



## Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions

Grit Mehnert, Franziska Leunert, Samuel Cirés, Klaus Jöhnk, Jacqueline Rücker, Brigitte Nixdorf, Claudia Wiedner

### ► To cite this version:

Grit Mehnert, Franziska Leunert, Samuel Cirés, Klaus Jöhnk, Jacqueline Rücker, et al.. Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. *Journal of Plankton Research*, 2010, 32 (7), pp.1009. 10.1093/plankt/FBQ033 . hal-00585776

**HAL Id: hal-00585776**

**<https://hal.science/hal-00585776>**

Submitted on 14 Apr 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions

Journal:	<i>Journal of Plankton Research</i>
Manuscript ID:	JPR-2010-021.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	06-Mar-2010
Complete List of Authors:	Mehnert, Grit; Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Limnology of Stratified Lakes Leunert, Franziska; Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Limnology of Stratified Lakes Cirés, Samuel; Universidad Autónoma de Madrid, Departamento de Biología Jöhnk, Klaus; Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Limnology of Stratified Lakes Rücker, Jacqueline; Brandenburg University of Technology, Chair of Freshwater Conservation Nixdorf, Brigitte; Brandenburg University of Technology, Chair of Freshwater Conservation Wiedner, Claudia; Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Limnology of Stratified Lakes
Keywords:	Cylindrospermopsis raciborskii, Aphanizomenon ovalisporum, climate change, competition, ecophysiology



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Competitiveness of invasive and native cyanobacteria from temperate  
freshwaters under various light and temperature conditions**

Grit Mehnert<sup>a,\*</sup>, Franziska Leunert<sup>a</sup>, Samuel Cirés<sup>c</sup>, Klaus Jöhnk<sup>a</sup>, Jacqueline Rücker<sup>b</sup>, Brigitte  
Nixdorf<sup>b</sup> and Claudia Wiedner<sup>a</sup>

<sup>a</sup> Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Limnology of  
Stratified Lakes, Alte Fischerhütte 2, D-16775 Stechlin, Germany

<sup>b</sup> Brandenburg University of Technology, Chair of Freshwater Conservation, Seestraße 45, D-15526  
Bad Saarow, Germany

<sup>c</sup> Departamento de Biología, C/Darwin, 2, Universidad Autónoma de Madrid, 28049 Madrid, Spain

\* Corresponding author. Tel.: +49-33082-69922; fax: +49-33082-69917  
E-mail address: g.mehnert@igb-berlin.de

**Keywords:** *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, climate change,  
competition, ecophysiology

## Abstract

Some tropical cyanobacteria have spread to temperate freshwaters during the last decades. To evaluate their further development in temperate lakes we studied the temperature, and light, dependent growth of three invasive (*Cylindrospermopsis raciborskii*, *Anabaena bergii*, *Aphanizomenon aphanizomenoides*) and three native (*Aphanizomenon gracile*, *Aphanizomenon flos-aquae*, *Anabaena macrospora*) cyanobacterial species (Nostocales) from German lakes. We also included one potentially invasive (*Aphanizomenon ovalisporum*) Nostocales species. We conducted semi-continuous culture experiments and a microcosm experiment along a natural light gradient. Temperature data were used to design a model to simulate the development of selected species according to three temperature scenarios (past, present and future). Native species had significantly higher growth rates than invasive species and the potential invader *A. ovalisporum* at low temperatures ( $\leq 10^{\circ}\text{C}$ ), while the opposite was true at high temperatures ( $\geq 35^{\circ}\text{C}$ ). Maximum growth rates of *A. ovalisporum*, *A. aphanizomenoides* and *C. raciborskii* were clearly higher than those of *A. bergii* and the native species. Regarding light-dependent growth, significant differences were found between single species but not between all native and invasive species. The model simulation demonstrates a shift in dominance from the native *A. gracile* in the historic scenario to *C. raciborskii* populations in the future scenario, in which also the potential invader *A. ovalisporum* is able to establish populations in temperate lakes. Our findings suggest that any further temperature increase would promote the growth and development of Nostocales species in general, and that of the invasive species in particular, and would enable a more northward expansion of *A. ovalisporum*.

## Introduction

In the course of global warming, many species could expand their range from lower to higher latitudes (Parmesan, 2006). In the case of prokaryotes including freshwater cyanobacteria it has been stated that 'Everything is everywhere, but, the environment selects' (Baas Becking, 1934), from which it was concluded that cyanobacteria were cosmopolitan (Fogg et al., 1973). Nowadays, it is well established that not only cosmopolitan cyanobacterial species, but also species with holarctic or pantropical distributions exist (Komárek, 1994; Hoffmann, 1996). *Cylindrospermopsis raciborskii* (Woloszynská) Seenayya & Subba Raju 1972 is a prime example of a cyanobacterial species that has spread from tropical and subtropical to temperate latitudes of the northern and southern hemisphere over the last decades (Padisák, 1997; Wiedner et al., 2007). In Germany, *C. raciborskii* is now widespread (Stüken et al., 2006) and accounts for a considerable proportion of the phytoplankton biovolume (Nixdorf et al., 2003; Mischke, 2003). Two other cyanobacteria, *Anabaena bergii* Ostefeld 1908 and *Aphanizomenon aphanizomenoides* (Forti) Horecká &

Komáreck 1979, were recently reported for the first time in German freshwaters (Stüken et al., 2006) and other temperate regions, e.g. North Carolina/USA (Moisander et al., 2002), Slovakia (Hindák, 2000) and Poland (Stefaniak and Kokocinski, 2005). These three invasive cyanobacteria of the order Nostocales are able to fix molecular nitrogen and can survive unfavourable conditions as akinetes (resting stages).

Such invasive species are of general ecological interest as they can have a major impact on community composition and ecosystem processes (Sala et al., 2000; Strayer et al., 2006). The successful establishment of an invasive species in a new habitat depends on its invasive potential and the invasibility of the native community (Lonsdale et al., 1999). In this context, the competitive potential of invasive species against native species that are overlapping in an ecological niche is of crucial importance. Since all three invasive species belong to the order Nostocales, their niche most likely overlaps with that of native Nostocales species. In temperate freshwaters, the invasive species are most abundant in polymictic shallow lakes (Stüken et al., 2006), where they co-exist with a great variety of native Nostocales of the genus *Anabaena* and *Aphanizomenon*, among which *Aphanizomenon gracile* is the most frequent and abundant (Mischke and Nixdorf, 2003; Rücker et al., 2007). However, the invasive species can also be found, albeit less abundantly, in stratified lakes (Stüken et al., 2006), where the most frequent Nostocales are *Aphanizomenon flos-aquae* and several taxa of the genus *Anabaena*.

Temperature and light intensity, the two abiotic factors that vary most between latitudes, are key factors in the regulation of Nostocales growth and, presumably, of range expansion of tropical and subtropical species. Previous studies have shown that light is the main growth-limiting factor of Nostocales populations in temperate freshwaters, where they were out-competed by Oscillatoriales under light limitation and high nutrient conditions (Zevenboom, 1982; Scheffer, 1998; Wiedner et al., 2002). Light is also a growth-limiting factor for *C. raciborskii* populations in temperate waters (Wiedner et al., 2007). Ecophysiological studies show that *C. raciborskii* require high light intensities (80 to 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for maximum growth (Shafik et al., 2001; Briand et al., 2004); this is similar to the light intensities (100 to 110  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) required by *Aphanizomenon* species (Gentile and Maloney, 1969; Uehlinger, 1981). However, Padisák and Reynolds (Padisák and Reynolds, 1998) consider *C. raciborskii* a shade-tolerant species due to its low light saturation parameter ( $I_K$ ).

Cyanobacteria are known to have higher temperature optima than other phytoplankton species (Reynolds and Walsby, 1975; Paerl and Huisman, 2009). However, our knowledge about species specific differences in the temperature requirements within the cyanobacteria community is still incomplete. Temperature plays an important role in the onset of Nostocales akinete germination in spring (Kim et al., 2005; Rücker et al., 2009) and in their vegetative growth during the season.

Earlier increases in water temperatures in spring as well as a rise in the entire temperature amplitude were found to promote the development of *C. raciborskii* in temperate freshwaters (Wiedner et al., 2007). Minimum temperatures, for example, required for growth of *C. raciborskii*, as determined in field and laboratory studies, range from 15°C to 22°C (Dokulil and Mayer, 1996; Shafik et al., 2001; Mischke et al., 2003). The optimum growth temperature ( $T_{opt}$ ) for *C. raciborskii*, as determined in culture experiments, ranges from 29 to 31°C (Shafik et al., 2001; Briand et al., 2004), which is slightly higher than that for *Aphanizomenon* and *Anabaena* species, namely, 20 to 28°C (Foy et al., 1976; Uehlinger, 1981). However, as the published results are based on a mixture of different field and culture studies and culture methods, data comparisons are difficult. So far, no direct comparison of *C. raciborskii* with other Nostocales species in terms of temperature and light-dependent growth has been published. Moreover, there are only few studies available on the ecology of *A. aphanizomenoides* (Moisander et al., 2002; Stefaniak and Kokocinski, 2005), and none for *A. bergii*. Data about temperature-dependent growth of *Aphanizomenon ovalisporum*, a potentially invasive Nostocales species currently restricted to the Mediterranean region of Europe (Gkelis et al., 2005; Quesada et al., 2006), are restricted to strains from Lake Kinneret (Israel), where Hadas and co-workers (Hadas et al., 2002) estimated that its  $T_{opt}$  ranges between 26 and 30°C.

