenburg area over a period of three years, 2007–2009. Seasonal dynamics of cyanobacteria and cyanotoxins were studied in seven lakes, of which long-term data already existed. Additionally, seven other lakes were studied three times during the summer months.

Data collection: Lakes were sampled at their deepest point. Mixed samples from the whole water column in polymictic lakes and of the epilimnion in stratified lakes were prepared by taking samples at half-meter intervals with a 2.3 L Limnos sampler. Cyanobacterial composition and biovolume were analyzed using an aliquot of the mixed sample fixed with Lugol's solution and studied by inverse microscope according to Utermöhl (1958) and Rott (1981). Other aliquots were used to determine concentrations of total phosphorus (TP), dissolved inorganic phosphorus (DIP), total nitrogen (TN), ammonia (NH_4–N), and nitrate (NO_3-N) according to standard methods (DEV 1976–2009). Secchi depth (SD) and depth profiles of photosynthetically active radiation through the water column were measured using two spherical quantum sensors on each sampling date. The mean intensity of photosynthetically active radiation in the mixed water column (I_{mix}) was calculated as described in Wiedner *et al.* (2007). Vertical temperature profiles were measured using a multiparameter probe.

Data were integrated in an existing data base on long-term development of phytoplankton of 33 lakes. These data were analyzed with multivariate statistical approaches to identify main predictor variables of cyanobacteria performance and to evaluate threshold values for cyanobacterial dominance.

Results demonstrating long-term changes in the biovolume and species composition of cyanobacteria in three lakes with declining trophic status are presented in Chapter 1.1. The outcome of the statistical analysis on the impact of global warming and trophic status on the predominance of Nostocales is given in Chapter 1.2. Finally, results on the occurrence of cyanotoxins are presented in Chapter 1.3.

1.1 Long-term population dynamics of Nostocales – Case studies of three lakes over 16 years

Jacqueline Rücker

Study side

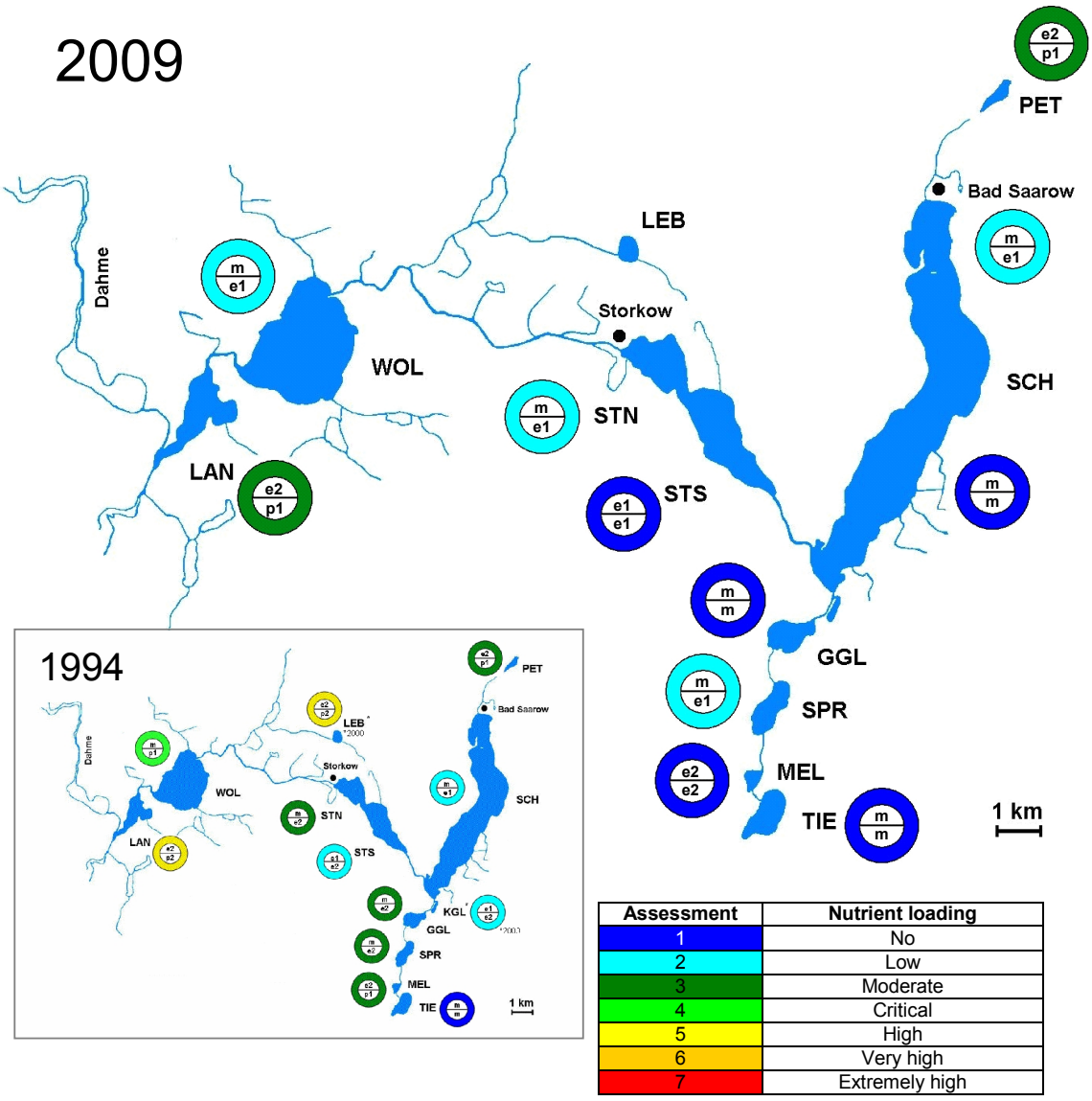
Long-term population dynamics of Nostocales were studied in the deep dimictic Lake Scharmützelsee and two shallow polymictic lakes, Langer See and Melangsee. Morphometric characteristics of the three lakes are presented in Table 1.1. The lakes are located in the catchment of River Dahme (Figure 1.1, see also Chapter 6.2). The water flows from the South through the lakes of lake chain Glubigseenkette (Tiefer See (TIE); Melangsee (MEL); Springsee (SPR); Großer Glubigsee (GGL) to Lake Scharmützelsee (SCH) and then westwards via channels through Storkower See (STS, STN) and Wolziger See (WOL) to Langer See (LAN).

Table 1.1. Morphometric characteristics of the studied lakes. (z_{mean} = mean depth, z_{max} = maximum depth)

Lake	Abbr.	Area (km^2)	Volume ($10^6 m^3$)	z_{mean} (m)	z_{max} (m)	Catchment (km^2)	Mixis
Scharmützelsee	SCH	12.1	108.23	9.0	29.5	110	di
Melangsee	MEL	0.12	0.17	1.6	2.1	4	poly
Langer See	LAN	1.21	3.27	2.1	3.8	392	poly

Results and discussion

After 1990, point sources of nutrient input into the surface waters were eliminated, the treatment of waste water was improved and the intensity of agricultural production was reduced. These reconstruction measures in the catchment resulted in decreasing nutrient load and improving water quality (Nixdorf *et al.* 2009). The coloured circles in Figure 1.1 indicate the assessment of trophic state in comparison to the potential natural state based on lake morphometry according to the method of LAWA (1999). The small inserted figure represents the status in 1994 illustrating the higher trophic state of all lakes in the catchment (except Tiefer See) and the higher degree of deviation from the potential natural state. In 2009, half of the stratified lakes reach the potential natural mesotrophic state while the other half is still eutrophic (e1). Among the polymictic lakes, two already reach the potential natural eutrophic state (e1 or e2), one deviates by one degree, and two by two degrees. Although the trophic index according to LAWA (1999) indicates quite good water quality the phytoplankton-based assessment of water quality according to the EU Water Framework Directive (Mischke and Nixdorf 2008) yields only moderate to poor water quality (compare Nixdorf *et al.* 2009 and Chapter 6.2).



Assessment	Nutrient loading
1	No
2	Low
3	Moderate
4	Critical
5	High
6	Very high
7	Extremely high

Figure 1.1. Map of Scharmützelsee-region with lakes studied by BTU Cottbus. The coloured rings represent the assessment according to LAWA (1999) based on the deviation between potential natural state (upper letter) and the present trophic state (lower letter) in 2009 compared to 1994 (small, inserted figure). Abbreviations of lake names are given in the text. The colour of the ring represents the deviation between the potential natural state (upper letter) and the present trophic state (lower letter). m=mesotrophic; e=eutrophic; p=polytrophic.

Figure 1.2 illustrates the trophical development on the basis of annual means of total phosphorus (TP) and total nitrogen (TN) for the three intensively studied lakes. Although the load reduction occurred rather abruptly as described for Lake Scharmützelsee in Nixdorf *et al.* (2009) especially due to elimination of point sources, these changes resulted in a more gradually reduction of lake-intern P concentrations in all lakes. In Lake Scharmützelsee, TP decreased from about 60 to 25 µg TP L^{-1} (annual means), in Lake Melangsee from 70 to 50 µg TP L^{-1}, and in Lake Langer See from 120 to 60 µg L^{-1}.

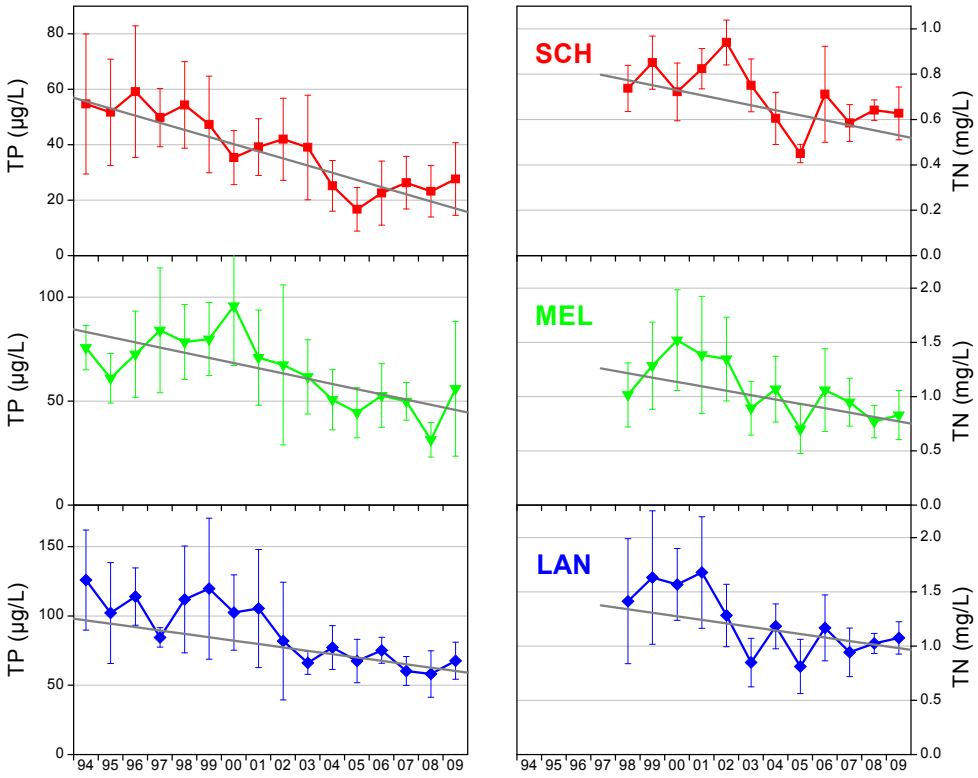


Figure 1.2. Annual means of total phosphorus (TP; left) and total nitrogen (TN; right), for lakes Scharmützelsee (SCH), Melangsee (MEL) and Langer See (LAN) from 1994–2009.

Regarding the development of TN in Lake Langer See a strong decline of concentrations from 1.5 mg L^{-1} beginning in 2001 to a lower level of about 1 mg L^{-1} since 2003 is striking. For the same time span, the total phytoplankton biovolume in the vegetation period (April to October) shows a drastic reduction by about 10 mm^3 L^{-1} compared to the period before year 2000, and the portion of cyanobacteria on total phytoplankton biovolume decreased from 80 % in the 1990s to values between 50 and 70 % since 2001 (Figure 1.3). This coincides with an important change in cyanobacterial species composition, i.e. with a decline of *Planktothrix agardhii*, the former dominant cyanobacterium (Oscillatoriales) in Langer See (Figure 1.4). The biovolume of other cyanobacterial species was not affected by these changes resulting in a relative increase of Nostocales compared to Oscillatoriales.

In Lake Melangsee TN concentrations reached a lower level between 0.5 and 1 mg L^{-1} since 2003. Considerably lower total phytoplankton biovolume was observed since 2005 (Figure 1.3). The portion of cyanobacteria on total phytoplankton biovolume in the vegetation period decreased from about 65 until 2006 to less than 40 %. During the same time span, the biovolume of fine filamentous Oscillatoriales was much lower compared to the previous years while the biovolume of Chroococcales increased (Figure 1.5). The biovolume of *Aphanizomenon* was – similar to Lake Langer See – almost unchanged during the study period. The high interannual variations of the biovolume of *Cylindrospermopsis raciborskii* are striking. The possible causes of this phenomenon are discussed in Chapter 3.1.

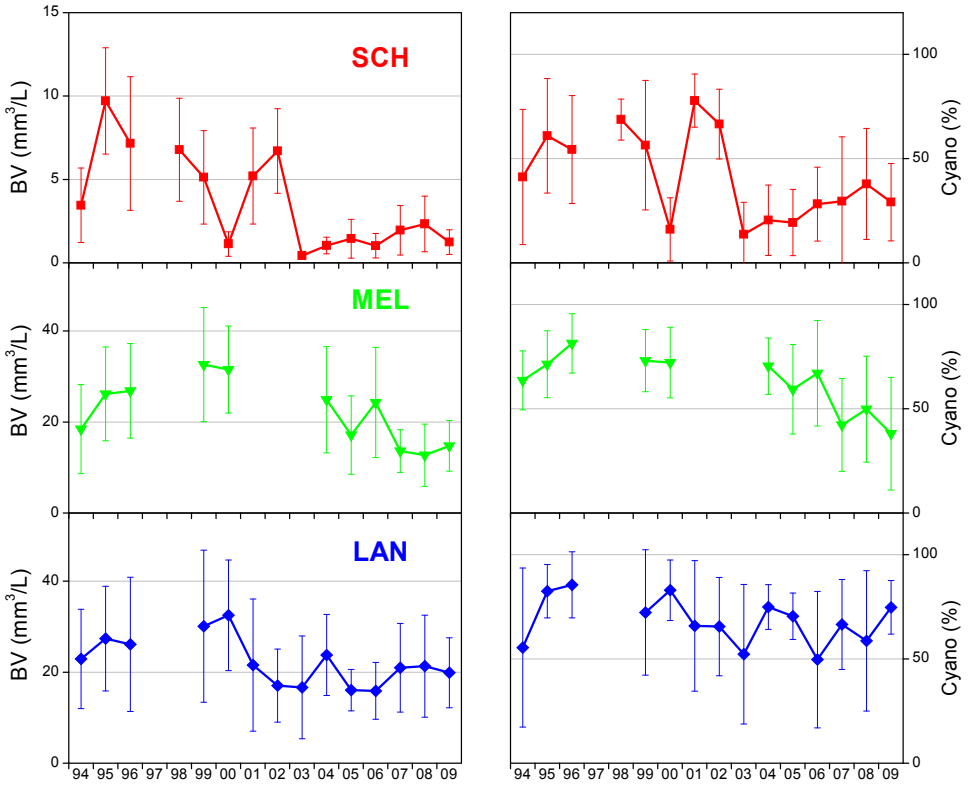


Figure 1.3. Means of the vegetation period (April–October) of total phytoplankton biovolume (BV; left) and portion of cyanobacteria on total phytoplankton biovolume (Cyano; right) for lakes Scharmützelsee (SCH), Melangsee (MEL) and Langer See (LAN) from 1994–2009.

In Lake Scharmützelsee the reduction of TN concentrations was less pronounced compared to the shallow lakes (from 0.8 to 0.6 mg L^{-1}; Figure 1.3), but the phytoplankton biomass dropped to a new level below 4 mm^3 L^{-1} in 2003. The portion of cyanobacteria on total phytoplankton biovolume in the vegetation period decreased from about 60 to 30–45 % (Figure 1.3). Oscillatoriales (*Planktothrix agardhii*, *Pseudanabaena* and *Limnothrix* species (the latter summarized as "fine filamentous Oscillatoriales") almost disappeared since 2003. Among Nostocales species shifted from *Aphanizomenon gracile* to *Aphanizomenon flos-aquae* and *Anabaena* species could be observed. The number of *Anabaena* species increased from two before the year 2000 to 11 nowadays. The most frequent species of this genus are *Anabaena flos-aquae*, *A. macrospora* and *A. lemmermannii*. The species variety and the biovolume of Chroococcales increased as well. Consequently, in Lake Scharmützelsee an absolute increase of *Anabaena* species and *Aphanizomenon flos-aquae* could be observed as well as an increase in the relative portion of Nostocales.

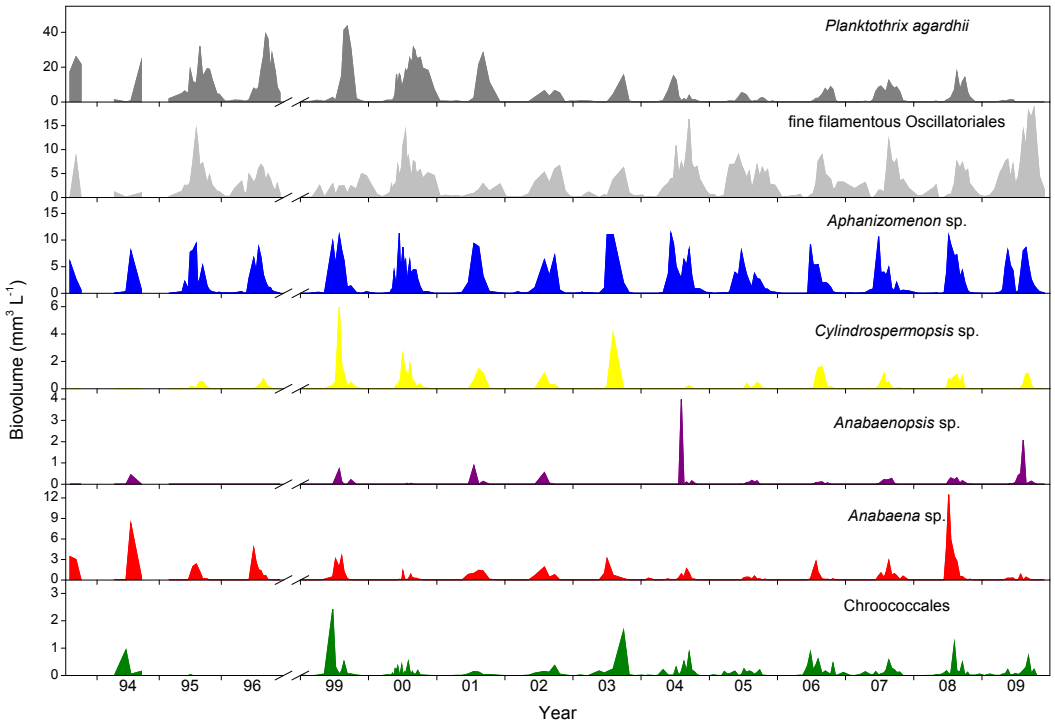


Figure 1.4. Biovolume of the most important genera or groups of cyanobacteria in Lake Langer See from July 1993 to December 2009. "Fine filamentous Oscillatoriales" comprises the genera *Pseudanabaena*, *Limnothrix* and *Planktolyngbya*.

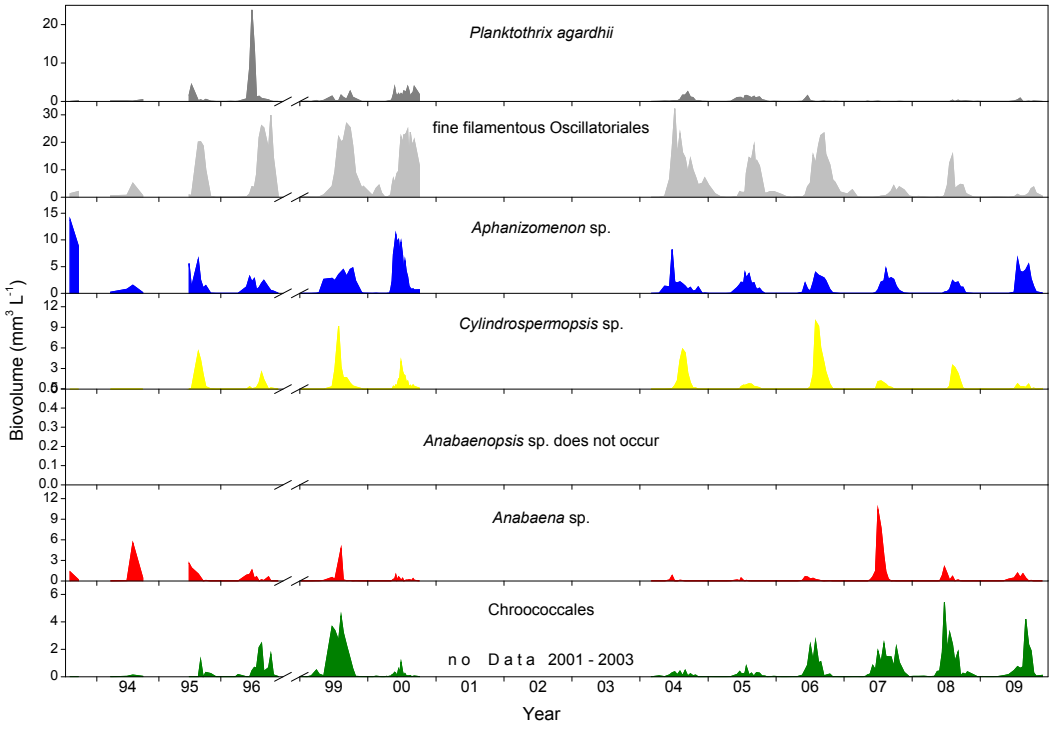


Figure 1.5. Biovolume of the most important genera or groups of cyanobacteria in Lake Melangsee from July 1993 to December 2009. "Fine filamentous Oscillatoriales" comprises the genera *Pseudanabaena*, *Limnothrix* and *Planktolyngbya*.

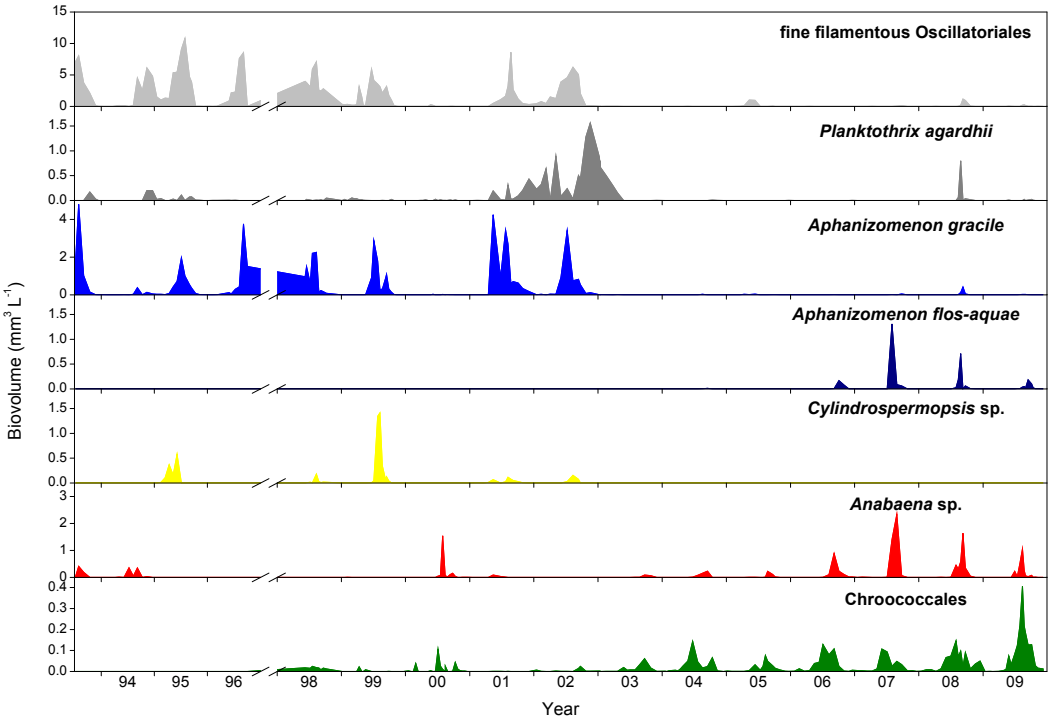


Figure 1.6. Biovolume of the most important genera or groups of cyanobacteria in Lake Scharmützelsee from July 1993 to October 2009. “Fine filamentous Oscillatoriales“ comprises the genera *Pseudanabaena*, *Limnothrix* and *Planktolyngbya*.

1.2 Impact of global warming and trophic status on the predominance of Nostocales in 33 lakes

Claudia Wiedner, Carola Wagner, Jacqueline Rücker

Introduction

Worldwide Cyanobacteria became dominant in many lakes due to excessive nutrient enrichment and eutrophication (e.g. Vollenweider and Kerekes 1980, Smith and Schindler 2009). Major efforts to enhance the ecological quality of lakes by reducing phosphorus concentrations during the last decades (e.g. Sas 1989) have decreased cyanobacterial biovolumes in many lakes (e.g. Søndergaard *et al.* 2002). However, it was also found that decreasing and increasing nutrient concentrations evoke different responses under different conditions. For example, phosphorus concentrations promoting cyanobacterial dominance in the process of eutrophication are higher than those observed under conditions of re-oligotrophication (e.g. Reynolds 1992, Scheffer 1998). The last decades were also characterized by climatic changes that affected freshwaters and their biota in multiple ways. It has been hypothesized that cyanobacteria generally benefit from these climate changes (for review, see Paerl and Huisman 2009). Many freshwater systems are presently subject to changes in trophic status as well as global warming, and it is argued that global warming is more likely to counter nutrient load reduction efforts than to reinforce re-oligotrophication in lakes (e.g. Jeppesen *et al.* 2005, 2007). However, our understanding of the response of different cyanobacterial taxa to contemporary environmental changes is still poor.

Aim

This study aimed to improve the understanding of recent and future developments in various cyanobacterial taxa in different lake types in the course of changes in trophic status as well as global warming. We therefore performed bivariate correlation, canonical correspondence and classification tree analyses to analyze data on cyanobacterial occurrence and environmental variables collected during the last 16 years from 33 German lakes that differ in terms of morphology and trophic status. The impacts of temperature, nutrients and light on the biovolume of different cyanobacterial taxa were elucidated and thresholds for key variables predicting increasing or decreasing contributions of certain taxa to the total cyanobacterial community were ascertained.

Methods

We analyzed a data set originating from 31 lakes located in the North German Plain that differ in terms of morphology and trophic status. Two of the lakes (Scharmützelsee and Storkower See) have two lake basins, the morphology and trophic status of which differ so greatly that they were treated as separate lakes, increasing the total number of lakes studied to 33. The lakes were sampled from 1993 to 2008. Morphometric characteristics of the lakes and the number of analyzed data sets per lake are given in Table 1.2. For statistical analysis, the lakes were divided into three groups: very shallow polymictic lakes (vsp) with mean depths less than 4 m, shallow polymictic/dimictic lakes (spd) with mean depths of 4 to 6.5 m and temporary periods of thermal stratification, and deep dimictic lakes (dd) with mean depths of 9 m and more. To focus on cyanobacterial dominance we restricted data analysis to seasonal data sets collected from the beginning of May to the end of October, which corresponds to the period of thermal stratification in dimictic lakes. For data collection see Chapter 1.1.

The most important cyanobacteria in the studied lakes belong to the orders Nostocales and Oscillatoriales. Accordingly, the following five cyanobacterial taxa were included in the statistical analyses: *Aphanizomenon* (*A. gracile, A. flos-aquae*, *A. issatschenkoi* and *A. aphanizomenoides*), *Anabaena* (15 species), *Cylindrospermopsis raciborskii*, *Planktothrix agardhii* and fine filamentous Oscillatoriales (comprising species of the genera *Pseudanabaena*, *Limnothrix* and *Planktolyngbya* that had to be summarized here because unambiguous taxonomic separation of individual species was not always possible). As species of the order Chroococcales usually made only a minor contribution to the total cyanobacterial biovolume, they were excluded from the statistical analyses.

Statistical analysis

Differences in lake characteristics – After determining the distributions of abiotic parameters, biovolumes of the different cyanobacterial taxa, and contributions of these taxa to the total cyanobacterial biovolume for each lake type (box plots; Figure 1.8), Kruskal-Wallis tests followed by Dunn's multiple comparisons (GraphPad Prism, Version 4) were used to test for differences in distributions of the aforementioned variables between the three lake types (Figure 1.8).

Correlation analysis – The most commonly used method of extracting monotonic relations between variables is correlation analysis, which is a valuable tool to gain first information on effects of important factors. We used the nonparametric Kendall's τ_B correlation coefficient (Helsel and Hirsch 2002) to reveal bivariate relationships between biovolumes of cyanobacteria taxa and abiotic variables. However, as this simple method is unable to extract singular and combined effects of interacting forces, it may over- or underestimates certain factors.

Table 1.2. Morphometric characteristics (surface area, volume, mean and maximum depth) of the studied lakes. The lake type, number (N) of lakes and samples per lake studied are indicated.

Lake type	Lake name	Obser-vations N	Area (km^2)	Volume (10^6 m^3)	Mean depth (m)	Max. depth (m)
very shallow polymictic (13)	Langer See	141	1.6	3.3	2.1	3.5
	Melangsee	116	0.1	0.2	1.6	2.4
	Petersdorfer See	59	0.2	0.5	1.6	3.0
	Die Lieps	16	4.3	9.7	2.2	3.8
	Storkower See, S-basin	9	2.3	8.8	3.8	8.0
	Kutzingsee	6	0.3	0.8	2.4	6.0
	Kleiner Zeschsee	4	0.2	0.6	2.6	5.6
	Vielitzsee	3	1.1	1.6	1.5	3.0
	Bützsee	3	2.2	2.2	1.0	4.0
	Petzniksee	3	0.7	1.6	2.2	4.0
	Rahmer See	3	0.8	1.9	2.4	4.0
	Braminsee	3	0.7	1.0	1.5	2.2
	Zierker See	1	3.5	5.7	1.6	3.5
shallow poly/ dimictic (12)	Stolpsee	43	3.8	-	-	12.0
	Scharmützelsee, N-bay	34	1.4	7.5	5.3	7.9
	Wolziger See	27	5.2	32.0	5.5	13.0
	Grosser Glubigsee	14	0.6	2.6	4.6	12.0
	Storkower See, N-basin	9	1.4	6.6	4.7	11.4
	Feldberger Haussee	9	1.4	6.4	4.8	12.5
	Großer Plessower See	4	3.2	20.8	6.5	13.4
	Motzener See	4	2.0	12.7	6.2	13.5
	Pätzer Vordersee	4	1.7	10.3	6.2	18.5
	Zermützelsee	3	1.2	5.4	4.3	7.0
	Moderfitzsee	3	0.6	3.1	5.2	5.5
	Scharmützelsee, SAA, middle basin	3	2.0	11.7	6.0	9.5
deep dimictic (8)	Scharmuetzelsee, RIE, S-basin	115	12.1	108.2	9.0	29.5
	Tiefer oder Grubensee	27	0.6	7.0	11.6	22.9
	Springsee	19	0.6	5.6	9.5	18.1
	Stechlinsee (Tiefenboje)	19	4.2	96.9	22.8	68.0
	Schmaler Luzin	9	1.4	21.0	14.5	33.5
	Scharmützelsee, DIE, S-basin	3	8.6	100.9	11.8	22.0
	Ruppiner See	3	8.1	66.0	8.2	23.0
	Tollensesee	3	17.9	315.9	17.7	31.2
(33)	Total	713				

Canonical correspondence analysis (CCA) was used to explain variations in the cyanobacterial community composition and environmental conditions (using Canoco and CanoDraw for Windows, version 4.5; TerBraak and Smilauer 2002) not revealed by simple correlation analysis. CCA extracts theoretical variables (ordination axes) from the data assuming a unimodal response pattern of taxa to a linear combination of environmental factors, resulting in the representation of data in a lower-dimensional space. In the two-dimensional biplots presented in Figure 1.8, the taxon position gives a more detailed picture of the dependencies of cyanobacteria on abiotic factors. We selected the following options for CCA analysis: no transformation of taxa data, no weighting of taxa or samples, biplot scaling focused on inter-taxa distances, test for significance of canonical axes using Monte Carlo test with 999 permutations. All CCA analyses converged with canonical axes that were significant.

Classification tree analysis (CTA) was used to elucidate deeper aspects relating to changing environmental conditions and their potential interactions or the existence of certain threshold values predictive of cyanobacterial performance, which cannot be extracted from correlation analysis or CCA alone. CTA (De' Ath and Fabricius 2000) reveals the fine structure of dependencies by extracting the most important factors and threshold values explaining the population level of certain taxa among other things. The contribution of individual taxa to the total cyanobacterial biovolume was assessed in 5 % increments ranging from 10 to 40 percent. A dichotomous variable indicating whether the respective percentage was exceeded (value=1) or not (value=0) was then assigned to each tier. Only those taxa that contributed significantly to the cyanobacterial population were selected. *Cylindrospermopsis raciborskii*, which usually contributes less than 10 % to the total cyanobacterial biovolume (see Figure 1.7), was therefore excluded from the analysis. Classification trees were calculated for all dichotomous variables (for details, see Wagner and Adrian 2009) using SPSS version 15.0 for Windows.

Results

The ranges of physical and chemical parameters and the biovolumes of cyanobacterial taxa in the studied lakes are illustrated in Figure 1.7. Significant differences between lake types occurred in the ranges of nutrient concentrations (TP, TN and DIP) as well as cyanobacterial biovolumes that were highest in very shallow polymictic lakes , mid-range in shallow polymictic/dimictic lakes and lowest in deep dimictic lakes. The proportion of *Anabaena* increased progressively from very shallow polymictic to deep lakes, whereas that of *P. agardhii* decreased. Temperatures were slightly but significantly lower in deep lakes than in very shallow polymictic lakes.

Results of the bivariate correlation analysis are presented in Table 1.3. Temperature affected the abundance of all Nostocales in very shallow polymictic lakes, that of *Aphanizomenon* and *Anabaena* alone in dimictic to polymictic lakes, and that of *Anabaena* in deep dimictic lakes. Strong correlations between biovolume and I_{mix} were observed in

Oscillatoriales in shallow polymictic/dimictic and in deep lakes. All taxa showed positive correlations between biovolume and TN concentration, which was persistently higher in deep dimictic lakes.

Results of the canonical correspondence analysis (CCA) are presented in Figure 1.8. In very shallow polymictic lakes, the three nostocalean taxa are situated closer together and their ecological requirements are mostly determined by the factors weighing on axis 2, particularly high temperature and high nitrogen concentration. Inversely, *P. agardhii* and fine filamentous Oscillatoriales, which are located at high distances from nostocalean species and thus have different ecological requirements, are foremost determined by high concentrations of TP and low I_{mix}, respectively.

Results of the classification trees for a >25 % contribution of a given cyanobacterial taxon to total cyanobacterial biovolume are summarized in Figure 1.9. This scheme clearly illustrates differences in the primary factors and threshold values between lake types and cyanobacterial taxa. Water temperature was the primary factor predicting a >25 % contribution of *Aphanizomenon* and *Anabaena* to total cyanobacterial biovolume in very shallow polymictic lakes and of *Aphanizomenon* in shallow polymictic/dimictic lakes and for *Aphanizomenon* in shallow polymictic/dimictic lakes. I_{mix} was the major predictive factor only in shallow polymictic/dimictic lakes for *Anabaena* and fine filamentous Oscillatoriales. TP concentration was the primary factor yielding a >25 % contribution of *P. agardhii* in the shallow lakes types, of fine filamentous Oscillatoriales in very shallow polymictic and deep dimictic lakes, and of *Anabaena* in deep dimictic lakes. TN >538 µg L^{-1} increased the probability of a >25 % contribution of *Aphanizomenon* in deep dimictic lakes. In general, critical nutrient thresholds were highest in very shallow polymictic lakes, mid-range in shallow polymictic/dimictic lakes, and lowest in deep dimictic lakes.

Conclusion

The results of all three statistical analyses demonstrate similar pattern on the impacts of physical parameters and nutrient concentrations on the abundance of different cyanobacterial taxa in different lake types.

Nostocales are strongly correlated with water temperature. Their portion on total cyanobacteria biovolume increases with inclining temperatures. Effects of temperature on Nostocales are strongest in very shallow polymictic lakes.

Strongly positive correlations between nitrogen and biovolume were observed in all Nostocales in shallow lakes and in *Aphanizomenon* and Oscillatoriales in deep lakes.

Oscillatoriales are strongly correlated with TP concentrations.

In conclusion our results demonstrate that Nostocales will benefit from global warming and are less affected by declining phosphorous and nitrogen concentrations. Oscillatoriales are foremost controlled by phosphorous concentrations.

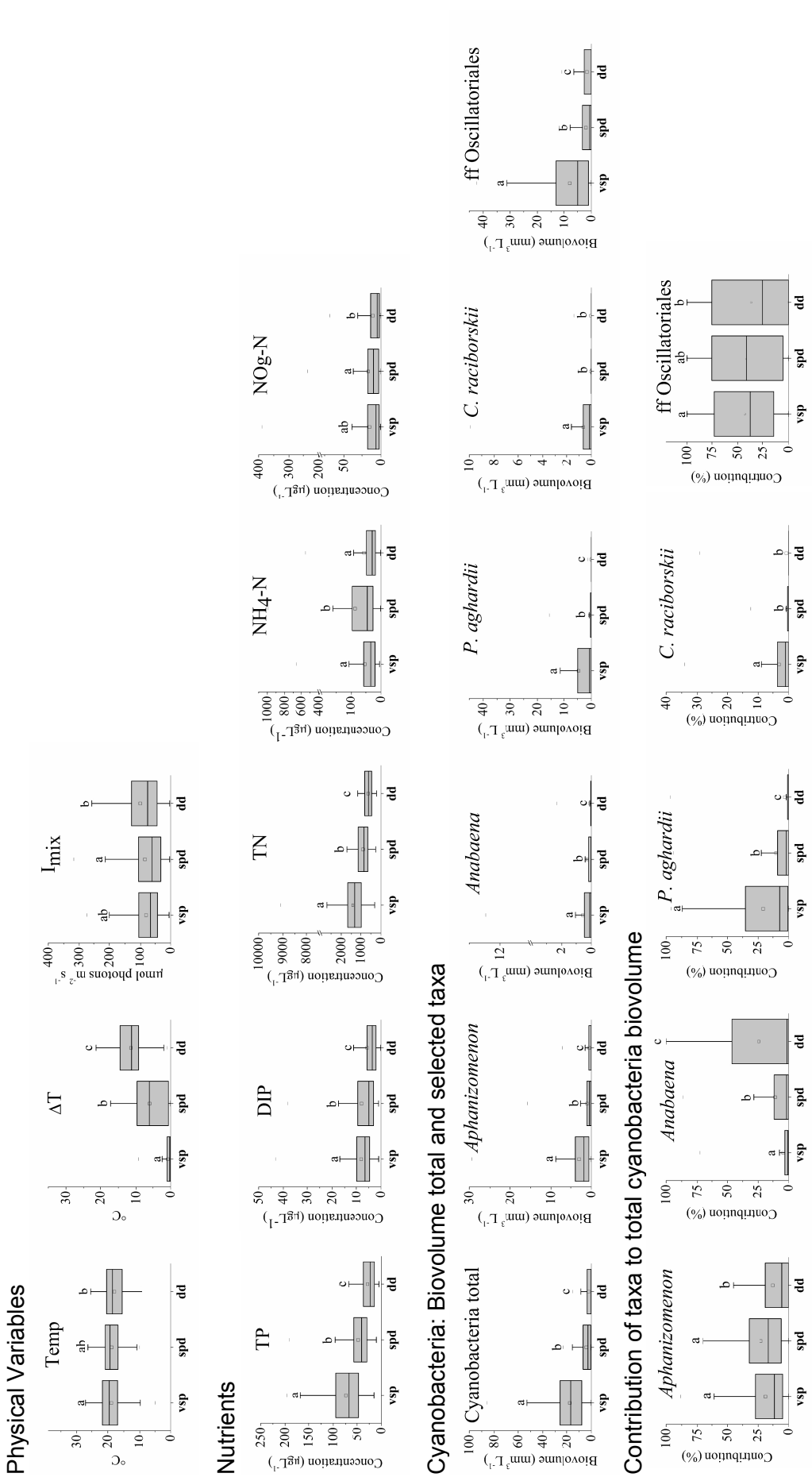


Figure 1.7. Distribution of environmental parameters, biovolume of cyanobacteria taxa as well as the contribution of individual taxa to total cyanobacteria biovolume in the three lake types (vsp = very shallow polymictic, spd = shallow poly-/dimictic, dd = deep dimictic lakes). (Temp = Temperature, ΔT = water temperature difference between surface and bottom, I_{mix} = mean light intensity in the mixed layer, TP = total phosphorus, DIP = dissolved inorganic phosphorus, TN = total nitrogen, NH_4-N = ammonium, NOg-N = sum of nitrate and nitrite).

Table 1.3. Bivariate correlations between biovolume of individual cyanobacterial taxa with temperature (T), temperature difference between lake surface and bottom (ΔT), mean photosynthetic radiation in the mixed water column (I_{mix}) and concentrations of the nutrients total phosphorus (TP), dissolved inorganic phosphorus (DIP), total nitrogen (TN), ammonium (NH_4-N), and the sum of nitrate and nitrite (NOg-N) according to lake type. Cases where Kendall's τ_B correlation coefficient was significant at the 0.05 (*) and 0.01 (**) level are marked accordingly.

Lake type/Cyanobacterial taxa	T	ΔT	I_{mix}	DIP	TP	NOg-N	NH_4N	TN
very shallow polymictic								
Aphanizomenon	.139(**)		-.198(**)		.319(**)	-.105(**)	-.166(**)	.232(**)
Anabaena	.179(**)				.168(**)	-.081(*)		.079(*)
C. raciborskii	.098(**)		-.125(**)	.171(**)	.113(**)	-.093(**)	-.205(**)	.265(**)
P. agardhii			-.255(**)		.445(**)	-.202(**)	-.209(**)	
ff Oscillatoriales		-.082(*)	-.091(**)	.134(**)		-.089(*)	-.146(**)	.240(**)
shallow poly-/dimictic								
Aphanizomenon	.148(**)		-.272(**)		.125(*)			.205(**)
Anabaena	.156(**)	-.122(*)						
C. raciborskii			-.156(**)					
P. agardhii		-.206(**)	-.323(**)		.432(**)		.282(**)	.266(**)
ff Oscillatoriales			-.355(**)	-.110(*)				.241(**)
deep dimictic								
Aphanizomenon			-.297(**)	.192(**)	.332(**)			.361(**)
Anabaena	.102(*)		.172(**)	-.108(*)	-.322(**)			-.262(**)
C. raciborskii			-.195(**)		.191(**)	-.152(**)	-.171(**)	.303(**)
P. agardhii	-.124(*)	-.213(**)	-.285(**)	.203(**)	.273(**)	.117(*)		.295(**)
ff Oscillatoriales			-.322(**)	.245(**)	.401(**)			.409(**)

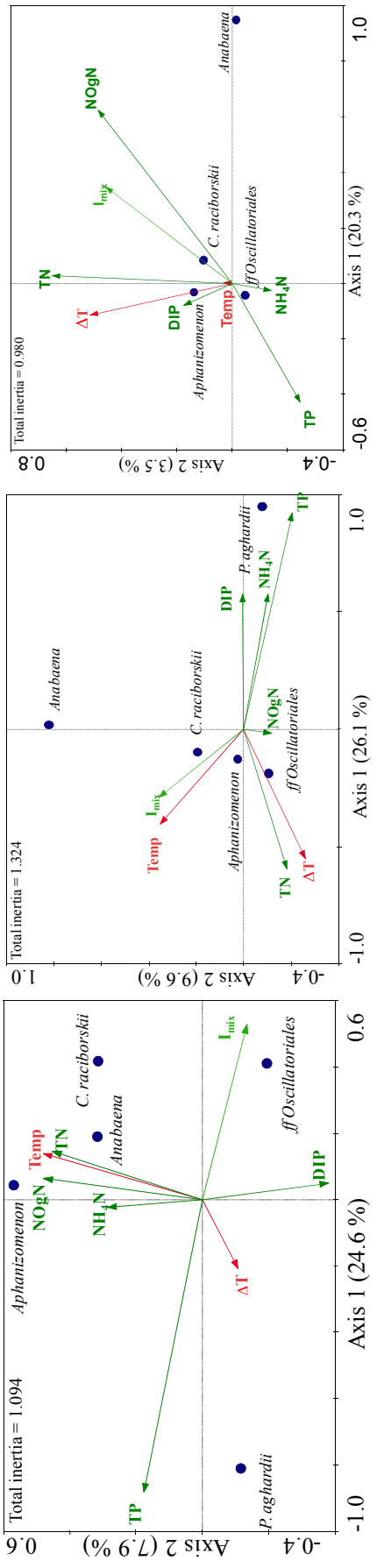


Figure 1.8. Biplots showing the association of selected cyanobacterial taxa and environmental parameters in (a) very shallow polymictic lakes (b) shallow poly-/dimictic lakes and (c) in deep dimictic lakes based on a canonical correspondence analysis (CCA). Environmental parameters related to trophic status are illustrated in green (TP = total phosphorus, DIP = dissolved inorganic phosphorus, TN = total nitrogen, NH_4-N = ammonium, NOg-N = sum of nitrate and nitrite), those related to climate in red (Temp = water temperature, ΔT = water temperature difference between surface and bottom), and mean light intensity in the mixed layer (I_{mix}) in light green.