In summary, current knowledge about the temperature and light-dependent growth of invasive and potentially invasive species in relation to native Nostocales species is insufficient to assess their competitive potential and predict their development in temperate freshwaters as a consequence of climate change. Therefore, the aim of the present study was to determine the temperature and light-dependent growth of three invasive and three native Nostocales species from German freshwater as well as of one potentially invasive species. Three approaches were used:

- (i) Semi-continuous culture experiments to study temperature and light-dependent growth of one to four different strains of each species
- (ii) Microcosm experiment to assess the growth of one strain of each native and invasive species along a natural light gradient in a shallow lake
- (iii) A simple mathematical model based on growth as a function of temperature (as determined in the culture experiments) was created to simulate the seasonal course of biovolume development and community composition for one native species (*A. gracile*), one invasive species (*C. raciborskii*) and one potentially invasive species (*A. ovalisporum*) at three different temperature scenarios: past, present and future.

## Method

### Strains

1  
2 140 Strains of three invasive species (*Cylindrospermopsis raciborskii*, *Anabaena bergii*,  
3  
4 141 *Aphanizomenon aphanizomenoides*) and three native species (*Aphanizomenon gracile*,  
5  
6 142 *Aphanizomenon flos-aquae*, *Anabaena macrospora*) from Northeast Germany and one potentially  
7  
8 143 invasive Spanish species (*A. ovalisporum*) were used (Table I). German strains were isolated as  
9  
10 144 described by Preußel (Preußel et al., 2006), and the Spanish strain as described by Wormer  
11 145 (Wormer et al., 2008).  
12  
13 146

14 147 **Culture experiments**

16 148 Temperature dependent growth of one strain of each species (Table I) was determined at seven  
17  
18 149 temperatures between 10°C and 35°C at 80 µmol photons m<sup>-2</sup> s<sup>-1</sup> for all species, and additionally at  
19  
20 150 40°C for *A. aphanizomenoides* (22C4-9) and *A. ovalisporum* (UAM290). Light dependent growth  
21  
22 151 of all strains was determined at six or more light intensities between 17 and 57 µmol photons m<sup>-2</sup> s<sup>-1</sup>  
23  
24 152 at 15°C and 20°C (Table I).

25 153 Growth experiments were performed using 100 mL culture volume in 250 mL Erlenmeyer flasks,  
26  
27 154 which were continuously shaken at 80-100 rpm. Z8-medium (Haande et al., 2008) modified with  
28  
29 155 164 nM biotin, 30 nM cyanocobalamine and 119 nM thiamine hydrochloride was used for  
30  
31 156 culturing. The experiments were carried out in growth chambers (BINDER, KBW 400, Germany)  
32  
33 157 with dimmable illumination (OSRAM, Lumilux 18W/865XT Cool Daylight, Germany) simulating  
34  
35 158 12 h:12 h light:dark photoperiods. The cultures were grown in a semi-continuous fashion according  
36  
37 159 to the turbidostat principle (Paasche, 1975; Nicklisch, 1992). The biovolume was measured every  
38  
39 160 second day and the every second day increase of biovolume was removed by dilution with Z8. The  
40  
41 161 biovolume determination was carried out using a UV/VIS spectrophotometer (Lambda 2, Perkin  
42  
43 162 Elmer, USA) at 750 nm. The cultures were diluted every second day to an optical density (OD) of  
44  
45 163 0.1.  
46  
47 164

46 165 *Growth rate and curve fitting*

48 166 As biomass is correlated to OD, the specific growth rate ( $\mu$ ) was calculated from the change of  
49  
50 167 biomass over time ( $\Delta t$ ):

51  
52  
53 168 
$$\mu = \ln\left(\frac{X_{\Delta t}}{X_0}\right) \cdot \frac{1}{\Delta t} , \tag{1}$$
  
54  
55

56 169 where  $X_{\Delta t}$  is biomass after cultivation time  $t$ ,  $X_0$  is biomass after dilution and  $\Delta t$  is the time between  
57  
58 170 two dilutions.

59 171 If biomass increase was equal to biomass loss after dilution, steady state was assumed. The mean  
60  
172 specific growth rate as well as the standard deviation was calculated from at least three consecutive



measurements of the specific growth rates per steady state. This was done for each strain and at all combinations of light and temperature.

Curve fitting of light-dependent growth rate was performed according to the exponential model of Mitscherlich (Mitscherlich, 1909) modified using a  $\beta$ -factor describing photoinhibition:

$$\mu(I) = A \cdot \left( 1 - \exp\left(-\frac{(\alpha + \beta) \cdot (I - I_0)}{A}\right) \right) - \beta(I - I_0) \quad (2)$$

$$A = \mu_{\max} \cdot \left( \frac{\alpha + \beta}{\alpha + \beta \cdot \ln\left(\frac{\beta}{\alpha + \beta}\right)} \right), \quad (3)$$

where  $\mu$  is the specific growth rate,  $\mu_{\max}$  the maximum specific growth rate,  $\alpha$  the initial slope at  $I = I_0$  with light intensity  $I$  and compensation light intensity  $I_0$ . If the factor of photoinhibition,  $\beta$ , is  $\beta = 0$ , then  $A = \mu_{\max}$  and the enhanced exponential model reduces to the model of Mitscherlich.

Irradiance at onset of light saturation,  $I_K$ , was calculated as  $I_K = \mu_{\max} / \alpha$ .

Curve fitting of the temperature-dependent growth rates was performed according to Jöhnk (Jöhnk et al. 2008):

$$\mu(T) = \mu_{\max} \cdot \left( 1 + b_i \left( (R_{1i}^{T-T_{opt,i}} - 1) - \frac{\ln(R_{1i})}{\ln(R_{2i})} \cdot (R_{2i}^{T-T_{opt,i}} - 1) \right) \right), \quad (4)$$

where  $\mu(T)$  is the specific growth rate at temperature  $T$ ,  $\mu_{\max}$  the maximum specific growth rate at optimal growth temperature  $T_{opt,i}$  and  $b_i$ ,  $R_{1i}$ ,  $R_{2i}$  are parameters describing the form of the optimum growth curve.

The slope,  $\alpha(T) = \partial\mu/\partial T$ , of the temperature-dependent growth curves was determined at  $T = 10^\circ\text{C}$ .

The  $Q_{10}$  value,  $Q_{10} = \mu(T+10)/\mu(T)$ , is the rate of change as a consequence of increasing the temperature by  $10^\circ\text{C}$  with  $\mu(T+10)$  is the specific growth rate at a temperature increase of  $10^\circ\text{C}$  and  $\mu(T)$  is specific growth rate at initial temperature.

### Microcosm experiment

The microcosm experiment was carried out using a mixed culture including three native and three invasive species (Table I). Pre-cultures were grown as batch cultures in 500 mL Erlenmeyer flasks with a culture volume of 300 mL. Z8 medium was used as described above. The pre-cultures were incubated at  $21^\circ\text{C}$  under  $59 \pm 15 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  provided by daylight fluorescent tubes (Phillips TL-D 36W/25-740) with 10 h:14 h light:dark photoperiod.



1  
2 201 Approximately equal parts of biovolume (assessed from correlations between OD and biovolume,  
3  
4 202 data not shown) of the pre-cultures were mixed and diluted to a final biovolume of  $28 \text{ mm}^3 \text{ L}^{-1}$ . The  
5  
6 203 pooled sample was filled in 2-L bottles (Nalgene) and incubated for four days at depths of 0.1, 0.2,  
7  
8 204 0.4, 0.6, 0.8, 1.0 and 1.6 m in a shallow experimental pond in the Scharmützelsee region. The  
9  
10 205 bottles were aerated daily. Subsamples from the starting suspensions and from each bottle were  
11  
12 206 taken after 4 days of incubation and preserved for phytoplankton biovolume analyses. The  
13  
14 207 biovolume was determined according to the method of Utermöhl (Utermöhl, 1958).  
15  
16 208 The mean water temperature during the experiment was  $19.7 \pm 0.4^\circ\text{C}$ . Global radiation was  
17  
18 209 measured with a cosine-corrected quantum sensor (MQS-B, Heinz Walz GmbH, Germany). The  
19  
20 210 vertical attenuation coefficient ( $K_d$ ) was calculated using photosynthetically active radiation (PAR)  
21  
22 211 measurements obtained at two defined depths with two spherical quantum sensors (LI-193 SA, LI-  
23  
24 212 COR Bioscience, USA). PAR at different depths was calculated from  $K_d$ , light intensity at the water  
25  
26 213 surface ( $I_0$ ) and depth ( $z$ ).  
27  
28 214 The specific growth rate ( $\mu$ ) was determined as the change in biovolume from beginning to end of  
29  
30 215 the experiment as described above (Eq. 1). Curve-fitting of light-dependent growth rates was  
31  
32 216 performed using Equation 2. The negative growth rate of *A. flos-aquae* at  $254 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$   
33  
34 217 was not included in the fit. *A. macrospora* growth rates were not fitted as Eq. (2) was not suitable  
35  
36 218 for them.

37  
38 219  
39 220 **Simulation model**

40  
41 221 We used a simple model to point out the effects of differences in growth rates for different  
42  
43 222 Nostocales species on the seasonal course of population biovolume. In contrast to a simulation  
44  
45 223 model describing the full life cycle of Nostocales cyanobacteria (Jöhnk et al., 2010, unpublished  
46  
47 224 data), we only included temperature-dependent growth (neglecting nutrient and light limitation) and  
48  
49 225 mortality rates from that model. Changes in pelagic population,  $C [\text{mm}^3 \text{ L}^{-1}]$ , are thus described by  
50  
51 226 growth and loss processes:

52  
53 227 
$$\frac{dC}{dt} = \mu(T)C - [m + m_{\text{dens}} C]C \quad . \quad (5)$$
  
54  
55 228

56  
57 229 Temperature-dependent growth was simulated using growth functions  $\mu(T)$  for three species,  
58  
59 230 *A. gracile*, *C. raciborskii*, and *A. ovalisporum*, determined by laboratory experiments (Eq. 4).  
60  
231 Model calibration provided a mortality rate  $m = 0.06 \text{ d}^{-1}$  and a density-dependent loss rate,  $m_{\text{dens}} C$ ,  
232 with  $m_{\text{dens}} = 0.01 \text{ d}^{-1} \text{ L mm}^{-3}$ , which is equivalent to a carrying capacity of  $K = \mu / m_{\text{dens}}$ . Loss rates  
233 are in good agreement with data published by Reynolds (1984). Changing the mortality rates within  
234 a feasible range does not significantly change the characteristics of the dynamics described  
afterwards, although the size of the population densities changes in response to changing carrying

capacities. The populations were initialized at a threshold temperature of  $T_{\text{threshold}} = 12^{\circ}\text{C}$ , when overwintering akinetes in the sediments are germinating (Rücker et al., 2009). In our simplified model, this is represented by a starting population of

$$C(t_{\text{start}}) = C_0, \quad \text{where} \quad t_{\text{start}} = \text{day}(T \geq T_{\text{threshold}}). \quad (6)$$

Inoculum values,  $C_0$ , for the three species were chosen as ratios of 1 to 1/3 to 1/10 of  $10^{-4} \text{ mm}^3 \text{ L}^{-1}$ , representing spring conditions.

The model is driven by seasonal changes in water temperature of a lake. Here, we chose the mean seasonal course (averaged over a period of 14 years, 1994 – 2007) of Lake Melangsee (Table I).

Simulations for current mean water temperatures (CWT) were complemented by scenarios for historic (CWT minus  $2^{\circ}\text{C}$ ) and future mean water temperatures (CWT plus  $4^{\circ}\text{C}$ ) representing possible future climate change conditions.

## Statistical analysis

The investigated species were divided into four groups, native (*A. gracile*, *A. flos-aquae*, *A. macrospora*), invasive (*C. raciborskii*, *A. bergii*, *A. aphanizomenoides*), deep oligotrophic lake (*A. flos-aquae* and *A. macrospora*) and shallow eutrophic to polytrophic lake species (*A. gracile* and invasive species), for the statistical analysis. *A. ovalisporum* was not included in the statistical analysis. Statistically significant differences between the groups were assessed using the t-test for independent samples and the Welch test of non-homogeneous variance. The Mann-Whitney U-test was used to compare growth rates at  $35^{\circ}\text{C}$ . All statistical tests were two-tailed.

## Results

### Culture experiments

#### Temperature-dependent growth

Native and invasive species differed significantly in their growth rates at very low ( $10^{\circ}\text{C}$ ) and very high ( $35^{\circ}\text{C}$ ) temperatures (Fig. 1). At  $10^{\circ}\text{C}$ , native cyanobacteria grew at rates of  $0.06$  to  $0.15 \text{ d}^{-1}$ , whereas invasive cyanobacteria did not grow. At  $35^{\circ}\text{C}$ , native cyanobacteria ceased to grow, whereas the invasive cyanobacteria grew at rates of  $0.09$  to  $0.32 \text{ d}^{-1}$ .

The growth curves of *A. aphanizomenoides*, *C. raciborskii* and *A. bergii* exceeded those of *A. gracile*, the main competitor of these invasive species in shallow lakes, at  $16^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$  and  $31^{\circ}\text{C}$  (Fig. 1). Below these temperatures *A. gracile* grew faster than invasive species. *Aphanizomenon aphanizomenoides* and *C. raciborskii* had clearly higher maximum growth rates than native species (Table II).

The invasive cyanobacteria had significantly higher  $Q_{10}$  values between  $15$  to  $25^{\circ}\text{C}$  ranging from  $2.0$  to  $3.2$  than native cyanobacteria with values of  $1.4$  to  $1.7$  ( $\text{df} = 4$ ;  $t = 3.025$ ;  $p \leq 0.05$ ). Their

growth curves had significantly higher initial slopes ( $\alpha = 0.024$  to  $0.035$ ) than growth curves of native cyanobacteria ( $\alpha = 0.010$  to  $0.011$ ) ( $df = 4$ ;  $t = 5.151$ ;  $p \leq 0.01$ ). Native and invasive species did not differ significantly in their  $Q_{10}$  values between  $20$  to  $30^{\circ}\text{C}$  ( $df = 2.210$ ;  $t = 1.936$ ;  $p > 0.05$ ) and their  $T_{opt}$  ( $df = 4$ ;  $t = 1.999$ ;  $p > 0.05$ ). Optimal growth temperatures of native and invasive species were not significantly different ( $df = 4$ ;  $t = 1.999$ ;  $p > 0.05$ ). The lowest  $T_{opt}$  was measured for the two species from the deepest lake (*A. flos-aquae* and *A. macrospora*) (Table II). The Spanish strain (*A. ovalisporum*) did not grow at  $10^{\circ}\text{C}$  like the invasive species. Compared to all other species its growth curve is characterized by a low initial slope ( $\alpha = 0.014$ ), by the highest growth rate at  $35^{\circ}\text{C}$  ( $\mu = 0.34$ ), by the highest  $T_{opt}$  ( $T_{opt} = 32.8^{\circ}\text{C}$ ) and highest  $Q_{10}$  values between  $15$  to  $25^{\circ}\text{C}$  ( $Q_{10} = 3.6$ ) (Table II).

Light-dependent growth

The highest maximum growth rates at  $15^{\circ}\text{C}$  were achieved by strains of *A. gracile* (Figs. 2 A and 3). Growth rates of all invasive species were significantly lower than those of *A. gracile* ( $df = 9$ ;  $t = 3.781$ ;  $p \leq 0.01$ ) but equivalent to those of *A. flos-aquae* and *A. macrospora* ( $df = 9$ ;  $t = 0.667$ ;  $p > 0.05$ ). The highest growth rates among the invasive species were achieved by the *A. aphanizomenoides* strains. Initial slopes of the growth curves and  $I_K$  values did not differ significantly for the invasive species and *A. gracile* ( $p > 0.05$ ) but significantly for *A. flos-aquae* and *A. macrospora* ( $p \leq 0.05$ ). Initial slopes of the growth curves of the species from the oligotrophic deep lake were slightly higher than those of the species from eutrophic shallow lakes, and their  $I_K$  values were lower (Fig. 3). At  $20^{\circ}\text{C}$ , *A. flos-aquae* and *A. macrospora* had significantly lower maximum growth rates, higher  $\alpha$  and lower  $I_K$  values than the other species ( $p \leq 0.001$ ) (Figs. 2 B and 3). *A. gracile* did not differ significantly from invasive cyanobacteria in their maximum growth rates at  $20^{\circ}\text{C}$  ( $df = 8$ ;  $t = -0.891$ ;  $p > 0.05$ ) as well as in their  $\alpha$  and  $I_K$  values ( $p > 0.05$ ).

Microcosm experiment

In mixed culture, *A. aphanizomenoides* clearly reached the highest growth rates, followed by the other two invasive species, *C. raciborskii* and *A. bergii*, as well as by the native species, *A. gracile*, which had comparable growth rates (Fig. 4 A). The two species from the deep oligotrophic lake, *A. flos-aquae* and *A. macrospora*, had the lowest growth rates. A similar pattern in growth was found in the laboratory experiment at  $20^{\circ}\text{C}$  (Fig. 2 B). Slight differences between the two approaches were reflected in the shape of the growth curves: The initial slope of the growth curve of *A. aphanizomenoides* was distinctly higher and that of *A. flos-aquae* was distinctly lower in the microcosm experiment (Fig. 4 B). Unlike the invasive species, the growth rates of the native species

declined at high irradiance levels. This kind of grouping was not observed in the laboratory experiments. Growth rates of all strains determined in the microcosm experiment were lower than those determined in the laboratory culture experiments.

### Simulation of seasonal dynamics

Simulations show that compared to *A. gracile*, the biovolume of *C. raciborskii* is much lower at lower historic temperatures (Fig. 5, upper panel), nearly equal at current temperatures (Fig. 5, middle panel), and higher at higher future temperatures (Fig. 5, lower panel). The potential invader *A. ovalisporum* is virtually absent in the colder scenario, reaching (undetectable) low values at current temperatures and blooms during future temperature increases, as can be expected based on its higher  $T_{opt}$  (Table II). In the future scenario, the vegetation time of all three species is increased.