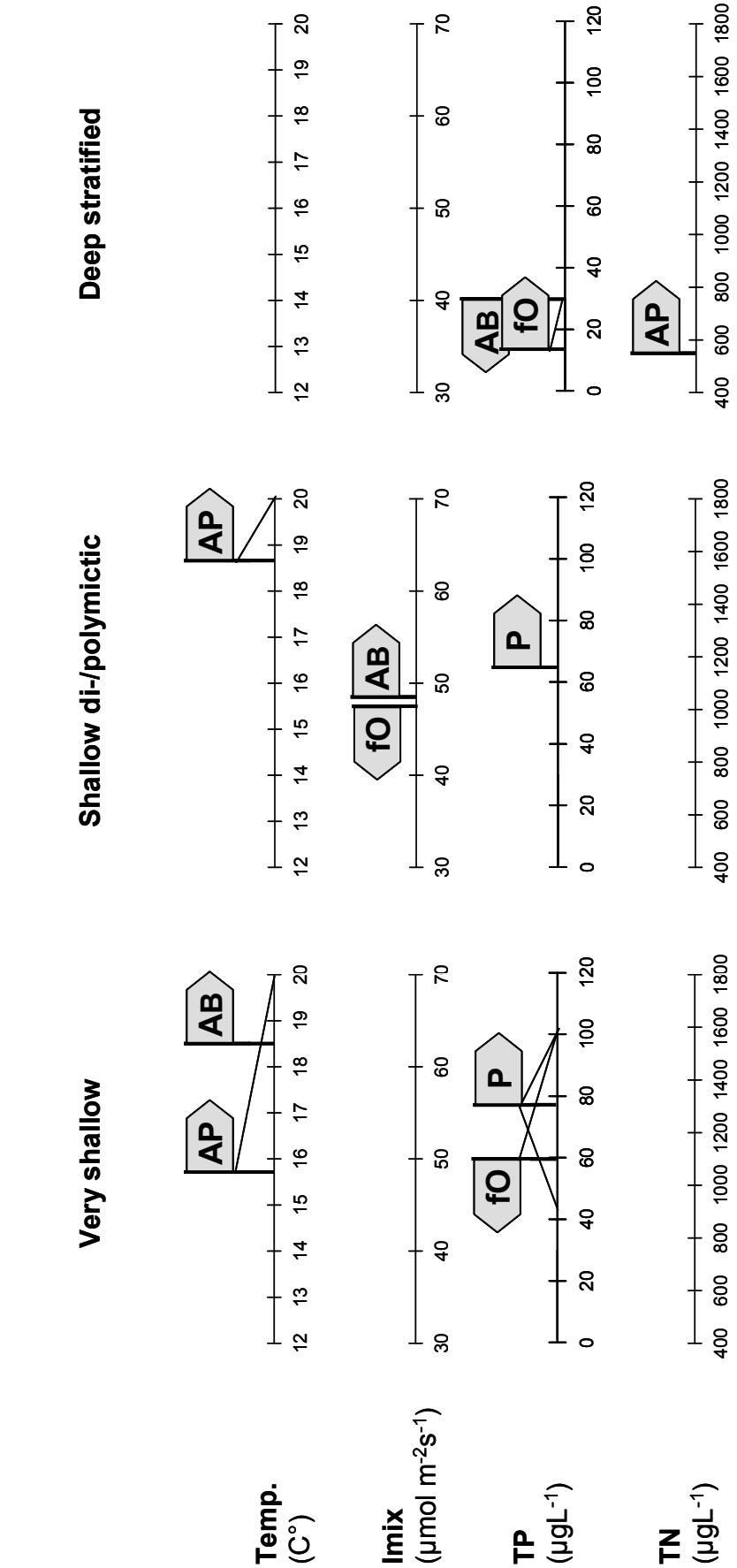


Figure 1.9. Illustration of threshold values for primary predictors indicating increasing or decreasing (indicated by arrow direction) probability of the four cyanobacterial taxa – *Aphanizomenon* (AP), *Anabaena* (AB), *P. agardhii* (P), and fine filamentous Oscillatoriales (fO) – to exceed 25 % of total cyanobacterial biovolume in the three lake types studied.

1.3 Cyanobacterial hepato- and neurotoxins in the studied lakes

Jutta Fastner

The first survey for cyanobacterial toxins in Germany 1995/96 had identified microcystins (MC) as the most frequent toxins, whereas anatoxin-a (ATX) and paralytic shellfish poisons (PSP) were much less abundant (Fastner *et al*. 2001, Chorus *et al*. 2001). Cylindrospermopsin (CYN) had not been investigated in this survey, but was found to be as frequent as microcystins in a survey in 2004 (Fastner *et a*l. 2007).

To comprise potential changes in toxin composition due to changing environmental conditions (i.e. decreasing eutrophication, climate change) as hypothesized in this study, we screened 14 lakes (12 of them had been already investigated in 1995/96) in 2007–2009 for the presence of MC, CYN, ATX and PSPs.

This is the first study in Germany in which all relevant groups of cyanobacterial hepato- and neurotoxins – MC, CYN, ATX and PSPs – were analysed simultaneously.

Materials and methods

Sampling

14 lakes located in northeast Germany (see Chapter 1.1) were sampled from 2007–2009 whereas sampling frequency ranged from infrequent sampling in only one year to bi-weekly over three years. Sampling was performed at the deepest site. Aliquots were taken for phytoplankton analyses and for preparing filters and filtrates for toxin analyses.

Toxin analyses

For extraction of the toxins from filters either 75 % aqueous methanol for microcystins or 75 % acetonitrile-water-formic acid (75:14.9:0.1) for CYN, ATX and PSP was used.

Filters were extracted twice with 1.5 ml at room temperature. Each extraction step included 10 min ultrasonication, followed by shaking for 1 h and centrifugation. The supernatants were combined and dried by vacuum centrifugation. Prior to HPLC analysis extracts were resolved in either 50 % methanol for microcystin analyses or in 75 % aqueous acetonitrile or the analyses of CYN, ATX and PSPs.

For the determination of dissolved CYN, ATX and PSPs 1 ml of water was dried by vacuum centrifugation. Dissolved MC was not analysed as our previous studies and a large body of literature showed this toxin to occur largely cell-bound, with the exception of rather rare and short-lived events such as bloom lysis.

Microcystin were analysed by HPLC-PDA as described in detail previously (Fastner *et al.* 2001). CYN, ATX and PSPs analyses by LC-MS/MS were carried out on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a API

4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Framingham, MA) equipped with a turbo-ionspray interface. The extracts were separated using a 5 µm TSK gel Amide-80, 2 x 250 mm column (Tosohaas, PA, USA) at 30 °C. The mobile phase consisted of water (A) and acetonitrile-water (95:5, B) both containing 2.0 mM ammonium formate and 3.6 mM formic acid (pH 3.5) at a flow rate of 0.2 ml min^{-1}. For the analysis of multiple toxins (cylindrospermopsin, anatoxin-a, paralytic shellfish poisons) the following gradient programme was applied: 75 % B for 5 min, 75 %–65 % B over 1 min, hold for 13 min, 65–45 % over 4 min, hold for 10 min (Dell'Aversano *et al*. 2004). The injection volume was 10 µl. The mass spectrometer was operated in the selected reaction monitoring mode (SRM) for the detection and quantification of the following toxins as described in Dell'Aversano *et al*. (2004): cylindrospermopsin (CYN), anatoxin-a (ATX), saxitoxin (STX), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX) and decarbamoylneosaxitoxin (dcNEO), gonyautoxin-1, -2, -3, -4, -5 (GTX 1, -2, -3, -4, -5), decarbamoylgonyautoxin (dcGTX-3, -3), *N*-sulfogonyautoxins-1, -2 (C1, C2). Standard curves were established for all the toxins (CYN and PSP standards were obtained from National Research Council, Halifax, Canada and anatoxin-a from Tocris, UK) and analysed in a line with the unknowns (one calibration curve after 20 unknowns).

Results and discussion

Types of toxins detected

All toxins investigated in this study were detected either solely or in various combinations (Table 1.4). However, from some types of toxins – especially MC and PSP – numerous congeners of varying toxicities exist, and their differentiation can be important for risk assessment.

Among the microcystin variants primarily MC-LR, MC-RR, MC-YR, a HtyR variant and demethylated variants of MC-RR and MC-LR were detected (Figure 1.10). It is known from our previous study in 1995/96 and many other studies that *Microcystis* produces mainly MC-RR, MC-YR and MC-LR, while the dominant microcystins associated with *Planktothrix agardhii* are demethylated MC variants (e.g. Fastner *et al*. 1999).

These microcystins were also observed in 2007 and comparison with the data from 1996 even revealed a similar share of the single MC variants (Figure 1.10). This suggests a continuous persistence of certain chemotypes (i.e. genotypes producing a distinct pattern of toxins) and composition of these chemotypes in these lakes. Though changes in dominance of certain chemotypes can occur any time, the environmental conditions driving such shifts are still unclear.

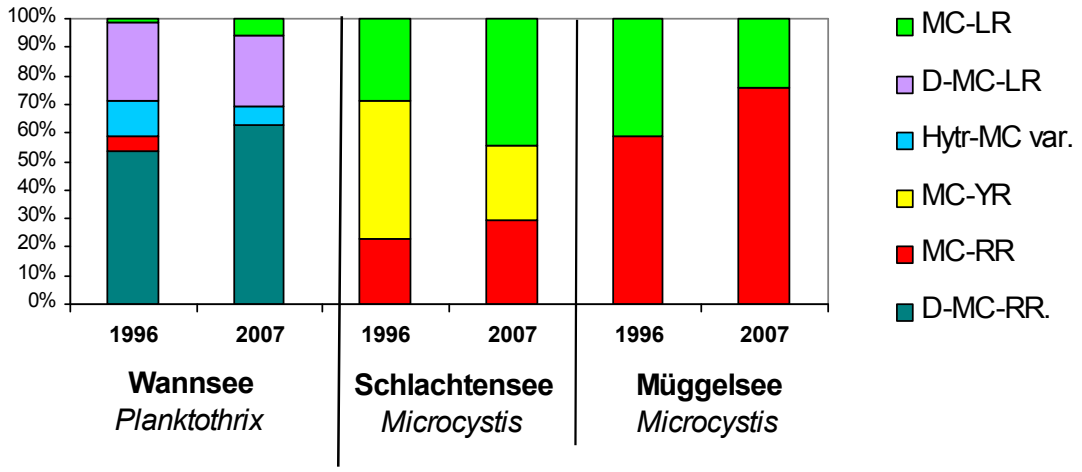


Figure 1.10. Microcystin variants detected in samples dominated by either *Microcystis* sp. or *Planktothrix agardhii* from different lakes in 1996 and 2007.

From the 13 PSPs analysed (see Materials and Methods) 4 congeners were detected regularly in both field samples and isolated strains thereof: GTX5, STX, dcSTX and NEO (see also Chapter 2.1): STX and NEO are the two most toxic PSPs, while dcSTX is less toxic and data on the toxicity of GTX 5 do not exist. Though PSP concentrations measured in this study are far below hazardous concentrations (see below) the prevalence of the most toxic PSP congeners in the field samples is important for risk assessment in case producer organisms proliferate in the future, causing higher concentrations to occur.

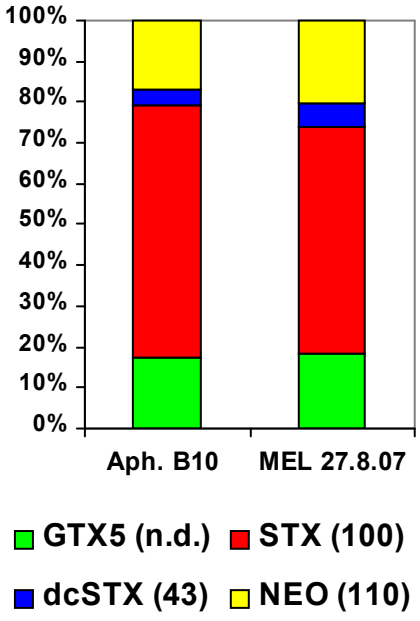


Figure 1.11. Relative share of PSP congeners detected in a field sample from Melangsee in 2007 and in the strain *Aphanizomenon* spp. B10 isolated thereof. Numbers in parentheses indicate the relative toxicity (%) of these congeners.

Figure 1.11 shows an example of PSP patterns from Melangsee in 2007 and of an *Aphanizomenon* strain isolated from this lake. Both samples show a very similar share of GTX5, STX, dcSTX and NEO, indicating this strain to be an important PSP producer in the lake. Data on PSP producing organisms in Europe are scarce but *Aphanizomenon* from Portugal have been found to show PSP patterns similar to those of the strains

isolated in this study, indicating a wide distributions of certain chemotypes – a phenomenon likewise observed previously for microcystin chemotypes (Dias *et al.* 2002).

In addition to CYN also the occurrence of deoxy-cylindrospermopsin was analysed in this study, although no quantitative data could be established due to the lack of a commercially available standard. Deoxy-cylindrospermopsin seems almost as abundant as CYN: it was detected in many samples with higher concentrations of CYN.

Frequency of occurrence

Cyanotoxins occurred in most of the 14 lakes investigated (Table 1.4). Only in two lakes no cyanotoxins were found, while the others showed the presence of 1–4 types of toxins. In five lakes all types of toxins were detected, and this is in part due to the high sampling frequency of these lakes as this increases the chance to pick up also infrequent occurrence of toxins.

Table 1.4. Lakes sampled from 2007–09: trophic status and presence of different hepato- and neurotoxins: **+** toxin detected, - toxin not detected.

Lake	Trophy	No of samples	MC	CYN	ATX	PSP
Tiefer See	oligo	4	-	-	-	-
Stechlinsee	oligo-meso	26	-	-	-	-
Schlachtensee	meso	12	+	-	-	-
Tegeler See	meso	15	+	+	-	-
Tollensesee	meso	3	-	+	-	+
Schmaler Luzin	meso	11	-	+	-	+
Wannsee	poly	14	+	+	-	-
Haussee	eu	12	+	+	-	+
Müggelsee	eu	7	+	+	+	-
Petersdorfer See	meso	38	+	+	+	+
Scharmützelsee	meso	29	+	+	+	+
Melangsee	eu	40	+	+	+	+
Stolpsee	meso	29	+	+	+	+
Langer See	eu	45	+	+	+	+

Table 1.5 summarizes the frequency of toxin occurrence per lake (n = 14) and per sample (n = 289). CYN and MC occurred in over 70 % of the lakes, PSPs were almost as abundant, while ATX was somewhat less frequent. Due to the above mentioned bias caused by differences in sampling frequency, this result primarily shows the wide distribution of cyanobacteria producing different types of toxins in these lakes.

Table 1.5. Frequency of toxin occurrence in 14 lakes studied from 2007–09.

	Positive lakes/ total number of lakes (n)	Frequency per lake (%)	Positive samples/ total number of samples (n)	Frequency per sample (%)
MC	10/14	71	103/272	38
CYN	11/14	79	174/289	61
ATX	6/14	43	70/289	24
PSP	8/14	62	61/289	21

Results on a per sample basis show that in these lakes CYN was the most abundant toxin (61 %, Table 1.5), followed by MC (38 %), ATX and PSPs (24 and 21 %). however this result is biased against MC because the detection method for MC was substantially less sensitive than for the other toxins.

CYN in these lakes most probably originates from Nostocalean cyanobacteria, however, recent findings show that also in the order Oscillatoriales more CYN producers may be present than previously suspected (Mazmouz *et al*. 2010). Importantly, this study is the first screening study in the Northern hemisphere which found CYN at least as abundant as microcystins and more frequently than the other toxins. The few studies screening for multiple toxins from Italy and USA both found microcystins much more frequently than CYN and ATX (Boyer 2008, Hedman *et al*. 2008, Messineo *et al*. 2009).

However, the results of our re-analysis of samples we had stored from 1995/96 for CYN demonstrate that already then, CYN was as frequent as microcystins (Figure 1.12). The twelve lakes analysed both in 1995/96 and 2007–09 all showed presence of CYN. This result confirms that of 2004 where 50 % of 127 lakes where tested positive for CYN (Fastner *et a*l. 2007). The presence of CYN in Germany is thus not a “new”, but an “old” problem. This is not surprising, as *Aphanizomenon* – currently the main producing organism identified – is a widespread and frequent part of the phytoplankton community in Europe.

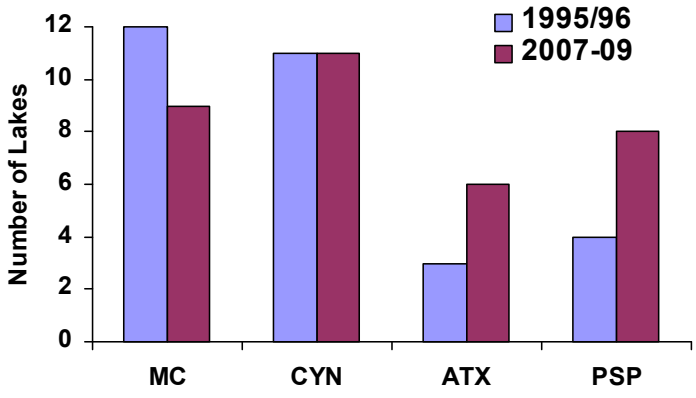


Figure 1.12. Cyanotoxin occurrence in 12 lakes sampled in 1995/96 and 2007–09. Columns represent number of lakes tested positive.

While the occurrence of CYN seems unaltered, the comparison with data from 1995/96 on a per lake basis shows a tendency of MC to occur less frequently and of ATX and

PSP to occur more frequently (Figure 1.12). However, the comparison of the two studies is biased by differences in sampling and analytical frequencies as well as differences in method sensitivity. Furthermore, not all lakes changed in their trophic state and thus toxin occurrence during the last decade. This limits conclusions of a general trend for all lakes from this data set.

Toxin concentrations

Total toxin concentrations found in the investigation period differed between types of toxins (Figure 1.13). Microcystin concentrations were clearly higher with maximal concentrations of ~ 18 µg L^{-1} followed by CYN (max. ~ 5 µg L^{-1}), ATX (max. ~ 0.9 µg L^{-1}) and PSPs (max. 0.7 µg L^{-1}).

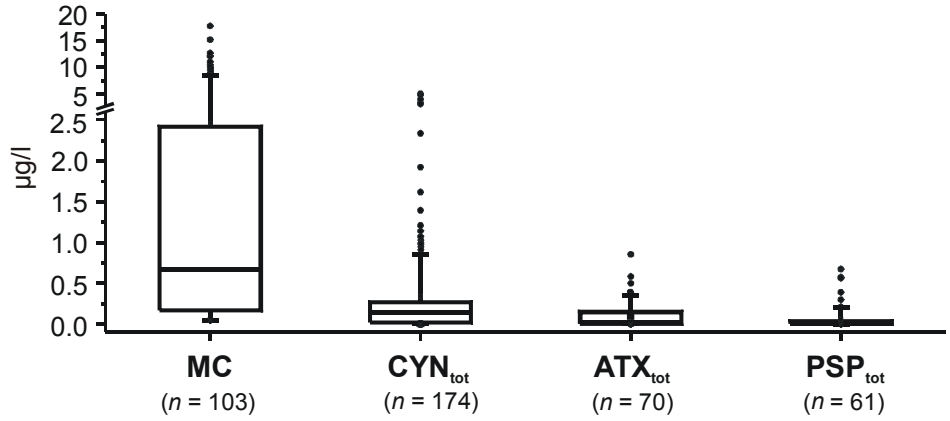


Figure 1.13. Box-Whisker plots of toxin concentrations in positive samples from 2007–09. Solid dots represent data outside the 10th and 90th percentile.

Though microcystin production by Nostocalean cyanobacteria of the genus *Anabaena* can not be excluded, field data suggest that microcystins predominantly originate from *Microcystis* and *Planktothrix* (e.g. Langer See, Wannsee) in the lakes investigated. It can thus be concluded that CYN is the most abundant toxin produced by Nostocalean cyanobacteria.

The comparison with data from 1995/96 revealed that the concentrations of microcystins are conspicuously lower in 2007/08, while those of anatoxin-a were similar (Figure 1.14). Quantitative data for CYN and PSP are not available for 1995/96, but CYN concentrations up to 12 µg L^{-1} found in 2005 (Rücker *et al*. 2007) show that CYN can occasionally be as abundant as microcystins in the lakes from northeast Germany.

Detailed evaluation of long term data clearly shows a reduction of phosphorus and consequently a reduction of microcystin concentrations in some lakes (e.g. Langer See). However, in other lakes such as Wannsee (data not shown) the lower microcystin concentrations observed in 2007/2008 can be attributed to the exceptionally low cyanobacterial biomass which in turn was probably due to unstable weather conditions, as in this lake since 1995/1996 phosphorus concentrations did not decrease to levels limiting phytoplankton biomass and thus impacting on cyanobacterial dominance.

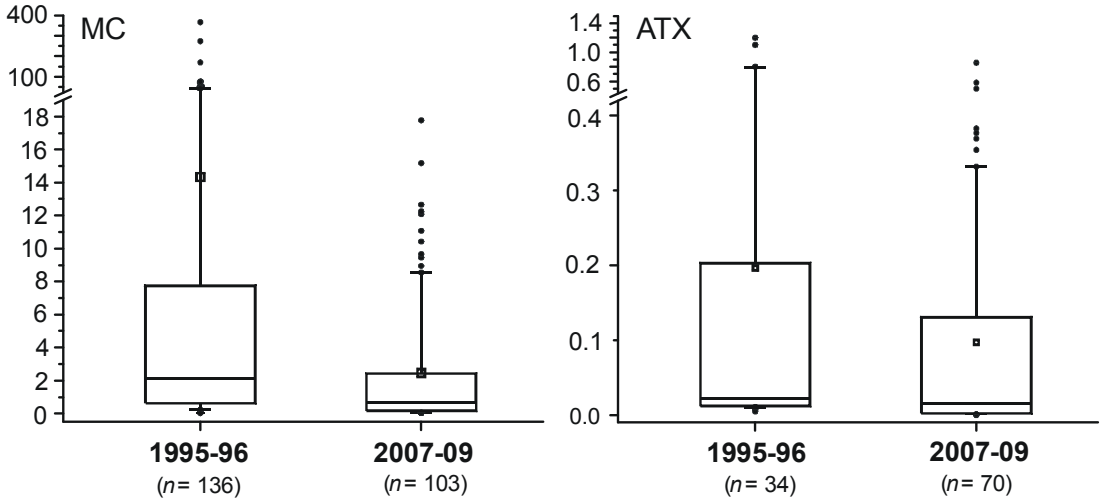


Figure 1.14. Box-Whisker plots of total toxin concentrations in positive samples from 1995/96 and 2007–09. Left panel: MC; right panel ATX; solid dots represent data outside the 10th and 90th percentile.

The annual variability of cyanotoxin occurrence in relation to that of toxin-producing cyanobacteria is shown for the example of Stolpsee 2007–2008 (Figure 1.15).

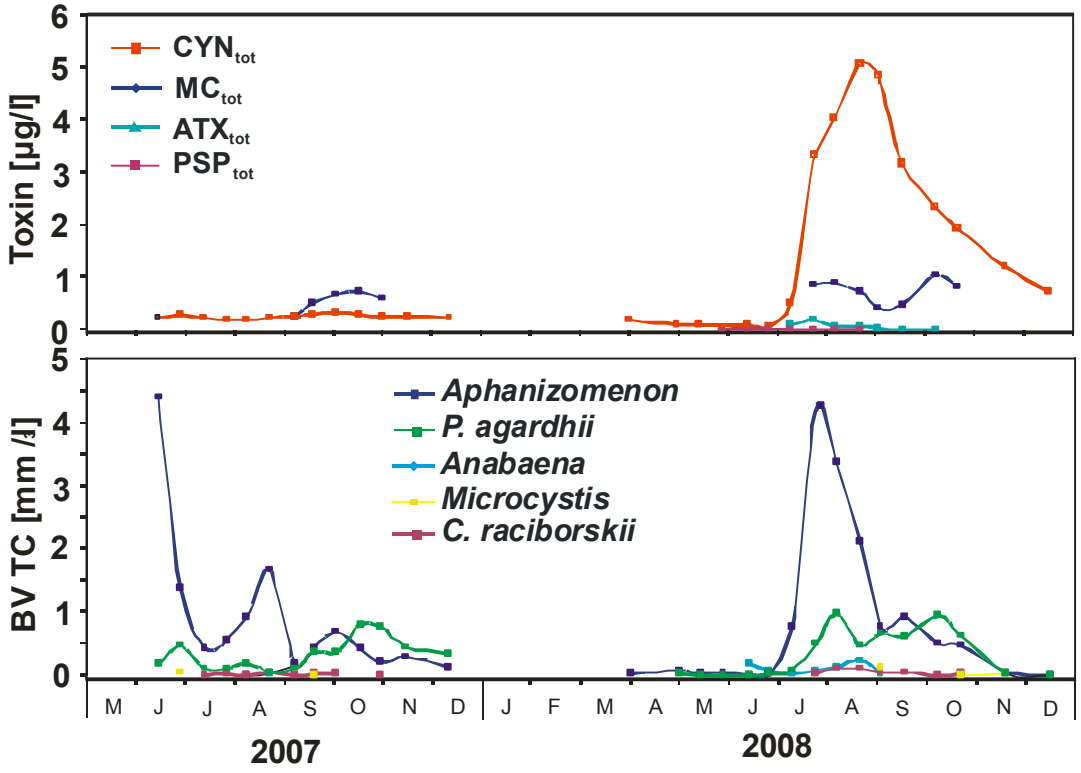


Figure 1.15. Toxin concentrations (upper panel) and potentially toxic cyanobacteria (lower panel) in Stolpsee 2007–2008.

While in 2007 only CYN and MCs in low concentrations could be detected in Stolpsee, in 2008 all of the investigated toxins were found, with CYN showing the highest concentrations of up to ~ 5 µg L^{-1} (Figure 1.15). In both years, numerous potentially toxin-producing cyanobacteria were present in the lake, of which *Aphanizomenon* spp. (primarily *Aph*. *gracile*) and *P. agardhii* showed highest biovolumes (Figure 1.15). Identifying toxin producers from field data is difficult as the course of the population and toxin concentrations can be uncoupled due to changes in toxin-producing genotypes, however, the presence of MC variants typical for *P. agardhii* as well as the similar patterns of occurrence over time for MC and *P. agardhii* show this to be the major microcystin producer in Stolpsee. *Aphanizomenon gracile* is most probably the CYN-producer in Stolpsee 2008 as biovolume and the particulate CYN are related (Figure 1.15, Figure 1.17). In contrast, in 2007 CYN concentrations were low and any relationship between *Aphanizomenon* and CYN was lacking. We assume this most likely to be due to a low amount of CYN-producing genotypes in 2007, and investigations of their relative quota using molecular methods would be the way forward for clarification.

An important result in Stolpsee 2008 is, that the high CYN concentration found in summer only declined slowly and that ~ 1 µg L^{-1} CYN was still present in December, after almost all cyanobacterial populations had died off (Figure 1.15). The reason for that is the high and persisting fraction of dissolved CYN (Figure 1.17). This example clearly shows monitoring/surveillance schemes based only on current amounts of cyanobacterial biomass (as first alert level) may miss risks due to dissolved CYN.

Particulate vs. dissolved toxins

It is known from our previous and other studies that especially CYN, but also ATX and PSPs can be present largely in the dissolved fraction of the water body. This is different to microcystins which are mainly cell-bound (i.e. particulate) and released only during cell lysis; i.e. in healthy populations usually only 1–2 % of microcystins are dissolved in the water (which is why the dissolved fraction of microcystins was not included in this study).

As expected, the dissolved fraction of CYN and PSP, and occasionally also of ATX, was often substantially higher (up to almost 100 % in some cases) than the particulate fraction (Figure 1.16).

The dissolved toxins showed differences in their persistence: while ATX and PSP disappeared quite rapidly together with the particulate fraction (data not shown), CYN often showed a high persistence in the water bodies investigated.

As shown exemplarily for Stolpsee 2008 the dissolved fraction peaked after the particulate one and then declined only slowly (Figure 1.17). The role of different factors such as biodegradation, degradation by sunlight, adsorption and dilution for the disappearance of CYN *in situ* is not yet understood, though first results indicate that biodegradation of CYN as well as degradation by UV light seems to play a minor role in lake water (see Chapter 5, Wörmer *et al.* 2008, Wörmer *et al*. 2010).

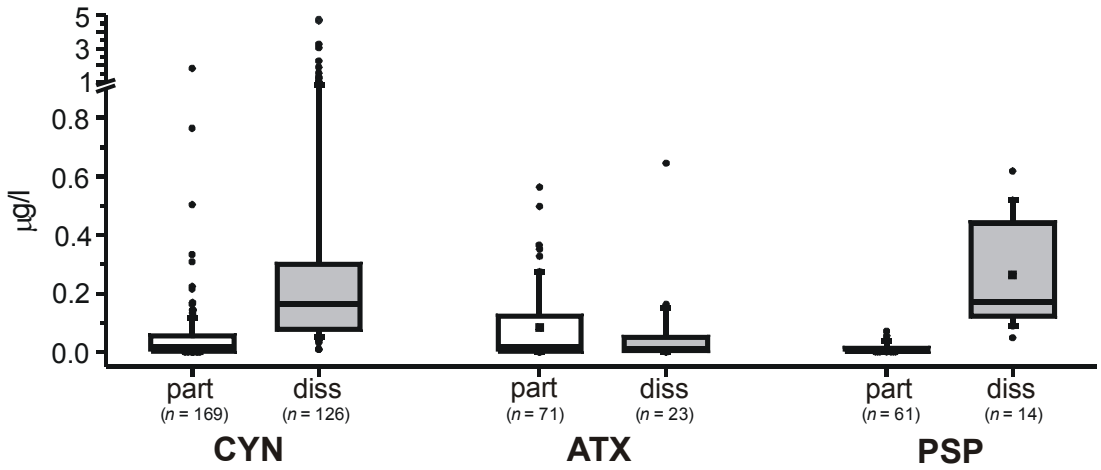


Figure 1.16. Box-Whisker plots of particulate and dissolved concentrations of CYN, ATX and PSPs in positive samples from 2007–09. Solid dots represent data outside the 10th and 90th percentile.

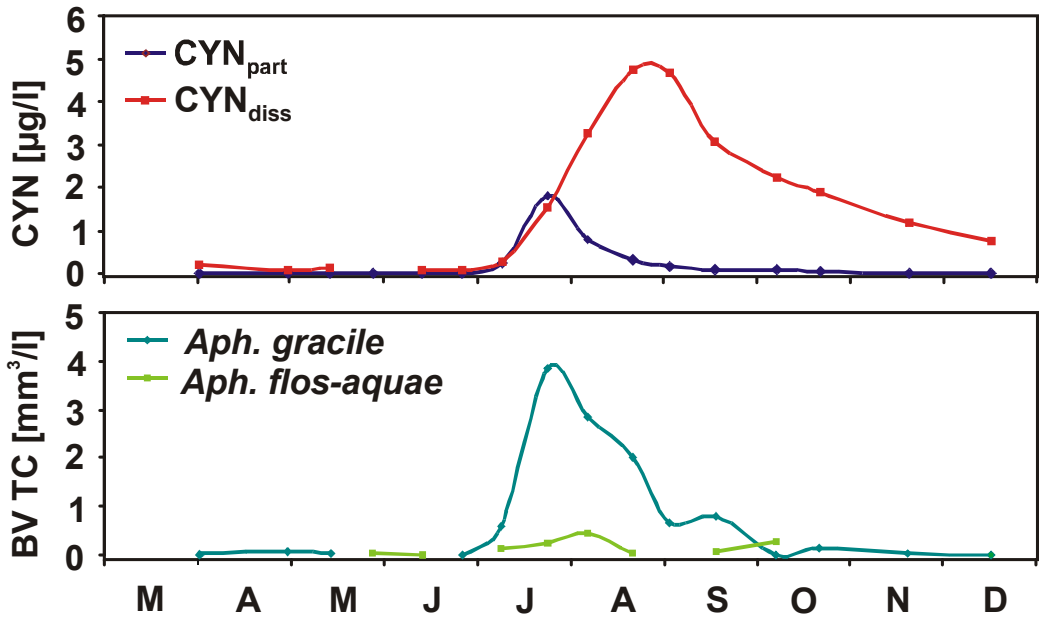


Figure 1.17. Concentrations of particulate and dissolved CYN (upper panel) and *Aphanizomenon* species (lower panel) in Stolpsee 2008.

The high shares of dissolved toxins, and especially those of persisting ones such as CYN, may have implications for other biota in the water body, as their exposure to this fraction is inevitable, while ingestion of the particulate one depends on uptake of cyanobacterial cells.

This issue is also of importance for the drinking-water production as cell-bound toxins such as microcystins can be removed to a large extent by flocculation/filtration, while the elimination of dissolved toxins may require other techniques (see also Chapter 5).

Consequently the high persistence of dissolved CYN eventually leading to year-round presence of this toxin in water bodies requires different monitoring schemes as for cell-bound toxins to pick up risks.

Conclusions

This study has shown that among the toxins produced by Nostocalean cyanobacteria CYN is the most frequent and abundant toxin in the lakes investigated. ATX and PSPs were not only less frequent, but also occurred in lower concentrations.

Giving the fact that microcystins have declined as a consequence of phosphorus and thus cyanobacteria reduction in some lakes, CYN may become more important than microcystins in those lakes if Nostocalean cyanoabcteria will increase further. Already to date CYN can occur in concentrations similar to those of microcystins.

A key result is that special attention should be given to CYN due to the high share of dissolved toxin together with its high persistence in water bodies. Monitoring schemes need to be adapted, as those for cell-bound toxins based on cyanobacterial biomass may miss risks due to dissolved CYN.

2. Identification of toxin producers and regulation of toxin production

2.1 Further clarification of species and chemotype diversity within the genus *Aphanizomenon, Anabaena* and *Anabaenopsis*

Andreas Ballot

Isolation of strains

Hepatotoxic cylindrospermopsin (CYN), neurotoxic paralytic shellfish poisoning (PSP) toxins and neurotoxic anatoxin-a (ATX) have been detected in several northeast German lakes in the last two decades (Chorus 2001). During the preceding CYLIN- and during the NOSTOTOX-project CYN, PSP toxins and ATX were detected in field samples from several lakes (e.g. Scharmützelsee, Melangsee, Langer See) (see Chapter 1.3). In the CYLIN-project it was demonstrated that *Aphanizomenon flos-aquae* strains are able to produce CYN (Preußel *et al.* 2006). The CYN-producing strains were isolated from the German lake Melangsee (Preußel *et al.* 2006). PSP toxin and ATX production in German lakes could not be related to specific cyanobacterial taxa and sources of PSP toxins and ATX have yet to be identified in German water bodies. Studies from Finland, Portugal, Brazil, New Zealand and the United States have shown that strains of *Aphanizomenon* sp., *Anabaena* sp. and other filamentous cyanobacteria like *Lyngbya wollei* are able to produce PSP toxins or ATX (Rapala *et al.* 1993, Carmichael *et al.* 1997, Pereira *et al.* 2004, Wood *et al.* 2007, Kellmann *et al.* 2008).

In our studies we put the focus on nostocalean cyanobacteria since most of the known PSP toxin and ATX producing cyanobacteria belong to this group and they are widely distributed in northeast German lakes.

Potentially toxin-producing cyanobacteria are often characterized by the co-existence of toxin-producing strains and non-toxin-producing strains. To find such toxin-producing strains we isolated a high number of filaments from different nostocalean species from different lakes. Isolations were carried out from samples taken from eight selected lakes (Table 2.1). Using a microcapillary, single colonies or filaments of A*phanizomenon* spp., *Anabaena* spp. and *Anabaenopsis* spp. were isolated, washed 5 times and transferred to microtiter plates containing 300µL Z8 medium (Kotai 1972). After successful growth they were transferred to larger culture vessels (Figure 2.1).

In 2007 and 2008 a total of 159 monoclonal strains belonging to the genus *Aphanizomenon* and 45 monoclonal strains belonging to the genus *Anabaena* and *Anabaenopsis* were successfully isolated from German lakes Langer See, Melangsee, Nehmitzsee, Petersdorfer See, Scharmützelsee, Springsee, Stechlinsee and Stolpsee. The IGB culture collection contains 60 additional *Anabaena*, *Aphanizomenon*, and *Cylindrospermop-*

sis strains isolated during former projects. The sampled lakes, taxon name, isolation dates and number of isolated strains are shown in Table 2.1.

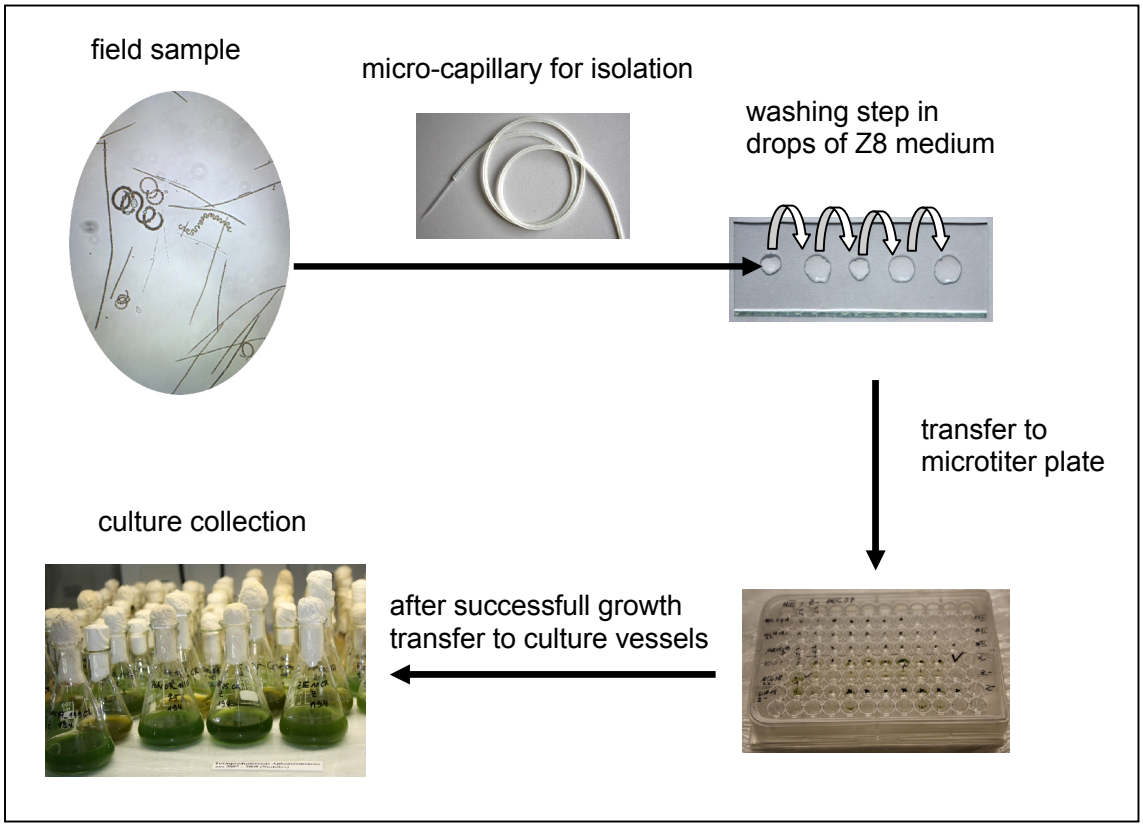


Figure 2.1. Isolation and cultivation of monoclonal cyanobacterial strains from field samples.

Table 2.1. Number of *Aphanizomenon* and *Anabaena* strains in culture isolated in 2007 and 2008 from different lakes.

	Taxon			
Lake	***Aphanizomenon* spp.**	***Anabaena* spp.**	***Anabaenopsis* spp.**	**Year of isolation**
Langer See	**25**	**9**	**3**	**2008**
Melangsee	**15**	**3**	**-**	**2008**
Nehmitzsee	**-**	**5**	**-**	**2008**
Petersdorfer See	**3**	**-**	**-**	**2007**
Scharmützelsee	**11**	**5**	**-**	**2007, 2008**
Springsee	**1**	**-**	**-**	**2007**
Stechlinsee	**20**	**20**	**-**	**2007, 2008**
Stolpsee	**84**	**-**	**-**	**2007, 2008**
Total	**159**	**42**	**3**	

Strain characterization

Morphotype

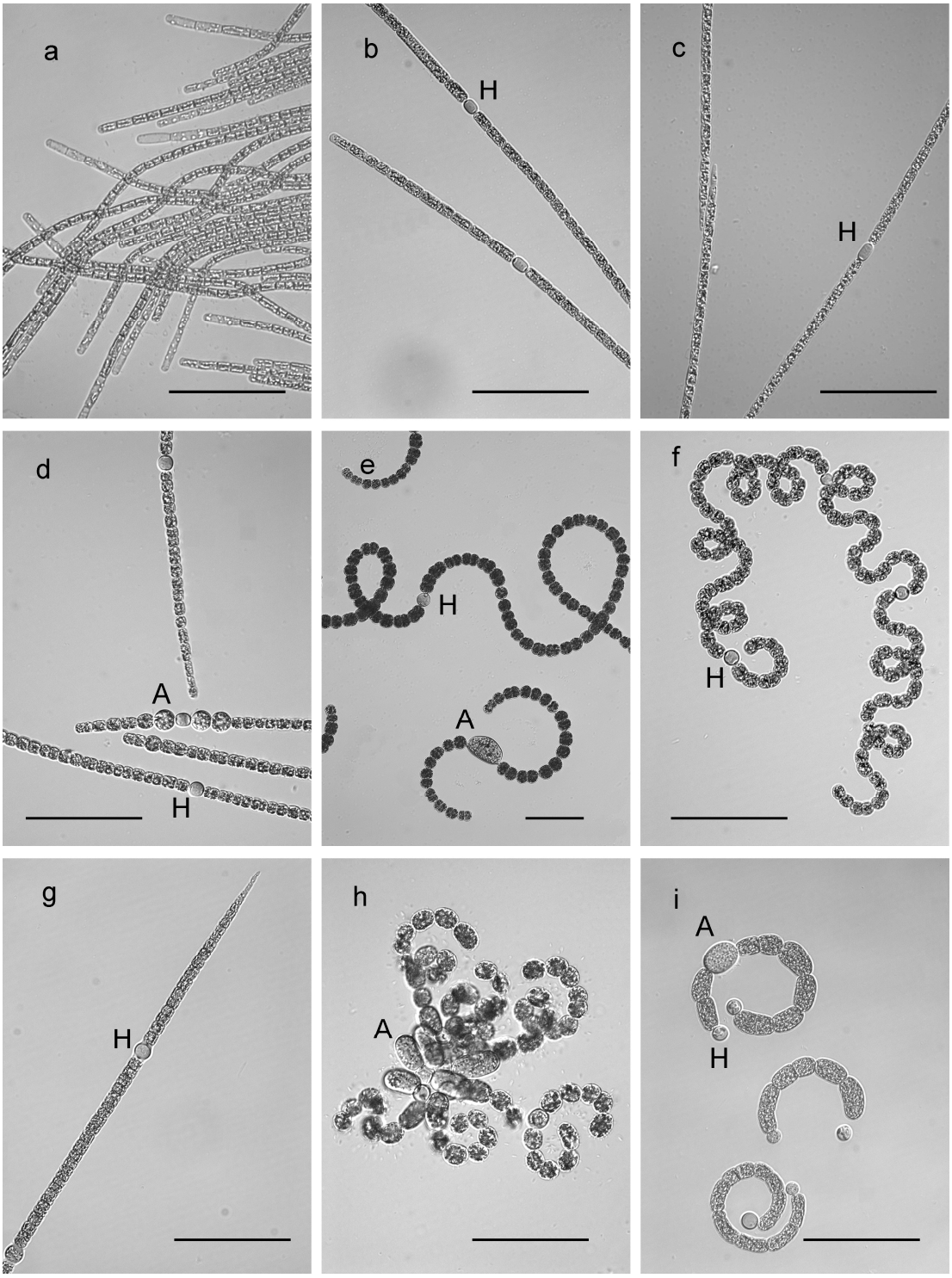


Figure 2.2. Micrographs of different Nostocalean strains investigated in this study. a) *Aphanizomenon flos-aquae*, b) *Aphanizomenon gracile*, c) *Aphanizomenon issatschenkoi*, d) *Aphanizomenon aphanizomenoides*, e) *Anabaena planktonica*, f) *Anabaena flos-aquae*, g) *Anabaena bergii*, h) *Anabaena lemmermannii*, i) *Anabaenopsis elenkinii*. A = akinet, H = heterocyst, Scale bars indicate 50µm.