### Discussion

The aim of this study was to determine which of the native, invasive and potentially invasive species are stronger competitors under various temperature and light conditions. Our culture experiments clearly demonstrated that native cyanobacterial species were superior competitors at low temperatures and invasive cyanobacterial species at high temperatures.

The  $T_{opt}$  of native and invasive Nostocales species do not differ significantly. The  $T_{opt}$  of 27.8°C for *C. raciborskii* coincided with values of 28 to 31°C reported for *C. raciborskii* isolates from different latitudes (Briand et al., 2004). This also holds true for the two native *Aphanizomenon* species, the  $T_{opt}$  of which were 24.7°C and 27.1°C in this study compared 25°C reported for *A. flos-aquae* RALFS f. *gracile* LEMM. (Foy et al., 1976) and 28°C reported for *A. flos-aquae* var. *klebahnii* ELENK. (Uehlinger, 1981) in other studies. In this study, *A. ovalisporum* had the highest  $T_{opt}$  of 32.8°C, which is also higher than  $T_{opt}$  of 26 to 30°C reported for strains from Lake Kinneret (Hadas et al., 2002). This high  $T_{opt}$  is advantageous as ponds and reservoirs in the Mediterranean region can reach temperatures over 30°C during summer months (A. Quesada, Madrid, personal communication).

Overall, high  $T_{opt}$  values suggest that Nostocales tolerate the low temperatures of their habitats rather than are adapted to them. Tang and co-workers (Tang et al., 1997) published similar observations for cyanobacteria from polar freshwater ecosystems. The  $T_{opt}$  of all Nostocales were higher than the mean water temperatures of their habitats during their vegetation period. Therefore, all Nostocales would benefit from rising temperatures. This is in agreement with the recently confirmed concept of global expansion of cyanobacterial blooms favoured by global and regional warming (Paerl and Huisman, 2009). *Cylindrospermopsis raciborskii*, *A. aphanizomenoides* and *A. ovalisporum* achieved the highest maximum growth rates at high temperatures. Hence, these

three species would benefit most from any increase in water temperature. Below 20°C the lowest growth rates were measured for *A. ovalisporum*. This may be the reason why *A. ovalisporum* is not present in German freshwaters yet.

Contrary to our assumption, the invasive Nostocales species are not superior competitors for light. Values of their light-dependent growth parameters ( $\alpha$ ,  $I_K$ ) were similar to those of *A. gracile*.  $I_K$  values for *C. raciborskii* (38 to 101  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were much higher in the present study than those (15 to 26  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) reported in the literature (Shafik et al., 2001; Briand et al., 2004). This might be due to differences in culturing methods since the low  $I_K$  values in the literature resulted from the exponential growth phase of batch cultures started at a much lower trichome density (OD 750 nm ~ 0.002) and determined at higher temperatures (25°C and 27°C) than those in the semi-continuous culture experiments of our study. As we considered the relative differences between native and invasive species, these absolute differences played a minor role in our overall conclusions.

*Aphanizomenon flos-aquae* and *A. macrospora*, the two species isolated from a deep oligotrophic lake, had a significantly lower optimal light intensity and were more stenoecious in regard to light than the species from the eutrophic shallow lakes.

The results of the microcosm experiment (at 19°C) are consistent with the main results of the culture experiments (at 20°C): The most powerful competitors were *A. aphanizomenoides*, followed by *C. raciborskii*, *A. gracile* and *A. bergii*; *A. flos-aquae* and *A. macrospora* could not compete with the other species. This shows that results obtained for single strains using artificial irradiance in the laboratory can be transferred to community level where species co-occur under natural light conditions. Although this study is restricted to the effects of light and temperature on cyanobacterial growth and competition, it can be noted that buoyancy enables the co-occurrence of Nostocales species with different light requirements. For example, under stratified conditions, *Anabaena* species can adjust their buoyancy to maintain a near surface position, while *C. raciborskii* can compete more effectively by remaining at greater depths. In shallow turbid waters, Nostocales species can regulate their buoyancy only under calm weather conditions.

Since there is increasing evidence that cyanobacterial populations are composed of different ecotypes or subpopulations (Becker et al., 2002; Johnson et al., 2006; Rohrlack et al., 2008), we included different strains of each species (except for *A. macrospora*) in the light experiment to estimate the variance in growth between strains. Figure 2 clearly illustrates that strains of some species such as *A. aphanizomenoides* or *A. flos-aquae* show a nearly identical growth response at different light intensities while others, such as *A. gracile* vary considerably in their growth, especially at high light intensities. In our study differences between species were more pronounced



than differences between strains. However, variability between strains should be taken into account when extending results obtained for one strain to the whole population.

An interesting result of our study is the fact that *A. aphanizomenoides* reached the highest growth rates in all experiments. This is contradictory to its low abundance in the field (Stüken et al., 2006). Therefore, the development of *A. aphanizomenoides* field populations may be limited by other factors as nutrients, or it may just be at the beginning of its population development in German freshwaters.

Overall, our study showed that temperature has a major impact on the competition between native and invasive cyanobacteria. Our results suggest that native species can begin to build up populations earlier during the season. This is confirmed by field studies showing that populations of *A. gracile* occur earlier in the season from temperatures of 13°C (J. Rücker, Bad Saarow, personal communication), while populations of *C. raciborskii* occur later in the season when the water temperature exceeds 17°C (Mischke, 2003; Wiedner et al., 2007; Dokulil and Mayer, 1996). Our results also suggest that *C. raciborskii* could outcompete *A. gracile* in summer when temperatures rise above 20°C. So far the population size of *C. raciborskii* does not exceed that of *A. gracile* (Wiedner et al., 2008). However, this could change if the water temperature increases by a further 4.0°C in the future (scenario A1F1, IPCC 2007). Our simulation clearly demonstrates a shift from past dominance by *A. gracile* to *C. raciborskii* in the future. The simulation also indicates that the Mediterranean species *A. ovalisporum* could undergo massive population growth under future climate conditions. Moreover, the simulation illustrates that the seasonal growth period of all Nostocales will be prolonged in the future scenario.

Other factors, such as nutrient concentration must also be considered in regulating the growth and competition of cyanobacteria. For example during eutrophication of Lake Balaton (Hungary) in the 1970s, populations of *A. gracile* disappeared while first populations of *C. raciborskii* and *A. aphanizomenoides* had been established. After re-eutrophication *A. gracile* reappeared and now co-occurs with the two invasive species (Padisák and Reynolds, 1998). In general, Nostocales are superior competitors under nitrogen shortage, because they can fix atmospheric nitrogen. However, it is unknown whether they differ in their ability to fix N<sub>2</sub> or to use dissolved nitrogen. Concerning the efficiency of phosphate use, *C. raciborskii* is known to be a strong competitor under phosphate limitation (Isvánovics et al., 2000; Spröber et al., 2003; Posselt et al., 2009). However, to complete the picture on the competitiveness of invasive and native cyanobacteria their growth under various nutrient concentrations must be compared in further laboratory studies.

Finally changes in the cyanobacterial community are of concern, because cyanobacteria can produce different toxins. For example, a survey in German lakes showed that hepatotoxic microcystins, which are mainly produced by *Microcystis* or *Planktothrix* occur in high

1  
2 409 concentrations, while neurotoxic as saxitoxins or anatoxins, which are produced by Nostocales,  
3  
4 410 occur less frequent and in much lower concentrations (Fastner et al., 1999). More recently it was  
5  
6 411 shown that also the toxin cylindrospermopsin is widely distributed in German lakes (Fastner et al.,  
7  
8 412 2007), where it is produced by *A. flos-aquae* and *A. gracile* (Preußel et al., 2006) and occurs in  
9  
10 413 concentrations that are in the same order of magnitude as those of microcystins (Rücker et al.,  
11 414 2007). An increase in the abundance of Nostocales species could result in an increase of anatoxin,  
12  
13 415 saxitoxin and cylindrospermopsin concentrations. Moreover, an introduction of the Mediterranean  
14  
15 416 *A. ovalisporum* could massively enhance cylindrospermopsin concentrations in temperate lakes,  
16 417 because all isolated strains of this species produce cylindrospermopsin (Banker et al., 1997;  
17  
18 418 Quesada et al., 2006), whereas only a very small portion of *A. flos-aquae* and *A. gracile* isolated  
19  
20 419 from German lakes can produce this toxin.

21  
22 420  
23 421 **Conclusion**

24  
25 422 In summary, we conclude that any further increase in water temperature would promote the  
26  
27 423 development of all of the investigated Nostocales species in general, and that of the invasive species  
28  
29 424 in particular, and would enable a more northward expansion of *A. ovalisporum*.  
30  
31 425 A shift in the portion and abundance towards Nostocales in cyanobacterial communities would most  
32 426 likely be accompanied by a shift in the predominating cyanotoxins.

33  
34 427  
35  
36 428 **Acknowledgments**

37  
38 429 We thank Marén Lentz, Monika Degebrot and Andrea Launhardt for their reliable work in the  
39  
40 430 laboratory, and Simone Klein, Ingo Henschke, Gudrun Lippert, Ute Abel, Jessica Ramm for their  
41 431 substantial help with the microcosm experiment and Anette Tworeck (LBH, Freiburg) for  
42  
43 432 phytoplankton analysis. Karina Preußel kindly supplied cyanobacterial strains. We also thank two  
44  
45 433 anonymous reviewers for helpful comments. Suzyon O’Neal Wandrey proofread the English draft  
46  
47 434 of the manuscript. The study was funded by the German Ministry of Education, Science and  
48  
49 435 Research (BMBF) and the KompetenzZentrum Wasser Berlin gGmbH (KWB) with financial  
50 436 support of Veolia Water and Berlin Water Enterprise (BWB).



## References

- Baas Becking, L.G.M. (1934) *Geobiologie of inleiding tot de milieukunde*. Van Stockum & Zoon, The Hague.
- Banker, R., Carmeli, S., Hadas, O., Teltsch, B., Porat, R., and Sukenik, A. (1997) Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from Lake Kinneret, Israel. *J Phycol* **33**, 613-616.
- Becker, S., Fahrbach, M., Boger, P., and Ernst, A. (2002) Quantitative tracing, by Taq nuclease assays, of a *Synechococcus* ecotype in a highly diversified natural population. *Appl Environ Microbiol* **68**, 4486-4494.
- Briand, J.F., Leboulanger, C., Humbert, J.F., Bernard, C., and Dufour, P. (2004) *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, or global warming? *J Phycol* **40**, 231-238.
- Christen, T. (2006) Horizontale Unterschiede trophierelevanter Parameter in großen Seen - Ihre Ausprägung und Bedeutung für die Trophiebewertung. Diploma Thesis, BTU Cottbus, Bad Saarow, Chair of Freshwater Conservation.
- Dokulil, M.T., and Mayer, J. (1996) Population dynamics and photosynthetic rates of a *Cylindrospermopsis-Limnethrix* association in a highly eutrophic urban lake, Alte Donau, Vienna, Austria. *Algological Studies* **83**, 179-195.
- Fastner, J., Neumann, U., Wirsing, B., Weckesser, J., Wiedner, C., Nixdorf, B., and Chorus, I. (1999) Microcystins (hepatotoxic heptapeptides) in German fresh water bodies. *Environ Toxicol* **14**, 13-22.
- Fastner, J., Rucker, J., Stuken, A., Preussel, K., Nixdorf, B., Chorus, I. et al. (2007) Occurrence of the cyanobacterial toxin Cylindrospermopsin in northeast Germany. *Environ Toxicol* **22**, 26-32.
- Fogg, G.E., Stewart, W.D.P., Fay, P., and Walsby, A.E. (1973) *The blue-green algae*. Academic Press, London.
- Foy, R.H., Gibson, C.E., and Smith, R.V. (1976) The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. *Br Phycol J* **11**, 151-163.
- Gentile, J.H., and Maloney, T.E. (1969) Toxicity and Environmental Requirements of a Strain of *Aphanizomenon Flos-Aquae* (L) Ralfs. *Can J Microbiol* **15**, 165-173.
- Gkelis, S., Moustaka-Gouni, M., Sivonen, K., and Lanaras, T. (2005) First report of the cyanobacterium *Aphanizomenon ovalisporum* Forti in two Greek lakes and cyanotoxin occurrence. *J Plankton Res* **27**, 1295-1300.
- Haande, S., Rohrlack, T., Ballot, A., Roberg, K., Skulberg, R., Beck, M. and Wiedner, C. (2008) Genetic characterisation of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) isolates

- 1  
2 472 from Africa and Europe. *Harmful Algae* **7**, 692-701.
- 3  
4 473 Hadas, O., Pinkas, R., Malinsky-Rushansky, N., Shalev-Alon, G., Delphine, E., Berner, T. et al.  
5 474 (2002) Physiological variables determined under laboratory conditions may explain the bloom of  
6 475 *Aphanizomenon ovalisporum* in Lake Kinneret. *Eur J Phycol* **37**, 259-267.
- 7  
8  
9 476 Hindak, F. (2000) Morphological variation of four planktic nostocalean cyanophytes - members of  
10  
11 477 the genus *Aphanizomenon* or *Anabaena*? *Hydrobiologia* **438**, 107-116.
- 12  
13 478 Hoffmann, L. (1996) Geographic distribution of freshwater blue-green algae. *Hydrobiologia* **336**,  
14 479 33-40.
- 15  
16 480 IPCC (2007) Summary for Policymakers. In Solomon, S., D. Qin, M. Manning, Z. Chen, M.  
17  
18 481 Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.), *Climate Change 2007: The Physical*  
19  
20 482 *Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the*  
21  
22 483 *Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United  
23 484 Kingdom and New York, NY, USA.
- 24  
25 485 Istvanovics, V., Shafik, H.M., Presing, M., and Juhos, S. (2000) Growth and phosphate uptake  
26  
27 486 kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in throughflow  
28  
29 487 cultures. *Freshwater Biol* **43**, 257-275.
- 30  
31 488 Jöhnk, K.D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P.M., and Stroom, J.M. (2008)  
32 489 Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biol* **14**, 495-512.
- 33  
34 490 Jöhnk, K.D., Brüggemann, R., Rucker, J., Luther, B., Simon, U., Nixdorf, B., and Wiedner, C.  
35  
36 491 (2010) Modelling life cycle and population dynamics of Nostocales (cyanobacteria). Unpublished  
37 492 data.
- 38  
39 493 Johnson, Z.I., Zinser, E.R., Coe, A., McNulty, N.P., Woodward, E.M.S., and Chisholm, S.W.  
40  
41 494 (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental  
42  
43 495 gradients. *Science* **311**, 1737-1740.
- 44  
45 496 Kim, B.H., Lee, W.S., Kim, Y.O., Lee, H.O., and Han, M.S. (2005) Relationship between akinete  
46 497 germination and vegetative population of *Anabaena flos-aquae* (Nostocales, Cyanobacteria) in  
47 498 Seokchon reservoir (Seoul, Korea). *Arch Hydrobiol* **163**, 49-64.
- 48  
49  
50 499 Komárek, J. (1994) Current trends and species delimitation in cyanoprokaryote taxonomy.  
51  
52 500 *Algological Studies* **75**, 11-29.
- 53  
54 501 Lonsdale, W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*  
55 502 **80**, 1522-1536.
- 56  
57 503 Mischke, U. (2003) Cyanobacteria associations in shallow polytrophic lakes: influence of  
58  
59 504 environmental factors. *Acta Oecol - Int J Ecol* **24**, S11-S23.
- 60  
505 Mischke, U., and Nixdorf, B. (2003) Equilibrium phase conditions in shallow German lakes: How  
506 Cyanoprokaryota species establish a steady state phase in late summer. *Hydrobiologia* **502**, 123-

- 132.
- Mitscherlich, E.A. (1909) Das Gesetz vom Minimum und das Gesetz des abnehmenden Bodenertrags. *Landw. Jhrb.* **38**, 537-552.
- Moisander, P.H., McClinton, E., and Paerl, H.W. (2002) Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microb Ecol* **43**, 432-442.
- Nicklisch, A. (1992) The interaction of irradiance and temperature on the growth rate of *Limnethrix redekei* and its mathematical description. *Arch Hydrobiol Suppl* **91** (Algological Studies 63), 1-18.
- Nixdorf, B., Mischke, U., and Rücker, J. (2003) Phytoplankton assemblages and steady state in deep and shallow eutrophic lakes - an approach to differentiate the habitat properties of Oscillatoriales. *Hydrobiologia* **502**, 111-121.
- Paasche, E. (1975) Growth Of The Plankton Diatom *Thalassiosira Nordenskioeldii* Cleve At Low Silicate Concentrations. *J Exp Mar Biol Ecol* **18**, 173-183
- Padisák, J. (1997) *Cylindrospermopsis raciborskii* (Wolozynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. *Arch Hydrobiol Suppl* **107**, 563-593.
- Padisak, J., and Reynolds, C.S. (1998) Selection of phytoplankton associations in Lake Balaton, Hungary, in response to eutrophication and restoration measures, with special reference to the cyanoprokaryotes. *Hydrobiologia* **384**, 41-53.
- Paerl, H.W., and Huisman, J. (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ Microbiol Rep* **1**, 27-37.
- Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol Syst* **37**, 637-669.
- Posselt, A.J., Burford, M.A., and Shaw, G. (2009) Pulses of Phosphate Promote Dominance of the Toxic Cyanophyte *Cylindrospermopsis Raciborskii* in a Subtropical Water Reservoir. *J Phycol* **45**, 540-546.
- Preussel, K., Stüken, A., Wiedner, C., Chorus, I., and Fastner, J. (2006) First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. *Toxicon* **47**, 156-162.
- Quesada, A., Moreno, E., Carrasco, D., Paniagua, T., Wormer, L., De Hoyos, C., and Sukenik, A. (2006) Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. *Eur J Phycol* **41**, 39-45.
- Reynolds, C.S., and Walsby, A.E. (1975) Water-Blooms. *Biol Rev* **50**, 437-481.
- Reynolds, C.S. (1984) The ecology of freshwater phytoplankton. In Beck, E., Birks, H.J.B., and Connor, E.F. (eds), *Cambridge Studies in Ecology*. Cambridge University Press, Cambridge.