In a first approach the cultured strains were microscopically determined to the species level using classical morphological criteria. The *Aphanizomenon* strains could be classified as *Aphanizomenon flos-aquae*, *Aphanizomenon gracile, Aphanizomenon aphanizomenoides*, or *Aphanizomenon issatschenkoi* according to Komárek (2005) (Figure 2.2). The isolated *Anabaena* and *Anabaenopsis* strains were classified as *Anabaena bergii*, *Anabaena flos-aquae*, *Anabaena lemmermannii*, *Anabaena macrospora*, *Anabaena spiroides* and *Anabaenopsis elenkinii* according to Komárek and Komárkova (2006) and Komárková-Legnerová and Eloranta (1992) (Figure 2.2).

Here is to note that the determination of strains at the species level is not in all cases feasible. In culture strains may develop features which are different from those shown by members of similar species found in natural samples. In these cases a determination to genus level only is conducted.

Molecular systematics

To support the morphological determination of the cyanobacterial strains phylogenetic investigations were conducted. For the characterization of the genetic variability of the isolated strains the intergenic spacer of the phycocyanin operon (PC-IGS) and the *rbcLX* gene (ribulose-1,5-bisphosphate carboxylase/oxygenase subunit L and chaperonin-like protein X gene) were analyzed as genetic marker. Both locations were chosen and combined in a concatenated tree because they include highly variable genetic regions, which make studies between closely related cyanobacterial species possible. Another genetic marker often used is the 16S rRNA gene. In comparison to the PC-IGS-region in the 16S rRNA gene the number of variable positions is low. Therefore the 16S rRNA gene it is not as suitable to study the relationship between closely related species like the investigated *Aphanizomenon* or *Anabaena* strains.

Although morphologically our *Aphanizomenon* spp., *Anabaena* spp. and *Anabaenopsis* sp. strains can be clearly distinguished, the morphological differentiation is only partly supported by our phylogenetic investigations. In our concatenated phylogenetic tree using PC-IGS and *rbcLX* as genetic markers *Anabaena bergii* is closer related to *Anabaenopsis elenkinii* than to other *Anabaena* spp. and *Aphanizomenon flos-aquae* is closer related to *Anabaena* spp. than to other *Aphanizomenon* spp. Only *Aphanizomenon issatschenkoi, Aphanizomenon aphanizomenoides* and *Aphanizomenon gracile* are located in clearly separated clusters (Figure 2.3).

A phylogenic tree is depicted in Figure 2.3 which shows the relationship between *Aphanizomenon Anabaena*, and *Anabaenopsis* strains from seven different lakes using the PC-IGS and *rbcLX* region as genetic markers.

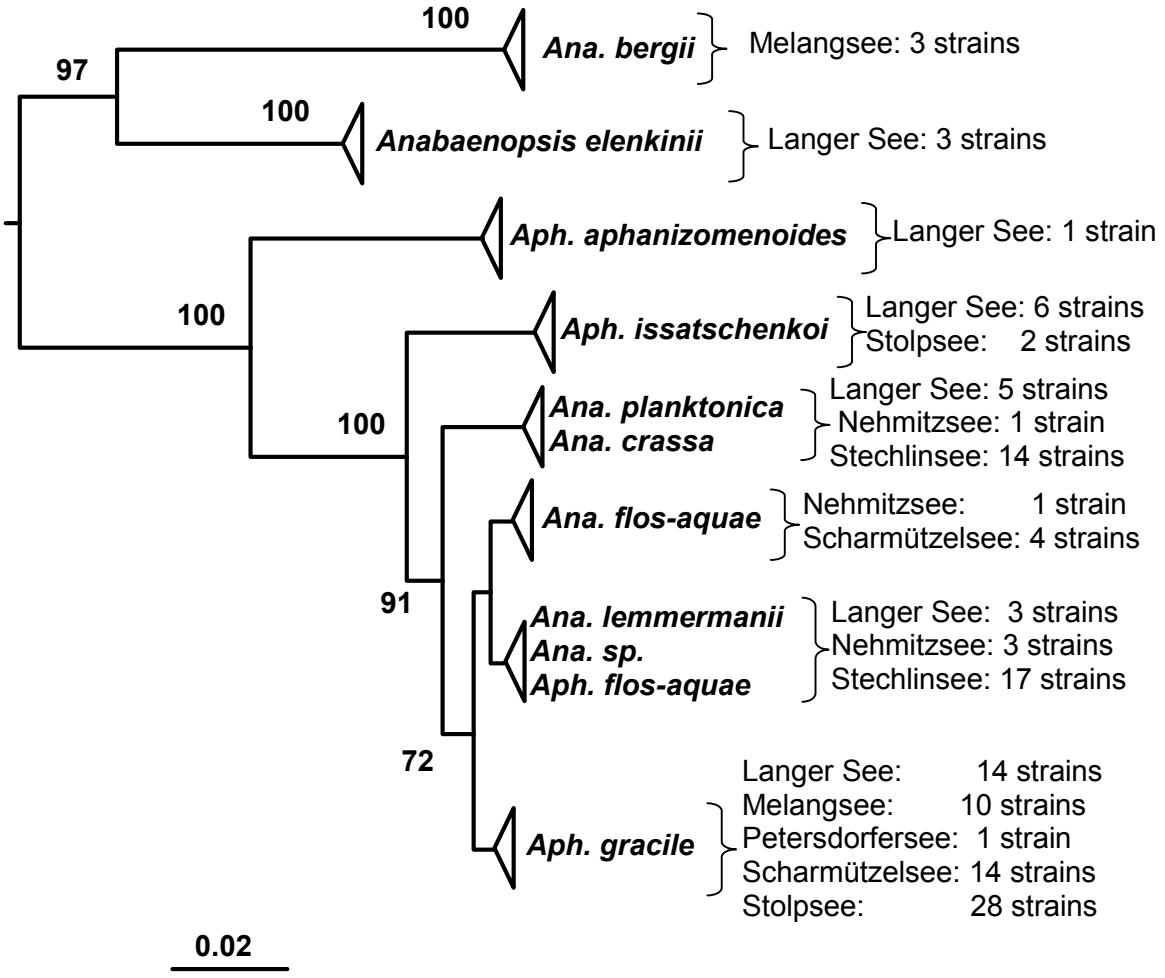


Figure 2.3. Phylogenetic tree (Neighbour-joining) based on concatenated PC-IGS and *rbcLX* sequences of 130 nostocalean strains from different lakes (Langer See, Melangsee, Nehmitzsee, Petersdorfer See, Scharmützelsee, Stechlinsee, Stolpsee). Bootstrap values above 50 are included. Bar indicates 2 % sequence divergence. *Ana =Anabaena*, *Aph. = Aphanizomenon.*

Chemotypes

Cylindrospermospsin-, paralytic shellfish poisoning toxin- and anatoxin-a-producing strains

CYN-, PSP-, and ATX-producing cyanobacteria can be detected using molecular or biochemical/chemical tools.

a) Molecular detection of CYN-, PSP-toxin- and ATX-encoding genes

To detect potential CYN- ,PSP-toxin-, or ATX-producers, all of our 204 *Aphanizomenon*, *Anabaena*, and *Anabaenopsis* strains were investigated for the possession of the cylindrospermospin gene cluster, the saxitoxin gene cluster and the putative anatoxin gene cluster.

Detection of CYN encoding genes in nostocalean strains

To find the CYN-gene cluster in a cyanobacterial strain the primers M13 and M14 according to Schembri *et al.* (2001) can be used. These primers amplify a peptide synthetase (PS) gene involved in CYN biosynthesis. All 204 nostocalean strains isolated in 2007 and 2008 were investigated for the potential of CYN-production and tested negative for the PS gene as genetic marker.

Detection of PSP toxin encoding genes in nostocalean strains

PSP toxin-producing cyanobacteria possess a saxitoxin gene cluster. The structure of this gene cluster was described by Kellmann (2008). On this basis we were able to design primers which make a fast detection of the *sxtA* gene of the saxitoxin gene cluster possible (Ballot *et al.* 2010a). *SxtA* is a gene encoding a polyketide synthase (PKS) like structure. Like the above mentioned peptide synthetase, polyketide synthases are involved in cyanobacterial secondary metabolite biosynthesis of eg. PSP toxins, ATX and CYN.

Hundred seventy nine of the 204 strains investigated for the potential of PSP toxin production tested negative using *sxtA*-gene as genetic marker The *sxtA* gene was detected in 25 *Aphanizomenon*, *Anabaena* and *Anabaenopsis* strains. Only 14 *Aphanizomenon gracile* strains also tested positive for PSP toxins using LC-MS/MS. Seven strains were isolated from L. Melangsee and seven strains from L. Scharmützelsee (Figure 2.4 a). Our findings are the first detection of PSP toxin-producing cyanobacterial strains in German water bodies (Ballot *et al.* 2010a).

Detection of putative ATX encoding genes in nostocalean strains

Anatoxin-a-producing cyanobactera most likely possess an anatoxin-gene cluster. Cadel-Six *et al.* (2009) were the first to find evidence of a putative anatoxin gene cluster in strains of *Oscillatoria* sp. The primers described by Cadel-Six *et al.* 2009 detect a gene sequence encoding a polyketid-synthase (PKS), which is putatively involved in the biosynthesis of anatoxin-a. The primers mentioned by Cadel-Six (2009) are specific for *Oscillatoria* sp. and according to our studies not suitable for anatoxin-a producing *Aphanizomenon issatschenkoi* strains. We therefore designed primers to amplify the corresponding gene sequence in *Aphanizomenon issatschenkoi* strains (Ballot *et al.* 2010b).

All 204 strains were investigated for the potential of anatoxin-a production using newly developed primers. One *A. issatschenkoi* strain isolated from Lake Stolpsee exhibited amplification of a PKS encoding gene sequence of the putative anatoxin biosynthesis gene cluster (Ballot *et al.* 2010b).

The PCR analyses of the *sxtA* gene and of the PKS encoding gene putatively involved in ATX biosynthesis of *Aphanizomenon* spp. strains is shown in Figures 2.4 a and b.

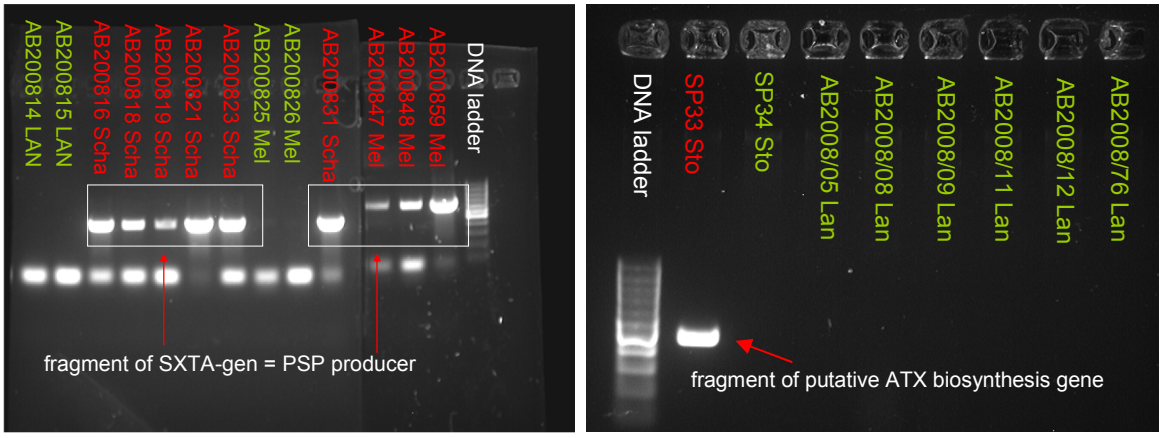


Figure 2.4. a) PCR analysis of 13 *Aphanizomenon gracile* strains for the presence of the *sxtA* gene fragment. **b)** PCR analysis for PKS encoding gene putatively involved in ATX biosynthesis of 8 *Aphanizomenon issatschenkoi* strains from two German lakes. (AB2008/14 = strain number, LAN = Langer See, Mel = Melangsee, Scha = Scharmützelsee, Sto = Stolpsee). Red marked strains show a positive result, green marked strains show a negative result.

b) Toxin detection by ELISA

The isolated strains were tested with a specific cylindrospermopsin-ELISA and a specific Saxitoxin-ELISA (Enzyme-Linked-Immuno-Sorbent-Assay) (Abraxis, Warminster, USA) for the production of cylindrospermopsin or saxitoxin. The used ELISA tests are direct competitive ELISA based on the recognition of an antigen by specific antibodies. The antigen, when present in a sample and an antigen-enzyme conjugate complex compete for the binding sites of rabbit-antigen antibodies in the solution. The antigen antibodies are then bound by a second antibody (sheep anti-rabbit) and immobilized on a plate. After a washing step and addition of a substrate solution a colour signal is produced. The intensity of the colour is inversely proportional to the antigen in the sample.

Saxitoxin-ELISA

Saxitoxin production was confirmed with the Saxitoxin-ELISA for all 14 *Aphanizomenon* strains which exhibited PCR amplification for sxtA gene (Ballot *et al.* 2010a). All remaining 190 nostocalean strains tested negative using Saxitoxin-ELISA

CYN-ELISA

All 204 nostocalean strains investigated showed negative results using the CYN-ELISA.

c) Toxin detection by LC-MS/MS

All 204 nostocalean strains isolated were checked with LC-MS/MS for production of CYN, PSP toxins and ATX.

CYN

All 204 *Aphanizomenon* spp., *Anabaena* spp. and *Anabaenopsis* spp. strains investigated tested negative for CYN using LC-MS/MS.

PSP toxins

Fourteen *Aphanizomenon gracile* strains which exhibited amplification of the *sxtA* gene and which tested positive using Saxitoxin-ELISA were shown to produce PSP-toxins using LC-MS/MS. In all strains the 4 PSP toxin variants Gonyautoxin 5 (GTX5), Saxitoxin (STX), decarbamoyl-Saxitoxin (dcSTX) and Neosaxitoxin (NEO) were detected. The seven strains from Lake Scharmützelsee produced mainly GTX5 and the seven strains from Lake Melangsee produced mainly NEO (Figure 2.5).

Hundred-ninety *Aphanizomenon* spp., *Anabaena* spp. and *Anabaenopsis* spp. strains tested negative for PSP toxins using LC-MS/MS.

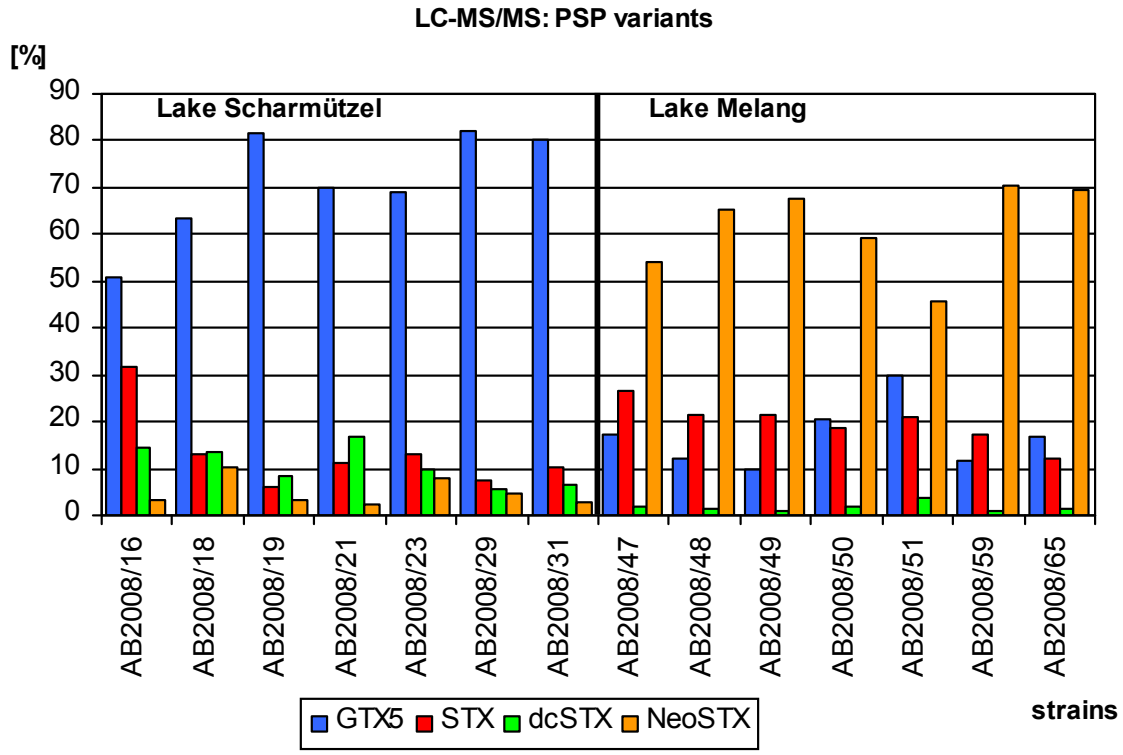


Figure 2.5. Ratio (%) of PSP toxin variants in 14 *Aphanizomenon gracile* strains from lakes Melangsee and Scharmützelsee, as determined with LC-MS/MS.

ATX

In environmental samples taken from several northeast German lakes in 2007 and 2008 the neurotoxin anatoxin-a (ATX) was detected. Accordingly, all 204 *Aphanizomenon*, *Anabaena* and *Anabaenopsis* strains isolated in the NOSTOTOX project were investigated for production of ATX with LC/MS-MS. One cyanobacterial strain, *Aphanizomenon issatschenkoi* strain SP33 isolated from Lake Stolpsee was confirmed to produce ATX. This supports the detection of a PKS gene putatively involved in ATX biosynthesis. The ATX concentration found in the culture of *A. issatschenkoi* strain SP33 was 2354 µg g^{-1}

fresh weight. No sources for ATX production have been identified in German water bodies to date. Hence, *A. issatschenkoi* is the first cyanobacterial species proven as ATX producer in German water bodies. The other 203 nostocalean strains did not produce ATX including seven other *Aphanizomenon issatschenkoi* strains isolated from Lake Stolpsee and Lake Langer See. The morphological determination of ATX producing *A. issatschenkoi* strain SP33 and non-ATX producing *A. issatschenkoi* strains from lakes Langer See and Stolpsee was supported by phylogenetic investigations using PC-IGS and *rbcLX* gene sequencing (Figure 2.3).

2.2 Toxin production and growth of native *Aphanizomenon* strains at changing environmental conditions – Results from culture experiments

Jutta Fastner, Uwe Hammer, Christine Beusch, Karina Preußel

The amount of toxins found in a water body depends on i) the biomass of (potentially) toxin-producing cyanobacteria, ii) the abundance of toxin-producing genotypes and iii) the toxin content of the producing genotypes. Environmental factors influence all these processes in multiple ways, and an understanding of the impact of each factor is helpful for estimating potential concentrations and associated risks.

Here we present data on the growth and toxin production of several *Aphanizomenon* strains (isolated from lakes in Brandenburg, Germany) under different environmental conditions (esp. temperature and light). The toxins produced by these strains are cylindrospermopsin (CYN) and paralytic shellfish poisons (PSP). Unfortunately, isolation of a native ATX producer was successful only at the end of the project, thus toxin production of a native ATX producer could not be investigated as intended.

Materials and methods

The *Aphanizomenon* strains were grown in slightly modified Z8 medium with semi-continuous dilutions every second day in a 12 h/12 h light-dark-cycle and with continuous shaking. The cultures were grown at all combinations of the following temperatures and light intensities: 16, 20, 25 and 30 °C; 10, 20, 60 and 100 µE m^{-2} s^{-1}. All cultures were cultured under the condition tested for at least 2 weeks to ensure that they were adapted, i.e. in steady state, before taking samples for toxin analyses.

Growth rates were calculated from optical density (OD) values of the cultures measured photometrically at 750 nm. As initial culture density for each growth period an OD = 0.1 was set. For estimation of cyanobacterial biovolume of the samples, biovolume versus optical density curves were prepared by microscopic counting and measuring of at least 10 different samples of each strain. The cyanobacterial biovolume of the samples was used to estimate biovolume related toxin concentrations.

Samples for analyses of intra- and extracellular CYN and PSP were analysed as described in detail previously and in this report (Chapter 1.3, Fastner *et al.* 2007, Ballot *et al*. 2010a).

Results and discussion

Influence of temperature and light – cylindrospermopsin

Specific growth rates of the CYN producing *Aphanizomenon* strains ranged from 0.06 to 0.26 d^{-1} under the conditions applied (Figure 2.6). In the range from 10–100 µE m^{-2} s^{-1} and up to 20°C these growth rates are similar to those of other native *Aphanizomenon flos-aquae* and *Aph. gracile* strains investigated in NOSTOTOX (see Chapter 3.3). Mean values of total CYN per biomass amount 0.6 µg mm^{-3} for strain 10E9 and 1 µg mm^{-3} for strain 22D11 (mean of all treatments), with strain 22D11 having a higher share of extracellular CYN than strain 10E9 (mean 42 % and 27 %, respectively).

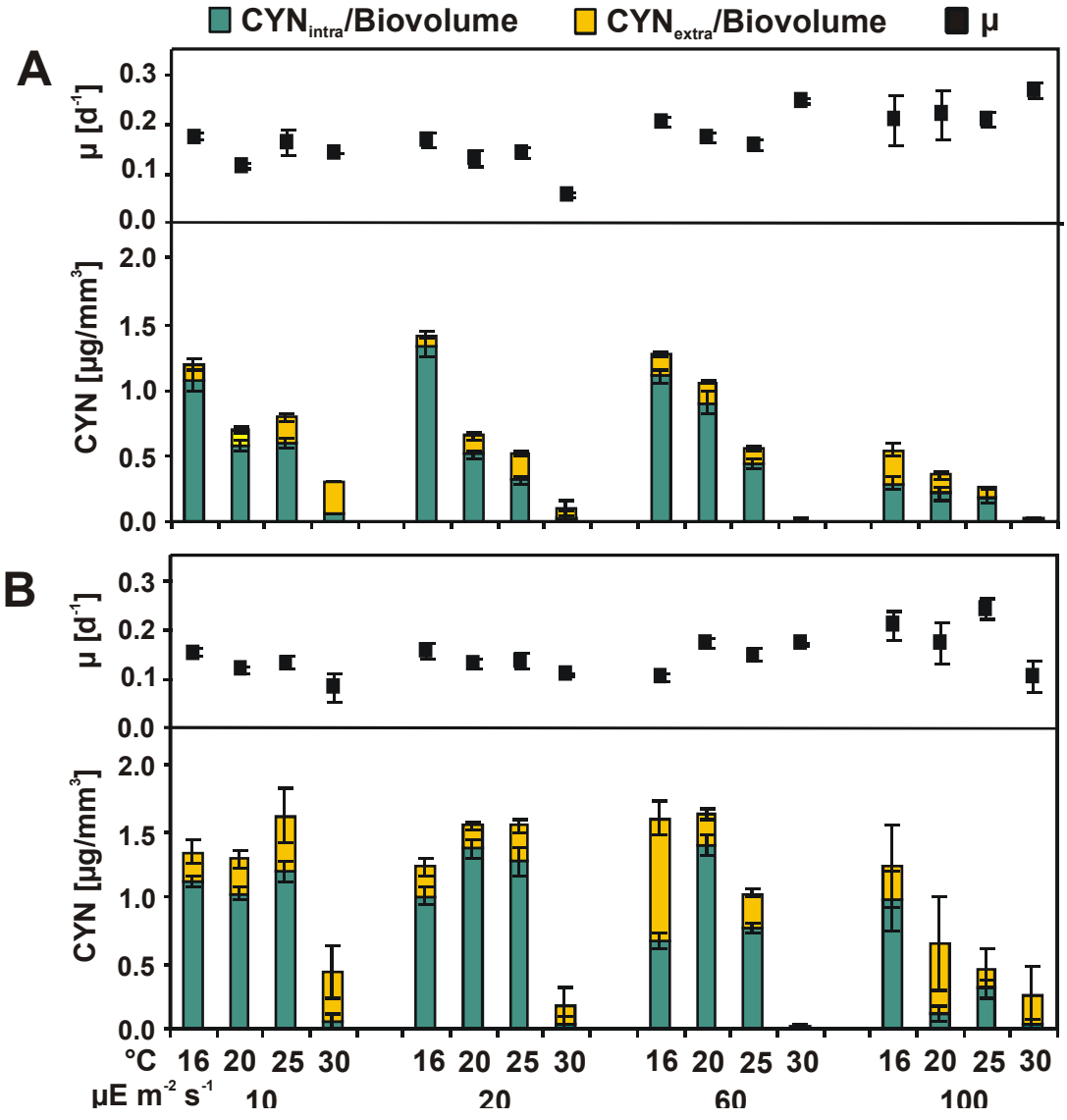


Figure 2.6. Growth rates (µ) and CYN content (µg mm^{-3}) in *Aphanizomenon* 10E9 (A) and 22D11 (B) under different temperature and light conditions. Growth rates, intracellular and extracellular CYN concentration are given as means (n = 3) with standard deviation (From Preußel *et al*. 2009, supplemented).

Similarities of the temperature and light dependent growth patterns between both CYN-producing strains 10E9 and 22D11 (Figure 2.6) include a tendency towards lower growth rates at 30°C at low light (up to 20 µE m^{-2} s^{-1}), while at higher light intensities growth rates tended to increase with temperature, an exception being strain 22D11 at 30°C and 100 µE m^{-2} s^{-1}.

In contrast, the production of CYN showed a more pronounced response to temperature, especially for *Aphanizomenon* 10E9: at all four light intensities tested, the CYN content continuously decreased with increasing temperature down to almost undetectable intracellular concentrations at 30°C (Figure 2.6 A). Low concentrations of dissolved CYN were still measured at 30°C at the lower light intensities, under which growth rates decreased; however, no dissolved CYN was detected at 30°C and higher light intensities under which growth rates increased. This is most probably due to the fact that the dissolved CYN was not completely diluted out during the adaptation time, as the lower growth rates required less dilution. Strain 22D11 showed a roughly similar pattern, although at the lower light intensities a marked change in CYN occurred only at 30°C. Again, the presence of dissolved CYN at 30 °C can be explained with the low growth rates (and thus culture dilution rates) at this temperature (Figure 2.6 B). Our results show the temperature dependence of the CYN-production of native *Aphanizomenon* strains from Germany to be similar to that described for *Cylindrospermopsis raciborskii* from Australia (Saker *et al*. 2000), i.e. with an almost total shut-down at the top end of the temperature range under which the organisms show growth.

The results show that growth and CYN-production is uncoupled, which is in contrast to findings for microcystins where growth and MC-production have been found to be closely related (Long *et al*. 2001, Wiedner *et al*. 2003). Throughout most of the range of growth conditions tested, the variation of the cellular CYN content proved to be in a similar range as found for microcystins (i.e. factor 2-4), with the difference that CYN production appears to be totally shut-down at 30°C.

Influence of temperature and light – paralytic shellfish poisons

Numerous PSP-producing *Aphanizomenon gracile* were isolated in this study (see Chapter 2.1), among which three (AB2008/16, AB2008/23, AB2008/59) were used to study light- and temperature dependent growth and toxin-production.

The total PSP content was similar for strains AB2008/23 and AB2008/59 (mean 0.29 and 0.26 µg mm^{-3}) and somewhat lower for AB2008/16 (0.17 µg mm^{-3}), but overall it attained maximally only half of the CYN content of the other *Aphanizomenon* strains.

All strains produce 4 congeners of PSPs GTX 5, STX, DcSTX and NEO (see Chapter 2.1, Materials and methods), with their ratio being strain-specific (Figure 2.7). Interestingly, the intra- and extracellular PSPs profiles were different from each other, i.e. in all strains the intracellular content of NEO was higher than the extracellular, while for DcSTX the extracellular concentration was higher (Figure 2.7). Possible explanations of these differences in the profiles are selective release mechanisms from the cells or congener specific bacterial or chemical transformation and degradation processes in the

medium (Jones and Negri 1997, Smith *et al*. 2001). The finding of a temperature dependent change in the PSP profile in a Portuguese strain of *Aphanizomenon* by Dias *et al*. (2002) was occasionally also observed in this study (data not shown), however, no uniform pattern of a temperature dependence could be found.

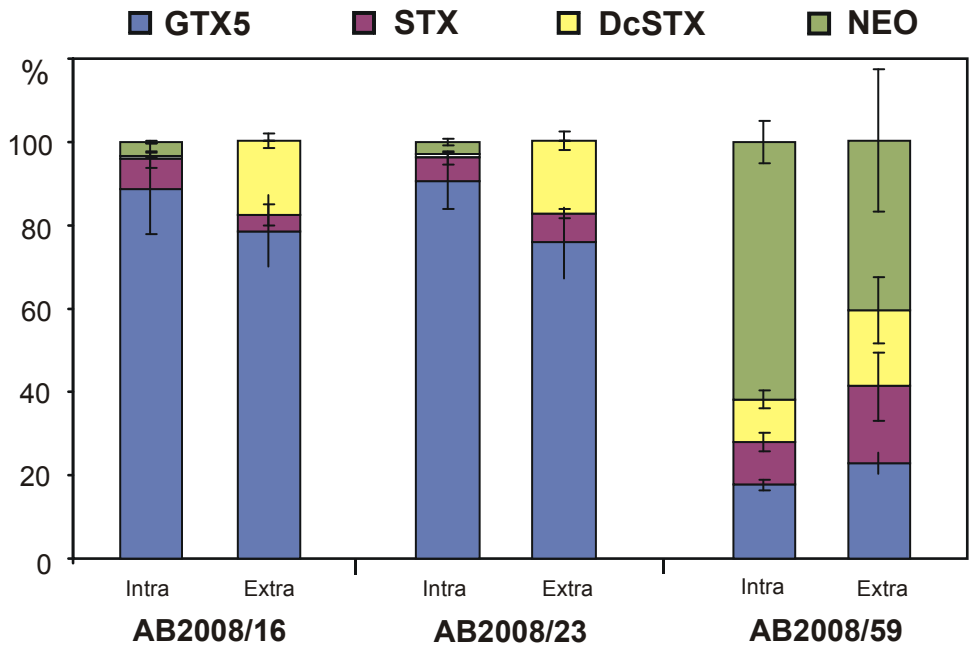


Figure 2.7. Intra- and extracellular relative share of PSP congeners in *Aphanizomenon* strains AB2008/16, AB2008/23 and AB2008/59 at 60 µE and 25°C.

Specific growth rates of the PSP producing *Aphanizomenon gracile* strains ranged from 0.01 to 0.29 d^{-1} and are thus in a similar range as those of the CYN-producing *Aphanizomenon* strains and other non-toxic native *Aphanizomenon* under the conditions applied (Figure 2.8, see Chapter 3.3).

All three strains showed a clear light- and temperature dependent growth (Figure 2.8) with growth rates increasing from 10 to 60 µE m^{-2} s^{-1}. At light intensities ≥ 16 µE m^{-2} s^{-1}, growth rates were highest at 25°C and decreased again at 30°C. Under conditions of low light (10 µE m^{-2} s^{-1}) all three strains failed to grow at high temperature (30°C) and showed reduced growth rates already at 25 °C. Similar results for temperature were found in the NOSTOTOX growth experiments for native *Aph. gracile* and *Aph. flos-aquae*, however, these species showed much higher growth rates at light intensities >100 µE m^{-2} s^{-1} (Chapter 3.3).

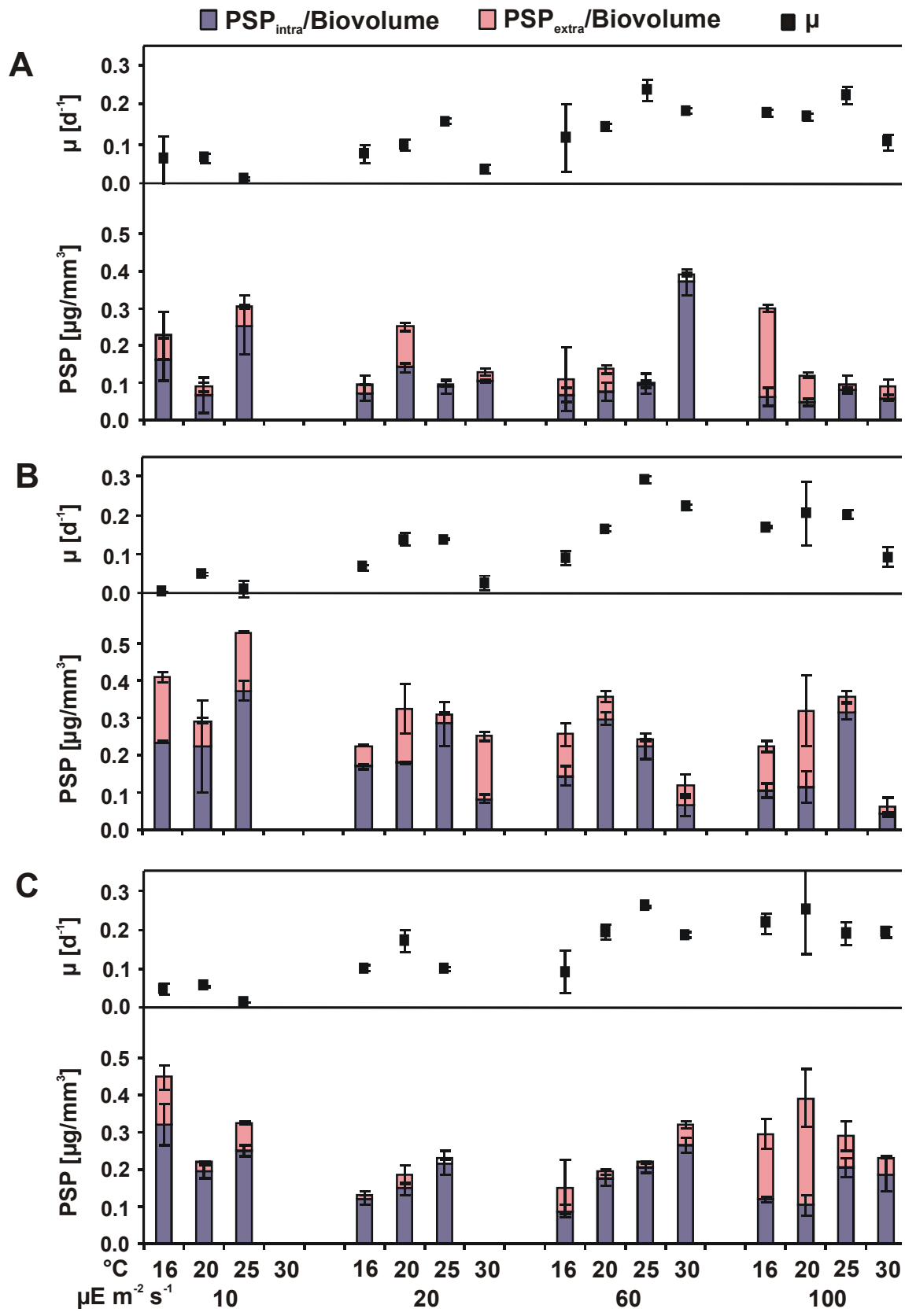


Figure 2.8. Growth rates (μ) and PSP content (sum of all congeners; μg mm^{-3}) in *Aphanizomenon gracile* AB2008/16 (A), AB2008/23 (B) and AB2008/59 (C) under different temperature and light conditions. Growth rates, intracellular and extracellular PSP concentration are given as means (*n* = 3) with standard deviation.

In contrast to these growth rate patterns, responses of PSPs to changes in light and temperature were strain-specific (Figure 2.8). None of the strains showed clear trends in the response of their PSP-content to light intensity. Strain AB2008/16 also showed no consistent PSP-pattern in relation to temperature (Figure 2.8 A). Concerning temperature, the intracellular PSP content of strain AB2008/23 tended to increase between 16–25 °C and drop at 30°C, with extracellular PSP being very variable (Figure 2.8 B). AB2006/59 showed a more pronounced increase of PSP content (largely intracellular) especially at 20 and 60 µE m^{-2} s^{-1} and no decrease at 30°C (Figure 2.8 C).

The differences between strains observed here may in part reflect different optima for toxin production (as also seen above for CYN-production). Some of the scatter in the data for PSP-content may also be attributable to problems in cultivation of *Aphanizomenon*: formation of lumps occurred especially under the more extreme conditions. Furthermore, scatter in the ratio of intra- and extracellular PSPs may be due to differences in release, transformation or degradation processes which themselves or that of the involved bacteria may be temperature or light dependent.

In summary, the variations found for the intracellular PSP content mainly range between a factor 2–3 and are thus in a similar range as found for microcystins and cylindrospermopsin. Similarly to our results Dias *et al*. (2002) found an about two-fold increase in the cellular PSP content from 22°c to 28° C in an Portuguese strain of *Aphanizomenon*.

Influence of nutrients

Experiments with nitrogen and phosphorus are still ongoing at the point of finalising this report with ATX-producing *Anabaena flos-aquae* SAG 30.87, CYN-producing strains *Aphanizomenon* 10E9, 22D11, 30D11, and PSP producing strain AB2008/16 (data not shown). All strains show similar growth rates with either nitrate as N-source or without nitrogen, and for four strains this applied also to the growth with ammonia as N-source. However, two strains (10E9, 30D11) failed to grow with ammonia as N-source. Time-consuming attempts to achieve growth of these strains like e.g. change of media, slow adaptation to ammonia containing media, failed to make these grow. In earlier studies we had already observed the failure of native strains of *C. raciborskii* to grow with ammonia, and other authors have observed the same phenomenon for some cyanobacterial strains (Del Campo and Quesada, pers. communication).

Conclusions

The experiments revealed an influence of temperature on both growth rates and the production of CYN and PSPs in native *Aphanizomenon* strains. In contrast, light influenced primarily the growth rates.

The results from the culture experiments suggest that with an increase in water temperature up to 25°C the production of CYN might decrease while that of PSPs would increase. However, the variations observed are only in a range of factor 2–3 and may be counteracted by other factors such as nutrient availability either promoting or preventing growth. It is more than likely that the genotype composition as well as the biomass can determine the actual toxin concentration to a larger extent than the variations in the toxin production observed.

The only exception is the observed strong decrease (or even cessation) of the production of CYN – and in part also for PSP – at 30°C, however, water temperatures are unlikely to increase that strongly in the future.

3. Life cycle regulation and competiveness of Nostocales

3.1 Life cycle of Nostocales – Results of a field study at Lake Melangsee and germination experiments

Jacqueline Rücker

Introduction

Cyanobacteria of the order Nostocales may produce two types of specialized cells: heterocytes for nitrogen fixation and akinetes that serve as resting stages. Akinete formation enables cyanobacteria to survive adverse conditions such as droughts in tropical and subtropical regions or winters in temperate zones. Akinete formation is assumed to be a prerequisite for the invasion of originally tropical species to temperate climatic zones (Kaplan-Levy *et al.* 2010) where Nostocales have an annual life cycle (Figure 3.1): During summer, vegetative filaments grow and form akinetes in the water column (Figure 3.1 A). After degradation of the vegetative cells (Figure 3.1 B), akinetes (Figure 3.1 C) settle to the lake bottom where they overwinter and germinate (Figure 3.1 D) the following year.

The overall aim of this study was to improve our understanding on the ecology of the invasive Nostocales *Cylindrospermopsis raciborskii* in temperate regions. The long-term studies on phytoplankton dynamics at lakes Langer See and Melangsee (Chapter 1.1, Figures 1.4 and 1.5) revealed that *C. raciborskii* population size vary greatly between years (Wiedner *et al.* 2007). The hypothesis for the intense *field study* in the NOSTOTOX-project was that the size of the inoculum, i.e. the number of *C. raciborskii* akinetes deposited in the sediment, determines the population size in the following year. This was found for *C. raciborskii* populations in Lake Balaton, Hungary (Padisák, 2003), where calculations based on the number of primary (freshly hatched) filaments in the water column gave a rough estimate of the minimum inoculum size. To proof this hypothesis we conducted a field study at Lake Melangsee. This is the first study on the seasonal patterns and morphological features of all life cycle stages of *C. raciborskii* in a natural habitat. We compared *C. raciborskii* to native Nostocales of the genus *Aphanizomenon* in order to answer the following questions:

- When and under which conditions Nostocales form akinetes (resting cells) in the pelagic?
- Is the seasonal dynamic of the pelagic akinete production reflected by a corresponding dynamic of the abundance of akinetes deposited in the sediment surface?
- Is there a loss of akinetes in the course of the winter?
- When and under which conditions do the akinetes germinate?
- Does the number of akinetes in spring (size of inoculum) determine the size of Nostocales population in the following summer?

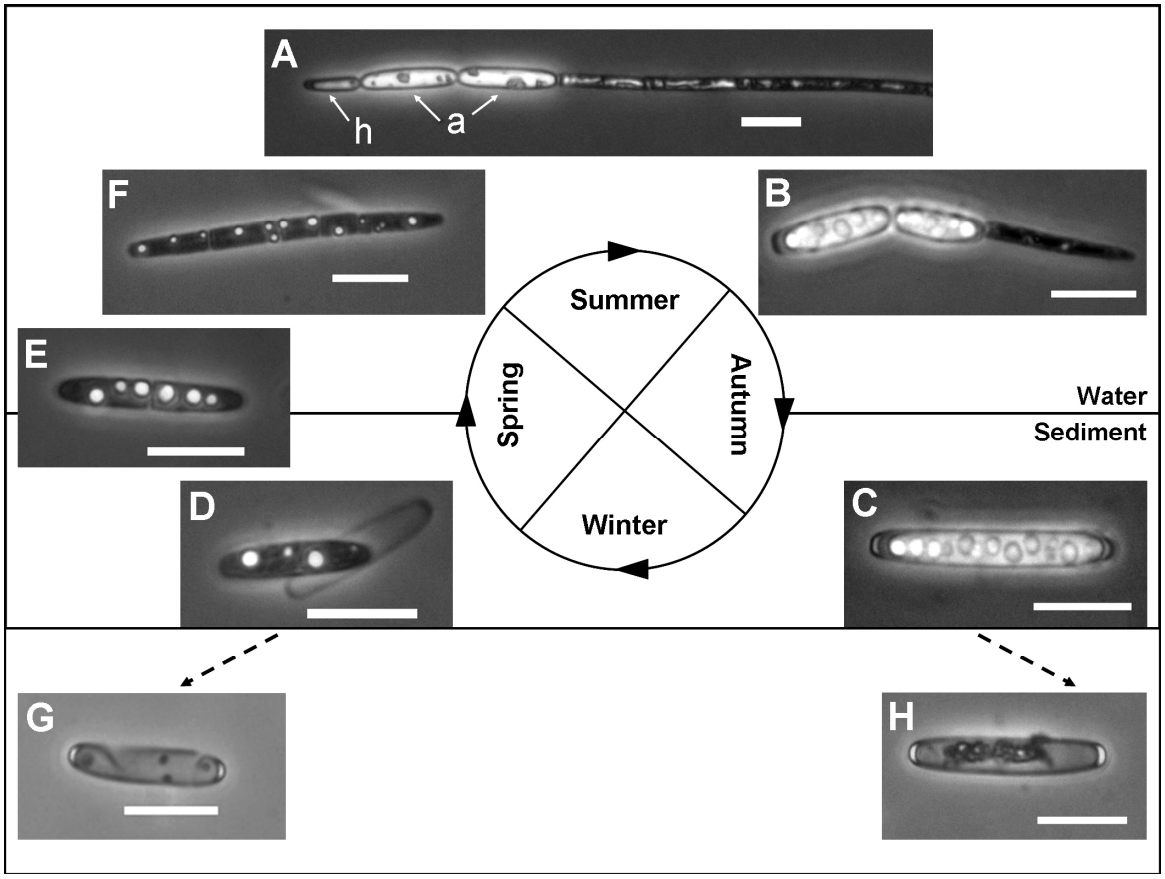


Figure 3.1. Schematic life cycle of Cylindrospermopsis raciborskii in the temperate climatic zone illustrated using phase contrast microscopic images of formaldehyde-fixed net samples (A, F) and sediment samples (B-E, G-H) of Lake Melangsee. **A:** Pelagic filament with two akinetes (a) and one heterocyte (h), **B:** Akinete with rests of vegetative cells attached sinking to the lake bottom, **C:** Free akinete either free-floating in the pelagic zone or deposited in the sediment, **D:** Germination: the germling is hatching out of the akinete shell, **E:** Germling after hatching, **F:** Older germling/young filament without specialized cells, **G:** Empty akinete shell, **H:** Non-intact akinete with irregularly shaped endospore. Scale bars, 10µm (from Rücker *et al.* 2009).