- 1  
2 542 Rohrlack, T., Edvardsen, B., Skulberg, R., Halstvedt, C.B., Utkilen, H.C., Ptacnik, R., and  
3  
4 543 Skulberg, O.M. (2008) Oligopeptide chemotypes of the toxic freshwater cyanobacterium  
5  
6 544 *Planktothrix* can form subpopulations with dissimilar ecological traits. *Limnol Oceanogr* **53**, 1279-  
7  
8 545 1293.
- 9 546 Rücker, J., Stüken, A., Nixdorf, B., Fastner, J., Chorus, I., and Wiedner, C. (2007) Concentrations  
10  
11 547 of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes.  
12  
13 548 *Toxicon* **50**, 800-809.
- 14 549 Rücker, J., Tingwey, E.I., Wiedner, C., Anu, C.M., and Nixdorf, B. (2009) Impact of the inoculum  
15  
16 550 size on the population of Nostocales cyanobacteria in a temperate lake. *J Plankton Res* **31**, 1151-  
17  
18 551 1159.
- 19  
20 552 Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R. et al. (2000)  
21  
22 553 Biodiversity - Global biodiversity scenarios for the year 2100. *Science* **287**, 1770-1774.
- 23 554 Scheffer, M. (1998) Ecology in shallow lakes. In Usher, M.B., DeAngelis, D.L., and Manly, B.F.J.  
24  
25 555 (eds), *Population and Community Biology Series*. Chapman & Hall, London.
- 26  
27 556 Shafik, H.M., Herodek, S., Presing, M., and Vörös, L. (2001) Factors effecting growth and cell  
28  
29 557 composition of cyanoprocaryote *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba  
30  
31 558 Raju. *Arch Hydrobiol Suppl* **140**, 75-93.
- 32 559 Spröber, P., Shafik, H.M., Presing, M., Kovacs, A.W., and Herodek, S. (2003) Nitrogen uptake and  
33  
34 560 fixation in the cyanobacterium *Cylindrospermopsis raciborskii* under different nitrogen conditions.  
35  
36 561 *Hydrobiologia* **506**, 169-174.
- 37  
38 562 Stefaniak, K., Kokocinski, M. (2005) Occurrence Of Invasive *Cyanobacteria* Species In Polimictic  
39  
40 563 Lakes Of The Wielkopolska Region (Western Poland). *Oceanol Hydrobiol Stud* **34** (Suppl. 3), 137-  
41  
42 564 148.
- 43 565 Strayer, D.L., Eviner, V.T., Jeschke, J.M., and Pace, M.L. (2006) Understanding the long-term  
44  
45 566 effects of species invasions. *Trends Ecol Evol* **21**, 645-651.
- 46 567 Stüken, A., Rücker, J., Endrulat, T., Preussel, K., Hemm, M., Nixdorf, B. et al. (2006) Distribution  
47  
48 568 of three alien cyanobacterial species (Nostocales) in northeast Germany: *Cylindrospermopsis*  
49  
50 569 *raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. *Phycologia* **45**, 696-703.
- 51  
52 570 Tang, E.P.Y., Tremblay, R., and Vincent, W.F. (1997) Cyanobacterial dominance of polar  
53  
54 571 freshwater ecosystems: Are high-latitude mat-formers adapted to low temperature? *J Phycol* **33**,  
55  
56 572 171-181.
- 57 573 Uehlinger, U. (1981) Experimental studies on the autecology of *Aphanizomenon flos-aquae*  
58  
59 574 (Experimentelle Untersuchungen zur Autökologie von *Aphanizomenon flos-aquae*). *Arch Hydrobiol*  
60  
575 *Suppl* **60**, 260-288.
- 576 Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int*

1  
2 577 *Ver Theor Angew Limnol* **9**, 1-38.

3  
4 578 Wiedner, C., Nixdorf, B., Heinz, R., Wirsing, B., Neumann, U., and Weckesser, J. (2002)

5 579 Regulation of cyanobacteria and microcystin dynamics in polymictic shallow lakes. *Arch Hydrobiol*  
6 580 **155**, 383-400.

7  
8  
9 581 Wiedner, C., Rücker, J., Brüggemann, R., and Nixdorf, B. (2007) Climate change affects timing and  
10 size of populations of an invasive cyanobacterium in temperate regions. *Oecologia* **152**, 473-484.

11 582 Wiedner, C., Rücker, J., Fastner, J., Chorus, I., and Nixdorf, B. (2008) Seasonal dynamics of  
12  
13 583 cylindrospermopsin and cyanobacteria in two German lakes. *Toxicon* **52**, 677-686.

14 584 Wormer, L., Cires, S., Carrasco, D., and Quesada, A. (2008) Cylindrospermopsin is not degraded  
15  
16 585 by co-occurring natural bacterial communities during a 40-day study. *Harmful Algae* **7**, 206-213.

17  
18 586 Zevenboom, W. (1982) N<sub>2</sub>-Fixing Cyanobacteria: Why They Do Not Become Dominant In  
19  
20 587 Shallow Hypertrophic Lakes. *Aquat Ecol* **16**, 289-290.  
21  
22 588

1  
2 589  
3  
4 590  
5  
6 591  
7  
8 592  
9  
10 593  
11 594  
12  
13 595  
14  
15 596  
16  
17 597  
18 598  
19  
20 599  
21  
22 600  
23  
24 601  
25  
26 602  
27 603  
28  
29 604  
30  
31 605  
32  
33 606  
34  
35 607  
36 608  
37  
38 609  
39  
40 610  
41  
42 611  
43 612  
44  
45 613  
46  
47 614  
48  
49 615  
50  
51 616  
52 617  
53  
54 618  
55  
56 619  
57  
58 620  
59 621  
60  
622

**Table and Figure legends**

Table I: Studied strains: their origin (poly = polymictic; di = dimictic) and the experiments in which they were used (T = temperature-dependent growth, L = light-dependent growth, M = microcosm experiment).

(a) Isolated as described by Preußel (2007). (b) Isolated as *A. flos-aquae* but designated here *A. gracile* as its morphology in culture is similar to that of 16D11 and ZIE23AFA. (c) Maximum depth. (d) Northern Bay of Scharmützelsee, which can be considered a separated water body (Christen 2006)

Table II: Parameters of temperature-dependent growth calculated using Equation (4), where  $R^2$  is the coefficient of determination,  $\mu_{max}$  the maximum growth rate,  $T_{opt}$  the optimum growth temperature,  $\alpha$  the slope at 10°C [ $d^{-1} \mu mol photons^{-1} m^2 s$ ], and  $Q_{10}$  the temperature coefficient between 15 to 25°C and 20 to 30°C, respectively.

Fig. 1 Temperature-dependent growth rates and growth curves at 80  $\mu mol photons m^{-2} s^{-1}$ . 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.

Fig. 2 Light-dependent growth rates and growth curves at 15°C (A) and 20°C (B). Error bars represent standard deviation of the mean growth rate. Each symbol represents one strain.

Fig. 3 Maximum growth rate ( $\mu_{max}$ ), initial slope ( $\alpha$ ) and light saturation parameter ( $I_K$ ) for individual strains of each species from light-dependent growth curves at 15°C (left) and 20°C (right).

Fig. 4 (A) Microcosm experiment: Growth rates and growth curves (dashed lines) along a natural light gradient. In the case of *A. macrospora*, growth rates without curve fitting are shown. (B) Comparison of light-dependent growth curves derived from microcosm (dashed lines) versus laboratory experiment at 20°C (solid lines).

Fig. 5 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.

For Peer Review



Tables

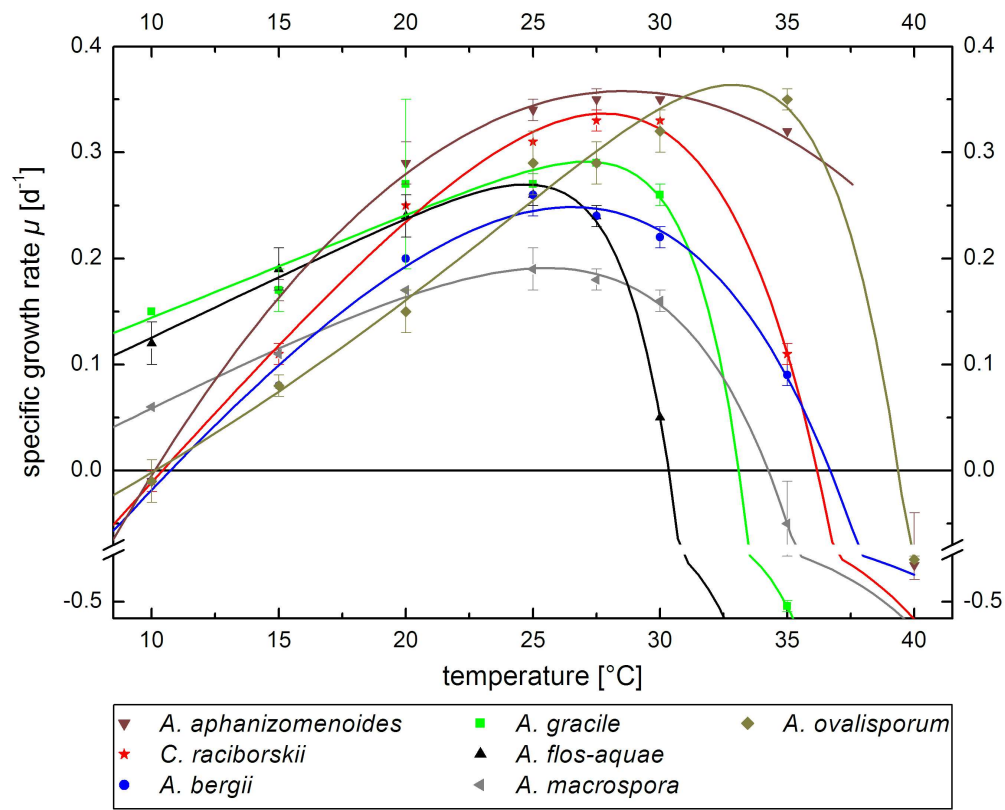
Table I: Studied strains: their origin (poly = polymictic; di = dimictic) and the experiments in which they were used (T = temperature-dependent growth, L = light-dependent growth, M = microcosm experiment).

Species	Strain	Lake of origin	Mean lake depth [m]	Mixis type	Exp. performed
<b>Invasive species</b>					
<i>C. raciborskii</i>	19F6 <sup>a</sup>	Melangsee (13°59'18" E, 52°09'40" N)	1.7	Poly	L
<i>C. raciborskii</i>	ZIE11CR	Zierker See (13°02'12" E, 53°21'44" N)	1.6	Poly	L
<i>C. raciborskii</i>	24G7 <sup>a</sup>	Werbellinsee (12°56'37" E, 52°55'27" N)	3.5 <sup>c</sup>	Poly	L, T, M
<i>C. raciborskii</i>	26D9 <sup>a</sup>	Rangsdorfer See (13°24'14" E, 52°17'19" N)	1.5	Poly	L
<i>A. bergii</i>	LIE02AB	Lieps (13°09'30" E, 53°27'07" N)	2.3	Poly	L, T, M
<i>A. bergii</i>	ZIE26AB	Zierker See	1.6	Poly	L
<i>A. aphanizomenoides</i>	22C4-9 <sup>a</sup>	Heiliger See (13°04'18" E, 52°24'44" N)	6.6	Di/ Poly	L, T, M
<i>A. aphanizomenoides</i>	22F6-1 <sup>a</sup>	Heiliger See	6.6	Di/ Poly	L
<b>Native species</b>					
<i>A. gracile</i>	ZIE23AFA	Zierker See	1.6	Poly	L
<i>A. gracile</i>	30D11 <sup>a, b</sup>	Petersdorfer See (14°04'27" E, 52°18'52" N)	1.9	Poly	L
<i>A. gracile</i>	16D11 <sup>a</sup>	Scharmützelsee, Northern Bay <sup>d</sup> (14°02'46" E, 52°14'22" N)	7.9 <sup>c</sup>	Di/ Poly	L, T, M
<i>A. flos-aquae</i>	ST115AF	Stechlin (13°01'40" E, 53°09'08" N)	22.8	Di	L, T, M
<i>A. flos-aquae</i>	ST86AF	Stechlin	22.8	Di	L
<i>A. macrospora</i>	ST195AS	Stechlin	22.8	Di	L, T, M
<b>Potentially invasive species</b>					
<i>A. ovalisporum</i>	UAM290	Juan Carlos First Park, Spain (3°36'59" W, 40°27'42" N)	1.5	Poly	T

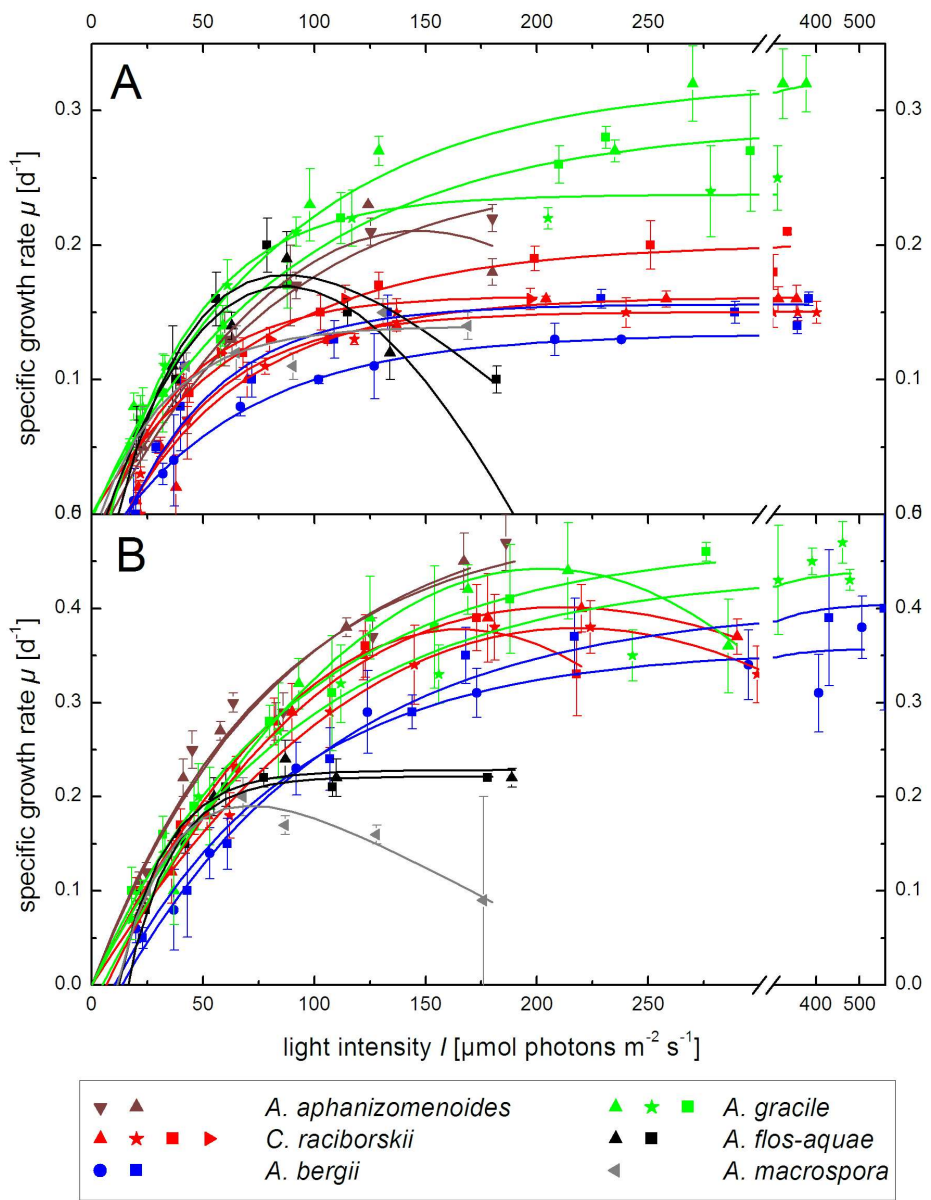
(a) Isolated as described by Preußel (2007). (b) Isolated as *A. flos-aquae* but designated here *A. gracile* as its morphology in culture is similar to that of 16D11 and ZIE23AFA. (c) Maximum depth. (d) Northern Bay of Scharmützelsee, which can be considered a separated water body (Christen 2006)

Table II: Parameters of temperature-dependent growth calculated using Equation (4), where  $R^2$  is the coefficient of determination,  $\mu_{max}$  the maximum growth rate,  $T_{opt}$  the optimum growth temperature,  $\alpha$  the slope at 10°C [ $\text{d}^{-1} \mu\text{mol photons}^{-1} \text{m}^2 \text{s}$ ], and  $Q_{10}$  the temperature coefficient between 15 to 25°C and 20 to 30°C, respectively.