Some aspects of the field study were supplemented by experiments. First, in spring 2007, an *in situ germination experiment* was carried out in Lake Melangsee to proof the hypothesis that the decrease of akinete number in the sediment observed in spring results from germination. For this experiment sediment was incubated with filtrated lake water in transparent beakers during the germination period. Caged in the beakers, the germlings can not drift away after getting buoyant as it is supposed for the open water. This experiment could also give information on the germination conditions in the field.

Secondly *germination experiments* were carried out under defined conditions in the laboratory with natural sediment samples of Lake Melangsee to figure out the temperature and light dependence of germination of *Cylindrospermopsis raciborskii* and *Aphanizomenon* spec. akinetes, and to study the further development of germlings to filaments with specialized cells.

Methods

Field study. A description of the study side, the water sampling, determination of abiotic background parameters and phytoplankton biovolume is given in Chapter 1. The abundance of akinetes in the uppermost sediment layer was studied from 2005–2007 fortnightly (April–Oct.) to monthly (Nov.–March). At each sampling date, three sediment cores of 60 mm diameter were taken using a Kajak-Brinkhurst corer. The uppermost 2 cm of the cores were sliced and mixed. This mixed sediment sample was preserved with formalin (4 % final concentration) for the later enumeration of akinetes. For the enumeration of akinetes, the fixed sediment samples were diluted 1:100 with Lugol's solution (100 mL cold boiled tape water with 6 droplets of Lugol's solution). The diluted samples were filled into a Limnos phytoplankton counting chamber and counted under an inverted microscope at 400fold magnification. Two chambers were prepared for each sample and the mean value was calculated. Two categories of akinetes were distinguished: i) intact akinetes with regularly shaped and coloured endospore (Figure 3.1 C). Usually the endospore contains granules and fills the exospore (akinete shell) almost completely. ii) Non intact akinetes which comprises empty shells (Figure 3.1 G) and akinetes less than half filled by an endospore, mostly not regularly shaped (Figure 3.1 H).

During the ice free period data logger were installed at the routine sampling point which recorded the water temperature continuously in 0.5, 1 and 1.5 m depth. Meteorological data were provided by the Meteorological Observatory of German Weather Service in Lindenberg, which is situated 10.5 km apart from the lake.

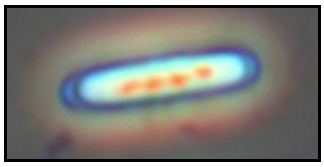
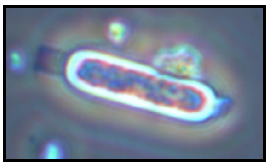
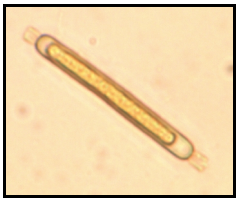
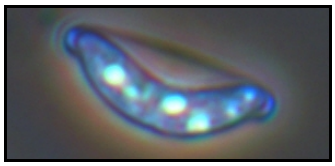
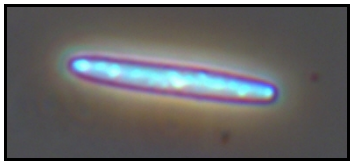
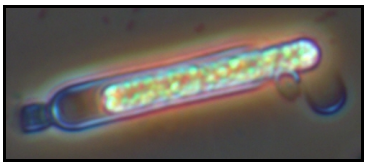
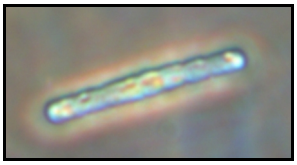
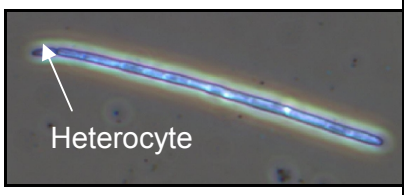
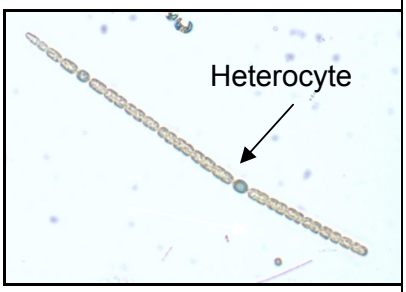
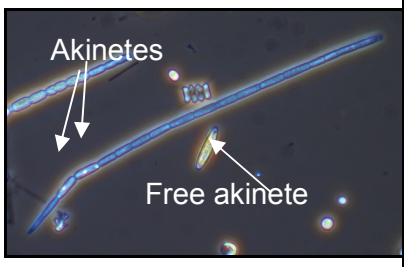
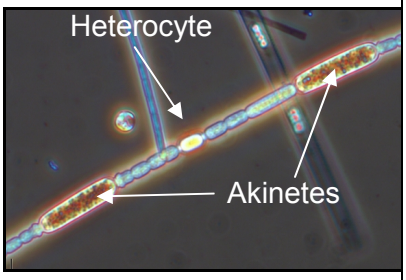
In situ germination experiment. 1.5 g fresh sediment sampled from Lake Melangsee at 10.04.07 was placed in a transparent beaker (volume app. 130 mL) and filled to the top with cold (4 ^{o}C) filtrated (membrane filters, pore size 0,45 µm) lake water. The beaker was covered with a semipermeable membrane (Pall Corporation Supor®200; 0.2 µm) which was fixed with a ring. 15 beakers prepared in this way were incubated in 1.5 m depth in Lake Melangsee. Each 2 – 4 days one beaker was harvested. The content was fixed with Lugol's solution and investigated under an inverse microscope as described for the field study. A description of the different morphological states is given in Table 3.1.

In vitro germination experiments. The sediment sampled during the field study of akinete dynamics in Lake Melangsee was stored in the dark at 4 ^{o}C until the experiments. 7 g of sediment was mixed with 600 mL culture medium (N-free MIII according to Nicklisch, 1992) and the suspension was split up and placed in two Erlenmeyer flasks. The parallels were cultivated over approximately two weeks in the same light intensity of 130, 100, 50, 25, 10, 5 and 0 µmol photons m^{-2} s^{-1}, respectively. Experiments were carried out successively at 13, 15, 17 und 19 ^{o}C with the same sediment material. During the first five days of the experiment a subsample was taken from each flask and fixed with Lugol's solution every day. Later the cultures were sampled every 2–3 days.

Abundance and biovolume of Nostocales were estimated in Hydrobios plankton counting chambers under an inverse microscope as described above. Table 3.1 gives a descrip-

tion of the 4 developmental stages of *Cylindrospermopsis raciborskii* and *Aphanizomenon* spec. which were differentiated according to their morphology.

Table 1. Morphology of different developmental stages of *Cylindrospermopsis raciborskii* and *Aphanizomenon gracile* (Photographs by: S. Mummert, 400fold magnification).

Stage	Feature	*Cylindrospermopsis raciborskii*	*Aphanizomenon gracile*
1	Intact akinetes; germling and shell close together		
2	Growing akinetes; a space is developing between germling and shell.		
3	Akinetes in germination (above) and naked germling (below) in the size of akinetes (up to 30 µm)	 	 
4a	Young filaments (>30 µm) with Heterocytes, but without akinetes		
4b	Filaments (>30 µm) with heterocytes and akinetes		

Results

Field study. The most frequent Nostocales in Lake Melangsee are the originally tropical species *Cylindrospermopsis raciborskii* and *Aphanizomenon gracile* (see Chapter 1.1). The rare species *Aphanizomenon flos-aquae* and *A. issatschenkoi* could not be distinguished unambiguously from *A. gracile* based on akinete morphology. Therefore, all *Aphanizomenon* species were summarized. Akinetes of the genus *Anabaena* were found very rarely in the sediment until 2007.

To sum up Figure 3.2 shows the seasonal development of abiotic parameters, the biovolumes and net change rates of *C. raciborskii* und *Aphanizomenon* spec. in the pelagic as well as seasonal dynamics of its akinetes in the sediment. Filaments of both species were not detected in the pelagic in winter. Thus they overwinter as akinetes in the sediment and the germination is the starting point of the new population in spring. In Table 3.2 the dates when the species were first detected in the year are listed.

C. raciborskii starts to produce akinetes when the water temperatures start to decrease and the population maximum is passed. This connection is obvious in the years 2004 and 2006. In 2005 and 2007 *C. raciborskii* reached only a very small population size. These two years differ from 2006 by lower temperatures during the growth phase (Table 3.2). In contrast to *C raciborskii* the population size of *Aphanizomenon* spec. was almost the same in the years studied. This genus produced akinetes during the whole growing season (In 2004, akinete counting started in June.). The increase of the akinete number in the pelagic is reflected by a growing number of akinetes in the sediment. This process is strikingly for *C. raciborskii* in summer 2006 (Figure 3.2). In the course of autumn and winter a slight decrease of akinete number in the sediment surface was observed.

A pronounced and continuous decrease of the number of akinetes in the sediment was observed in the spring of every year. This period of negative net change rates of sediment akinete numbers is regarded as the germination period. It is marked as the red hatched area in Figure 3.2. The light and temperature conditions as well as the duration of germination periods are summarized for both species in Table 3.2. The results show that the native *Aphanizomenon* species germinate two weeks earlier (beginning of April) than the invaded *C raciborskii* (end of April). The intensity of underwater light is highest of the year during the germination period.

Depending on the pelagic population size the akinete number of *C. raciborskii* varied much more from year to year compared to *Aphanizomenon* spec. But the paradox is that the relatively large population in 2006 arose from a rather small inoculum whereas from rather high akinete numbers in the springs of 2005 and 2007 only small populations developed.

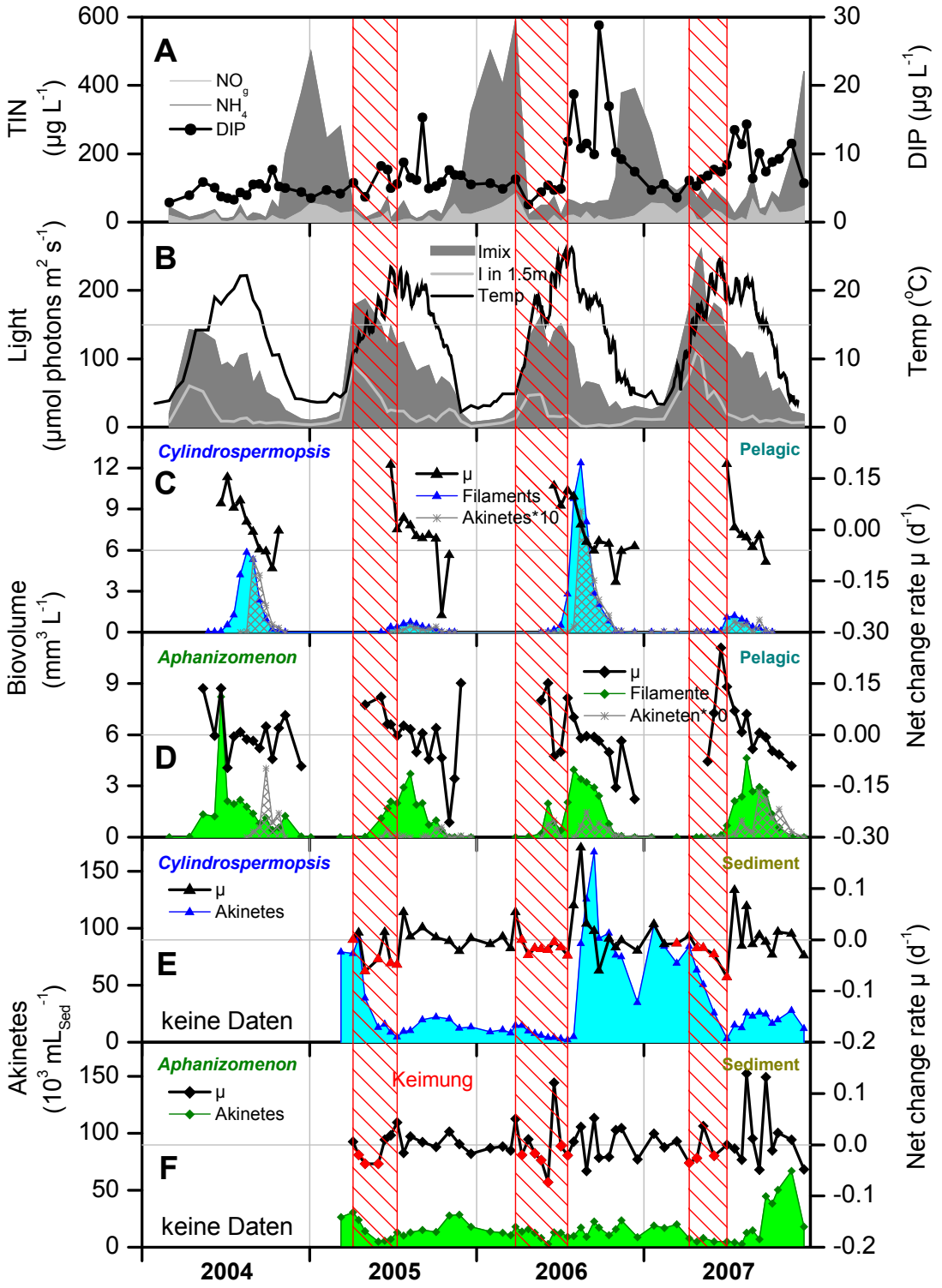


Figure 3.2. Dissolved inorganic nitrogen and phosphorus **(A)**, water temperature and underwater light supply in 1.5 m depth and the mixed layer (I_{mix}) **(B)**, total biovolume and net change rate as well as biovolume of akinetes (times 10) in the pelagic of *Cylindrospermopsis raciborskii* **(C)** and *Aphanizomenon* spec. **(D)**, abundance and net change rate of akinetes in the uppermost 2 cm of the sediment for *C. raciborskii* **(E)** and *Aphanizomenon* spec. **(F)** in Lake Melangsee 2004–2007. The red hatched area highlights the germination periods in spring characterized by negative net change rates of akinete number in the sediment.

Table 3.2. Dates, temperature (Temp.) and light (I) in 1.5 m depth for the germination periods (periods with negative net change rates of akinete numbers in the sediment). Dates, mean water temperature and underwater light supply in the mixed layer (I_{mix}) for the growth periods (period from the first detection of Cylindrospermopsis raciborskii (Cyli) and Aphanizomenon spec. (Apha) in the water column until the population peak) in Lake Melangsee 2005–2007.

	2005		**2006**		**2007**	
	Cyli	**Apha**	**Cyli**	**Apha**	**Cyli**	**Apha**
Germination period						
Start	21.04.05	08.04.05	24.04.06	09.04.06	15.04.07	19.03.07
End	11.06.05	12.06.05	22.07.06	10.06.06	10.07.07	02.05.07
First detection in the pelagic	06.06.05	07.04.05	06.06.06	24.04.06	04.06.07	07.05.07
Temp. in 1.5 m (^oC)						
at beginning and end	13,0 – 16,4	11,1 – 16,1	13,2 – 25,2	8,0 – 16,9	14,7 – 18,7	7,9 – 16,3
(Min ; Max)	(12,8 ; 19,1)	(11,1 ; 19,1)	(13,2 ; 26,0)	(8,0 ; 19,3)	(13,5 ; 24,6)	(5,4 ; 18,1)
I in 1.5 m (µmol phot. m^{-2} d^{-1})						
at beginning and end	50 – 21	73 – 19	43 – 5	24 – 9	73 – 6	47 – 85
(Min ; Max)	(20 ; 58)	(19 ; 75)	(5 ; 65)	(9 ; 65)	(6 ; 92)	(47 – 92)
Growth period						
Population max.	09.08.05	18.07.05	17.08.06	01.08.06	18.07.07	14.08.07
Max. biovolume (mm^3 L^{-1})	0,7	3,8	12,4	4,0	1,2	4,6
Mean temp. (^oC)	20,9	17,8	23,3	21,9	21,5	20,9
(Min ; Max)	(16,3 ; 24,8)	(12,5 ; 24,8)	(15,8 ; 27,1)	(12,6 ; 27,1)	(18,5 ; 24,9)	(15,9 ; 24,9)
I_{mix} (µmol phot. m^{-2} d^{-1})	117	141	112	138	119	128
(Min ; Max)	(79 ; 148)	(107 ; 185)	(48 ;143)	(99 ; 207)	(78 ; 162)	(74 ; 240)

In situ germination experiment. The highest biovolume of *Aphanizomenon* germlings was found in the beaker harvested already after two days of incubation (Figure 3.3). The number of akinetes decreased for *Cylindrospermopsis raciborskii* during the first two days as well but the biovolume of germlings peaked after 7 days, i.e. later than for the other species. After the fifth day of the experiment a strong increase of biovolume was observed for *Aphanizomenon* which reached the highest biovolume of 0.45 mm^3 L^{-1} on May 7th. Contrasting to this species no positive growth was observed for *C. raciborskii* after the 7th sampling day. The reason for this growth stop could be an episodic decrease of the water temperature below 15 ^oC after the 18th of April 2007 (Figure 3.3).

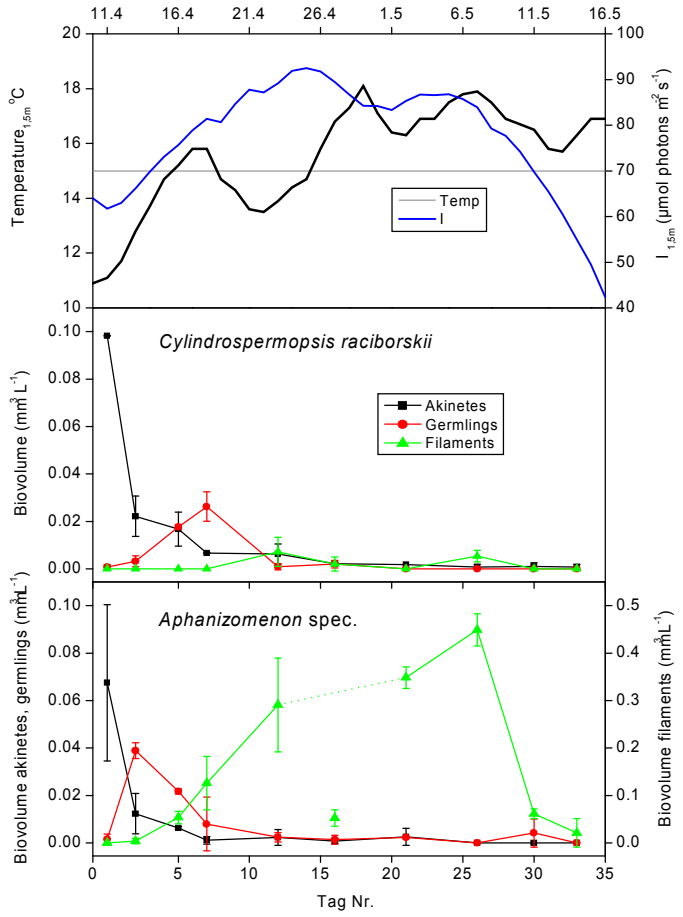


Figure 3.3. Biovolume of akinetes, germlings and filaments of *Aphanizomenon* spec. (below) and *Cylindrospermopsis raciborskii* (middle) of sediment samples incubated in the lake. Course of temperature and light intensity (I) in 1.5 m depth (above) during the in situ experiment carried out from 11th of April to 14th of May 2007 in Lake Melangsee.

<u>*Germination experiments in vitro.*</u> Both species - the native *Aphanizomenon* spec. as well as the invaded *C. raciborskii* - germinated at all temperatures, i.e. down to 13 °C and at all light intensities studied. Even in darkness germination was observed to a low extend. But these results can not be interpreted as germination in darkness since light could not be excluded completely when the experiments were started.

The time span when akinetes germinated and the germlings developed to filaments increased with decreasing temperatures, especially for *C. raciborskii* as it is shown in Figure 3.4 exemplarily for the light intensity of 130 µmol photons m^{-2} s^{-1}. Whereas the number of individuals (one filament or germling corresponds to one individual) stayed constant in the course of the experiment for *Aphanizomenon* spec. losses occurred with *C. raciborskii* at 13 °C (Figure 3.5).

Aphanizomenon spec. showed a positive correlation between light intensity and growth at all temperatures studied and light intensities above 25 µmol photons m^{-2} s^{-1}. In contrast to this, *C. raciborskii* did not exhibit positive growth rates at 13 and 15 °C. At 17 and 19 °C the growth rates were comparable to *Aphanizomenon* spec. but also negatively below 25 µmol photons m^{-2} s^{-1} (Figure 3.6).

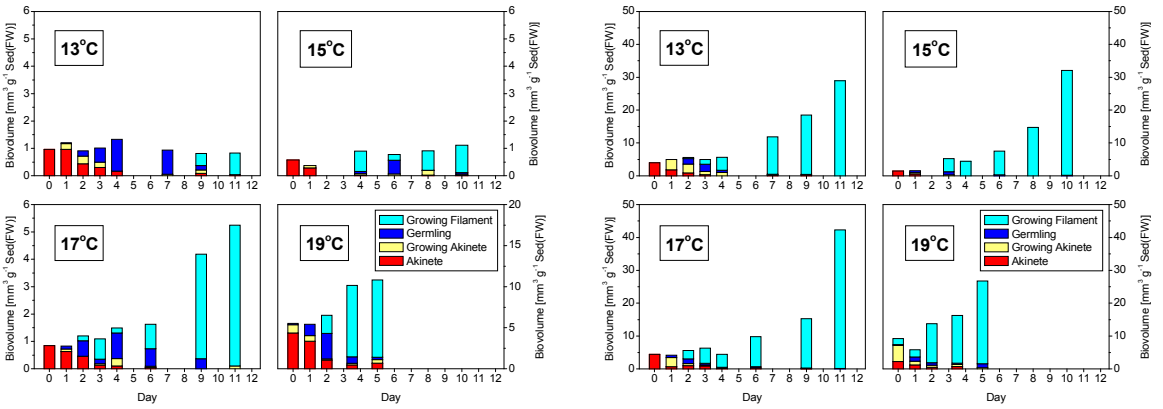


Figure 3.4. Biovolume of akinetes, growing akinetes, germlings and filaments of *Cylindrospermopsis raciborskii* (left) and *Aphanizomenon* spec. (right) originating from sediment of Lake Melangsee grown at 130 µmol photons m^{-2} s^{-1} and different temperatures.

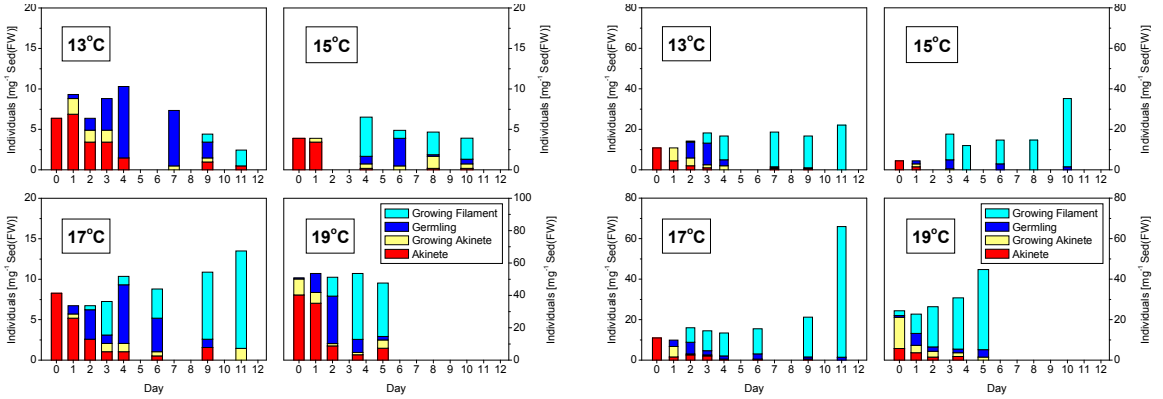


Figure 3.5. Abundance of akinetes, growing akinetes, germlings and filaments of *Cylindrospermopsis raciborskii* (left) and *Aphanizomenon* spec. (right) originating from sediment of Lake Melangsee grown at 130 µmol photons m^{-2} s^{-1} and different temperatures.

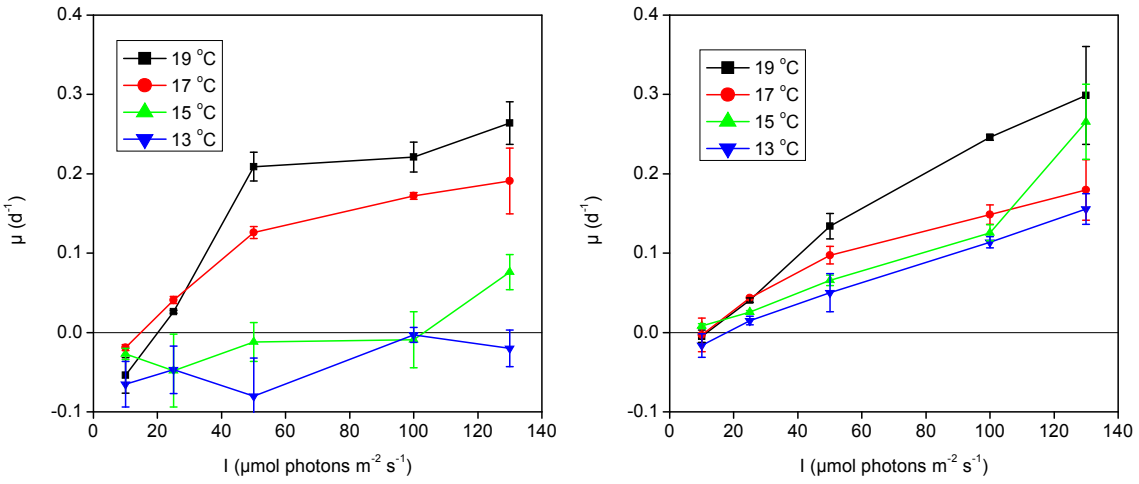


Figure 3.6. Mean growth rates (µ) of *Cylindrospermopsis raciborskii* (left) and *Aphanizomenon* spec. (right) reached in germination experiments at different temperatures in dependence of the light supply (I).

Discussion

The originally tropical cyanobacterium *Cylindrospermopsis raciborskii* germinates below 15 °C. This is the most important and coincident finding of the germination experiments, the long-term study, and the in situ experiment in Lake Melangsee. This germination temperature is lower than so far known from the literature (Wiedner *et al.* 2007). In contrast to the native *Aphanizomenon* species *C. raciborskii* did not show positive growth rates below 15 °C. This observation is consistent with the results of the experiments with semi-continuous cultures (Chapter 3.3). The second coincidence between germination studies and culture experiments is that below 25 µmol photons m^{-2} s^{-1} both species do not grow.

The strong year-to-year variation of the population size of *C. raciborskii* in Lake Melangsee (compare 1.1) can be explained by loss processes. After hatching the naked germlings are assumed to be more susceptible to temperature stress. The year 2006 with the highest ever observed biovolume of *C. raciborskii* differed by a constantly increasing water temperature from the years 2005 and 2007 when temperature dropped episodically below 15 °C, and the year 2006 was characterized by markedly higher water temperatures towards the end of the germination period. The negative influence of low temperature episodes on the growth of young hatched *C. raciborskii* germlings could also be proven by the in situ experiment in spring 2007. Consequently, the native *Aphanizomenon* species are stronger competitors compared to the invaded species of tropical origin under low temperatures.

Based on the long-term study of akinete abundance in the uppermost sediment layer it can be concluded that the growth conditions after germination are more important for the size of the developing population compared to the size of the inoculum (number of akinetes in the sediment at the beginning of germination period). But other impact factors like active and passive recruitment will be discussed in Chapter 3.2 to explain the discrepancy between inoculum size and population size.

3.2 Factors influencing the colonization of the pelagic by Nostocales in spring

Matthias Knie

For a better understanding of Nostocales inoculation by akinetes at the population start three studies/experiments were done concerning the timing of germination and the transfer of dispersal units into the pelagic water.

Dormancy ensures germination in spring

The dormant phase prevents akinetes (like seeds of higher plants) from germinating before winter. The earliest possible germination (end of dormancy) was estimated by monthly incubating fresh sediment from Lake Melangsee for six days (17 °C, 85 µE m^{-2} s^{-1}) from November 2009 until May 2010. It showed that germination success (percentage of filaments on the total amount of propagules (filaments plus akinetes)) increases in December/January and stayed high until May (Figure 3.7). As the dormant phase ended long before spring (Dec/Jan) climatic changes might lead to an earlier start of pelagic population growth.

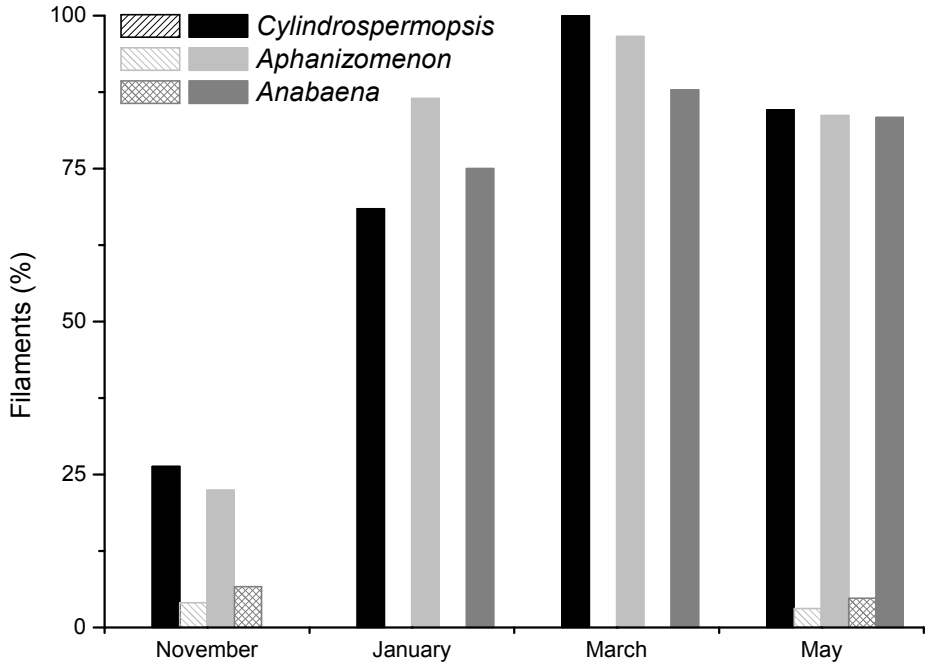


Figure 3.7. Percentage of filaments on the total amount of Nostocales propagules (sum of filaments and akinetes) before (hedged columns) and after (solid columns) an incubation for six days.

Active recruitment of buoyant young filaments

The recruitment (transfer of propagules to the pelagic water) was surveyed in two different approaches. First the active recruitment of germinated filaments by the help of gas vesicles was observed with a recruitment trap (Figure 3.8).

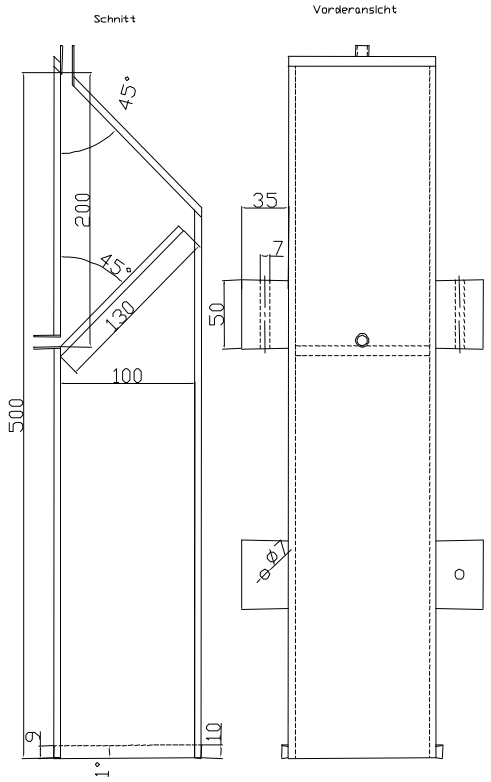


Figure 3.8. Cross section (left) and front side view (right) of the recruitment trap.

The trap was applied weekly for 3 days from April to June 2008 in Lake Melangsee. From the 4th of June on, the collecting vessel was poisoned with formalin (0.5 %) to reduce zooplankton grazing. Three Nostocales genera were observed during this survey (*Cylindrospermopsis*, *Anabaena* and *Aphanizomenon*). The detectable pelagic growth of *Anabaena* and *Aphanizomenon* started on 24.04.2008. The population of *Anabaena* increased over the whole period while the population growth of *Aphanizomenon* stagnated until 09.06.2008. For *Cylindrospermopsis* only a small pelagic population was detected at the end of the study period and no recruitment was observed. Recruitment of *Anabaena* and *Aphanizomenon* could not be detected prior a pelagic population was present (05.05.2008 for *Anabaena* and 19.05.2008 for *Aphanizomenon*) and filaments originated directly from the sediment contributed only for a small part of the population. The recruitment rates increased with increasing temperatures and decreasing light availability.

No pelagic population of Nostocales was observed during winter and recruitment from the akinete bank in the sediment is the only source for new filaments. At the onset of a fast growing population, like for *Anabaena*, a massive recruitment is expected, that

accounts for a large part of the starting biomass. The rise in recruitment rate together with increasing temperature and decreasing light availability support the amplifying role of positive buoyancy in population growth. But due to the late detected initial recruitment it seems unlikely that active recruitment by buoyancy is the main way for inoculation at the start of pelagic colonization.

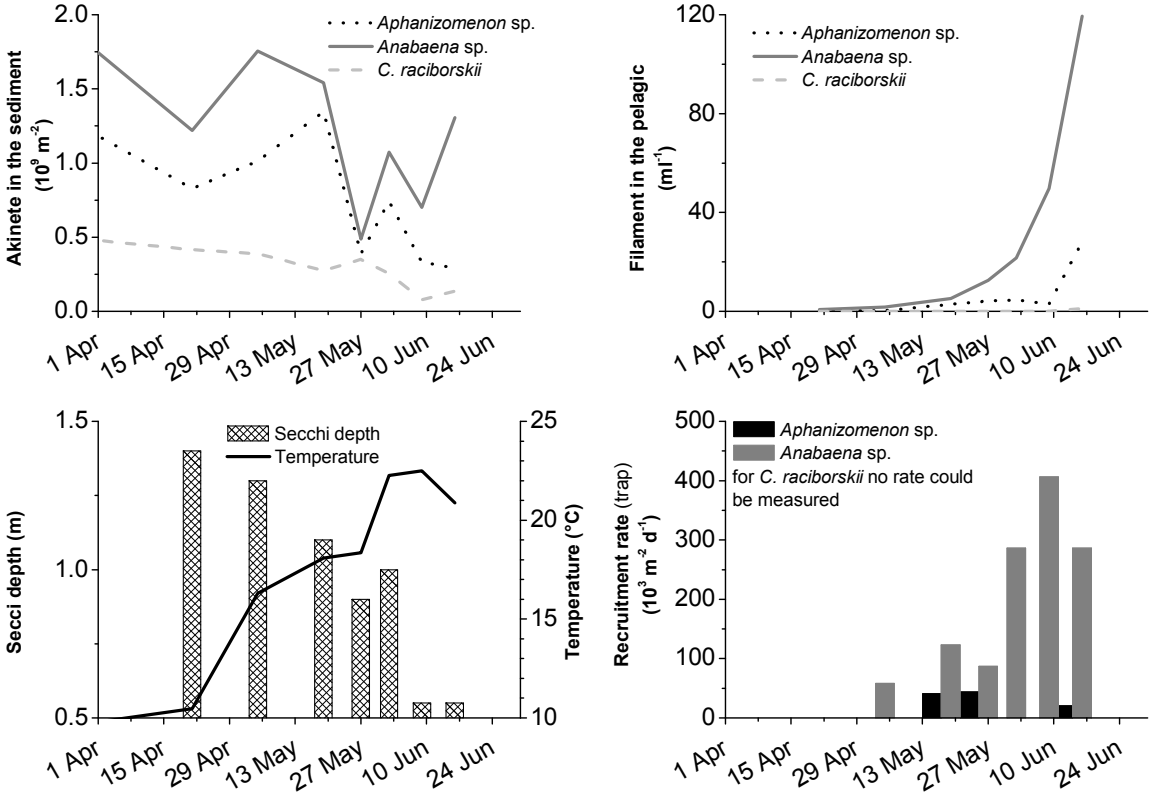


Figure 3.9. Abundance of akinetes in the sediment (A) and filaments of three genera of Nostocales in the pelagic (B). Recruitment rates (D), Secchi depth and water temperature above the sediment (C) in Lake Melangsee 2008.

Passive recruitment by resuspension

The second way to transfer of dispersal units to the pelagic water is the resuspension of sediment, akinetes and germinated filaments. Resuspended akinetes are supposed to germinate in the pelagic water. A special characteristic of Lake Melangsee is an algal mat that grows in winter/spring on the sediment surface. It has its developmental maximum in April and then degrades rapidly until end of May. The mat survives throughout the year as small patches. In the second survey, sediment cores were taken in April and November 2009, transferred to the lab and applied in an erosion chamber (Gust 1990, Figure 3.10).

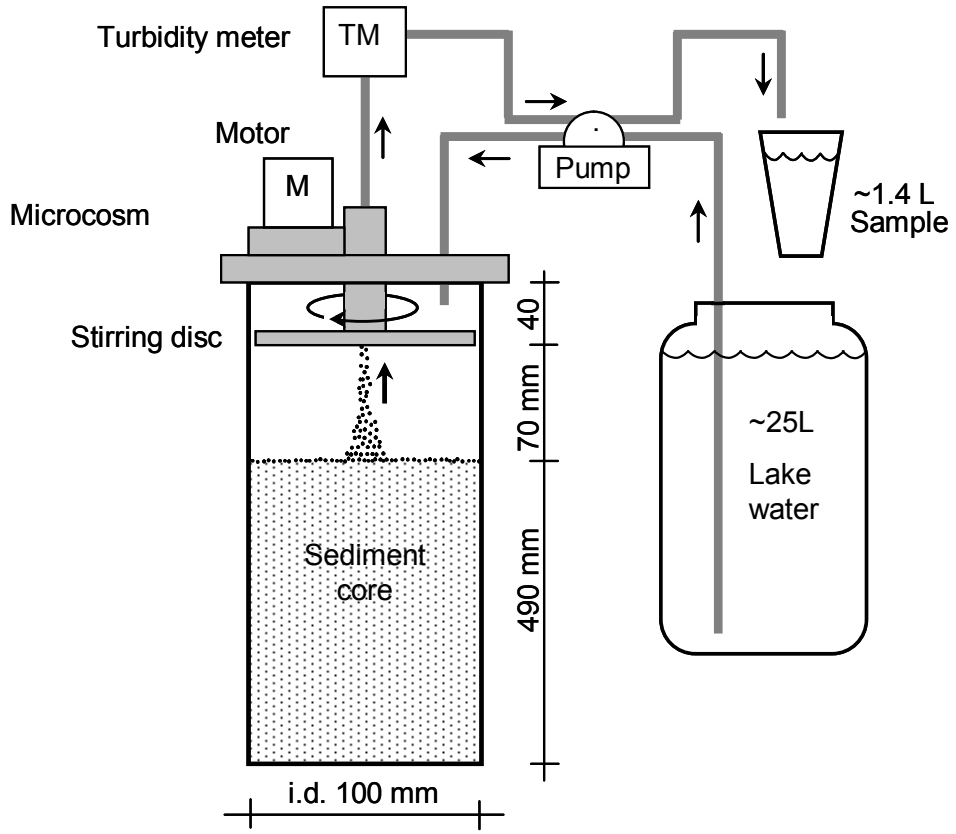


Figure 3.10. Schema of the erosion chamber with an applied sediment core.

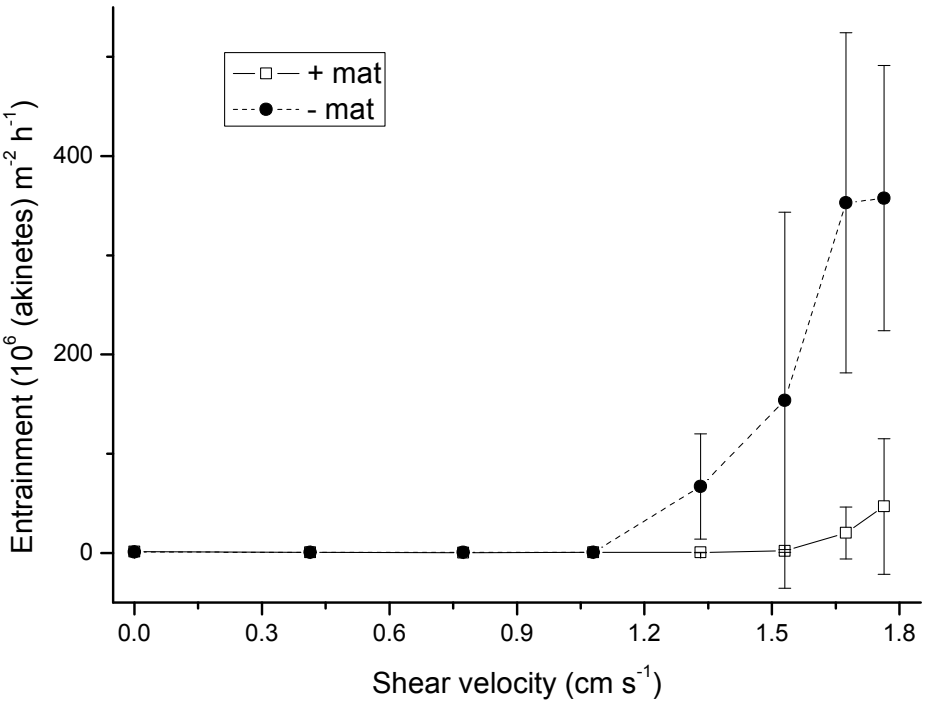


Figure 3.11. Entrainment of akinetes at different shear velocities.

The sediment surface of the April cores was covered with a thick mat of cyanobacteria while no mat was recognized macroscopically in November. In both months resuspension was only observed at high shear velocities. In presence of an algal mat resuspension started later, the critical shear velocity for akinetes was 1.5 cm s^{-1} in April and 1.3 cm s^{-1} in November. The maximum observed entrainment rate of akinetes was ten times higher in November (without algal mat) than in April (Figure 3.11).

Summary

Requirements for the start of pelagic Nostocales population growth are conditions that allow a successful germination of akinetes and the transfer of dispersal units (filaments and akinetes) to the pelagic water. Temperature premise for akinete germination differs slightly between the species. *Cylindrospermopsis* and *Aphanizomenon* for example start germinating at 13 °C. Light is essential for a successful germination in nearly all species. Although young filaments are able to swim up due to the buoyancy of their gas vesicles, resuspension provides larger amounts of dispersal units, because even buried akinetes were transferred to the pelagic water where light and temperature conditions are better. The conditions at the sediment surface can play a big role in the timing of Nostocales inoculation due to microphytobenthos covering and consolidating the sediment surface or bioturbation by macrozoobenthos and fish increasing the flux of dispersal units.

3.3 Competiveness of native and invaded Nostocales under various light and temperature conditions

Grit Mehnert

Aim

We investigated the light- and temperature-dependent growth of three invasive, three native and one potentially invasive cyanobacterial species to evaluate which species is a stronger competitor and to predict their further development in temperate freshwaters in the course of global warming.

Methods

For the study we have chosen the invasive species *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*, the native species *Aphanizomenon gracile*, *Aphanizomenon flos-aquae* and *Anabaena macrospora* and the potentially invasive species *Aphanizomenon ovalisporum* (Table 1). To consider strain-specific differences one to four strains per species were studied. Strains were isolated from different type of lakes (Table 1).

Strains were grown in 250 ml Erlenmeyer flasks containing 100 ml of Z8 medium (Kotai 1972) at a photoperiod of 12:12 h light:dark. The temperature-dependent growth was determined for one strain of each species (Table 1) from 10 to 35°C with an interval of 5°C at a light intensity of 80 µmol photons m^{-2} s^{-1}. The light-dependent growth was determined for all strains for at least six light intensities from 17 to 557 µmol photons m^{-2} s^{-1} at 15 and 20°C. To consider intraspecific variance within populations several strains of each species were used.

To determine the specific growth rate µ the cultures were grown in a semicontinuously according to the turbidostat principle, i.e. the cultures were diluted every second day to an optical density (OD) of 0.1. The OD was determined at 750 nm with a spectrophotometer. As the biomass is correlated to the OD (Kohl and Nicklisch 1988), the specific growth rate µ was calculated from the variation of the OD over a period of time (*t*):

$$\mu = \ln\left(\frac{X_t}{X_0}\right) \cdot \frac{1}{\Delta t}$$

X_t – biomass after cultivation time t, X_0 – biomass after dilution, Δt – time interval between two dilutions.

After a time of adaptation (transient state) the growth is in balance (steady state), i.e. the biomass increase is equal to the biomass loss after dilution. The mean specific growth

rate for each light intensity and temperature is calculated from the mean value of at least three specific growth rates per steady state.

Table 3.3. Studied strains: their origin (Poly = polymictic; Di = dimictic) and the experiments in which they were used (T = temperature-dependent growth, L = light-dependent growth).

Species	Strain	Lake of origin	Mean lake depth [m]	Mixis type	Exp. performed
Invasive species					
C. raciborskii	19F6	Melangsee	1.7	Poly	L
C. raciborskii	ZIE11CR	Zierker See	1.6	Poly	L
C. raciborskii	24G7	Werbellinsee	3.5	Poly	L, T
C. raciborskii	26D9	Rangsdorfer See	1.5	Poly	L
A. bergii	LIE02AB	Lieps	2.3	Poly	L, T
A. bergii	ZIE26AB	Zierker See	1.6	Poly	L
A. aphanizomenoides	22C4-9	Heiliger See	6.6	Di/ Poly	L, T
A. aphanizomenoides	22F6-1	Heiliger See	6.6	Di/ Poly	L
Native species					
A. gracile	ZIE23AFA	Zierker See	1.6	Poly	L
A. gracile	30D11	Petersdorfer See	1.9	Poly	L
A. gracile	16D11	Scharmützelsee, Northern Bay	7.9	Di/ Poly	L, T
A. flos-aquae	ST115AF	Stechlin	22.8	Di	L, T
A. flos-aquae	ST86AF	Stechlin	22.8	Di	L
A. macrospora	ST195AS	Stechlin	22.8	Di	L, T
Potentially invasive species					
A. ovalisporum	UAM290	Juan Carlos First Park, Spain	1.5	Poly	T

Results and discussion

Light-dependent growth

At 15°C the highest growth rates were achieved by the native species *A. gracile* (Figure 3.12 A, B). The invasive species had significantly lower growth rates than *A. gracile*, but they were equivalent to those of the native species *A. flos-aquae* and *A. macrospora*. At 20°C *A. flos-aquae* and *A. macrospora* had significantly lower maximum growth rates than the other species, which differed insignificantly in their maximum growth rates (Figure 3.12 C, D).

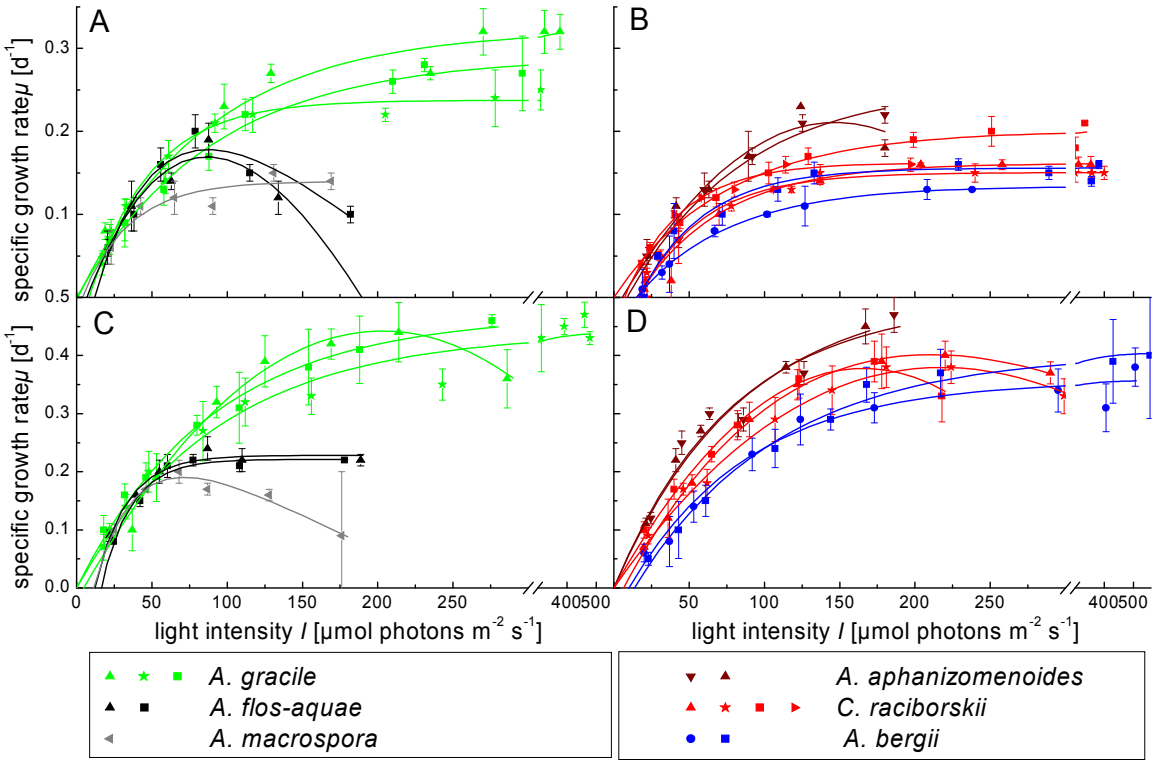


Figure 3.12. Light-dependent growth rates and growth curves of three native species (A, C) and three invasive species (B, D) at 15°C (A, B) and 20°C (C, D). Error bars represent standard deviation of the mean growth rate. Each symbol represents one strain.

At both temperatures the growth curves of *A. flos-aquae* and *A. macrospora*, the two species from the deep stratified lake, had significantly higher initial slopes and lower light saturation values than the species from the shallow lakes (*A. gracile* and the invasive species), while the latter species did not differ from each other in these two parameters (Figure 3.12).

Our results showed that the invasive Nostocale species were not superior competitors for light. Moreover, the two species from the oligotrophic stratified lake were more stenoecious in regard to light compared to the species from the eutrophic shallow lakes.

Since increasing evidence exists that cyanobacterial populations are composed of different ecotypes, we included several strains per species. Our results showed that strain-specific differences exist, e.g. growth of *A. gracile* at high light intensities (Figure 3.12 A, C). However, differences in growth between species were more pronounced than between strains.

Temperature-dependent growth

The invasive and native species differed significantly in their growth at low and high temperatures (Figure 3.13). At 10°C the native species grew with rates of 0.05 to 0.15 day^{-1}, while the invasive species were not able to grow. At 35°C it was vice versa: the invasive species grew with rates of 0.09 to 0.32 day^{-1}, while native species did not grow.

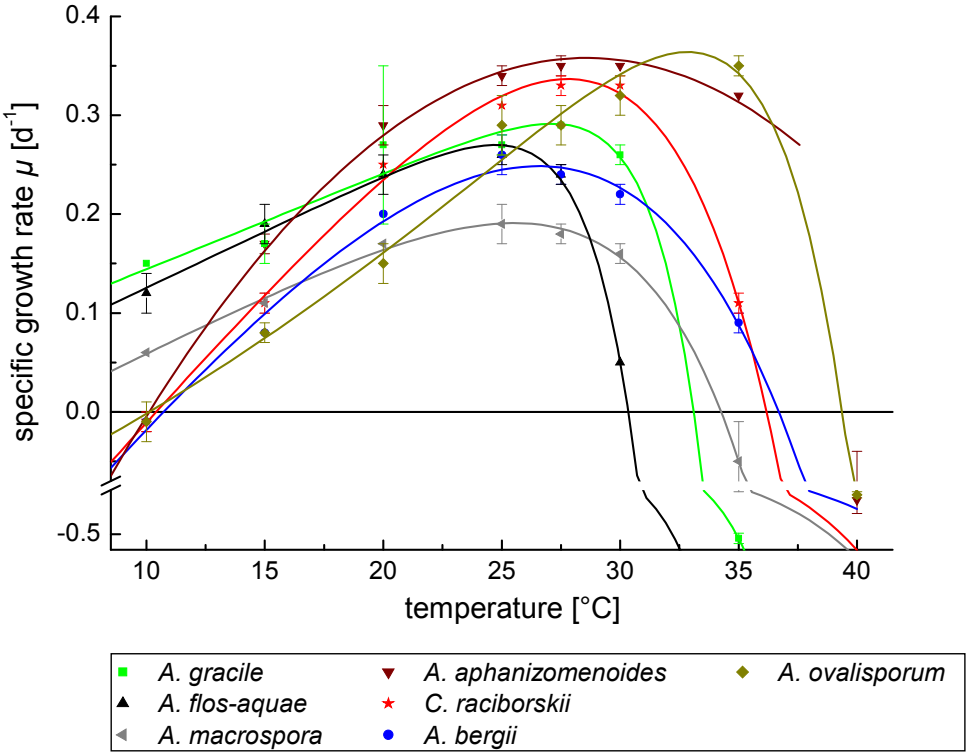


Figure 3.13. Temperature-dependent growth rates and growth curves at 80 µmol photons m^{-2} s^{-1}. The growth rate of *A. aphanizomenoides* at 40°C was not included in curve fitting. Error bars represent standard deviation of the mean growth rate. Each symbol represents one strain.

We considered the native species *A. gracile* and the invasive species, which occur often together in shallow lakes (Stüken *et al.* 2006), as direct competitors. *Aphanizomenon gracile* grew with higher growth rates than *A. aphanizomenoides* until 17°C, with higher growth rates than *C. raciborskii* until 20°C and with higher growth rates than *A. bergii* until 31°C. These results suggest that *A. gracile* can begin to build up its population earlier in the season. Indeed, we know from field observation that *A. gracile* occurred in the pelagial from on water temperatures of 13°C, while *C. raciborskii* occurred from on 15 to17 °C (Rücker *et al.* 2009, Wiedner *et al.* 2007). If temperatures rise above 20°C *A. gracile* could be outcompeted by *C. raciborskii*. The potentially invasive species *A. ovalisporum* possessed the lowest growth rate below 20°C. This might be the reason, why *A. ovalisporum* is not present in German freshwaters yet.

Furthermore, our results showed that native and invasive species did not differ significantly in their optimum growth temperature (Figure 3.13). The optimum growth temperatures of all species were higher than the mean water temperature during their growth season. We conclude that the cyanobacterial species tolerate the low temperatures of their habitat rather than to be adapted to them.

Overall, our results demonstrate that an increase in water temperature above 20°C in summer as well as an earlier warming of lakes in spring will favour the development of the nostocale cyanobacteria.

Simulation of seasonal dynamics

We developed a simple mathematical model, which is based on the temperature-dependent growth data (determined in the culture experiments) to simulate the seasonal population dynamics of a native species (*A. gracile*), an invasive species (*C. raciborskii*) and a potentially invasive species (*A. ovalisporum*) (Figure 3.14). Simulation was performed for three temperature scenarios: a historic scenario (-2°C), a presence scenario and a future scenario (+4°C). The simulation showed a shift in the dominated species with increasing temperature (Figure 3.14). While at the historic scenario *A. gracile* was the dominating species, *C. raciborskii* reached nearly equal biovolume at current temperatures and became dominant at the future scenario. *Aphanizomenon ovalisporum* was virtually absent in the historic scenario and rarely detectable at current temperatures, but it strongly increased its biovolume in the future scenario. In the future scenario the vegetation time of all three species increased.

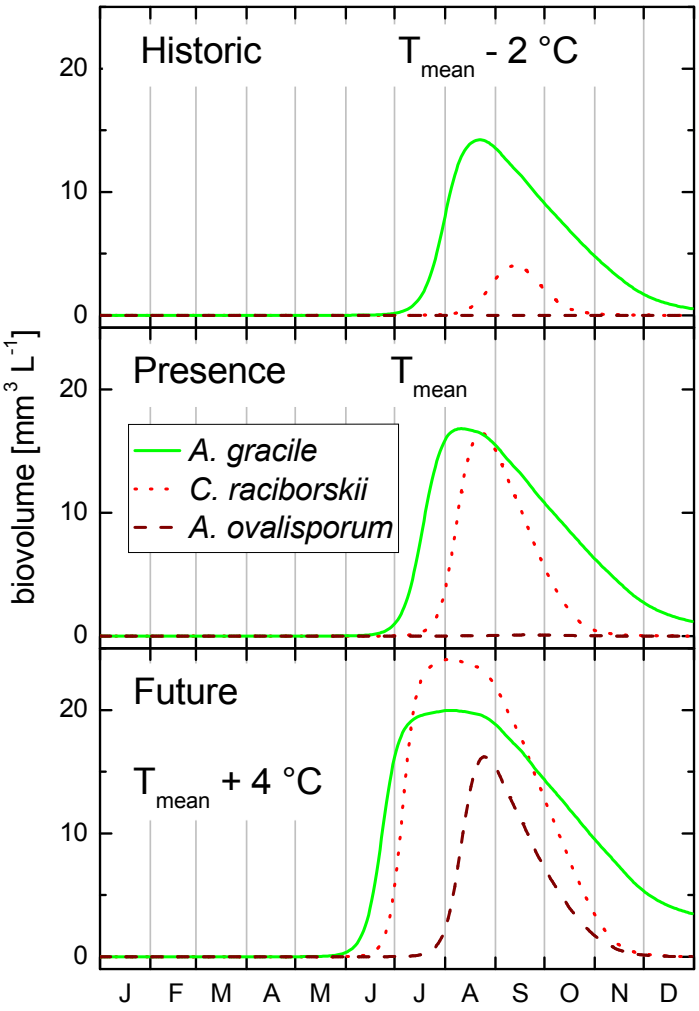


Figure 3.14. Simulated population dynamics. Upper panel: Historical scenario with seasonal water temperature 2°C below current mean values. Middle panel: Presence scenario using seasonal mean water temperatures. Lower panel: Future scenario with water temperature 4°C above current temperatures. Figure was modified after Mehnert *et al.* 2010.

The introduction of *A. ovalisporum* could massively enhance CYN concentration in temperate lakes, as all isolated *A. ovalisporum* strains were CYN producers (Banker *et al.* 1997; Quesada *et al.* 2006).

Conclusion

Based on our results we state that an increase in water temperatures during the last decades favoured the colonization of tropical cyanobacteria in temperate freshwaters. A further temperature increase of 4°C (IPCC 2007, scenario A1F1) would enhance the biomass of all investigated Nostocale species, would promote the development of *C. raciborskii* and *A. aphanizomenoides* in particular and would enable the establishment of new cyanobacterial species.

4. A mathematical model for process analysis, simulation and prediction of the occurrence of Nostocales

Klaus Jöhnk

Introduction

The purpose of this study was the creation of a mathematical model to simulate Nostocales population dynamics throughout their entire life cycle. Nostocales species modelled here have a very special strategy to survive harsh winter conditions. By forming resting stages, called akinetes, which sink to the bottom, they are able to overcome annual cold periods or they may even last for several years in the sediments before germination. The model inclusion of such an overwintering strategy avoids a reset of late winter/spring starting conditions for populations as is needed in most mathematical models tackling real environmental conditions.

This modelling tool can be used to analyze past, present and future population dynamics of different Nostocales species under changing climate and environmental conditions. Other models for the simulation of Nostocales life cycle so far are based on internal nutrient cell quota (Hense and Beckmann 2006; Hellweger *et al.* 2008). These models are difficult to calibrate to field data because nutrient cell quota can be determined for culture strains but not for field populations. Here we go for a more simple approach, taking into account nutrient limitation via a proxy variable. Nutrient storage of Nostocales is not accounted for in this model approach. It is thus limited to eutrophic lakes of temperate regions. Lake flushing is accounted for in loss rates for pelagic population and akinete population size. Again, grazing losses are kept constant around the year, unless there was evidence of a clear water phase. In those cases additional grazing was applied. The model is based on data regarding growth regulation, akinete production, survival and germination of Nostocales species obtained from laboratory studies (compare Chapter 3.3). Population dynamics is calibrated and validated with data from a long-term (13-year) field study of Nostocales population dynamics in a shallow eutrophic lake of the Northern temperate zone (Lake Melangsee in Germany, see Chapters 1.1 and 3.1). We selected *C. raciborskii* as model organism for which extensive data was available. To test the generality of the model other Nostocales species were simulated.

Sensitivity studies are mandatory to test how the model reacts on small changes in its parameter settings. We used a novel technique called the Hasse diagram technique (e.g. Brüggemann and Carlsen (2006). The basic principles are described in Brüggemann *et al.* (2001). The use of Hasse diagrams allowed us to rank the influence of the selected parameters on the simulation results.

To predict the future development of different Nostocales species under varying climate and environmental conditions, we ran different scenarios for real environmental data as well as generalised, mean-year-scenarios. Depending on the magnitude of supposed

temperature changes different species will cope better or worse with temperature increase or decrease. It is a matter of fact that population dynamics are not solely driven by climate change but also by changes in nutrient availability. The model is also used to assess those effects. Another interesting effect often overlooked is the variability of environmental conditions (other than an annual cycle) and the effects of events. These could be cold spells, heat waves, or as exemplified here, sporadic occurrence of clear water phases (or equivalent flushing events) which highly increase population loss during a short time period.

A detailed description of this life-cycle model and first simulation results can be found in a paper published in Environmental Modelling & Software (Jöhnk *et al.* 2011). Here, we only briefly describe simulation techniques and the mathematics behind our sensitivity study. In the following we will firstly describe the model basics and the sensitivity analysis (Material and Methods). Thereafter we will focus on simulation results for climate effects, nutrient effects, and a short introduction on timescales of invasion, or development times calculated with this model.

Material and Methods

Data

Simulations were driven by time-series data on mean water temperature, T, light supply in the mixed water column, I_{mix}, and Secchi depth, Z_{Secchi}. The calibration and validation procedure for the simulation model was based on data collected at Lake Melangsee, a small, shallow eutrophic lake situated in northeast Germany (mean depth 1.6 m, maximum depth 2.4 m, area 0.17 km^2. Data were collected over a period starting from 1995 until now. Here we used highly resolved counts for akinetes in the sediment and biovolume of Nostocales species in the pelagic. A more detailed description of the data is given in Rücker *et al.* (2009).

Model

The model is built for 2 dependent variables, pelagic population, C, expressed as biovolume per litre, and numbers of akinetes, A, in the sediments. The latter expressed as akinetes per volume of sediment.

The dynamics of the pelagic population of Nostocales was described by two ordinary differential equations for the species biovolume

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \mu_C(T, I_{\mathrm{mix}}, N)C - \left[m_C + m_{\mathrm{dens}}C + m_{\mathrm{cwp}}(Z_{\mathrm{Secchi}}, T)\right]C + \alpha_C\,\mathrm{germ}(I_{\mathrm{mix}}, T)A - \mathrm{form}(T)C \tag{1}$$

and the akinetes

$$\frac{\mathrm{d}A}{\mathrm{d}t} = -m_{\mathrm{A}}A + \alpha_A\mathrm{form}(T)C - \mathrm{germ}(I_{\mathrm{mix}}, T)A\,. \tag{2}$$

Here, the species biovolume (C, in mm^3 L^{-1}) changes due to vegetative growth

$$\mu_C(T, I_{\text{mix}}, N) = \mu_C^{\max} \, \text{rts}(T) \, \text{rls}(I_{\text{mix}}) \, \text{fds}(N) \tag{3}$$

depending on temperature (T), light (I_{mix}), and nutrients (N) split into separate limiting functions for temperature (rts), light (rls), and nutrients (fds). A detailed explanation of the processes can be found in (Jöhnk *et al.* 2011).

The equation for C (Eq 1) describes population development due to temperature, light, and nutrient dependent growth, loss terms consisting of a mortality rate, m_C, a density dependent loss rate, $m_{dens}C$, and an event driven loss term, m_{cwp}, depending on specific constellations described by Secchi depth, Z_{Secchi}, and water temperature. Akinete germination, α_C germ, will contribute positively to the pelagic population and akinete formation, form, depending on a temperature threshold in the lakes cooling phase, acts as loss term.

Equation 2 for akinetes is missing a growth term and includes only a simple loss rate, m_A. Formation of akinetes enters as positive rate and germination is a loss. α_C and α_A are conversion factors between biovolume and akinetes.

Since nutrients might not be well known in a lake we use a better known (or more often measured) proxy variable, the Secchi depth, to describe it. During times of high Secchi depth bioavailable phosphorus is plenty available, while during the growing season increasing total phosphorus means higher turbidity, decreasing fraction of bioavailable phosphorus, and shallower Secchi depth. The functional form between this proxy and nutrient limitation has to be chosen as some empirical function using as many parameters as necessary to describe data in the calibration process and is described here as,

$$\text{fds}(N) = \frac{N}{N + h}. \tag{4}$$

Expressed as a function of Secchi depth assuming a one to one relation between Secchi depth and bioavailable phosphorus and introducing an exponent as modifier, we arrive at the equation describing nutrient limitation in our model

$$\text{fds}(N) = \frac{1}{\left(1 + H / Z_{\text{Secchi}}\right)^n}. \tag{5}$$

Here H is the half-saturation point for nutrient limitation, and n an exponent.

The event base loss term in (Eq 1) is based on the fact that there might be additional grazing in spring (or flushing) due to a clear water phase. In shallow lakes the phenomenon of a clear water phase due to zooplankton grazing is not very common, but does occur sporadically in Melangsee and other lakes of the region (Deneke and Nixdorf 1999). We implemented this sporadic loss effect by parameterizing a temperature dependent period where enhanced zooplankton grazing takes place, thus introducing additional parameters: additional grazing rate, Secchi depth and temperature range of the occurrence of a clear water phase.

Only by introducing such an additional loss factor we were able to reproduce the measured data of the pelagic population of *C. raciborskii* and their akinetes in the sediments.

Solution techniques

The coupled set of ordinary differential equations was originally programmed using Matlab. For large calibration projects this is not suitable due to its slow performance. Thus, an enhanced version (CyliMod V2.x) was written using the high level language C++, running on the command line. This allowed for a speed-up of up to 10000 compared to the Matlab version. To use the model a C++ compiler is necessary to recompile for any changes made to parameters or scenario set-up.

The system at hand is non-stiff and thus can be solved with a multitude of one-step methods. Here I decided to use a matrix-exponential method (e.g. Jöhnk 1990), which is more accurate than an explicit Euler method and much faster than a step size controlled Runge-Kutta-Method. Tests with different truncation levels were performed for the selection of an optimal value with respect to CPU time and accuracy.

Sensitivity study

To test for parameter sensitivity we used the Hasse diagram technique, which ranks parameter based on a partial order mechanism. The method is explained in detail in Brüggemann and Carlsen (2006) and introduced for parameter sensitivity in ecological models in Jöhnk *et al.* (2011).

Results

In the following a cross section of model simulations is given. Firstly model calibration and sensitivity study are described using our model organism *C. raciborskii*. After that simulations for a second species, *A. gracile* are shown. The model was originally directed to solve the question, "*when did C. raciborskii invaded our lakes*". This is not easy to answer since there are combined effects of changes in water temperature as well as nutrient availability. To get an impression how these two forcing factors act, we show simulations for climate warming/cooling and changes in nutrients. To better answer the invasion question we simulated the development of single akinetes in a lake, using daily mean fields for temperature and Secchi depth, i.e. driving factors were mean annual cycles iterated over a period of 128 years. Finally an interesting question is, how long will it take that a small initial akinete seed will grow into a larger population exceeding some threshold value. As a basic test simulations for different climate/nutrient scenarios were run.

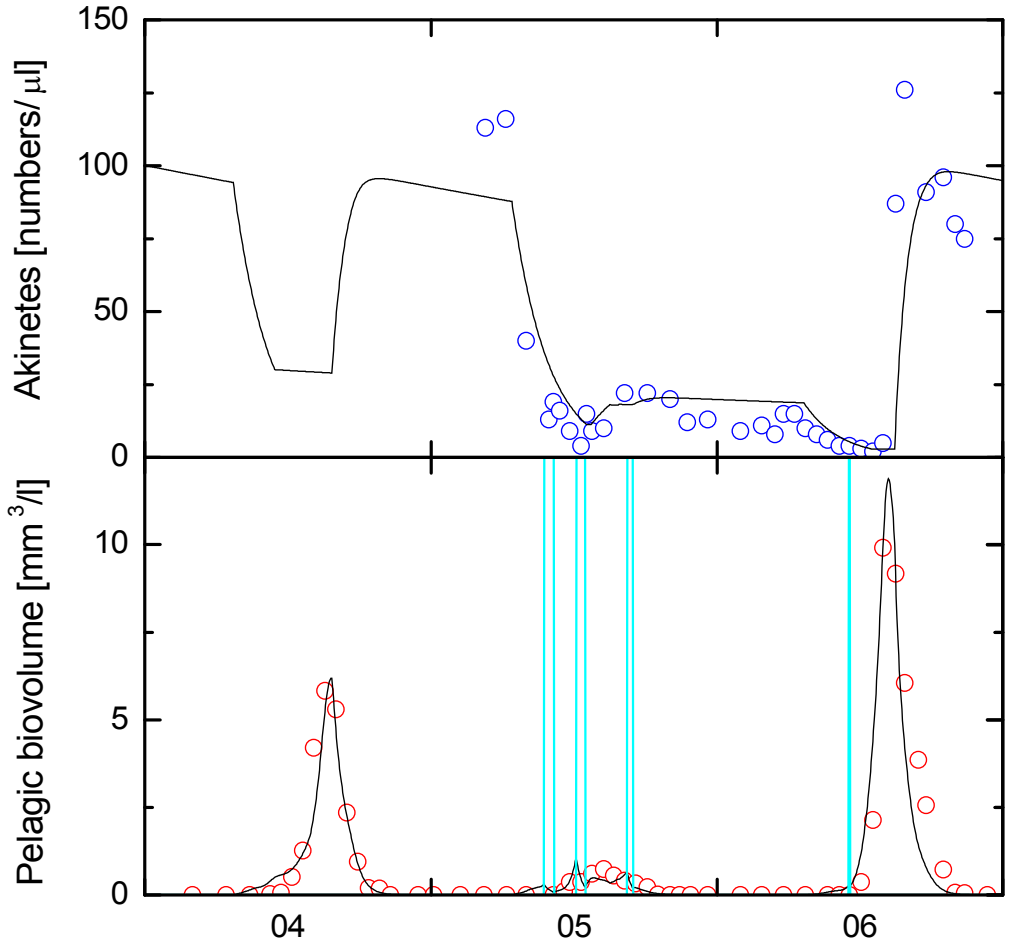


Figure 4.1. Simulated life cycle dynamics of *Cylindrospermopsis raciborskii*. Predicted values (lines) and measured values (dots) for the number of akinetes in the sediment and the biovolume of vegetative cells in the water column. Data for the years 2004–2006 were used for calibration.

Calibration

The calibration of the simulation model for the period 2004–2006 (Figure 4.1) is a nearly perfect fit to the measured data. And also the simulation of the full period of data for *C. raciborskii* (Figure 4.2) is fitting them very well. Figure 4.2 shows the periods of additional grazing as blue lines. These periods are very short, usually in the range of one or a few days. But they can have a dramatic effect on biomass production. Here we point to the years 2005 and 2007 were pelagic biovolume is quite low although the number of akinetes in the preceding winter are very high and (not shown) the water temperature was slightly above normal, thus perfect growth conditions. Our model is capable to simulate this unusual low biovolume. In a climate scenario simulation shown later, where we did not use these sporadic grazing events, the development of biovolume is quite different, leading to higher overall values and nearly equally high biovolumes in 2005–2007.

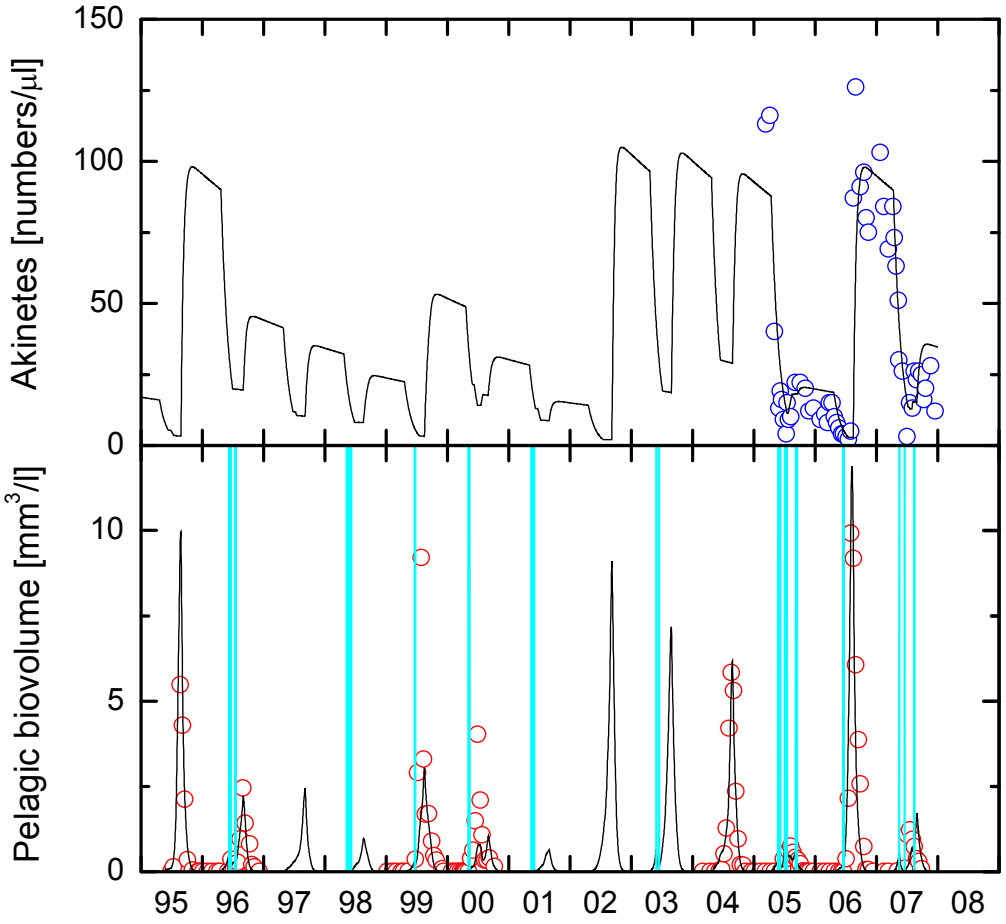


Figure 4.2. As in Figure 4.1, now showing the full simulation period 1995–2007. Included are the short periods of additional losses (blue lines).

Sensitivity

To get an impression on the effects of parameter changes we also did a sensitivity study. Changes in the annual course of biovolume due to variation of optimum growth temperature and temperature of akinete formation are illustrated in Figure 4.3. Changes in optimum temperature had tremendous effects. A 10 % decrease in optimum temperature, which would result in the start of growth earlier in the year and higher growth rates in summer, resulted in a more than two-fold increase in biovolume and akinete population size (Figure 4.3, dashed line). Inversely, a 10 % increase in optimum temperature would shift the favourable temperature range out of scope and the populations would die off. The effects of formation temperature were minor (Figure 4.3, right panel). A 10 % change in formation temperature resulted in a 10 % change in akinete population size, marginal changes in case of a 10 % decrease, and a drop in population size in case of a 10 % increase in temperature of akinete formation. However, this drop is not reflected in the generated akinete population of the following year. Ten percent changes in temperature optimum equals a change in temperature of about 3 degrees Celsius. Thus, such a dramatic shift as was shown here is very likely under proposed global warming conditions.

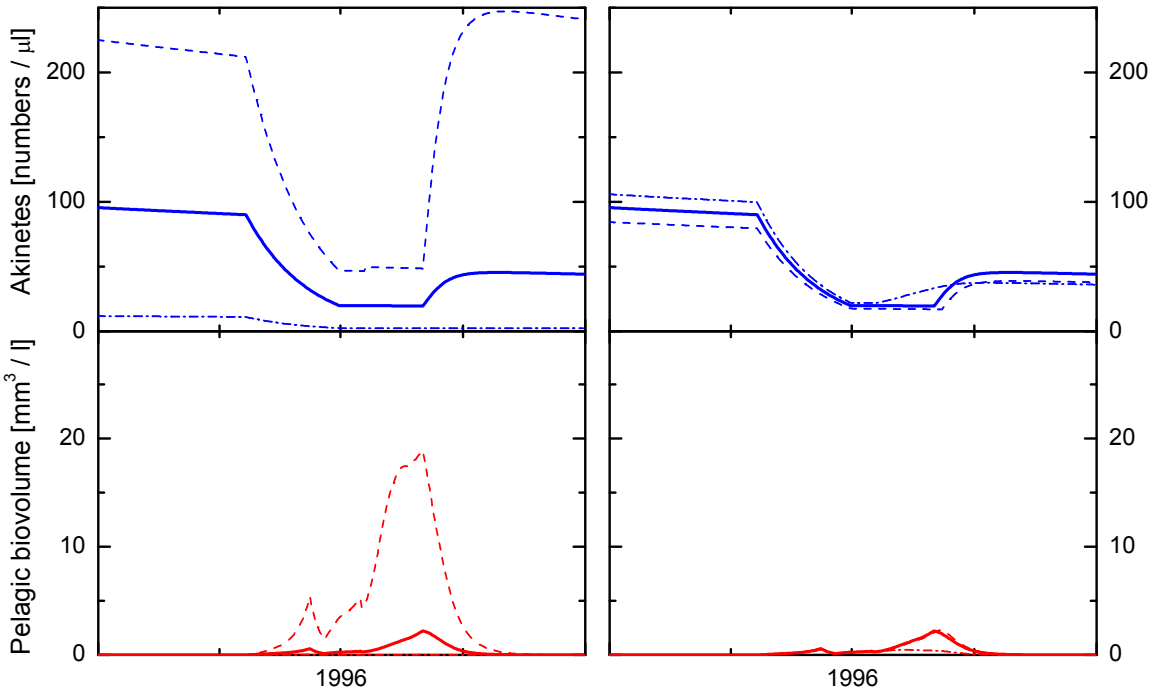


Figure 4.3. Sensitivity of model results to +/- 10 % variation of the optimum growth temperature, T_{opt} (left), and the temperature threshold for akinete formation, T_{form} (right). The upper and lower panels show changes in the number of akinetes and the biovolume of vegetative cells, respectively. Dashed and dash-dotted lines represent simulations with a 10 % decrease and a 10 % increase in parameter values, respectively.

Physiology

The model was developed with the goal to simulate the development of all different kinds of Nostocales cyanobacteria. Several sets of growth curves for light and temperature dependence were generated from laboratory data (Chapter 3.3). Since we do not have detailed data for all Nostocales cyanobacteria in our model lake Melangsee the calibration for other species is not as good as for *C. raciborskii*. The calibration/simulation for *A. gracile* is shown in Figure 4.4. The simulation is fully calibrated (no validation period). It could also be shown that the event based grazing effect has a less dramatic effect on *A. gracile*, i.e. calibration suggests that the additional grazing pressure is less than that for *C. raciborskii*, but cannot be neglected. In Figure 4.5 we show a small part of calibration results depicting the root mean square error for akinetes and pelagic population for several millions of different parameter settings. The graph is already restricted to an optimal area, where only values for additional grazing occur which are about half the size that for *C. raciborskii*.

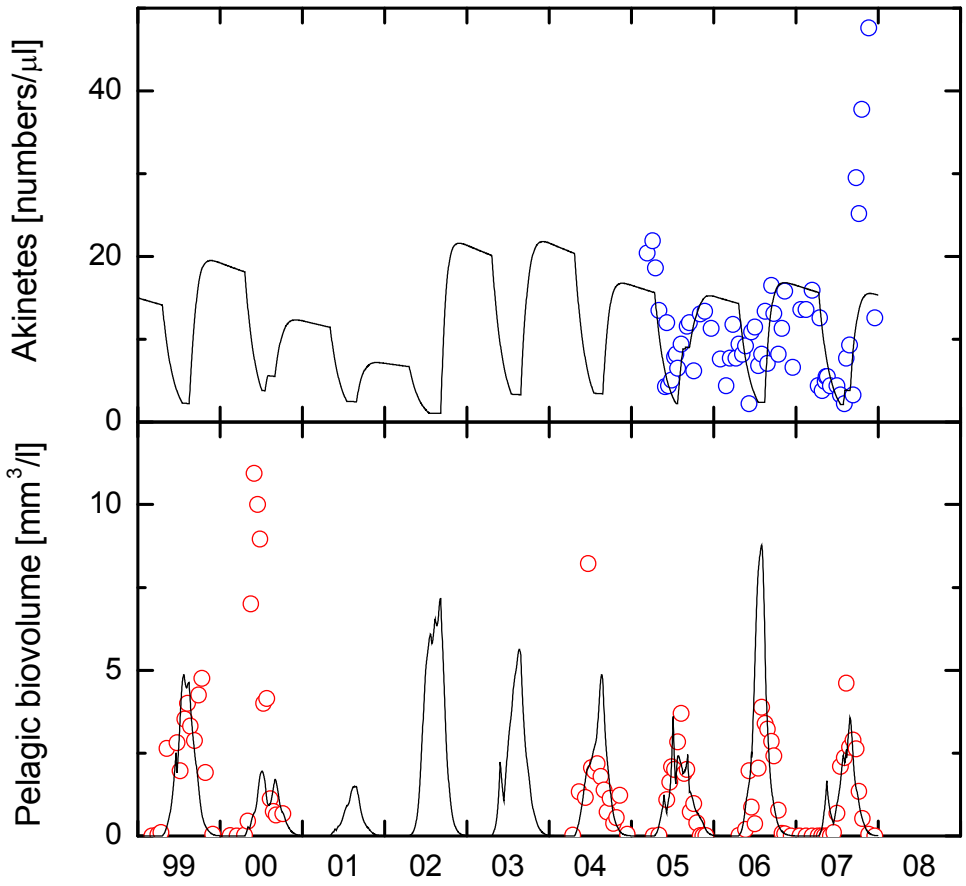


Figure 4.4. Simulated life cycle dynamics of *A. gracile*. Top panel: Predicted values (lines) and measured values (dots) for the number of akinetes in the sediment. Bottom panel: Biovolume of vegetative cells in the water column.

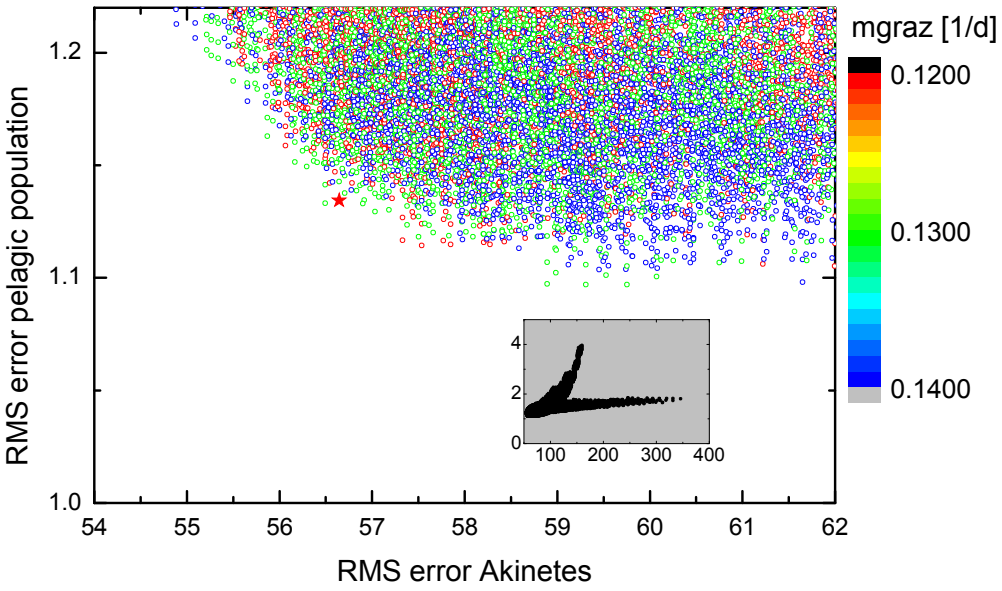


Figure 4.5. Calibration of additional loss rate. Dots represent one full simulation cycle aggregated into values for root mean square errors for akinetes and biovolume in the water column. Colors stand for different ranges of additional loss rate. The star points to the chosen calibration point.

Climate change scenarios

We simulated different climate (temperature) scenarios for different cyanobacteria species. They are based on simulations using the 13-year meteorology derived for Lake Melangsee. The main features were already discussed in Chapter 3.3 for the very simple model of temperature dependent growth. Those results translate one to one to the outcome of the more complicated simulation model. Here we show results for *C. raciborskii* (Figure 4.6) assuming that the additional grazing (or flushing) effect is not present – we have no knowledge how such a nearly random effect would appear in future scenarios. Increasing water temperatures would lead to an overall increased biovolume, while akinete numbers would not change much. A climate scenario with 1 degrees lower temperature would result in a quite fast decrease of pelagic biovolume and finally vanishing population of the high temperature adapted species (not shown here), where only 1 degree will lead to a very small biovolume which is only enhanced in warmer than usual years; variability of interannual maxima will decrease with increasing temperature. The same scenario used for *A. gracile,* which has a lower optimal temperature of growth, does have smaller biovolume with lower temperatures, but the differences are not that pronounced as with *C. raciborskii* (Figure 4.7).