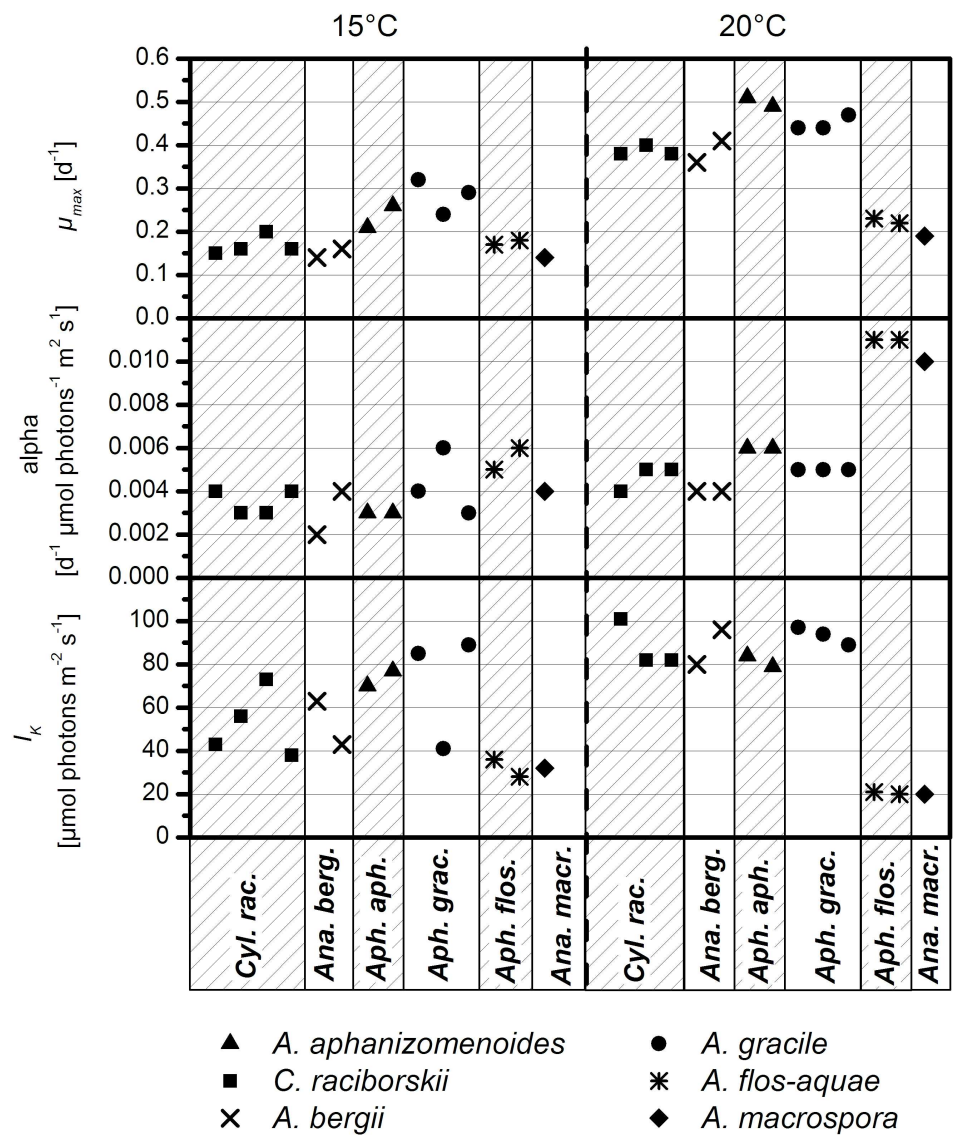
Species	$R^2$	$\mu_{max}$ [ $\text{d}^{-1}$ ]	$T_{opt}$ [°C]	$\alpha$ (10°C)	$Q_{10}$ (15:25°C)	$Q_{10}$ (20:30°C)
<i>C. raciborskii</i>	0.99	$0.34 \pm 0.01$	$27.8 \pm 0.9$	0.024	3.2	1.1
<i>A. bergii</i>	0.96	$0.25 \pm 0.01$	$26.5 \pm 1.3$	0.026	2.8	1.3
<i>A. aphanizomenoides</i>	0.98	$0.36 \pm 0.01$	$28.8 \pm 2.0$	0.035	2.0	1.2
<i>A. gracile</i>	0.99	$0.29 \pm 0.02$	$27.1 \pm 1.2$	0.010	1.6	1.0
<i>A. flos-aquae</i>	0.96	$0.27 \pm 0.01$	$24.7 \pm 0.8$	0.011	1.4	0.2
<i>A. macrospora</i>	0.99	$0.19 \pm 0.01$	$25.5 \pm 0.5$	0.011	1.7	0.9
<i>A. ovalisporum</i>	0.99	$0.36 \pm 0.02$	$32.8 \pm 0.9$	0.014	3.6	2.1



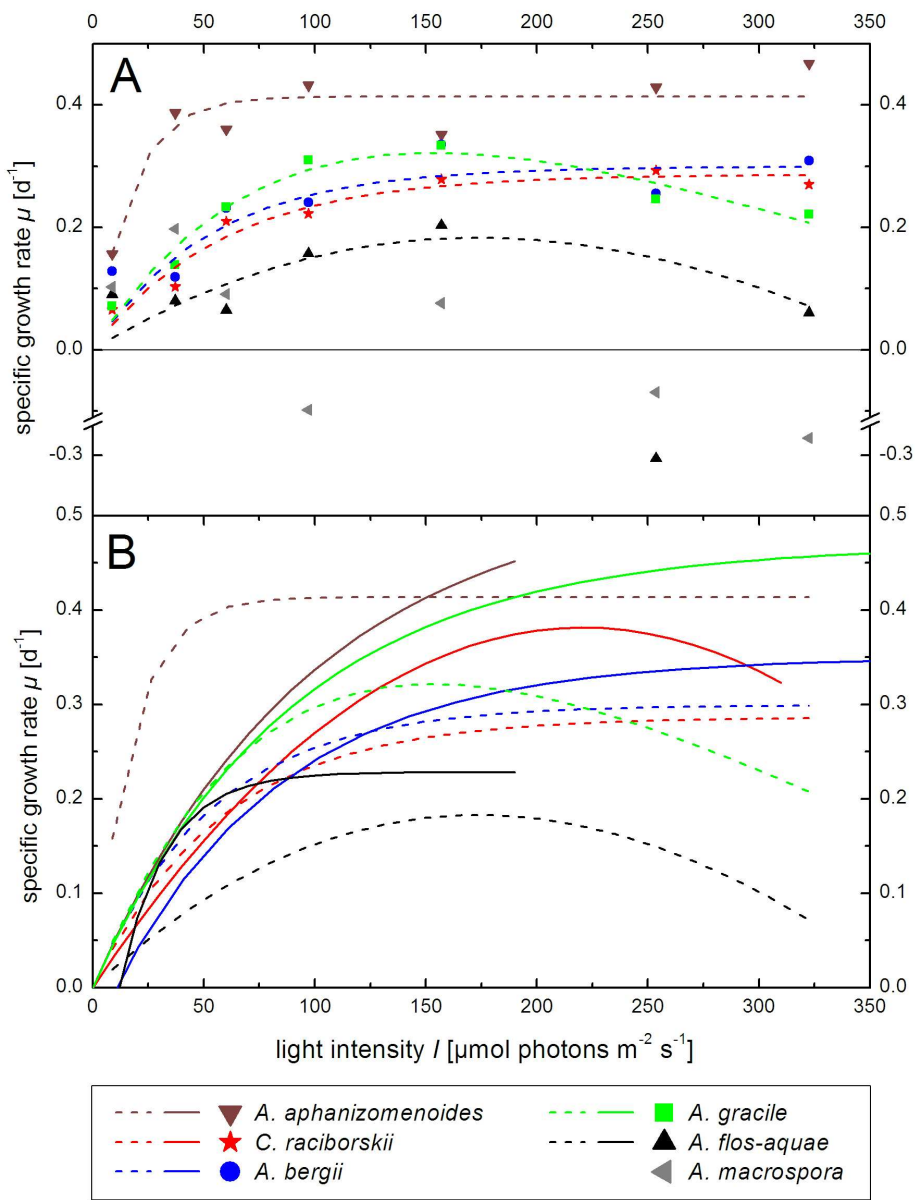
228x185mm (300 x 300 DPI)



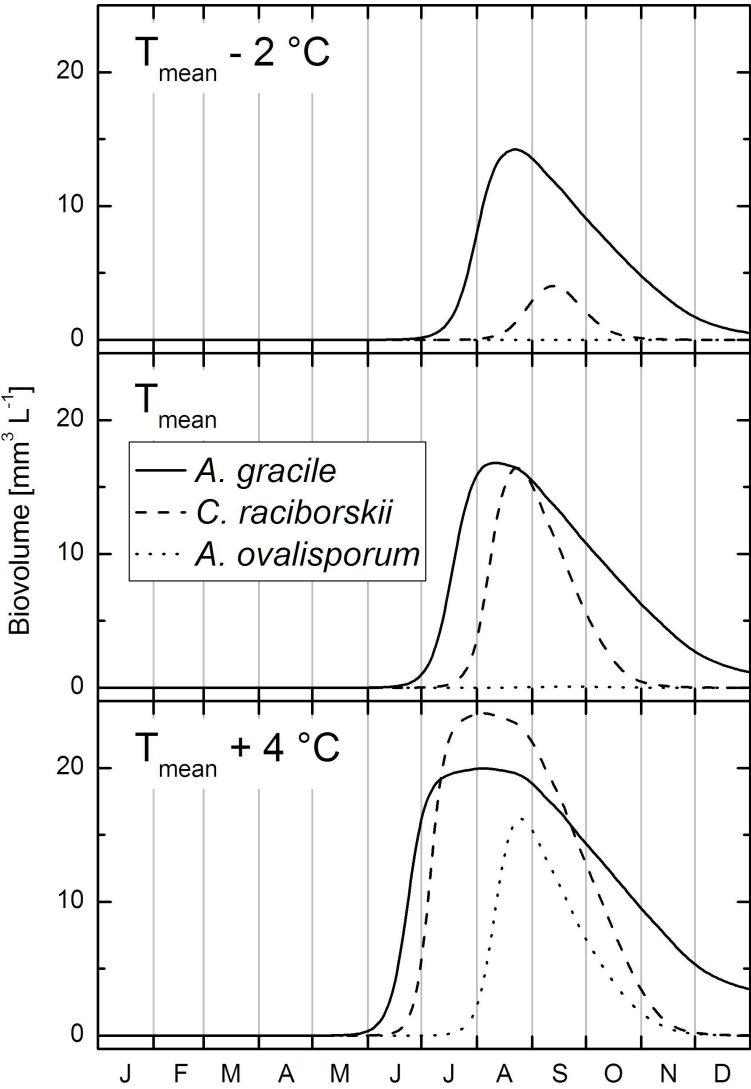
150x195mm (300 x 300 DPI)



237x270mm (300 x 300 DPI)



149x194mm (300 x 300 DPI)



187x275mm (300 x 300 DPI)