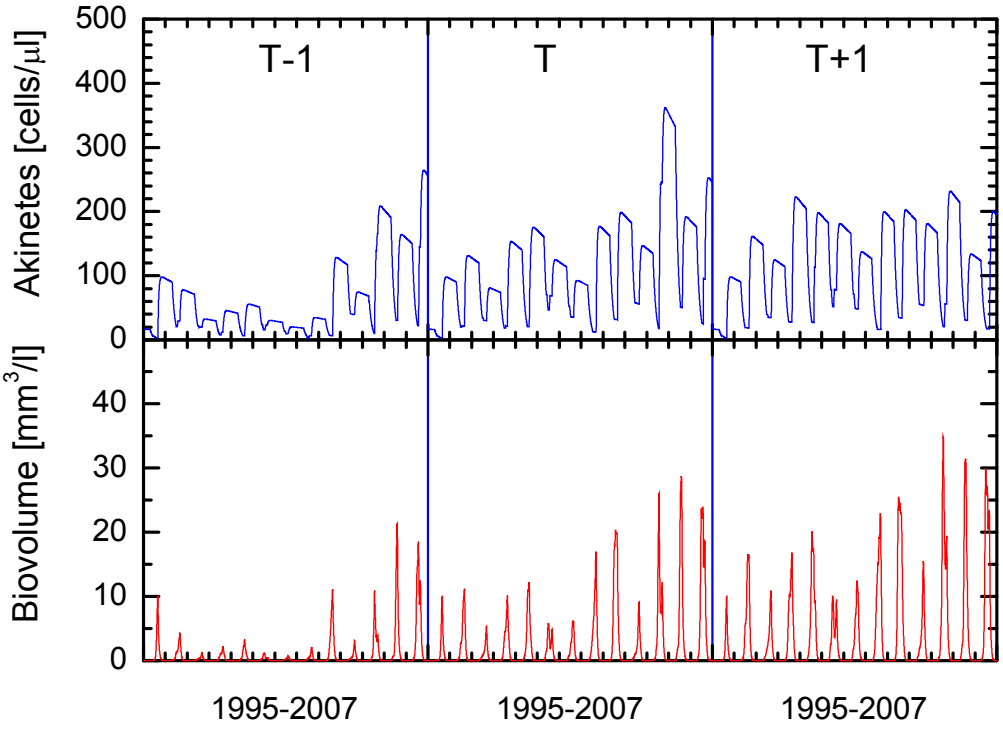


Figure 4.6. Effect of temperature changes on population dynamics. Shown are three sections, the first one uses a temperature 1 C lower than normal, the second section is the reference run, and the third rightmost section is the simulation with a 1 C increase in temperature. Results for *C. raciborskii*.

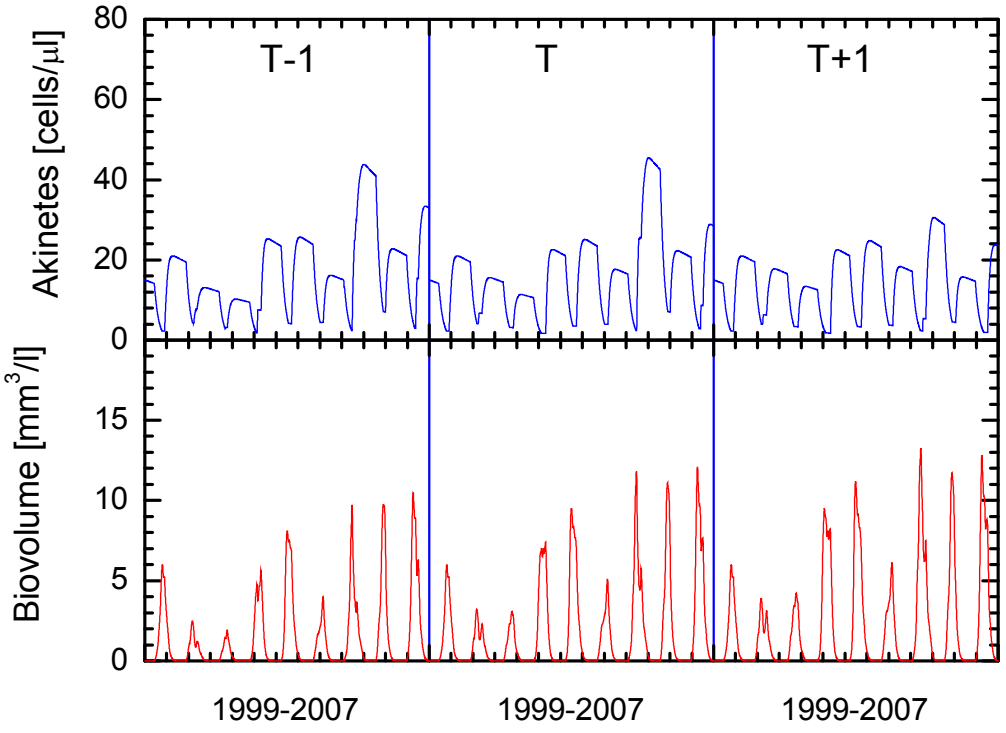


Figure 4.7. Effect of temperature changes on population dynamics. Shown are three sections, the first one uses a temperature 1 C lower than normal, the second section is the reference run, and the third rightmost section is the simulation with a 1 C increase in temperature. Results for *A. gracile*.

Nutrient effects

Here we show a simple scenario for nutrient effects on population dynamics. Since we describe nutrient availability via the proxy Secchi depth, a change in Secchi depth does not only change nutrient availability as described earlier, but also modifies light climate, i.e. I_{mix}. This can be corrected by a scaling factor derived from the new and old Secchi depth and the old value of I_{mix}. Results are equivalent to those without using this correction. Population size will be slightly larger than for runs without correction factor. To focus on pure nutrient effects, here we only show results for changes in Secchi depth related to nutrient uptake without changes in light availability.

Figures 4.8 and 4.9 show the simulation results for *C. raciborskii* and *A. gracile* in an environment with Secchi depths 0.2 m lower (less nutrients), and 0.2 m higher (more nutrients) than normal. As before, the graphs are based on simulations using the 13-year meteorology derived for Lake Melangsee. The changes due to these nutrient effect are larger than those for the +/- 1 degree temperature scenarios above. Again *A. gracile* can cope better with bad conditions and still shows some biovolume, while *C. raciborskii* would diminish under strong nutrient limitation.

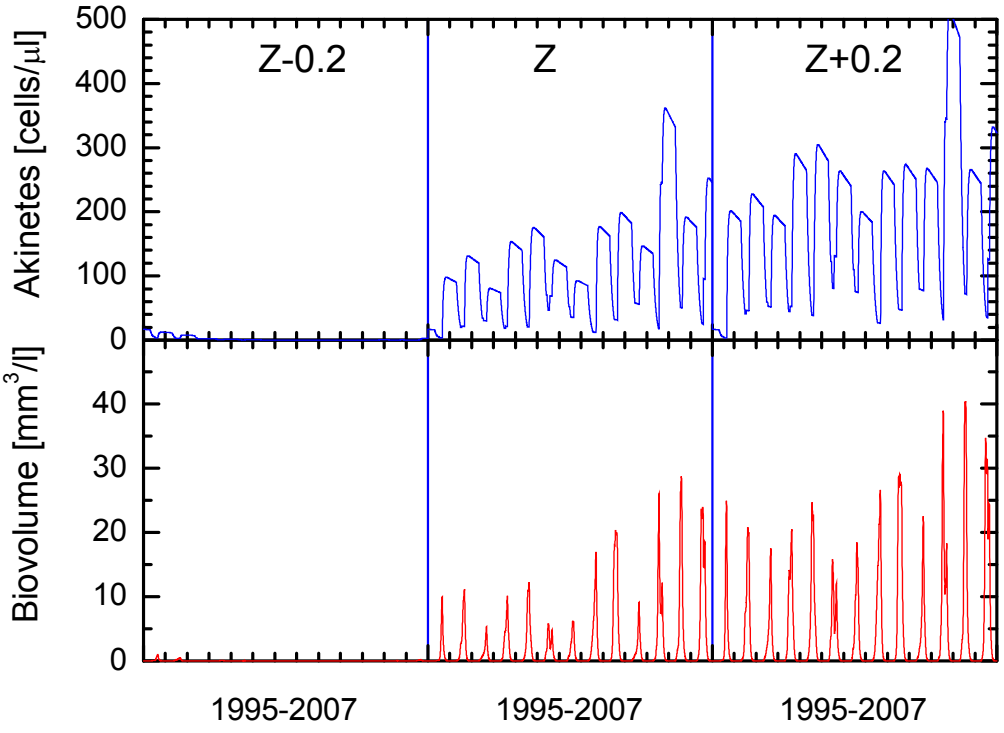


Figure 4.8. Effect of nutrient changes exemplified by changes in Secchi depth (Z). Left section Z-0.2m, middle section is reference run, right section is for Z+0.2 m. Results for *C. raciborskii*.

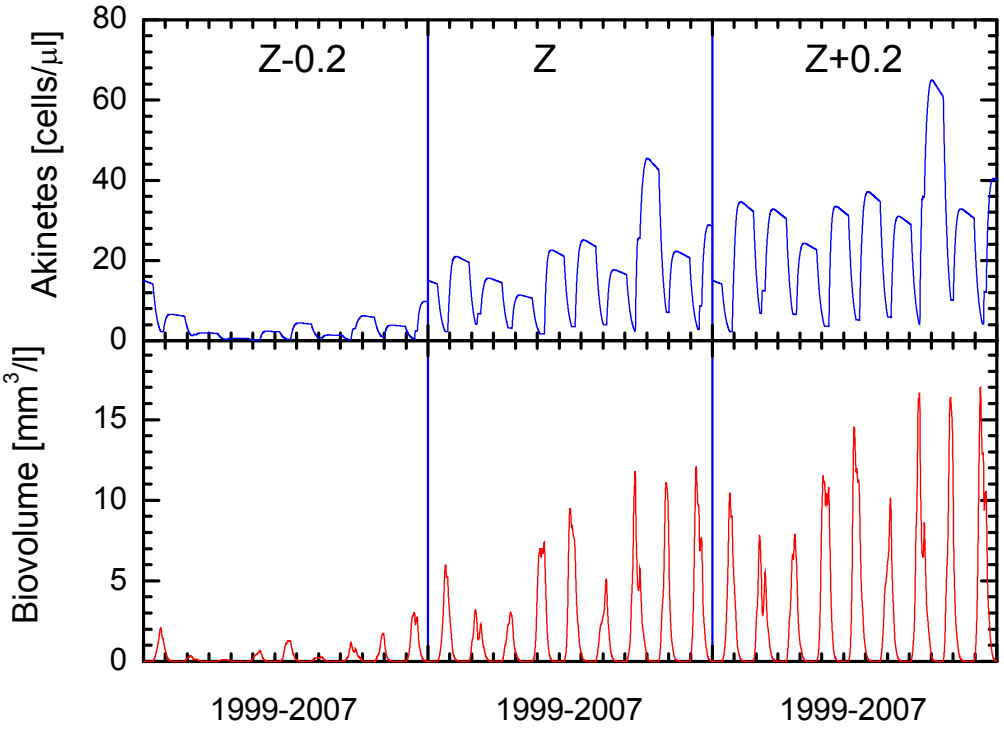


Figure 4.9. Effect of nutrient changes exemplified by changes in Secchi depth (Z). Left section Z-0.2m, middle section is reference run, right section is for Z+0.2 m. Results for *A. gracile*.

Mixed nutrient/temperature effects

To test for the combined effect of change in water temperature and nutrient availability runs with temperature changes of +/-1 and changes in Secchi depth of +/-0.2 m were made. Results are shown for *C. raciborskii* (Figure 4.10). From that it becomes clear that decreased nutrient availability (decreased Secchi depth) can be counteracted with increased temperature, i.e. even when nutrients are reduced a shallow lake might still show high biovolume of *C. raciborskii* under increased temperature conditions.

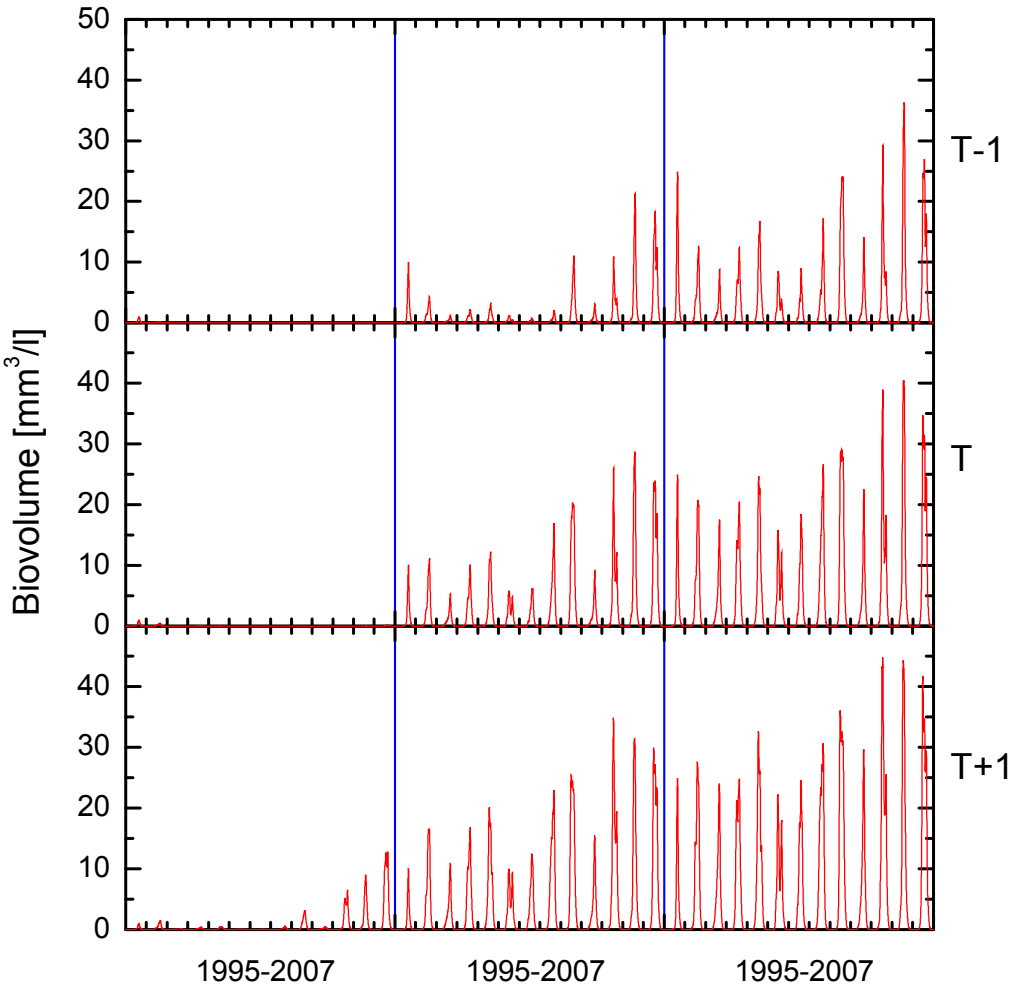


Figure 4.10. Mixed effect of changes in temperature (T+/-1 C) and in Secchi depth (Z+/-0.2 m). Results for *C. raciborskii*.

Akinete seeding (invasion)

The simulation runs shown here are based on a defined number of akinetes introduced into the lake. Under favorite conditions these akinetes will germinate and grow into a small pelagic population forming more akinetes, and so forth. The seeding with a single akinete might not be sufficient to build up a larger population due to threshold conditions in pelagic biovolume (population goes extinct whenever biovolume drops below the volume of a single filament in the lake volume). Seeding with 10 akinetes: It will take

13 mean years for the population to reach a threshold, here chosen to be 1 mm^3 L^{-1} (Figure 4.11). If temperature increases the development time drops to 6 years (T+2) and 4 years (T+4). *A. gracile* is less sensitive to temperature changes and will even build up a population for a colder climate (T-2) after 23 years (Figure 4.12). It should be mentioned that these development times are achieved for mean annual fields. Using more realistic, varying fields it is to be expected that due to "colder" years spread over the course of years populations will be "reset". Thus the figures given here are basically lower estimates of the development time.

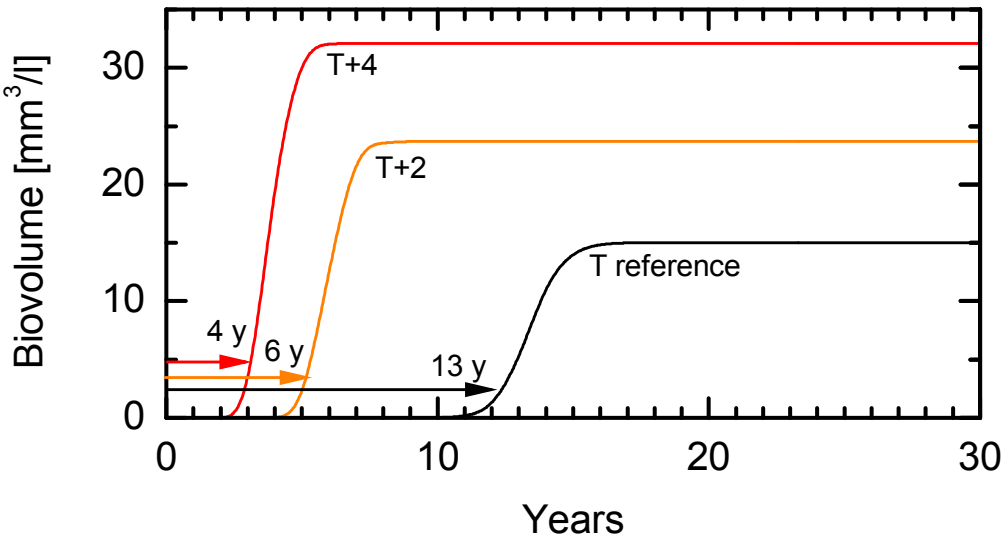


Figure 4.11. Annual maximum for *C. raciborskii* under changed temperature conditions starting from an initial akinete seed of 10 akinetes per lake area.

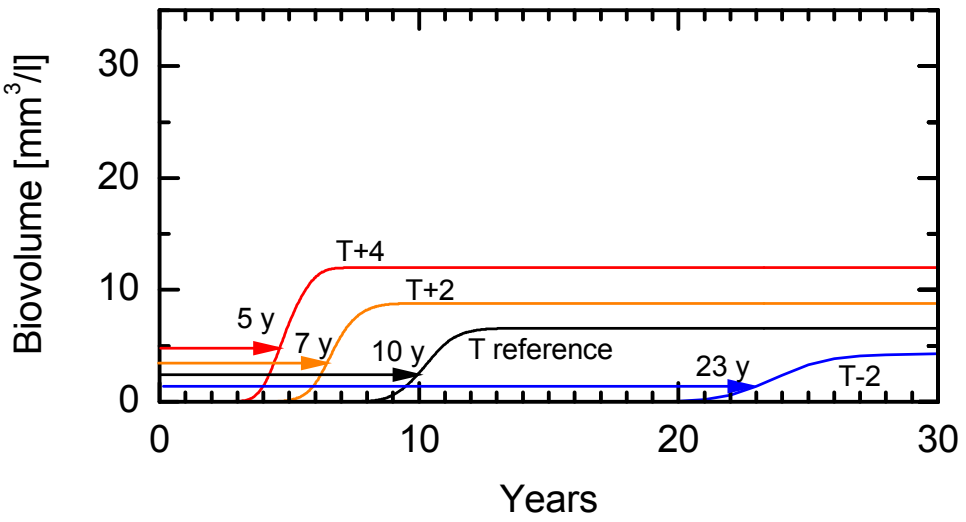


Figure 4.12. Same as Figure 4.11 for *A. gracile*.

The dependence on temperature changes and akinete seed in more general terms for ranges of initial akinetes between 1 and 2^{20} akinetes per lake area and temperature changes between -4 and +4 degrees Celsius are given in Figures 4.13 and 4.14. The development time increases with decreasing akinete seed and decreasing temperature. Below a certain temperature value, development time is either larger than 128 years (the simulation horizon) or the population is extinct.

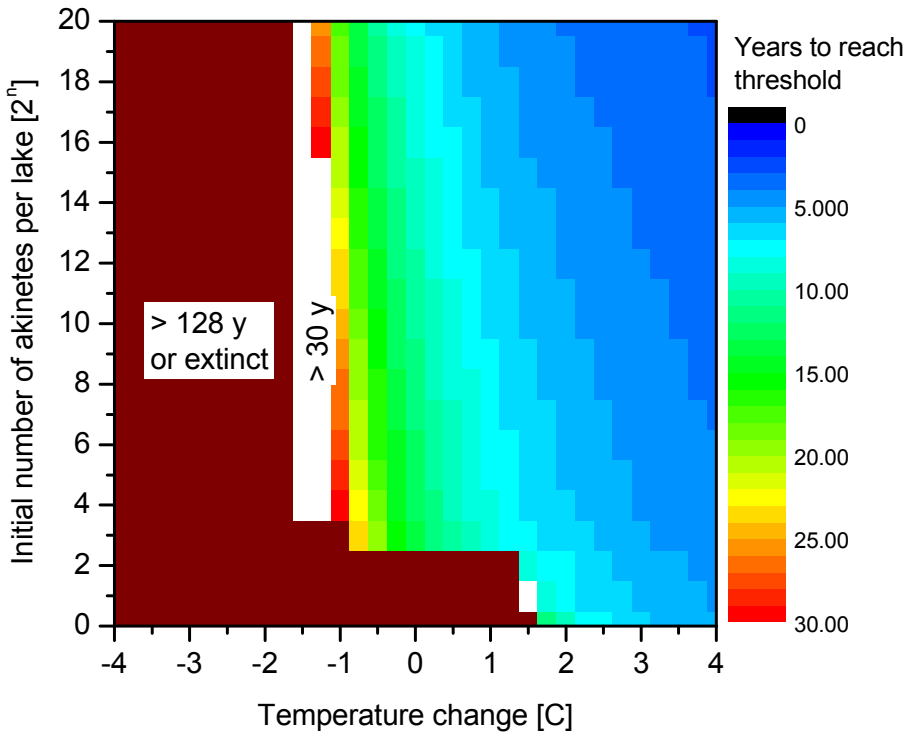


Figure 4.13. Development time to reach a threshold value of 1 $mm^3\ L^{-1}$ biovolume for *C. raciborskii* depending on temperature changes and initial akinete number per lake.

A last graph (Figure 4.15) wraps up the model organisms behavior for changes in temperature and nutrient availability. Here, a seed of 10 akinetes per total lake area was used. As can be expected, development times increase with decreasing temperature and decreasing nutrient level. The dependence shows a nonlinear behavior, which can be explained by the nonlinear nature of the model and the thresholds involved therein. As explained above using a simulation horizon of 128 years it cannot be decided whether a population goes extinct or has a very large theoretical development time whenever there was no passing of the threshold after 128 years. Using longer time series would smooth that picture. Using Figure 4.15 allows one to estimate development times of initial seeds of *C. raciborskii* for different levels of nutrient availability and temperature regimes.

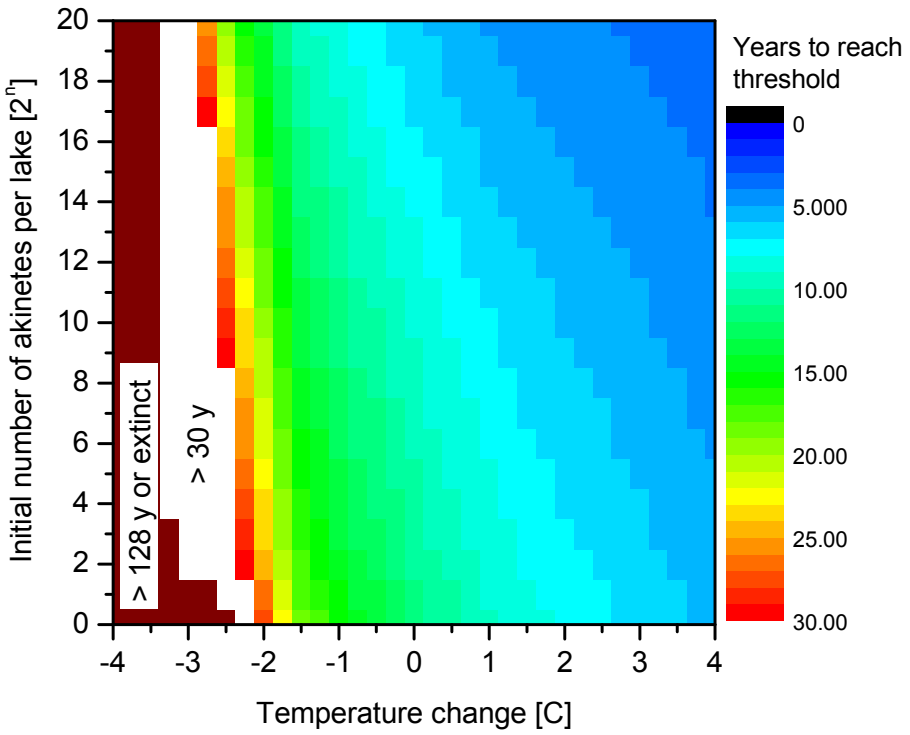


Figure 4.14: Same as Figure 4.13 for *A. gracile*.

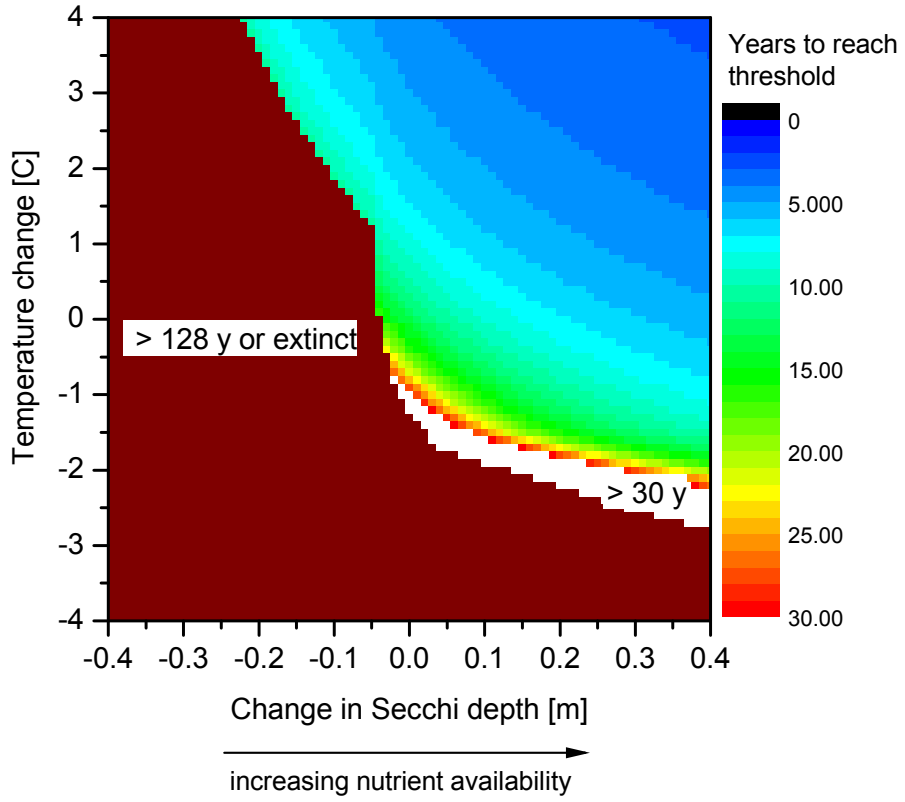


Figure 4.15: Development time to reach a threshold value of 1 $mm^3 L^{-1}$ biovolume for *C. raciborskii* depending on changes in nutrient availability and temperature for a seed value of 10 akinetes per lake.

Conclusion

Here we have developed a simulation model for Nostocales species, which allows for overwintering due to akinete formation and germination. Using the simulation model it is possible to estimate the course of Nostocales biolvolume over time depending on temperature conditions and nutrient levels in shallow lakes. For lake management this can provide answers to the question how a population will develop over future years assuming certain management options, like flushing using the concept of extra loss rates built into the model, reduction of nutrient levels using the concept of Secchi depth proxy. Also the effects of global warming can be further evaluated.

5. The fate of cylindrospermopsin and anatoxin-a during sediment passage

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The occurrence of Cylindrospermopsin (CYN) and Anatoxin-a (ATX) in surface waters has been reported throughout the world. A screening conducted in 127 lakes in north-eastern Germany revealed widespread occurrence of CYN in half of the investigated water bodies (Fastner *et al.* 2007). Rücker *et al.* (2007) found total concentrations in some of these lakes ranging up to 12.1 µg L^{-1}. In contrast to the well studied micro-cystins, a large fraction of CYN (68–98 %) occurs extracellularly, with peak concentrations in the water body towards the end of a bloom (Chiswell *et al.* 1999; Rücker *et al.* 2007). Due to its chemical stability and slow degradation (Chiswell *et al.* 1999) CYN shows a high persistence in many water bodies (Wörmer *et al.* 2008). These findings may have important implications for water authorities if concentrations in surface waters are in a health-relevant range (e.g. >1 µg L^{-1}, as proposed by Humpage and Falconer (2003) for drinking-water).

Even though the results of a previous and the current ATX screening study in north-eastern German lakes (Bumke-Vogt *et al.* 1999, Chapter 1.3) suggest ATX occurrence to be less frequent than CYN, concentrations in some lakes exceeded a proposed guideline value for drinking-water of 1 µg L^{-1} (Fawell *et al.* 1999). Hence, effective elimination of both CYN and ATX has to be ensured if contaminated surface waters are used for drinking-water production via sediment passage. Therefore, the aim of this subproject was to identify physico-chemical boundary conditions which are conducive to biotic and abiotic toxin elimination. In particular, we investigated the role of sediment preconditioning, the presence of dissolved organic carbon (DOC), temperature, redox conditions, and contact time in toxin degradation. Furthermore, we determined sediment characteristics relevant for toxin adsorption.

Experiments were conducted in laboratory scale (column and batch studies) as well as under near-natural conditions (enclosure facility, slow sand filter system) using the Federal Environment Agency's Facility for the simulation of river bank and slow sand filtration (SIMULAF) in Berlin-Marienfelde.

Sorption of CYN and ATX onto sediments

Sorption is largely controlled by the chemical properties resulting from the structure of the respective toxin. In environmentally relevant pH ranges, CYN occurs as a zwitterion (Meriluoto and Spoof (2008), Figure 5.1 a), while ATX occurs as cation at pH values <9.6 (Devlin *et al.* 1977; Figure 5.1 b).

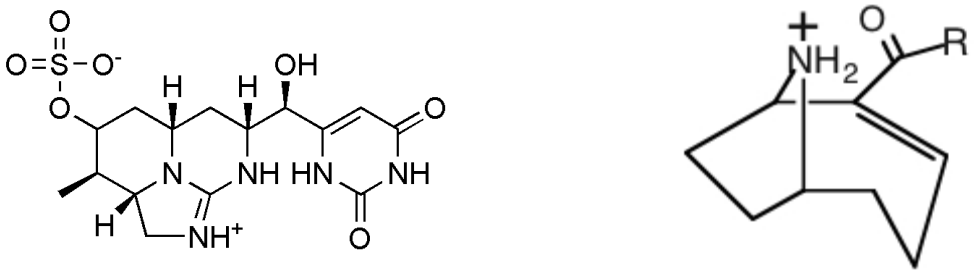

Figure 5.1. Molecular structure of **(a)** cylindrospermopsin and **(b)** anatoxin-a.

Sorption parameters were determined in a batch study in 0.01 M $CaCl_2$ solution according to the procedure described in the OECD guideline 106 with initial solution concentrations ranging from 1 to 46 µg L^{-1} toxin and a solid-solution-ratio of 1:5.

Anatoxin-a showed weakest sorption to sandy sediments (UBA (99 % sand); Mergel (89 % sand); GW (96 % sand)), medium sorption on silty-sandy sediments (Kyll (Figure 5.2 a), KHW) and highest sorption to clay-rich (SRW, 27 % clay, Figure 5.2 c) and organic-rich (organic mud 44.5 % C_{org}; Figure 5.2 b) sediments.

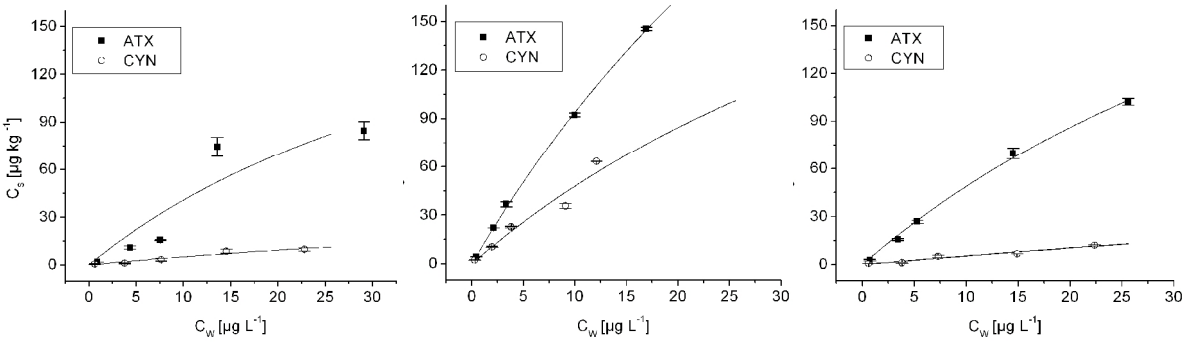

Figure 5.2. Sorption of CYN and ATX on sediments: **(a)** Kyll, **(b)** organic mud, and **(c)** SRW.

Sorption of ATX was mostly best described by the non-linear model according to Langmuir, whose sorption parameters of the analysed sediments are summarized in Table 5.1. In order to determine sorption-relevant parameters, linear and bivariate regression analyses were conducted.

Table 5.1. Sorption parameters for ATX and CYN (n. d.: not determined).

Sediment	Langmuir q_{max} [µg kg^{-1}]	
	ATX	**CYN**
Müggel (uS)	561	206
Organic mud (organic)	656	361
SRW (scU)	347	124
KHW (ufS)	330	27
Kyll (UfS)	254	39
GW (fS)	112	n. d.
Mergel (fS)	107	No sorption
UBA (mS)	47	No sorption
NM (mfS)	n. d.	19

Regression analysis revealed clay to be the substrate with the highest impact on ATX sorption (data not shown). Similar findings were observed for the cyanobacterial toxin nodularin in a batch study by Miller *et al.* (2005). At high concentrations, ATX sorption onto clay would be enhanced with increasing pH. This is due to an increasing negative surface charge of clay minerals with increasing pH leading to stronger electrostatic attraction of the ATX cation. The second important parameter affecting ATX sorption was the organic carbon content. However, with the slope of the regression equation amounting to only 1/5 and to 1/8 of the slope values of clay, it only plays a minor role. The strong linear correlations between the sorbed amount of ATX and the effective cation exchange capacity (0.827 for C_w = 1 µg L^{-1} and 0.756 for C_w = 30 µg L^{-1}) suggest sorption to occur through cation exchange as a main mechanism, a common sorption mechanism for organocations (Narine and Guy 1981). The authors report this sorption mechanism to make organocations very susceptible to changes in ionic strength. This means that increasing cation concentrations in solution would lead to a displacement of ATX from the exchange site, hence rendering it more mobile in the pore water.

Similarly to ATX, sorption of CYN was mostly best described by the non-linear Langmuir model (Table 5.1). Cylindrospermopsin showed no sorption on strongly sandy sediments (UBA, Mergel). While CYN sorption to organic matter (organic mud, 44.5 % C_{org}, Figure 5.2 b) was very high, sorption to silty-sandy sediments such as Kyll (Figure 5.2 a), KHW, and NM was low. Regression analysis revealed organic C as the main parameter controlling CYN sorption. A separate sorption experiment which showed only limited exchangeability of CYN by Ca suggests a small fraction of CYN (approx. 20 % throughout the measured concentration range) to be bound via cation exchange. The remaining fraction is most likely sorbed via a less specific mechanism such as hydrogen bonding. This proposed sorption mechanism would render CYN less susceptible to desorption from exchange sites. These findings may provide one possible explanation for the observed high persistence of CYN in surface waters: sorption onto particulate organic matter, which occurs ubiquitously in waters, may protect CYN from microbial degradation.

As CYN shows very little sorption high breakthrough has to be expected in sediments unless further environmental factors are conducive to CYN degradation. Even though ATX shows much stronger sorption than CYN, ATX desorption from sediments may have to be expected, especially if water hardness is high.

Retention and degradation of cylindrospermopsin in waters and sediments – Results from laboratory studies

Retention and degradation of CYN in a flow setting were determined with column experiments. Unless otherwise stated we used two types of natural sandy sediments and surface water (filtered over a 1.2 µm membrane) obtained from the Federal Environment Agency's slow sand filter pond. The pond water has been abstracted from a quartenary aquifer and Fe and Mn have been removed by microbial precipitation prior to its infiltration into the pond system. The ground water sediment (fS, content of fines: 4 %) was obtained from an aquifer north of Berlin (2.6–4.4 m below top ground surface) and the filter sand (mS, content of fines: 1 %) from one of the slow sand filters of the Federal Environment Agency's facility for technical-scale slow sand and bank filtration experiments (SIMULAF) in Berlin. Flow-through column experiments (FT) were used to study CYN retention as a function of sediment texture (i.e. content of fines 1 % and 4 %), flow rate, initial concentration, and the presence of DOC. Closed-loop column experiments (CL) were conducted to investigate CYN degradation as a function of the following parameters: sediment preconditioning, the presence and composition of dissolved organic carbon (DOC), temperature, and redox conditions. Initial concentrations amounted to 10 ± 2.8 µg L^{-1} (with the exception of the application of the lysed cell extract (165 µg L^{-1})).

Retention in sediments

Cylindrospermopsin showed tracer-like mobility under the investigated conditions. Retention in sandy sediments proved negligible regardless of the content of fines, flow rate, initial concentration, and the presence of DOC (R values ranging from 1.00 to 1.14).

Degradation in water

Cylindrospermopsin showed constant concentrations in the water phase for all investigated conditions during the length of the experiment, i.e. at room temperature (during 40 days), at 10°C (during 70 days), in the absence (during 40 days) and presence of DOC (during 8 (aquatic DOC) to 40 days (DOC from lysed cells), under aerobic (during 40 days) as well as anaerobic conditions (during 113 (pond water) to 140 days (anoxic groundwater)). This confirms the already observed high persistence of CYN in natural water (Wörmer *et al.* 2008).

Degradation in sediments

*The role of the lag-phase and the presence of DO*C. Virgin sediments, i.e. sediments without prior contact to CYN, showed a lag phase of approximately 20 days prior to CYN

degradation (data not shown). Preconditioned sediments showed no lag phase (Figure 5.3). The composition of dissolved organic carbon proved important for the lag phase: while aquatic dissolved organic matter (DOM) revealed no lag phase, the presence of easily degradable organic substances which are released during the lysis of cyanobacterial cells, induced a lag phase of 2 to 3 days (Figure 5.3). This may be attributed to the presence of competing substrates, i.e. more easily available C-sources such as biopolymers (i.e. carbohydrates, peptides), and low-molecular neutral amphiphilics. These compounds are preferentially metabolized by the microorganisms. These findings are in agreement with Smith *et al.* (2008) who propose degradation competition between natural organic matter (NOM) and cyanobacterial toxins, with NOM being the primary substrate for microbial degradation due to its occurrence in higher concentrations (mg L^{-1} as opposed to mg L^{-1} for toxins).

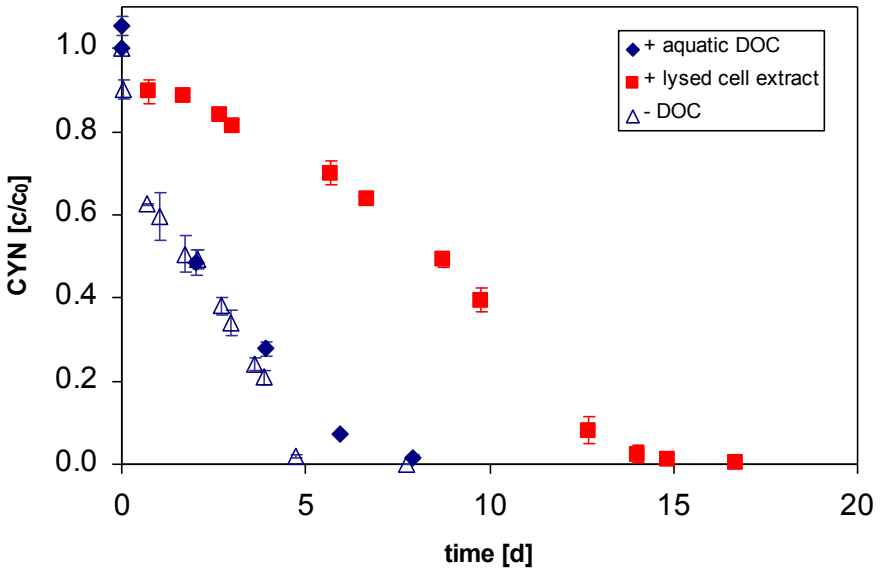

Figure 5.3. Normalized CYN concentrations determined in closed-loop column experiments using preconditioned filter sand after the addition of aquatic DOC (initial CYN conc.: 13 µg L^{-1}), a lysed cell extract (initial CYN conc.: 265 µg L^{-1}), and without any additional carbon substrates (initial CYN conc.: 14 µg L^{-1}). Error bars depict one standard deviation of duplicate experiments.

The addition of DOC to virgin sediments resulted in a shortening of lag phases from 20 days to 10 days suggesting the availability of DOM to enhance bacterial population growth with the consequence of a faster start of CYN degradation. In the presence of DOC, preconditioning did not only result in the abolition of the lag phase, but also in faster (1^{st} order) degradation than in virgin sediments (zero order; $T_{1/2\ (virgin)}$ = 5 days, $T_{1/2\ (precond)}$ = 1.3 days).

Temperature effect. Cylindrospermopsin degradation at 10°C was decelerated by a factor of 10 (zero order degradation parameters at 20°C and 10°C: $\kappa_{20°C}$ = 0.18 day^{-1}, $\kappa_{10°C}$ = 0.018 day^{-1}; $T_{1/2\text{-}20°C}$ = 2.4, $T_{1/2\text{-}10°C}$ = 18.3), most likely due to lower microbial activity.

Redox conditions. Cylindrospermopsin degradation was studied in a nitrogen-flushed glovebox (oxygen content ≤ 0.4 %) using anoxic groundwater sampled on-site from a quartenary aquifer and preconditioned sediments (i.e. from previous experiments under oxic conditions). Prior to the start of the experiment columns have been circulated with anoxic groundwater until redox equilibrium was reached (± 50 mV). While in the sandy groundwater sediment (4 % of fines, 0.04 % C_{org}, redox potential during the experiment: 260 to 440 mV) there was no CYN degradation over a period of 3 months, concentrations decreased slowly in the filter sand sediment (1 % of fines, 0.7 % C_{org}, redox potential during the experiment: –170 to –329 mV). After a lag phase of 20 days, degradation yielded half-lives of 24 days (± 21 %) until day 113, when a (normalized) residual concentration of 0.45 (± 9 %) was reached. Sorption in both sediments could be ruled out based on results from FT experiments. These findings demonstrate very clearly that the effect of anoxic conditions is not consistent for different sediments and is most likely controlled by other parameters. One possible explanation might be the higher content of organic C (possibly in the form of detritus present in the filter sand) that would lead to enhanced microbial activity (through the supply of electrons) leading to a reduction of Fe- and Mn-(hydr)oxides resulting in lower redox potentials. However, the role of organic carbon in CYN degradation under anoxic conditions cannot be clarified with this data set and hence requires further investigations.

Our data obtained under laboratory conditions demonstrates efficient CYN removal through degradation under oxic conditions, however, high breakthrough has to be expected (i) if sediments have not had any previous contact to CYN and (ii) under anoxic conditions.

Cylindrospermopsin elimination in waters and sediments studied under near-natural conditions

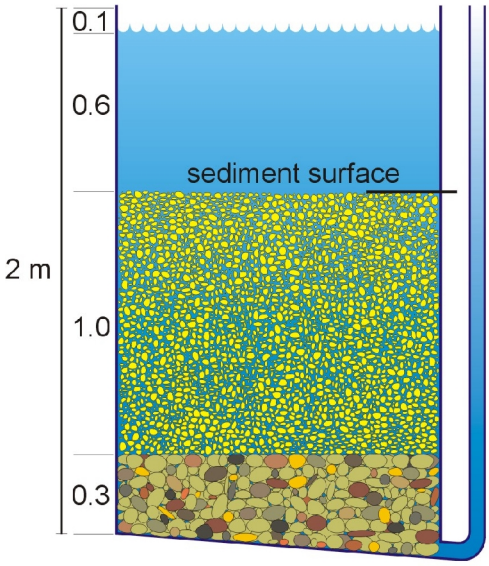

Figure 4. Cross-sectional view of an enclosure column.

Experiments under near natural conditions were conducted in the enclosure facility and the slow sand filter system of the Federal Environment Agency's Experimental Site in Berlin-Marienfelde (SIMULAF).

Experiments in the enclosure facility

The enclosure facility consists of water-saturated sediment columns (Figure 5.4), with a sediment layer of 1 m thickness and a texture of medium sand. Pond water that has percolated the sediment can be sampled at the column outflow. Both water head and flow rates are adjustable. We conducted several experiments with a pulsed application and one experiment with

continuous CYN dosing during 6 weeks. We investigated the role of (i) filter velocity[1] (0.2 m d^{-1} (river bank filtration), 0.7 m d^{-1} (artificial groundwater recharge), 1.2 m d^{-1} (slow sand filtration)), (ii) anoxic conditions, and (iii) the effect of the "schmutzdecke" following a pulsed CYN application.

The effect of varying flow rates showed complete CYN breakthrough for scenarios such as artificial groundwater recharge and slow sand filtration. Only at the characteristic flow rate of river bank filtration 40 % of the initial CYN concentration was degraded ($T_{1/2}$ = 3.6 days). These results clearly demonstrate the importance of sediment contact time. Experiments conducted under anoxic conditions (O_2 content <0.5 mg L^{-1}) at flow rates of 8 l h^{-1} and 30 l h^{-1}, respectively, showed complete CYN breakthrough in both cases, suggesting no CYN degradation.

Similarly, there was no CYN elimination in the presence of a "schmutzdecke" (flow rate 10 l h^{-1}). These results are in contrast to observations reported for microcystins (Grützmacher *et al.* 2002). Microcystin removal was found to be enhanced in the presence of an intact "schmutzdecke".

In an experiment with continuous dosing, we observed a lag phase of approximately 6 days (Figure 5.5). Between day 6 and day 12, the elimination efficiency increased. From day 12 until the end of the experiment between 75 % and 95 % of the initial concentration was degraded. Half-lives (calculated at the end of the lag-phase) amounted to 4.0 days ± 30 % and are in a similar order of magnitude as observed during closed-loop column experiments (2.4 ± 2 %) conducted under laboratory conditions.

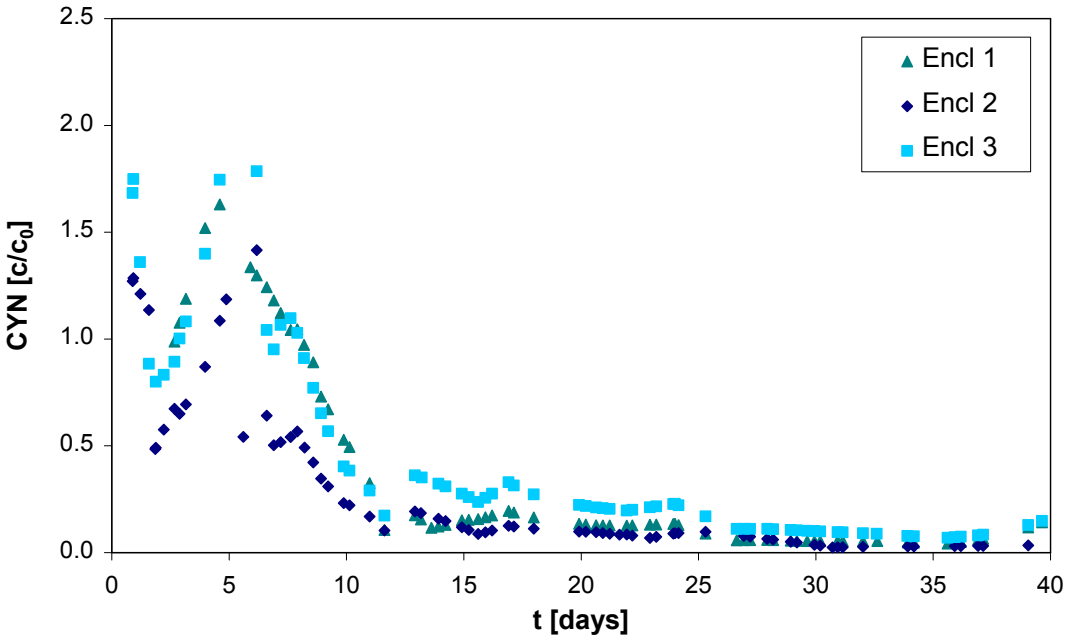

Figure 5.5. Normalized CYN concentrations determined in the outflow of enclosure columns following continuous dosing for 6 weeks (CYN concentrations in the supernatant: 2 ± 1 µg L^{-1}).

[1] with filter velocity being defined as discharge divided by area

Experiments in the slow sand filter ponds

We conducted two experiments in the slow sand filter ponds with a filter velocity according to a river bank filtration scenario (0.2 m d^{-1}), an intact "schmutzdecke" and pulsed CYN application. Details on the pond system are described in Grützmacher *et al.* (2002). In the first experiment (August 2009), we added dissolved CYN with an initial CYN concentration in the supernatant of 2.2 µg L^{-1}. In the second experiment, which was conducted at the beginning of October 2009, we added a complete culture of *Aphanizomenon flos-aquae D11* with an initial concentration of dissolved (i.e. extracellular) CYN of 2.9 µg L^{-1} in the supernatant. In both experiments supernatant and effluent have been sampled and analysed for CYN.

Addition of dissolved CYN. The supernatant showed no decline in CYN concentrations due to (a)biotic processes during the sampling period of four days (data not shown), confirming the previously observed persistence of CYN in water during lab experiments as well as results from other studies (for instance Wörmer *et al.* 2008). In the effluent, neither CYN retention nor degradation could be detected (data not shown). These findings are in agreement with results obtained from the enclosure experiment in the presence of a "schmutzdecke".

Addition of Aphanizomenon flos-aquae D11. Cylindrospermopsin concentrations in the supernatant followed the calculated dilution curve (Figure 5.6), suggesting no CYN elimination due to biotic or abiotic processes in the water (i.e. CYN concentrations only decrease due to dilution of inflowing water). Determination of the biovolume showed complete break-down of the culture (data not shown), presumably due to the drastic change in temperatures (20°C during culture cultivation and 14°C in the pond water). Despite of the release of cell pigments by lysed cells into the water, we could not detect any CYN degradation in the supernatant. These findings are in contrast to observations reported by Chiswell *et al.* (1999) who found CYN degradation in dam water in the presence of cell pigments with half-lives of 11 and 15 days. Results of CYN concentration in the effluent demonstrated no CYN retention and complete CYN recovery, indicating no CYN degradation and hence also no effect of the "schmutzdecke" (data not shown).

Our data obtained from experiments under near-natural conditions revealed contact time to be a crucial parameter in CYN elimination. In addition, preconditioning of sediments was found to be a crucial parameter in CYN degradation. The "schmutzdecke" did not enhance CYN removal.

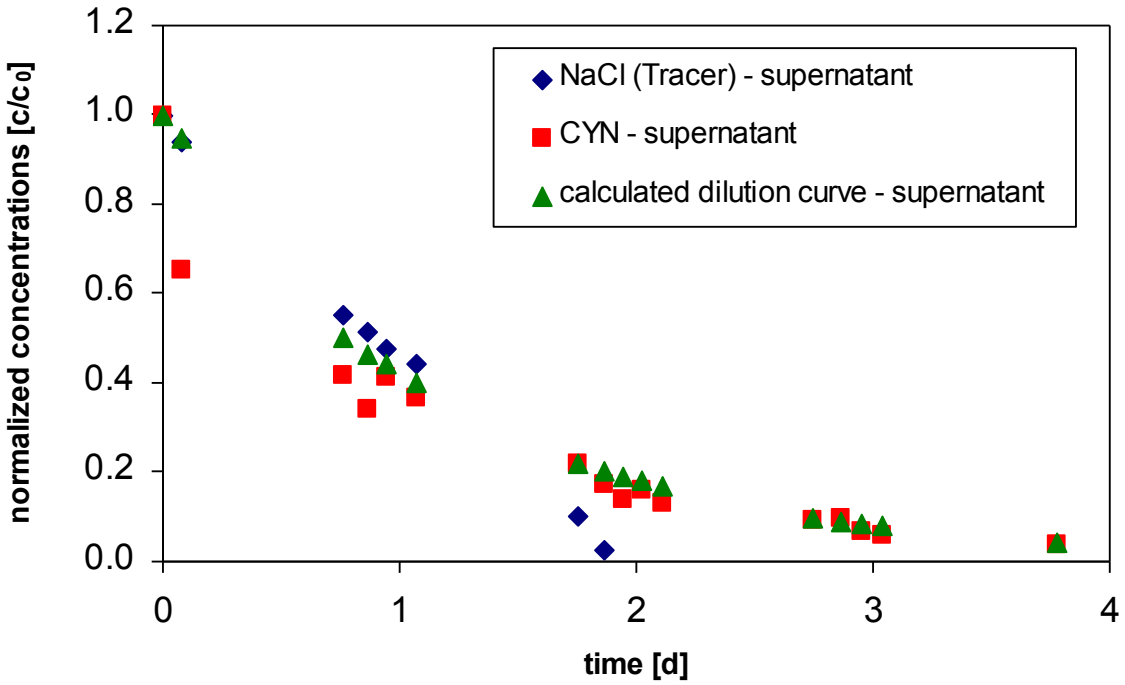

Figure 5.6. Normalized CYN concentrations determined in the supernatant of a filter sand following the addition of an intact culture of *Aphanizomenon flos-aquae D11* (dissolved CYN concentrations in the supernatant: 2.9 µg L^{-1}).

Retention and degradation of anatoxin-a in waters and sediments

Retention of ATX was studied by flow through column experiments using filter sand and surface water obtained from the SIMULAF (for details see above "Retention and Degradation of Cylindrospermopsin ...") with an initial ATX concentration of 19 µg L^{-1}. Results showed ATX to be much stronger retarded than CYN (R = 1.03 ± 3 %) with R-values amounting to 2.4 ± 2 %.

Anatoxin-a degradation under various physico-chemical conditions (in the presence of DOC, at 10°C (as opposed to room temperature) and under (an)aerobic conditions) was studied in batch experiments using field-moist filter sand (mS) and surface water (filtered over a 1.2 µm membrane) obtained from the SIMULAF (see above "Retention and Degradation of Cylindrospermopsin ...") and an initial ATX concentration of 10–15 µg L^{-1}. Anatoxin-a was determined in the aqueous phase of the supernatant. The experiments under anoxic conditions were conducted using anoxic groundwater that has been equilibrated with the filter sand prior to ATX addition. To distinguish between biotic and abiotic degradation ATX dissipation was determined in sterile (i.e. autoclaved) and non-sterile samples. Degradation was quantified by fitting a 1st order exponential degradation curve to the measured, normalized values (c/c_0; see equation (1) below), with y_0 being the residual concentration, A the initial concentration and κ_1 the degradation rate [d^{-1}]). Because of the strong sorption characteristics of ATX, in some cases, degradation was best described by a two-compartment model (equation (2)), which describes degradation processes between two compartments at different rates. In the first compartment, degra-

dation occurs rapidly due to the availability of the substrate to microorganisms. In addition, sorption may contribute to fast initial dissipation. In the second compartment, the toxin,is sorbed to sediment particles and hence degradation is controlled by the rate of desorption into the water phase (Henriksen *et al.* 2004). The two constants a_1 and a_2 express the quantitative partitioning between the two compartments, κ_1 and κ_2 denote degradation rates [d^{-1}]. Modelled parameters describing ATX degradation are summarized and discussed in the respective publication (Klitzke *et al.*, in prep.).

$$C(t) = y_0 + Ae^{-\kappa * t} \quad (1)$$

$$C(t) = a_1 e^{-\kappa_1 * t} + a_2 e^{-\kappa_2 * t} \quad (2)$$

In general, ATX was found to decompose much faster than CYN and without the formation of a lag phase. Photolytic ATX degradation (Stevens and Krieger 1991) could be ruled out as all samples were covered by aluminium foil. Under room temperature, biotic and abiotic degradation took place in both water and sediment. After approx. 28 days, half of the initial concentration (10–15 µg L^{-1}) was eliminated in the water (Figure 5.7 a). In contact with sediment the concentration dropped below 1 µg L^{-1} after 7 days (data not shown). The decrease in ATX concentrations in the sterile sediment samples suggests ATX sorption as an abiotic process for ATX removal (Figure 5.7 b). The pronounced difference between ATX concentrations in the sterile and non-sterile sediment samples indicates microbial degradation (Figure 5.7 b). While ATX degradation in sediment was decelerated in the presence of DOC, there was no change in degradation in the water (data not shown).

At 10°C ATX elimination in the sediment slowed down by approximately a factor of 2 (data not shown). Under anoxic conditions, ATX elimination in sediments was decelerated in a similar order as at 10°C (Figure 5.8). While under oxic conditions it took 2 to 3 days for concentrations in the sediment to drop below 0.2 it took about 20 days under anoxic conditions. This could be due to (i) lower microbial activity as microbes in the sediments needed to adapt to the changed redox conditions and (ii) fewer available sorption sites as reducing conditions would result in dissolution of sesquioxides. The strongly decreasing ATX concentrations in the water may most likely be attributed to a chemical and/or microbial reduction of the contaminant (Figure 5.8), with a plateau forming at $c/c_0 = 0.4$ indicating a possible depletion of potential electron donors. Interestingly, anoxic conditions appear to be more conducive to ATX elimination in the water than oxic conditions.

Our results demonstrated ATX removal in both water and sediments, with higher elimination in sediments than in water. Degradation takes place without a lag phase. Abiotic degradation (i.e. sorption) makes up a substantial part of ATX removal as already shown in our experiments on ATX sorption characteristics (see above “Sorption of CYN and ATX ...”).

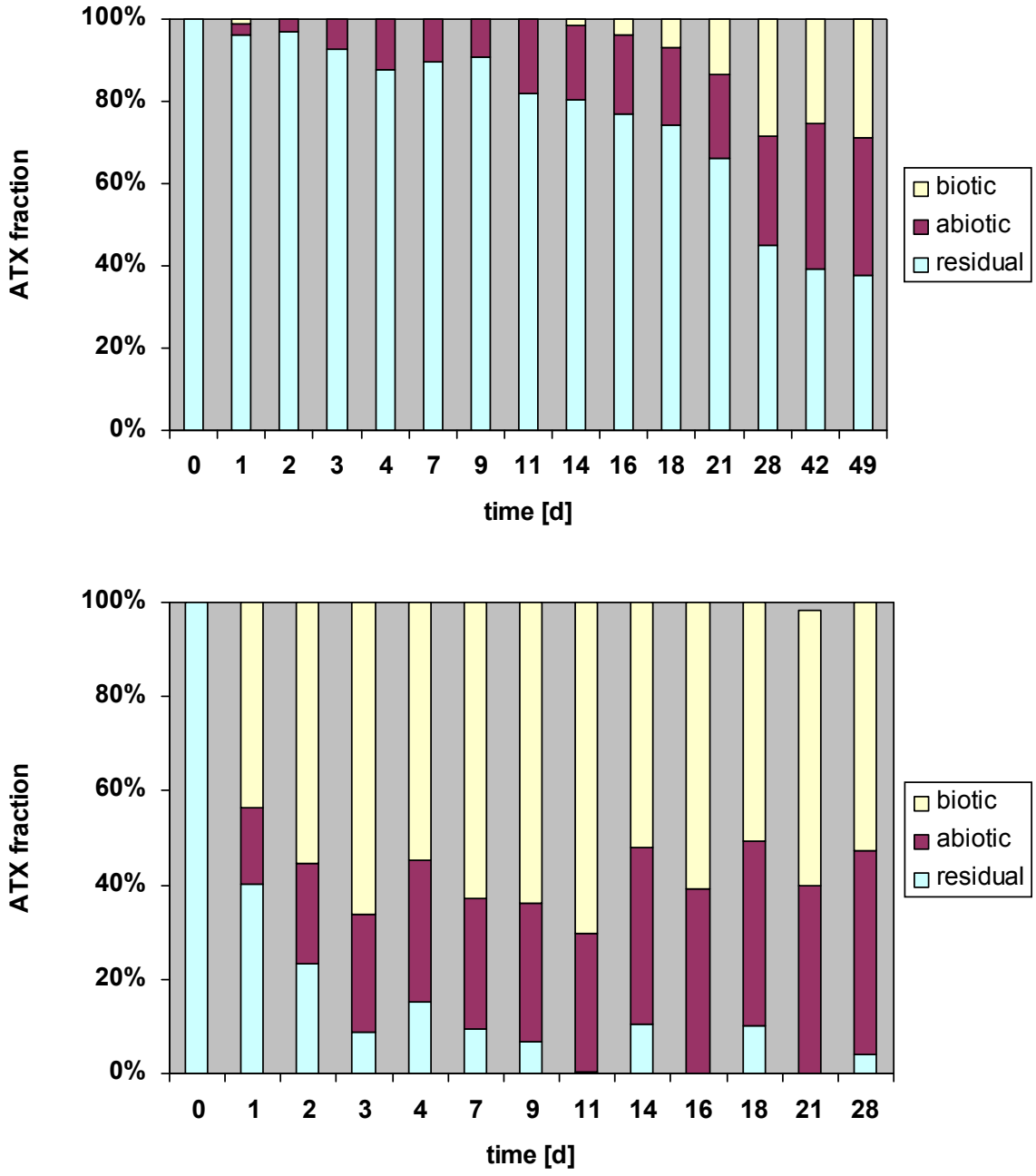

Figure 5.7. Percentage of biotic and abiotic ATX degradation and residual fraction in **(a)** pond water samples and **(b)** samples with sediment contact.

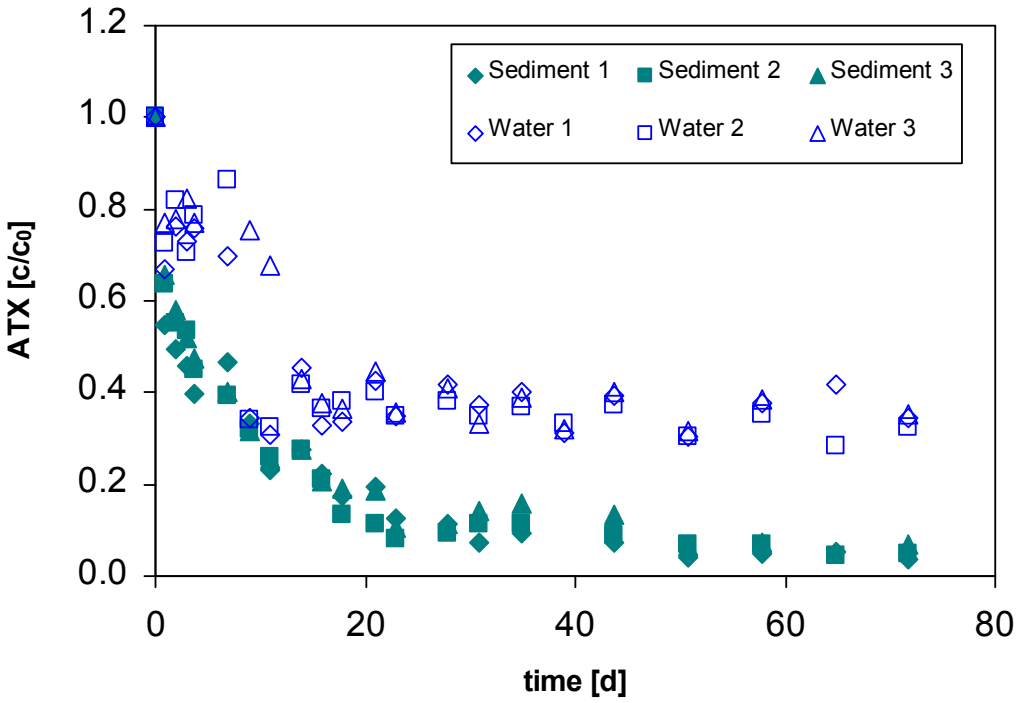

Figure 5.8. Normalized ATX concentrations in batch experiments under anoxic conditions in water and sediment samples.

Conclusions:

Risk assessment of toxin breakthrough during sediment passage

Our results showed sediment passage to be an efficient treatment for the removal of CYN and ATX from surface waters under a range of varying physico-chemical conditions.

Microbial degradation as opposed to sorption was identified as the main elimination pathway for CYN in sandy sediments. Key parameters for CYN removal in sediments are

- sediment preconditioning
- flow rate (i.e. 0.2 m d^{-1}; river bank filtration)
- the presence of dissolved organic carbon
- temperature
- oxic conditions

Cylindrospermopsin will be eliminated if sediments have been preconditioned and at low flow rates (i.e. 0.2 m d^{-1}; river bank filtration). At higher flow rates, shorter contact time with the sediment will not allow for CYN degradation and hence high breakthrough has to be expected. The presence of easily degradable organic carbon substrates delays CYN

degradation. At 10°C, degradation is decelerated by a factor of 10 in comparison to room temperature. Under anoxic conditions, CYN degradation was strongly retarded or does not take place at all. The processes governing CYN elimination under anoxic conditions are not yet fully understood. Even though CYN showed strong sorption onto organic sediments (“organic mud”) there was no CYN retention in the presence of the “schmutzdecke” consisting of plant debris. This suggests the composition of organic matter to be crucial in CYN sorption. Hence, CYN retention may vary significantly for different types of organic matter, especially as a function of degradation state.

Anatoxin-a is less stable in waters and sediments than CYN and eliminated not only through microbial degradation but also through sorption. In addition, sediment preconditioning is not as critical as for CYN. These findings allow for the conclusion of higher removal during sediment passage than for CYN. Further studies are on the way to investigate ATX retention and degradation in a flow setting.

6. Recommendations for water management and decision support systems

6.1 Consequences for public health

Ingrid Chorus

For public health, on the background of findings from previous projects the key outcome of NOSTOTOX is the consolidation – now on the basis of a wide range of samples and years observed – of the state of knowledge on the frequency of occurrence of a comprehensive set of cyantoxin groups and on their concentrations in north-western Germany. For human exposure the key outcomes are:

1. We may expect exposure to cylindrospermopsin (CYN) more frequently than to any other cyanotoxin: CYN occurred in 79 % of all lakes and 61 % of all samples investigated (see Chapter 2.2).
2. Although the frequency of microcystin occurrence has declined somewhat, we may still expect exposure to microcystins (MC) at a frequency almost as high as for CYN (MCs occurred in 71 % of the lakes and in 38 % of the samples).
3. While in some lakes MC-concentrations are decreasing in response to reduced trophic levels, we may still expect the highest cyantoxin concentrations from microcystins: in samples usually taken outside of scum areas, NOSTOTOX found a median of 0.67 µg L^{-1} – i.e. concentrations often in the range of the provisional WHO Guideline value of 1 µg L^{-1} for MC-LR for lifetime consumption of drinking-water (WHO 1998), with concentrations in the range of 10 µg L^{-1} not uncommon. In *Microcystis* scums, we still need to be prepared for concentrations in the range of milligrams per litre.
4. We may usually expect CYN in concentrations lower than those for MC (the NOSTOTOX median for CYN was 0.14 µg L^{-1}, while maxima may be in the same range at those for MC, i.e. several µg L^{-1}; see Chapter 2.2). However, the public health concentrations of regular exposure to CYN at these levels are less clear, as there is no WHO Guideline value to which we can relate these concentrations. On the basis of organ damage observed in a subchronic animal study with mice, Humpage and Falconer (2003) propose a guideline value of 1 µg L^{-1} for CYN in drinking-water, and Sukenik *et al.* (2006) come to a similar conclusion. A number of *in vitro* test results indicate genotoxicity, i.e. cellular mechanisms that suggest CYN may be carcinogenic (Žegura 2011), but whether or not the mechanisms picked up by the *in vitro* assays are relevant for whole mammals is not yet clear.
5. For substances with lacking or insufficient toxicological data for setting a guideline value, UBA's concept for assessing their occurrence in drinking-water (2003a; Dieter 2003) stipulates that an adequate precautionary level is 0.1 µg L^{-1}

if genotoxicity cannot be ruled out. This default value may trigger either a strategy to remove the source of contamination or an incentive to fund the toxicological research necessary for clarification (the result of which may well be "de-warning" and a higher tolerable concentration). Applying this assessment frame to CYN would currently indicate 0.1 µg L^{-1} to be an adequate level until new data either establish a genotoxicity-based guideline value or that CYN is not genotoxic and the TDI-based approach followed by Humpage and Falconer (2003) is adequate. These authors propose a guideline value for CYN of 1 µg L^{-1}.

6. For scums consisting primarily of Nostocales we cannot yet rule out the possibility of high levels of CYN, PSP or ATX. Additionally, in face of the pronounced acute toxicity of PSP and ATX with an onset of neurotoxic effects within minutes, these may present higher risks at lower concentrations. Laboratory culture results indeed show that for some CYN-producing *Aphanizomenon*-strains the CYN-quota per cell can be as high as the MC-quota per cell for MC-producing *Microcystis*- or *Planktothrix*-strains. However, in practice, these have not been observed, and the maximum CYN-concentration found in Germany to date is 12 µg L^{-1} (Rücker *et al.* 2007). Thus, in northwestern Germany, the toxin most likely to cause risks in scums continues to be MC in *Microcystis*-scums.
7. Particularly two candidate mechanism for the lack of very high CYN-concentrations merit further investigation: (i) blooms of *Aphanizomenon* forming heavy surface scums seem to be less common than *Microcystis* scums and (ii) where the population density of *Aphanizomenon* is high, such populations rarely consist chiefly of CYN-producing genotypes, thus rendering the mean cellular CYN quota much lower than that of the CYN-producers. If this can be ascertained as a typical pattern, we could confidently assess the risk of exposure to CYN at levels of mg L^{-1} as low.
8. We need to expect shares of CYN dissolved in the water to be substantially higher than those in cyanobacterial cells. This was the case in almost all NOSTOTOX samples and may explain low frequencies of CYN findings in other studies that did not include the dissolved fraction. In particular, we must expect dissolved CYN throughout autumn and well into winter, long after CYN-producers are no longer present, and findings from Australia indicate that concentrations may be highest above the sediments where conditions for degradation are poor (Everson *et al.* 2009); also Nostocales may accumulate there and release CYN as they are biodegraded. Biodegradation of CYN is apparently much slower than that of MC. Sediment passage may remove CYN effectively (primarily through biodegradation) if conditions are suitable (i.e. oxic and preconditioned sediments), but under some conditions breakthrough is likely to be more pronounced than previously found for MC (see Chapter 5).
9. We can expect exposure to the neurotoxic anatoxin-a (ATX) and saxitoxins (PSP, from paralytic shellfish poisoning) quite often: though their frequency in the NOSTOTOX lakes and samples was lower than that of CYN or MC, they did oc-

cur in about half of the lakes and one fourth of the samples investigated. Maxima found in NOSTOTOX scarcely exceeded 1 µg L^{-1} (see Chapter 2.2.). The scant toxicological evidence available to date does not indicate levels in the range of few µg L^{-1} to be of concern for human health (Fawell *et al.* 2004; Kuiper-Goodman *et al.* 1999), although chronic effects and potential mechanisms have not been sufficiently investigated to rule them out. Also (as for CYN) we cannot rule out high scum concentrations of ATX or PSP because laboratory cultures show that some strains may have high ATX or PSP cell quotas. Moreover, the levels causing acute symptoms are lower for ATX and PSP than for CYN or MC.

10. As for CYN, the fraction of PSP dissolved in water can be substantially higher than the particulate one (see Chapter 2.2), and high levels of dissolved ATX are also not uncommon. For PSP the few published data on the fate of some PSP in the field suggest a slow degradation rate (Jones and Negri 1997), while NOSTOTOX results show a much lower persistence of ATX and PSP as compared to CYN. Also, dissolved ATX proved readily amenable to sorption, chemical breakdown and biodegradation in sediment passage (see Chapter 5). Thus, for obtaining drinking-water by bank filtration, ATX and PSPs seem less likely to pose a health risk than CYN.

For overall cyanotoxin risk assessment and risk management in Germany, these key NOSTOTOX outcomes have the following implications:

For recreational exposure, any heavy cyanobacterial scum can be dangerous if it consists primarily of genotypes with a high cell quota of a cyanotoxin. Scum consumption has lead to animal deaths likely caused by MC, PSP and ATX in other countries (Chorus and Bartram 1999), and for Germany there is (largely unreported) evidence of dog deaths from scum ingestion (Chorus and Rösel 2000). Subacute repeated exposure to high cyanotoxin levels therefore remains an issue for establishing bathing water profiles as required by the EU Bathing Water Directive.

For this aim, to satisfy Article 8 of the EU Bathing Water Directive, the primary focus should remain on levels of total phosphorus, as these determine the amount of cyanobacterial biomass possible (i.e. the "carrying capacity" for phytoplankton biomass). Information on nitrate levels and N:P-ratios may be useful as indicator of potential nostocalean dominance. However, these ratios alone are not a reliable predictor, and where total phosphorus concentrations above 30–50 µg L^{-1} and/or Secchi-disc readings below 1-2 m render high cyanobacterial biomass a likely scenario, bathing sites should be monitored for cyanobacterial mass developments and scums. The UBA guideline for protecting bathing site users from exposure to cyanotoxins (UBA 2003b) calls for warning site users at high cyanobacterial biomass (i.e. biovolume >1 mm^3 L^{-1} or Chlorophyll-a >40 µg L^{-1} with dominance of cyanobacteria) and for temporary site closure at MC-concentrations >100 µg L^{-1}. While this MC-level has been criticised as high in international comparison (Chorus 2005), the NOSTOTOX results show that revision is important to include checking for CYN, ATX or PSPs if high cyanobacterial biomass consists of

Nostocales, with levels for temporary closure yet to be derived from acute exposure data available in the literature.

Exposure through drinking-water would not be to levels of acute or sub-acute relevance for any cyanotoxin, as in Germany drinking-water abstraction from scums is highly unlikely. Furthermore, where drinking-water supplies draw on eutrophic rivers, lakes or reservoirs, in most cases treatment plants will have advanced technologies in place to remove high DOC levels and other contaminants such as pesticides. While site-specific validation of their efficacy against cyanotoxins is important, the current knowledge base for cyanotoxin removal through treatment indicates that if processes are operated properly, the risk of cyanotoxin breakthrough is low.

The lakes studied in NOSTOTOX are not used as drinking-water source, but using the findings to depict a drinking-water scenario indicates the following: With the cyanotoxin concentration ranges found in NOSTOTOX and assuming cyanotoxins to break through bank filtration and/or treatment, maximum concentrations in the finished drinking-water could occasionally reach 1 µg L^{-1} for MC or CYN, but usually would be much lower. A public health assessment of this case would consider that guideline values such as the WHO-value for MC-LR are set to be protective at lifetime daily consumption of 1 litre of water. For almost all substances regulated for drinking-water, short-term exposure to higher concentrations of a given substance scarcely elevates the risk of health impacts, and higher levels can be transiently tolerated (WHO 2003, chapter 2.2.2). Tolerating them for a limited time period may be adequate to allow focussing all efforts on removing the source of contamination (e.g. excessive nutrient loading) rather than investing in less sustainable quick fixes (e.g. upgrading treatment). The German Drinking-water Ordinance therefore allows health authorities to permit an exceedance up to a concentration not causing a health risk at short-term exposure, provided a restoration plan is proposed and being implemented and the population affected is adequately informed (Trinkwasserverordnung 2001). A concentration not causing a health risk at short-term exposure may well be several-fold higher than a guideline value or standard for lifetime exposure through drinking-water and is set for the parameters regulated in the Drinking-water Ordinance by UBA (2003c).

Cyanotoxins typically occur only periodically, and these considerations highlight that health risks from exposure to CYN, ATX or PSPs through drinking-water are unlikely if cyanotoxin concentrations in drinking-water reservoirs do not exceed the concentration patterns found in NOSTOTOX.

On the other hand, this fortunately applies to the concentrations of almost all chemicals regulated in drinking-water: Among the wide range of chemicals (originating from industrial or natural sources, from human dwellings, agricultural activities or drinking-water treatment and installations) for which WHO gives guideline values or the EU Drinking Water Directive (EU DWD) has set standards, very few are actually found frequently in concentrations causing health risks. Nonetheless, for any substance occurring in drinking-water sources more than just occasionally, health authorities and water suppliers need guidance on tolerable concentrations, and frequency of occurrence is the key

criterion for deriving guideline values. For MC water suppliers can use the provisional WHO Guideline Value for MC-LR as default value for the sum of al MCs to validate that the control measures they have in place are sufficiently effective, and health authorities can use this value for surveillance.

For CYN, however, health authorities and water suppliers currently lack orientation. Some may use the level of 1 µg L^{-1} proposed by Humpage and Falconer (2003), while others may follow UBA (2003b) and use the value of 0.1 µg L^{-1} in face of evidence for potential genotoxicity. As NOSTOTOX typically found CYN in the concentration range between 0.1 and 1 µg L^{-1}, whether 0.1 or 1 µg L^{-1} can be tolerated in finished drinking-water may be a relevant issue, particularly in countries with a high share of nostocalean cyanobacteria in meso-eutrophic reservoirs.

A key outcome of NOSTOTOX for CYN thus is the demonstration of an urgent need for a guideline value for CYN in drinking-water against which concentrations can be assessed. Such a guideline value might best be placed in the WHO Guidelines for Drinking-water, rather than including a standard for CYN in European or national drinking-water legislation, as the revision of the EU DWD is likely to include the *Water Safety Plan* approach. This approach encompasses the requirement of assessing hazards challenging a given drinking-water supply system against the system's efficacy in controlling them – an approach that provides the necessary flexibility for assessing cyanotoxin risks. In contrast, a regulation requiring compliance to a mandatory standard would require careful definition of monitoring schemes to avoid surveillance responses that would not be adequate for cyanotoxin occurrence.

Whether or not guideline values are needed for PSPs and ATX remains unclear: The working-hypothesis gleaned from the early toxicological study with mice (Fawell *et al.* 1999) that chronic exposure is not likely to be a significant health concern has been upheld for more than 10 years without further evidence emerging to substantiate or disprove it. However, in face of the rather frequent occurrence of ATX and also of PSP, data from assays investigating potential long-term effects, e.g. on neuronal development, would indeed be important. For PSP an assessment of potential impacts of longer-term exposure to low concentrations in drinking-water is totally lacking. Potentially, an analysis of data available from paralytic shellfish poisoning is be a useful way forward to identify concentrations in drinking-water that would be of concern.

In conclusion, the NOSTOTOX results show that for recreational exposure health concerns remain high for MCs, while they are less likely for CYN, PSP and ATX. For drinking-water exposure they show that the levels found do not give rise to immediate concern of health hazards, but due to their frequent occurrence MC, CYN, PSP and ATX certainly require regulatory attention – as has been given to other substances found in drinking-water at similar or lower frequencies.

While ever-increasing lists of standards to monitor in drinking-water are not helpful, the Water Safety Plan approach promoted by WHO (and likely to be incorporated in the revision of the EU DWD) provides a platform for assessing and managing risks from the

hazards that are relevant in the specific supply system. In this context, WHO guideline values for cyanotoxins will be urgently needed to assess cyanotoxin concentrations and to set targets. Toxicological research to fill the data gaps currently precluding a guideline value for CYN is therefore a high priority, followed by research (possibly with sub-organismic test systems) to clarify whether we can uphold the working-hypothesis that sub-acute health risks from ATX and PSP are unlikely.

6.2 Recommendations for assessment and management of lakes

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Introduction

The phytoplankton community is considered to respond first to eutrophication pressures especially in lakes. According to the European Water Framework Directive (WFD; European Commission 2000) surface waters have to reach the good ecological status by 2015. The WFD defines high, good and moderate status for three phytoplankton quality elements that potentially can be used to assess the ecological status of lakes: Phytoplankton abundance and composition; phytoplankton biomass and planktonic bloom intensity and frequency. These three parameters were quantified in the German lake assessment system basing on the biocomponent phytoplankton (Mischke and Nixdorf 2008, Nixdorf *et al.* 2008).

At present, the international process of intercalibration of national methods of the European member states within the geographic groups (GIGs) is executed. Within this process certain thresholds for the concentration of chlorophyll *a* are agreed as a parameter for phytoplankton biomass by the European Commission (2008). The German biomass boundaries for phytoplankton in lakes are in agreement to this European decision results.

Within this chapter the assessment of water quality by the method of LAWA (1999) and the method according to WFD (Mischke and Nixdorf 2008) will be compared, and the reasons for the different evaluation of the same water body by different methods will be discussed. As an outcome of NOSTOTOX we show a different approach to consider cyanobacterial taxa in the trophic gradient and compare it to the WFD method. Finally, suggestions for lake management will be given exemplary for a model region, i.e. the Scharmützelsee region.

Methods

The German lake assessment system basing on the biocomponent phytoplankton. To carry out assessment, three to four metrics have to be used in the German system: total phytoplankton "biomass", "algal classes" and phytoplankton indicator taxa in a special lake index (PTSI). The results of each metric are combined by using lake-specific weighting factors to average the final lake index "Phyto-See-Index" (PSI), reflecting the quality

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status in the water body. Assessment requires specific reference conditions for each lake type which were developed also by paleolimnological investigations (Nixdorf *et al.* 2006).

Cyanobacterial taxa are considered in German lake assessment system in the algal class metric and as by indicator taxa in the metric PTSI. The biovolume of all cyanobacteria is assessed directly as a summer mean (July–October) and should be below 3.5 $mm^3 L^{-1}$ for good status in polymictic lowland lakes, and below 2.0 $mm^3 L^{-1}$ in stratified lowland lakes. Further algal groups (chyso-, chloro- and dinophytes) are assessed to produce the final score of the algal class metric.

In PTSI metric, cyanobacteria taxa indicate mainly bad and poor ecological status at eu- and hypertrophic conditions for Nostocales and Oscillatoriales and better ecological status for Chroococcales, except of the genera *Woronichinia* and *Merismopedia* and some of *Microcystis* species, which also indicate bad status. Examples for trophic anchor values (TAW) of the 59 indication taxa within the cyanobacteria are listed in Table 6.1 for polymictic and stratified natural lowland lakes according to the distribution of these taxa across a trophic gradient. These trophic scores are derived from the trophic index according to LAWA (1999; compare Chapter 1.1) as the pressure scale. The LAWA index is a trophic parameter allocating different weighted values of chlorophyll *a* concentration, Secchi depth and TP concentration within one trophic index in Germany. The LAWA index is not an element of the phytoplankton assessment, but the trophic anchor values (TAW) of the indicator taxa are calibrated in the same index range from oligotrophic to polytrophic (see Mischke and Nixdorf 2008). The TAW values of each indicator species contributes to the weighted average of the metric PTSI.

Because of the different reference conditions, the ecological meaning of the TAW values differs between the lake types: In stratified lowland lakes a TAW value below 2.5 indicates a good status (mesotrophic; Type 10, 13 (lake types according to Mathes *et al.* 2002) and a TAW value above 3.0 already a poor or bad status (eutrophic). For phytoplankton of polymictic lakes a TAW value below 3 is still a good status (slightly eutrophic; Type 11.1 / 11.2) and a TAW value only above 4 presents already a bad status (polytrophic).

The NOSTOTOX approach. Whereas the WFD method considers the absolute biomass and the presence of certain cyanobacterial taxa, in the NOSTOTOX project we also analysed its contribution to the total cyanobacterial biovolume (compare Chapter 1.2). The analysis of cyanobacterial community composition was based on the same dataset used in Chapter 1.2, but here we investigated the mean values for August and September reflecting stable mid summer phytoplankton population. All lakes were summarized irrespective of their morphometry. The area of probable occurrence of cyanobacterial taxa or groups in the trophic (TP) gradient was illustrated by Gauss fit functions (Figure 6.2).

Table 6.1. Selected indicator taxa within the cyanobacteria for polymictic (TAW_poly) and stratified lowland lakes (TAW_strat) according to the trophic anchor values (TAW) for polymictic lakes (redrawn from Mischke *et al.* 2008). The resulting ecological status is given as an example for some TAW values. (ff Oscill = fine filamentous Oscillatoriales)

Taxon name (Cyanobacteria taxa selected)	Cyano-bacteria group	TAW_poly German lake types 11.1 / 11.2 / 14	TAW_strat German lake type 10.1 & 13
Anabaenopsis elenkinii	Nostocales	6.12	4.72
Anabaenopsis arnoldii	Nostocales	6.12	4.72
Anabaena viguieri	Nostocales	6.12	
Anabaena compacta	Nostocales	6.11	
Planktolyngbya contorta	ff Oscill	6.05	
Limnothrix planctonica	ff Oscill	5.40	4.20
Woronichinia compacta	Chroococcal.	5.22	
Anabaena circinalis	Nostocales	5.20	3.25
Planktolyngbya limnetica	ff Oscill	5.18	3.51
Woronichinia naegeliana	Chroococcal.	5.16	2.66
Merismopedia tenuissima	Chroococcal.	5.13	1.56
Merismopedia punctata	Chroococcal.	5.13	1.56
Merismopedia minima	Chroococcal.	5.13	1.56
Merismopedia glauca	Chroococcal.	5.13	1.56
Merismopedia elegans	Chroococcal.	5.13	1.56
Planktothrix agardhii		5.03 (bad)	4.14 (bad)
Cylindrospermopsis raciborskii	Nostocales	4.97	4.80
Limnothrix redekei	ff Oscill	4.68	4.04
Pseudanabaena limnetica	ff Oscill	4.64	3.94
Anabaena spiroides	Nostocales	4.50	
Microcystis wesenbergii	Chroococcal.	4.25	
Aphanizomenon issatschenkoi	Nostocales	3.88	4.17
Aphanizomenon gracile	Nostocales	3.88 (poor)	3.38 (bad – poor)
Aphanizomenon flos-aquae	Nostocales	3.88	
Oscillatoria trichoides	ff Oscill	3.45	
Gomphosphaeria natans	Chroococcal.	3.26	
Gomphosphaeria aponina	Chroococcal.	3.26	
Anabaena crassa	Nostocales	3.00 (good – moderate)	
Microcystis viridis	Chroococcal.	2.36	2.50
Microcystis aeruginosa	Chroococcal.	2.28	3.06
Anabaena lemmermannii	Nostocales		1.50 (high)

The model region. The catchment Scharmützelsee region (area 393 km², runoff 2.54 L m^{-2} s^{-1}), population density 58 inhabitants km^{-2}) is intensively used for tourism, fishery, nature protection and has its own drinking water supply and waste water treatment (3000 m^3 d^{-1}). Land cover is the following: 51 % forest, 32 % agricultural, 10 % surface waters with about 20 lakes and 6 % urban areas. Main point sources of nutrient input have drastically decreased or even stopped since 1990. Concentrations of TP and TN have been reduced by about 50 % in most lakes since 1990 (compare Chapter 1.1).

Results

As an example for improvement of water quality we showed the development of trophic state in lakes of the Scharmützelsee region since 1994 in Chapter 1.1. Although the trophic index according to LAWA (1999) indicates that most stratified lakes reach already the potential natural state according to morphometry in 2009 the assessment method based on the biocomponent phytoplankton (Mischke and Nixdorf 2008) reveals only moderate water quality as it is shown for Lake Scharmützelsee in Figure 6.1.

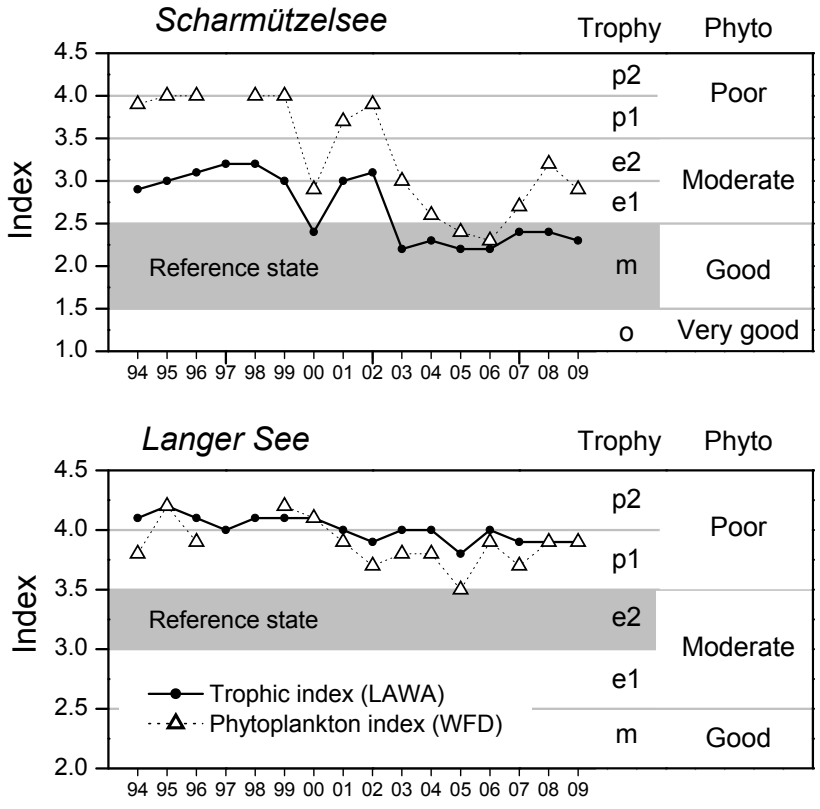

Figure 6.1. Comparison of trophic index according to LAWA (1999; Trophy; black dots) and phytoplankton based index according to EU Water Framework Directive (Phyto; open triangles) for lakes Scharmützelsee (above) and Langer See (below) from 1994–2009. Abbreviations give the trophic state, the grey area the reference state (morphometry) according to LAWA (m=mesotroph, e=eutroph, p=polytroph). “Good”, “moderate” and “poor” represent the assessment according to EU-WFD (Mischke and Nixdorf 2008).

The course of both indices goes parallel. Contrasting to this, in Lake Langer See both indices are in good agreement (Figure 6.1). Based on the rigorous assessment system according to WFD more than half of the 20 lakes in the catchment of Scharmützelsee region are at risk (Table 6.2) and will not reach good ecological water quality by 2015.

One important result of the NOSTOTOX project was the analyses of trophic behaviour of main cyanobacterial taxa and groups. In Figure 6.2 the contribution of different cyanobacteria to total cyanobacterial biovolume depending on TP concentration in the mixed column (epilimnion or mean water depth in shallow waters) in August and September is shown. Gauss fit functions for 56 lake-years from 18 lakes of different trophic state and morphometry was applied. Some taxa were grouped, like fine filamentous Oscillatoriales = ffOscill. (*Pseudanabaena limnetica, Limnothrix redekei, Planktolyngbya limnetica, Limnothrix planctonica*), *Anabaena*, *Chroococcales*.

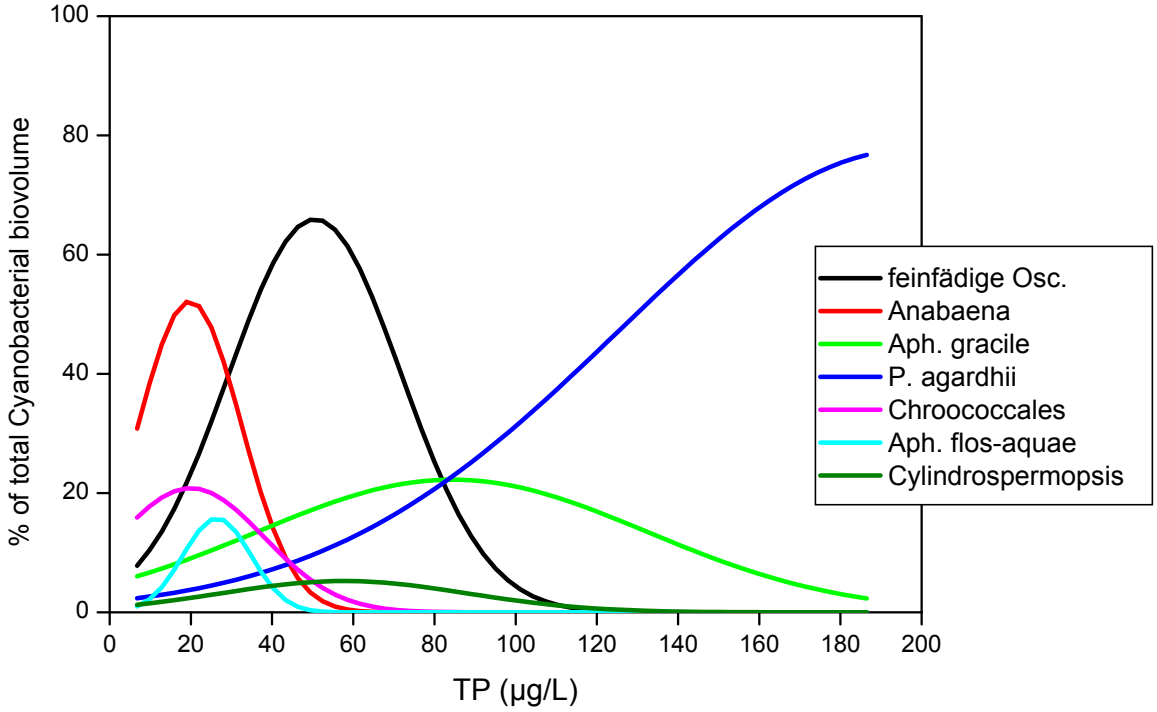

Figure 6.2. Contribution of different cyanobacteria to total cyanobacterial biovolume in August and September depending on TP concentration. Gauss fit functions for 56 lake-years from 18 lakes of different trophic state and morphometry. Fine filamentous Oscillatoriales (feinfädige Osc. = ff Oscill in Table 6.2) and all *Anabaena* species are grouped.

Project results are reflecting the following ranking according to trophy (from higher to lower TP and TN, last not shown here):

Planktothrix agardhii > *Aphanizomenon gracile* > fine filamentous Oscillatoriales > *Aphanizomenon flos-aquae* > *Anabaena*.

This is partly in contradiction to the results of the German assessment system (see Table 6.1) with the following ranking of taxa and groups:

Anabaenopsis elenkinii, *Anabaenopsis arnoldii*, *Anabaena viguieri* > fine filamentous Oscillatoriales > *Planktothrix agardhii* > *Aphanizomenon gracile* > *Anabaena crassa* & *lemmermannii.*

It is obvious that the taxa *Planktothrix agardhii* and *Aphanizomenon gracile* indicate the highest trophic state according to the project results, but *Aphanizomenon gracile* is characterized by a more moderate TAW in the German assessment system (3.38 for stratified lakes, 3.88 for polymictic lakes). Furthermore, the preference of *Anabaena* to lower trophy found in the NOSTOTOX project, is reflected only by the low TAW values of the species *Anabaena crassa* and *Anabaena lemmermannii*, but the PTSI assessment is much more worst in case of the other *Anabaena* species.

Discussion

Lake assessment. The main reasons for the worse evaluation of water quality by the phytoplankton based method according to WFD in comparison to the LAWA-method based on trophic parameters are the “bad” trophic anchor values for different cyanobacterial taxa. Another cause for these discrepancies can be found in the different available data sets. For example, the rarity of the Chroococcales *Woronichinia*, *Merismopedia* and some of *Microcystis* species in the NOSTOTOX data set may have caused the different TP-optima of the Chroococcales. Due to rare occurrence *Anabaena* species were summarized. Here further data collection is necessary for both studies. In case of *Aphanizomenon gracile* and *Anabaena* species modifications of the assessment system should be discussed and will be recommended according to the results of the project. Some species like *Aphanizomenon flos-aquae* are missing in the dataset for stratified lakes (WFD). There are significant gaps of knowledge from which the most notable are:

- reference conditions in different lake types
- threshold concentrations for high/good and good/moderate boundaries
- taxonomic indicators for measuring impacts of nutrient pressures
- establishment of supporting physicochemical conditions
- effect of seasonal variability on classification schemes
- ecological impact of nitrogen conditions (REBECCA 2005).

The important ecological question how the occurrence of toxic bloom forming cyanobacteria can be evaluated from an ecological point of view cannot be answered with the applied monitoring approaches. Recommended sampling procedure is 4 to 6 times during vegetation period (April to October or March to November, see Nixdorf *et al.* 2010). Up to now, neither blooms nor production of toxins can be quantified according to their ecological importance. Blooms of floating cyanobacteria (scums) are episodic events which can be observed and described, but not quantified during routine sampling. The ability of toxin production is a natural property of certain cyanobacteria and is not in-

duced by high trophic state or other anthropogenic pressure. The only way to minimize the risk of high toxin production is to reduce trophic pressure.

Very recently the EU-project WISER has made a suggestion how algal blooms can be reflected by evenness metric and by the total biovolume of cyanobacteria (Mischke *et al.* 2011). Central Baltic and Eastern Continental GIG have suggested that their assessment systems do represent the quality element as a whole and that there is no need for a more specific bloom metric (assessment systems include bloom metrics indirectly). Some countries have a cyanobacteria bloom metric (NL and BE) and others may consider adopting a bloom metric based on either % or absolute abundance of cyanobacteria.

Lake management. The risk of moderate to bad water quality for lakes in the Scharmützelsee region in particular and for lakes in the German lowlands in general is enhanced by effects of climate change esp. for shallow lakes concerning

a) water quantity by higher water level fluctuations due to lower discharge in dry summers and

b) water quality by timing and intensity of (toxic) cyanobacterial blooms, macrophyte mass developments and siltation.

For deep lakes, internal loading and high diffuse nutrient imports persist or gain relevance. The application of the MONERIS model for the quantification of diffuse loading in the Scharmützelsee region revealed that the main import is via groundwater and atmospheric deposition. The model allows scenario calculations to access the influence of changes in landuse and hydrology on nutrient import.

To counteract this eutrophication risk different complex measures of stabilizing water budget in the catchment and to influence matter and water retention mechanisms in the catchment and in the water bodies will be presented and compared. Water quantity management will include discharge of purified waste water into the very shallow lakes with water deficiencies, and the regulation and storage of water in the lakes, esp. using advantages of water connectivity in lake chains. Water quality can be influenced by nutrient retention in constructed wetlands, cropping of macrophytes and biomanipulation as well as other lake restoration measures. Suggested measures are summarized in Table 6.2.

For all lakes in the model area we observed that lake recovery from highly eutrophic conditions is delayed by ecological resistance to decrease of nutrient loading and P hysterese. Beside effects of global warming and more consideration of nitrogen as a potential limiting factor the main management strategy is focused on stabilization of lake ecosystems to get long-term success of restoration measures. “Pushing” of lakes (Jeppesen *et al.* 2007a, b) is recommended applying different lake type specific techniques (biomanipulation, flushing, precipitation). These methods include a drastical change of ecosystem conditions with consequences for the phytoplankton regime. One example for a climatic induced “pushing” of the food web was observed in Lake Lebbiner See after the strong winter 1995/96 when oxygen depletion under ice killed all fish. Zooplankton was very successful under these conditions and cyanobacteria disappeared for several years.

Table 6.2. Lakes "at risk" (not to achieve or maintain good ecological quality according to WFD, European Commission 2000) and measures to maintain or improve ecological quality.

Lakes		**Risk**	**Measures** **to maintain, stabilize or improve ecological quality**
deep lakes			
Tiefer See	TIE	No	(biomanipulation to stabilize good status)
Scharmützel-see	RIE	medium	improved agricultural practice support macrophytes, decrease nutrients & regulate N/P in the catchment, favour macrophytes or "sit and wait"…
Springsee	SPR	high	Al-precipitation, forced circulation, hypolimnetic withdrawl
Großer Glubigsee	GGL	medium	dredging – creation of sediment traps; favour macrophytes
Storkower See (N)	STNO	medium	Al-precipitation, decrease nutrients & regulate N/P in the catchment, favour macrophytes
Wolziger See	WOL	medium	use of waste water effluent after further treatment (surface flow constructed wetland); sediment traps
shallow lakes			
Kleiner Glubig-see	KGL	unknown	
Storkower See (S)	STSU	low	
Lebbiner See	LEB	high	dilution, biomanipulation, surface flow constructed wetland
Petersdorfer See	PET	high	control of input sources; Al-precipitation, food web control
Melangsee	MEL	high	flushing, dredging, food web control, "push"
Langer See	LAN	high	no measure – depends on quality of upward lakes of the chain
Groß Schaue-ner See	GSK	high	withdrawl of surface and treated waste water and filling up

Bibliography

Publications with NOSTOTOX results

In preparation

Wiedner, C., Wagner, C., Rücker, J., Jöhnk, K., Nixdorf, B.: Impact of global warming and trophic status on the predominance of different cyanobacteria taxa in different types of lakes in northern Germany.

Klitzke, S., Apelt, S., Fastner, J., Chorus, I.: Degradation of anatoxin-a in water and sediment.

Klitzke, S., Apelt, S., Fastner, J., Chorus, I.: Elimination of cylindrospermopsin during sediment passage under near-natural conditions.

Mehnert, G., Rücker, J., Nicklisch, A., Leunert, F., Wiedner, C.: Thermal and photoacclimation of lipophilic pigments in an invasive and a native cyanobacterium of temperate regions.

Knie, M., Kleeberg, A., Rücker, J.: Benthic mat counteracts recruitment of pelagic Nostocales from sediment akinetes.

Submitted

Ballot, A., Ramm, J., Rundberget, T., Hadas, O., Sukenik, A., Wiedner, C.: Occurence of non-toxic *Aphanizomenon ovalisporum* and *Anabaena bergii* strains in Lake Kinneret (Israel). (submitted to J. Plankton Res.)

Cirés, S., Wörmer, L., Timón, J., Wiedner, C., Quesada, A.: Cylindrospermopsin production and release by the potentially invasive cyanobacterium *Aphanizomenon ovalisporum* under temperature and light gradients. (submitted to Harmful Algae)

Klitzke, S., Fastner, J.: Cylindrospermopsin degradation in sediments – The role of temperature, redox conditions, and dissolved organic carbon. (submitted to Water Research)

In press

Apelt, S., Fastner, J., Klitzke, S. (2011): Einflussfaktoren auf die Dauer der Lagphase bei der Elimination von Cylindrospermopsin (CYN) im Sediment. Erweiterte Zusammenfassung der Jahrestagung der DGL 2010 in Bayreuth. Eigenverlag der DGL, in press.

Beusch, C., Fastner, J., Klitzke, S. (2011): Sorption der Cyanobakterientoxine Cylindrospermopsin und Anatoxin-a an verschiedenen Sedimenten. Erweiterte Zusammenfassung der Jahrestagung der DGL 2010 in Bayreuth. Eigenverlag der DGL, in press.

Published

2011

Jöhnk, K. D., Brüggemann, R., Rücker, J., Luther, B., Simon, U., Nixdorf, B., Wiedner, C. (2011): Modeling life cycle and population dynamics of Nostocales (cyanobacteria). Environ. Modeling Software 26: 669-677.

Klitzke, S., Beusch, C., Fastner, J. (2011): Sorption of the cyanobacterial toxins cylindrospermopsin and anatoxin-a to sediments. Water Research, 45, 1338-1346.

2010

Ballot, A., Fastner, J., Lentz, M., Wiedner, C. (2010): First report of anatoxin-a-producing cyanobacterium *Aphanizomenon issatschenkoi* in northeastern Germany. Toxicon 56(6): 964-971.

Ballot, A., Fastner, J., Wiedner, C. (2010): Paralytic shellfish poisoning toxin-producing cyanobacterium *Aphanizomenon gracile* in northeast Germany. Appl. Environ. Microbiol. 76(4): 1173-1180.

Baptista, M., Rücker, J., Brüggemann, R., Nixdorf, B. (2010): Seasonal succession and productivity determine phytoplankton diversity in lakes. Erweiterte Zusammenfassung der Jahrestagung der DGL 2009 in Oldenburg. Eigenverlag der DGL: 201-205.

Kaplan-Levy, R. N., Hadas O., Summers M. L., Rücker J., Sukenik, A. (2010): Akinetes: dormant cells of cyanobacteria. In: Lubzens, E., Cerdà, J., Clark, M. (eds.): Dormancy and resistance in harsh environments. Topics in Current Genetics, Vol. 21. Springer-Verlag. ISBN 978-3-642-12421-1: 5-28.

Klitzke, S., Apelt, S., Weiler, C., Fastner, J., Chorus, I. (2010): Retention and degradation of the cyanobacterial toxin cylindrospermopsin in sediments. The role of sediment preconditioning and DOM composition. Toxicon 55: 999-1007.

Knie, M., Rücker, J., Kleeberg, A. (2010): Trägt die Resuspension von Sediment zur Besiedlung des Pelagials durch nostocale Cyanobakterien bei? Erweiterte Zusammenfassung der Jahrestagung der DGL 2009 in Oldenburg. Eigenverlag der DGL: 206-210.

Mehnert, G., Leunert, F., Cirés, S., Jöhnk, K., Rücker, J., Nixdorf, B., Wiedner, C. (2010): Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. J. Plankton Res., 32:1009-1021.

Nixdorf, B., Hoehn, E., Riedmüller, U., Mischke, U., Schönfelder, I. (2010): Probenahme und Analyse des Phytoplanktons in Seen und Flüssen zur ökologischen Bewertung gemäß der EU-WRRL (III-4.3.1). Handbuch Angewandte Limnologie - 27. Erg.Lfg. 4/10: 1-24.

2009

Anu Mbunya, C., Mummert, S., Nixdorf, B., Rücker J. (2009): Invasive versus native Nostocales – Germination experiments with akinetes of *Cylindrospermopsis raciborskii* and *Aphanizomenon* spp. in natural sediment samples. Erweiterte Zusammenfassung der Jahrestagung der DGL 2008 in Konstanz. Eigenverlag der DGL, 364-368.

Klitzke, S., Apelt, S., Chorus, I. (2009): Retardation und Abbau von Cylindrospermopsin in der Sedimentpassage. Erweiterte Zusammenfassung der Jahrestagung der DGL 2008 in Konstanz. Eigenverlag der DGL: 445-448.

Klitzke, S., Apelt, S., Weiler, C., Fastner, J., Chorus, I. (2009): Fate of the cyanotoxin cylindrospermopsin in sediments. Geochimica et Cosmochimica Acta 73, No. 13S: A669.

Mischke, U., Nixdorf, B. (2009): Was wäre wenn: Phytoplankton-Bewertung nach EU-WRRL und Klimawandelszenarios. Erweiterte Zusammenfassung der Jahrestagung der DGL 2008 in Konstanz. Eigenverlag der DGL: 231-235.

Nixdorf, B., Rücker, J., Deneke, R., Grüneberg, B. (2009): Gewässer im Klimastress? Eutrophierungsgefahr in Seen am Beispiel der Scharmützelseeregion. Forum der Forschung 22: 99-106, Eigenverlag der BTU Cottbus, ISSN: 0947-6989.

Nixdorf, B., Vedder, F., Poltz, J., Mietz, O. (2009): Ressourceneffizienz (Chla/TP) des Phytoplanktons und Lichtregime in Flachseen. Erweiterte Zusammenfassung der Jahrestagung der DGL 2008 in Konstanz. Eigenverlag der DGL: 236-241.

Preussel, K., Wessel, G., Fastner, J., Chorus, I. (2009): Response of cylindrospermopsin production and release in *Aphanizomenon flos-aquae* (Cyanobacteria) to varying light and temperature conditions. Harmful Algae 8(5): 645-650.

Rücker, J., Tingwey, E. I., Wiedner, C., Mbunya Anu, C., Nixdorf, B. (2009): Impact of the inoculum size on the population of Nostocales cyanobacteria in a temperate lake. J. Plankton Res. 31: 1151-1159.

Wiedner, C., Rücker, J., Lenz, M., Nixdorf, B. (2009): Tropische Cyanobakterien in Deutschen Gewässern – Der Sprung ins kalte Wasser. Beiträge zum 5. Stechlinforum: Ökologische Konsequenzen auf Klimastress: 49-54.

2008

Wiedner, C., Rücker, J., Nixdorf, B., Chorus, I. (2008): Tropische Cyanobakterien in Deutschen Gewässern: Ursachen und Konsequenzen. In: Lozán, J. L., Graßl, H., Jendritzky, G., Karbe, L., Reise, K. (Hrsg.): Warnsignal Klima: Gesundheitsrisiken, Gefahren für Pflanzen, Tiere und Menschen: 253-256.

Literature

Ballot, A., Fastner, J., Wiedner, C. (2010a): Paralytic shellfish poisoning toxin-producing cyanobacterium *Aphanizomenon gracile* in northeast Germany. Appl. Environ. Microbiol. 76(4): 1173-1180.

Ballot, A., Fastner, J., Lentz, M., Wiedner, C. (2010b): First report of anatoxin-a-producing cyanobacterium *Aphanizomenon issatschenkoi* in northeastern Germany. Toxicon 56(6): 964-971

Banker, R., Carmeli, S., Hadas, O., Teltsh, B., Porat, R., Sukenik, A. (1997): Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from Lake Kinneret. Journal of Phycology 33: 613-616.

Boyer, G. L. (2008): Cyanobacterial toxins in New York and the lower Great Lakes ecosystems. Advances in Experimental Medicine and Biology 619: 153-165.

Brüggemann, R., Carlsen, L. (2006): Partial order in environmental sciences and chemistry. Springer, Heidelberg, pp. 406.

Brüggemann, R., Halfon, E., Welzl, G., Voigt, K., Steinberg, C. (2001): Applying the concept of partially ordered sets on the ranking of near-shore sediments by a battery of tests. Journal of Chemical Information and Computer Sciences 41: 918-925.

Bumke-Vogt, C., Mailahn, W., Chorus, I. (1999): Anatoxin-a and neurotoxic cyanobacteria in German lakes and reservoirs. Environ. Toxicol. 14(1): 117-125.

Carmichael, W. W.,Evan, W. R. ,Yin, Q. Q.,Bell, P., Modczydlowski, E. (1997): Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. Appl. Environ. Microbiol. 63: 3104-3110.

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(1): 155-161.

Chorus, I. (ed., 2001): Cyanotoxins: ocurrence, causes, consequences. Springer, Berlin, Heidelberg: 357 pp.

Chorus, I. (2005): Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries. WaBoLu Heft 02/05: 117 pp.

Chorus, I., Bartram, J. (eds., 1999): Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. Published on behalf of WHO by E & FN Spon / Chapman & Hall, London: 416 pp. Full text download: http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/

Chorus, I., Bumke-Vogt, C., Lindenschmidt, K.-E., Jamie, E. (2001): Cyanobacterial neurotoxins. In: Chorus, I. (ed.): Cyanotoxins: occurrence, causes, consequences. Springer, Berlin, Heidelberg: 37-45.

Chorus, I., Rösel, F. (2000): Toxische Cyanobakterien können für Hunde tödlich sein. Tierärztliche Praxis, Ausgabe Kleintiere und Heimtiere 28(4): 216.

De' Ath, G., Fabricius, D. K. E. (2000): Classification and regression trees: A powerful yet simple technique for ecological data analysis. Ecology 81: 3178-3192.

Dell'Aversano, C., Eaglesham, G. K., Quilliam, M. A. (2004): Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. J. Chromatogr. A 1028: 155-164.

Deneke, R., Nixdorf, B. (1999): On the occurrence of clear-water phases in relation to shallowness and trophic state: a comparative study. Hydrobiologia 408/409: 251-262.

DEV (1976–2009) (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). WILEY-VCH Verlag GmbH & Co. KGaA, Beuth Verlag GmbH; D11, E5, D9, L16.

Devlin, J., Edwards, O., Gorham, P., Hunter, N., Pike R., Stavric, B. (1977): Anatoxin-a, a toxic alkaloid from A*nabaena flos-aquae* NRC-44 h. Canadian J. Chem. 55: 1367-1371.

Dias, E., Pereira, P., Franca, S. (2002): Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA 31 (CYANOBACTERIA). J. Phycol. 38(4): 705-712.

Dieter, H. H. (2003): Kommentar zur Bewertung der Anwesenheit teil- oder nicht bewertbarer Stoffe im Trinkwasser aus gesundheitlicher Sicht. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 46: 245-248.

Eaglesham, G. K., Norris, R. L. (1999): Use of HPLC-MS/MS to monitor cylindrospermopsin, a blue-green algal toxin, for public health purposes. Environ. Toxicol. 14: 151-154.

EU-WRRL : 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 Europäischen Gemeinschaften L 327 vom 22. Dezember 2000. – EG WRRL

European Commission (2000): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Union L 327, Vol. 43.

European Commission (2008): Commission Decision of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration Exercise (notified under document number C(2008) 6016). Official Journal of the European Union L 332, Vol. 51: 20-44.

Everson, S., Fabbro, L., Kinnear, S., Eaglesham, G., Wright, P. (2009): Distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin in a stratified lake in north-eastern New SouthWales, Australia. Mar. Freshw. Res. 60: 25-33.

Fabbro, L. D.; Andersen, L. E.; Duivenvoorden, L. J. (2004): Critical aspects in the assessment of human health risk associated with cylindrospermopsin producing cyanobacteria – insights from Central Queensland, Australia. Presentation at the 6th Interational Conference on Toxic Cyanobacteria, Bergen, Norway.

Falconer, I. R. (2005). Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins. CRC Press, Boca Raton.

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. Environ. Toxicol. 16: 192-195.

Fastner, J., Erhard, M., Carmichael, W. W., Sun, F., Rinehart, K. L., Rönicke, H., Chorus, I. (1999): Characterization and diversity of microcystins in natural blooms and strains of the genera *Microcystis* and *Planktothrix* from German freshwaters. Arch. Hydrobiol. 145: 147-163.

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., Rücker, J., Stüken, A., Preußel, K., Nixdorf, B., Chorus, I., Köhler, A., Wiedner, C. (2007): Occurrence of the cyanobacterial toxin cylindrospermopsin in northeast Germany. Environ. Toxicol. 22: 26-32.

Fastner, J., Wirsing, B., Wiedner, C., Heinze, R., Neumann, U., Chorus, I. (2001): Microcystins and hepatocyte toxicity. In: Chorus, I. (ed.): Cyanotoxins: occurrence, causes, consequences. Springer, Berlin, Heidelberg: 22-37.

Fawell, J. K., Mitchell, R. E., Hill, R. E., Everett, D. J. (1999): The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. Hum. Exp. Toxicol. 18(3): 168-173.

Grützmacher, G., Böttcher, G., Chorus, I., Bartel, H. (2002): Removal of microcystins by slow sand filtration. Environ. Toxicol. 17(4): 386-394.

Gust, G. (1990): Method of generating precisely-defined wall shear stresses. US Patent Number: 4,973,1651990.

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) Seenaya and Subba Raju isolated form a domestic supply reservoir. Appl. Environ. Microbiol. 50: 1292-1295.

Hedman C. J., Krick, W. R., Karner Perkins, D. A., Harrahy, E. A., Sonzogni, W. C. (2008): New measurements of cyanobacterial toxins in natural waters using high performance liquid chromatography coupled to tandem mass spectrometry. J. Environ. Qual. 8; 37(5): 1817-24.

Hellweger, F. L., Kravchuk, E. S., Novotny, V., Gladyshev, M. I. (2008): Agent-based modeling of the complex life-cycle of a cyanobacterium (*Anabaena*) in a shallow reservoir. Limnology and Oceanography 53: 1227-1241.

Helsel , D. R., Hirsch, R. M. (2002): Statistical methods in water resources techniques of water resources investigations [Internet]. U.S. Geological Survey. Full text download: http://www.practicalstats.com/aes/aesbook/files/HelselHirsch.pdf

Henriksen, T., Svensmark, B., Juhler, R. K. (2004): Degradation and sorption of Metribuzin and primary metabolites in a sandy soil. J. Environ. Qual. 33(2): 619-627.

Hense, I., Beckmann, A. (2006): Towards a model of cyanobacteria life cycle – effects of growing and resting stages on bloom formation of N_2-fixing species. Ecological Modelling 195: 205-218.

Humpage, A. R., Falconer, I. R. (2003): Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. Environ. Toxicol. 18(2): 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 68: 739-753.

IPCC (2007): Summary for policymakers. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L. (eds.): Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jeppesen, E., Meerhoff, M., Jacobsen, B. A., Hansen, R. S., Søndergaard M., Jensen J. P., Lauridsen T. L., Mazzeo, N., Branco, C. (2007a): Restoration of shallow lakes by nutrient control and biomanipulation – the successful strategy varies with lake size and climate. Hydrobiologia 581: 269-285.

Jeppesen, E., Søndergaard, M., Jensen, J. P., *et al.* (2005): Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. Freshwater Biol. 50: 1747-1771.

Jeppesen, E., Søndergaard, M., Meerhoff, M., Lauridsen, T. L., Jensen, J. P. (2007b): Shallow lake restoration by nutrient loading reduction – some recent findings and challenges ahead. Hydrobiologia 584: 239–252.

Jöhnk, K. D. (1991): Analytische und numerische Untersuchungen der Eigenschwingungen des äußeren Erdkerns. Dissertation, TH Darmstadt, 196 p.

Jöhnk, K. D., Brüggemann, R., Rücker, J., Luther, B., Simon, U., Nixdorf, B., Wiedner, C. (2011): Modelling life cycle and population dynamics of Nostocales (cyanobacteria). Environmental Modelling and Software 26(5): 669-677.

Jones, G. J., Negri, A. P. (1997): Persistence and degradation of cyanobacterial paralytic shellfish poisons (PSPs) in freshwaters. Wat. Res. 31: 525-533.

Kaplan-Levy, R. N., Hadas, O., Summers, M. L., Rücker, J., Sukenik, A. (2010): Akinetes: dormant cells of cyanobacteria. In: Lubzens, E., Cerdà, J, Clark, M. (eds.): Dormancy and resistance in harsh environments. Topics in Current Genetics, Vol. 21. Springer-Verlag. ISBN 978-3-642-12421-1: 5-28.

Kellmann, R., Mihali, T. R. K., Jeon, Y. J., Pickford, R., Pomati, F., Neilan, B. A. (2008): Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl. Environ. Microbiol. 74: 4044-4053.

Komárek, J. (2005): Phenotypic diversity of the heterocytous cyanoprokaryotic genus *Anabaenopsis*. Czech Phycol., Olomouc. 5: 1-35.

Komárek, J., Komárkova, J. (2006): Diversity of *Aphanizomenon*-like cyanobacteria. Czech Phycol. 6: 1-32.

Komárková-Legnerová, J., Eloranta, P. (1992): Planktic blue-green algae (Cyanophyta) from central Finland (Jyväskylä region) with special reference to the genus *Anabaena*. Arch. Hydrobiol./Algol. Stud. 67: 103-133.

Kotai, J. (1972): Instructions for preparation of modified nutrient solution Z8 for algae. Publication B-11/69. Norwegian Institute for Water Research, Oslo.

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

Kuiper-Goodman, T., Falconer, I., Fitzgerald, J. (1999): Human health aspects. In: Chorus, I., Bartram, J. (eds.): Toxic cyanobacteria in water: a guide to public health consequences, monitoring and management. Published on behalf of WHO by E & FN Spon / Chapman & Hall, London: 113-154. Full text download: http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/

LAWA (Länderarbeitsgemeinschaft Wasser) (1999): Gewässerbewertung – stehende Gewässer. Vorläufige Richtlinie für eine Erstbewertung von natürlich entstandenen Seen nach trophischen Kriterien. Kulturbuch-Verlag, Berlin. 74 S.

Long, B. M., Jones, G. J., Orr, P. T. (2001): Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. Appl. Environ. Microbiol. 67: 278-283.

Mathes, J., Plambeck, G., Schaumburg, J. (2002): Das Typisierungssystem für stehende Gewässer in Deutschland mit Wasserflächen ab 0,5 km² zur Umsetzung der Wasserrahmenrichtlinie. In: Deneke, R., Nixdorf, B. (Hrsg.): Implementierung der EU-Wasserrahmenrichtlinie in Deutschland: Ausgewählte Bewertungsmethoden und Defizite. Aktuelle Reihe, BTU Cottbus 5/2002: 15-24.

Mazmouz, R., Chapuis-Hugon, F., Mann, S., Pichon, V., Méjean, A., Ploux, O. (2010): Biosynthesis of cylindrospermopsin and 7-epicylindrospermopsin in *Oscillatoria* sp. strain PCC 6506: Identification of the cyr gene cluster and toxin analysis. 76(15): 4943-4949.

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 and Reservoirs: Research and Management 5: 195-205.

Mehnert, G., Leunert, F., Cirés, S., Jöhnk, K. D., Rücker, J., Nixdorf, B., Wiedner, C. (2010): Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. Journal of Plankton Research 32: 1009-1021.

Meriluoto, J. A. O., Spoof, L. E. M. (2008): Cyanotoxins: sampling, sample processing and toxin uptake. In: Hudnell, H. K. (ed.): Cyanobacterial harmful algal blooms: state of the science and research needs. Springer, New York: 483-499.

Messineo, V., Bogialli, S., Melchiorre, S., Sechi, N., Lugliè, A., Casiddu, P., Mariani, M. A., Padedda, B. M., Corcia, A. D., Mazza, R., Carloni, E., Bruno, M. (2009): Cyanobacterial toxins in Italian freshwaters. Limnologica 39(2): 95-106.

Miller, M. J., Hutson, J., Fallowfield, H. J. (2005): The adsorption of cyanobacerial hepatoxins as a function of soil properties. J. Wat Health 3: 339-347.

Mischke, U., Carvalho, L., McDonald, C., Skjelbred, B., Solheim, A. L., Phillips, G., de Hoyos, C., Borics, G., Moe, J., Pahissa, J. (2011): Deliverable D3.1-2: Report on phytoplankton bloom metrics. EU-WISER Projekt (2009–2013): Water bodies in

Europe – Integrative Systems to assess ecological status and recovery. Full text download: http://www.wiser.eu/download/D3.1-2.pdf

Mischke, U., Nixdorf, B. (Hrsg., 2008): Gewässerreport (Nr. 10): Bewertung von Seen mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie. BTUC-AR 2/2008, Eigenverlag BTU Cottbus, ISBN 978-3-940471-06-2.

Mischke, U., Riedmüller, U., Hoehn, E., Schönfelder, I., Nixdorf, B. (2008): Description of the German system for phytoplankton-based assessment of lakes for implementation of the EU Water Framework Directive (WFD). In: Mischke, U., Nixdorf, B. (Hrsg.): Gewässerreport (Nr. 10). BTUC-AR 2/2008, Eigenverlag BTU Cottbus, ISBN 978-3-940471-06-2: 117-146.

Narine, D. R., Guy, R. D. (1981): Interactions of some large organic cations with bentonite in dilute aqueous systems. Clays and Clay Min. 29(3): 205-212.

Nicklisch, A., (1992): The interaction of irradiance and temperature on the growth rate of *Limnothrix redekei* and its mathematical description. Arch. Hydrobiol. 91 (Suppl., Algological Studies 63): 1-18.

Nixdorf, B., Hoehn, E., Riedmüller, U., Mischke, U., Schönfelder, I. (2010): Probenahme und Analyse des Phytoplanktons in Seen und Flüssen zur ökologischen Bewertung gemäß der EU-WRRL (III-4.3.1). Handbuch Angewandte Limnologie – 27. Erg. Lfg. 4/10: 1-24.

Nixdorf, B., Mischke, U., Hoehn, E., Riedmüller, U. (2005): Bewertung von Seen anhand des Phytoplanktons. Limnologie aktuell. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 11: 105-120.

Nixdorf, B., Mischke, U. Hoehn, E., Riedmüller, U. (2006): Leitbildorientierte Bewertung von Seen anhand der Teilkomponente Phytoplankton im Rahmen der Umsetzung der EU-Wasserrahmenrichtlinie. Bad Saarow. Full text download: http://www.laenderfinanzierungsprogramm.de/cms/WaBoAb_prod/WaBoAb/Vorhaben/LAWA/Vorhaben_des_Ausschusses_Oberflaechengewaesser_und_Kuestengewaesser_%28AO%29/OK_5.90/endbericht_1_190805.pdf

Nixdorf, B., Rektins, A., Mischke, U. (2008): Standards and Thresholds of the EU Water Framework Directive (WFD) – Phytoplankton and Lakes, Chapter 26. In: Schmidt, M., Glasson, J., Emmelin, L., Helbron, H. (eds.): Standards and thresholds for impact assessment series: environmental protection in the European Union. Vol. 3: 301-314.

Nixdorf, B., Rücker, J., Deneke, R., Grüneberg, B. (2009): Gewässer im Klimastress? Eutrophierungsgefahr in Seen am Beispiel der Scharmützelseeregion. Forum der Forschung 22: 99-106. Eigenverlag der BTU Cottbus, ISSN: 0947-6989.

Padisák, J. (2003): Estimation of minimum sedimentary inoculum (akinete) pool of *Cylindrospermopsis raciborskii*: a morphology and lifecycle based method. Hydrobiologia, 502: 389-394.

Paerl, H. W., Huisman, J. (2009): Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Reports 1: 27-37.

Pereira, P., Li, R., Carmichael, W. W., Dias, E., Franca S. (2004): Taxonomy and production of paralytic shellfish toxins by the freshwater cyanobacterium *Aphanizomenon gracile* LMECYA40. Eur. J. Phycol. 39: 361-368.

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.

Preußel, K., Wessel, G., Fastner, J., Chorus, I. (2009): Response of cylindrospermopsin production and release in *Aphanizomenon flos-aquae* (Cyanobacteria) to varying light and temperature conditions. Harmful Algae 8(5): 645-650.

Quesada, A., Moreno, E., Carrasco, D., Paniagua, T., Wörmer, L., de Hoyos, C., Sukenik, A. (2006): Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. Eur. J. Phycol. 41: 39-45

Rapala, J., Sivonen, K., Luukkainen, R., Niemelä, S. I., (1993): Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena*-strains – a laboratory study. J. Appl. Phycol. 5: 581-591.

REBECCA (2005): Relationships between pressures, chemical status, and biological quality elements. Analysis of the current knowledge gaps for the implementation of the Water Framework Directive.

Reynolds, C. S. (1992): Eutrophication and the management of planktonic algae: What Vollenweider couldn't tell us. In: Sutcliff, D. W., Jones, J. G. (eds): Eutrophication: Research and application to water supply. Freshwater Biological Association: 4-29.

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

Rücker, J., Stüken, A., Nixdorf, B., Fastner, J., Chorus, I., Wiedner, C. (2007): Concentrations of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes. Toxicon. 50(6): 800-809.

Rücker, J., Tingwey, E. I., Wiedner, C., Anu, C. M., Nixdorf, B. (2009): Impact of the inoculum size on the population of Nostocales cyanobacteria in a temperate lake. Journal of Plankton Research 31: 1151-1159.

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

Saker, M. (2000): Cyanobacterial blooms in tropical North Queensland water bodies. PhD-Thesis at James Cook University (Townsville).

Saker, M. L., Griffiths, D. J. (2000): The effect 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., Nogueira, I. C. G., Vasconcelos, C. M., Neilan, B. A., Eaglesham, G. K., Pereira, P. (2003): First report and toxicological assessment of the cyanobacterium *Cylindrospermopsis raciborskii* from Portuguese freshwaters. Ecotoxicol Environ Safety 55: 243-250.

Sas, H. (ed., 1989): Lake restoration by reduction of nutrient loading. Expectation, experiences, extrapolations. Academia Verlag Richardz, GmbH, St. Augustin.

Scheffer, M. (1998): Ecology of shallow lakes. Chapman & Hall, London, UK.

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.

Smith, E. A., Grant, F., Ferguson, C. M. J., Gallacher, S. (2001): Biotransformations of paralytic shellfish toxins by bacteria isolated from bivalve molluscs. Appl. Environ. Microbiol. 67(5): 2345-2353.

Smith, M. J., Shaw, G. R., Eaglesham, G. K., Ho, L., Brookes, J. D. (2008): Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. Environ. Toxicol. 23(3): 413-421.

Smith, V. H., Schindler, D. W. (2009): Eutrophication sciences: where do we go from here? Trends Ecol. Evol. 24: 201-207.

Søndergaard, M., Jensen, J. P., Jeppesen, E., Møller, P. H. (2002): Seasonal dynamics in the concentrations and retention of phosphorus in shallow Danish lakes after reduced loading. Aquatic Ecosystem Health and Management 5: 19-23.

Stevens, D. K., Krieger, R. I. (1991): Stability studies on the cyanobacterial nicotinic alkaloid anatoxin-A. Toxicon 29(2): 167-179.

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.

Sukenik, A., Rosin, C., Porat, R., Teltsch, B., Banker, R., Carmeli, S. (1998): Toxins from cyanobacteria and their potential impact on water quality of Lake Kinneret, Israel. Israel Journal of Plant Sciences 46: 109-115.

ter Braak, C. J. F., Šmilauer, P. (2002): CANOCO Reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power (Ithaca NY, USA), 500 pp.

Trinkwasserverordnung 2001: Verordnung über die Qualität von Wasser für den menschlichen Gebauch. http://bundesrecht.juris.de/trinkwv_2001/index.html

UBA (2003a): Bewertung der Anwesenheit teil- oder nicht bewertbarer Stoffe im Trinkwasser aus gesundheitlicher Sicht – Empfehlung des Umweltbundesamtes nach Anhörung der Trinkwasserkommission beim Umweltbundesamt. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 46´: 249-251. Full text download: http://www.umweltbundesamt.de/wasser/themen/downloads/trinkwasser/Empfehlung-Nicht-bewertbare-Stoffe.pdf

UBA (2003b): Empfehlung zum Schutz von Badenden vor Cyanobakterien-Toxinen. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 2003 46: 530-538.

UBA (2003c): Maßnahmewerte (MW) für Stoffe im Trinkwasser während befristeter Grenzwert-Überschreitungen gem. § 9 Abs. 6-8 TrinkwV 2001 – Empfehlung des Umweltbundesamtes nach Anhörung der Trinkwasserkommission beim Umwelt-

bundesamt. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 46, 707-710: Full text download: http://www.umweltbundesamt.de/wasser/themen/downloads/trinkwasser/MW-Empfehlung.pdf

Uehlinger, U. (1981a): Experimental studies on the autecology of *Aphanizomenon flos-aquae*. Arch. Hydrobiol. Suppl. 60: 260-288.

Uehlinger, U. (1981b): Zur Ökologie der planktischen Blaualge *Aphanizomenon flos-aquae* in Alpenrandseen. Schweiz. Z. Hydrol. 43: 69-88.

Utermöhl, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9: 1-38.

Vollenweider, R. A., Kerekes, J. (1980): OECD Cooperative programme for monitoring of iInland waters (eutrophication control). Synthesis Report, Paris.

Wagner, C., Adrian, R. (2009): Cyanobacteria dominance – quantifying the effects of climate change. Limnology and Oceanography 54: 2460-2468.

WHO (1998): Guidelines for drinking-water quality. Second edition, addendum to volume 2: Health criteria and other supporting information. World Health Organisation, Geneva.

WHO (2003): Guidelines for drinking-water quality. Third edition. World Health Organisation, 512 pp. http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/

Wiedner, C. (1999): Toxische und nicht-toxische Cyanobakterien in Gewässern der Scharmützelseeregion: Ihr Vorkommen in Gewässern unterschiedlicher Trophie und Morphometrie und Steuermechanismen ihrer Dynamik in polymiktischen Flachseen. Dissertation, Brandenburgische Technische Universität Cottbus.

Wiedner, C., Chorus, I., Fastner, J. (2001): The waterbodies surveyed for cyanotoxins in Germany. In: Chorus, I. (ed.): Cyanotoxins: occurrence, causes, consequences. Springer, Berlin, Heidelberg: 6-21.

Wiedner, C., Nixdorf, B. (2006): Microcystin produzierende Genotypen in Cyanobakterien-Populationen: Ihr Vorkommen, Regulationsmechanismen ihrer Dynamik und ihr Einfluss auf die Variabilität der Microcystinkonzentration. Jahresbericht, BMBF FKZ 0330533.

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., Rücker, J., Brüggemann, R., Nixdorf, B. (2007): Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia 152: 473-484.

Wiedner, C., Rücker, J., Fastner, J., Chorus, I., Nixdorf, B. (2008): Seasonal dynamics of cylindrospermopsin and cyanobacteria in two German lakes. Toxicon 52, 677-686.

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.

Wood, S. A., Rasmussen, J. P., Holland, P. T., Campbell, R., Crowe, A. M. L. (2007): First report of the cyanotoxin anatoxin-a from *Aphanizomenon issatschenkoi* (Cyanobacteria). J. Phycol. 43: 356-365.

Wörmer, L., Cirés, S., Carrasco, D., Quesada, A. (2008): Cylindrospermopsin is not degraded by co-occurring natural bacterial communities during a 40-day study. Harmful Algae 7(2): 206-213.

Wörmer, L., Huerta-Fontela, M., Cirés, S., Carrasco, D., Quesada, A. (2010): Natural photodegradation of the cyanobacterial toxins microcystin and cylindrospermopsin. Environ. Sci. Technol. 44(8): 3002-7.

Žegura, B., Štraser, A, Filipič, M. (2011): Genotoxicity and potential carcinogenicity of cyanobacterial toxins – a review. Mutation Research/Rev. in Mutation Research. DOI:10.1016/j.mrrev.2011.01.002.

Zevenboom, W., Mur, L. R. (1980): N_2-fixing cyanobacteria: why they do not become dominant in Dutch, hypertrophic lakes. Dev. Hydrobiol. 2: 123-130.