Population dynamics and akinete formation of an invasive and a native cyanobacterium in temperate lakes

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Most cyanobacteria of the order Nostocales in the temperate zone survive winters in the sediment as akinetes. The present study compared seasonal population dynamics and the formation of akinetes of the invasive cyanobacterium Cylindrospermopsis raciborskii with the native species Aphanizomenon gracile in three German lakes. The effect of light, temperature and growth rate on akinete formation was investigated in the field over a 3-year period and additionally in a culture study with nine different C. raciborskii and A. gracile strains. The results of the field study showed, for both species, a strong negative correlation between akinete formation and light and temperature, and a weaker negative correlation between akinete formation and the net growth rate of the populations. Akinete formation of the isolates was mainly affected by temperature and not by light intensity. The investigation of the seasonal population dynamics showed that the growth period of C. raciborskii in the pelagic zone was on average 64 days shorter compared with A. gracile. However, the amount of akinetes produced by C. raciborskii during 1 year was, in more than half of the cases, similar or higher than that produced by native A. gracile. The ratio of akinetes to total cell number was significantly higher for C. raciborskii compared with A. gracile, 9 and 2%, respectively. Therefore, we conclude that C. raciborskii populations in temperate lakes have a similar potential for an akinete-based life cycle performance as do the native A. gracile populations.
INTRODUCTION

Akinetes play an important role in the life cycle of cyanobacteria of the order Nostocales. Akinetes serve as resting stages to overcome unfavourable growth conditions, such as winters in temperate latitudes, drought or nutrient depletion (Olli et al., 2005; Becerra-Absalón and Tavera, 2009; Rücker et al., 2009).

An annual life cycle of a planktonic Nostocales of the temperate climate zone can be described as follows: at the end of summer, when decreasing light supply and temperatures prevent further growth, some cells differentiate into akinetes. When the vegetative population collapses, akinetes sink to the lake bottom. In the sediment akinetes take up nutrients and mature. Finally, in spring if the conditions are favourable akinetes germinate and begin to rise to the surface with the help of gas vacuoles. The pelagic population grows and the life cycle begins again (Hense and Beckmann, 2006; Kaplan-Levy et al., 2010).

However, not every temperate latitude Nostocales species survives the winter solely with akinetes in the sediment. For example, Nodularia spumigena hibernates partly as akinetes in the sediment and partly as vegetative cells in the water column. Some species even hibernate solely as vegetative cells in the pelagic zone, such as Aphanizomenon flos-aquae in the Baltic Sea (Suikkanen et al., 2010).

It is assumed that the ability to form akinetes is a prerequisite of tropical cyanobacteria like *Cylindrospermopsis raciborskii* for the successful colonization of the temperate zone, because they require high temperatures for growth and cannot survive as vegetative cells during winter. *Cylindrospermopsis raciborskii* is a potentially toxic, poleward spreading freshwater cyanobacterium of the order Nostocales (Padišák, 1997). Nowadays, it inhabits temperate freshwater worlds worldwide including Europe (Stüken et al., 2006; Kokocinski and Soiminen, 2012), North America (Hong et al., 2006) and New Zealand (Ryan et al., 2003). In temperate waters the growth period of *C. raciborskii* in the pelagic zone is restricted to the summer and the species hibernates as akinetes in the sediment (Padišák, 2003; Rücker et al., 2009). In tropical waters *C. raciborskii* is a perennial species that does not produce akinetes at all or very rarely (Saker and Griffiths, 2001; Haande et al., 2008; Everson et al., 2011).

Despite many studies on the growth of *C. raciborskii* (Istvánovics et al., 2000; Briand et al., 2004; Mehnert et al., 2010; Bonilla et al., 2012), the formation of akinetes and its regulation is still underexplored. So far it is known that temperature mediates the onset of the germination of akinetes in spring and that light controls the growth of the pelagic population of *C. raciborskii* (Wiedner et al., 2007). The field study by Rücker et al. (Rücker et al., 2009) showed that akinete formation starts when the *C. raciborskii* population reaches maximum cell abundance and the temperature drops from 25 to 20°C. Akinete formation was induced in a temperate Australian *C. raciborskii* isolate by an initial temperature shock and high irradiance (Moore et al., 2003, 2005).

Studies on the effects of environmental factors on akinete formation of other Nostocales species often provided contradictory results. Many authors have reported that light limitation is the major trigger of akinete development (Kaplan-Levy et al., 2010). In contrast, in *Anabaena circinalis* phosphate limitation appears to be the major trigger, while light limitation has no effect (van Dok and Hart, 1996). Low temperatures stimulate akinete formation in an *Anabaena flos-aquae* population and in a range of *Anabaena* isolates, whereas high temperatures induce akinete development in *Nodularia spumigena* isolates (Pandey, 1989; Li et al., 1997; Kravchuk et al., 2006). Some authors have postulated from the inconsistent results of the previous studies that a common physiological trigger, like decreased cell division or a low-energy content of the cell, induces akinete differentiation, not one single environmental factor (Rother and Fay, 1979; Adams and Duggan, 1999).

The population development of *C. raciborskii* in the temperate zone is assumed to benefit particularly from climate change, because under rising temperatures the invasive species is a stronger competitor compared with native Nostocales species like *Aphanizomenon gracile* (Mehnert et al., 2010). *Aphanizomenon gracile* is the most frequent and abundant Nostocales species in the lakes studied and other Northeast German lakes (Mischke and Nixdorf, 2003; Rücker et al., 2007). Both species are able to form akinetes and to fix molecular nitrogen. Thus, *A. gracile* and *C. raciborskii* overlap most likely in their ecological niche. Data on the life cycle of *A. gracile* are rare. Tezanos Pinto and Litchman (Tezanos Pinto and Litchman, 2010) reported that akinetes occur without any consistent pattern related to light availability in an *A. gracile* isolate.

The main aim of this study was to contribute to a better understanding of the life cycle performance of invasive *C. raciborskii* populations in comparison with native *A. gracile* populations in order to estimate the competitive ability and further development of *C. raciborskii* in the temperate zone.
Therefore, population growth and akinete formation of *C. raciborskii* and *A. gracile* in three German lakes over a 3-year period were monitored. Moreover, effects of temperature and light on akinete formation were analysed for the field populations and additionally studied in culture experiments with strains isolated from tropical and temperate waters.

**METHOD**

**Field study**

The study area is located in the Scharmützelsee region (52°25’ N; 14°05’ E) in Brandenburg (Germany), ~60 km to the south-east of Berlin. The study lakes are polymeric and shallow; relevant lake characteristics are given in Table I.

The three lakes have been sampled monthly to biweekly at their deepest point from January 2006 to December 2008. Mixed samples from the whole water column were prepared by taking samples at half-meter intervals with a 2.3-L Limnos sampler (Turku, Finland). Aliquots of the mixed samples were analysed to determine the concentrations of total phosphorus (TP) and chlorophyll *a* according to standard methods (DEV, 1976–1998). On each sampling date, depth profiles of water temperature at 0.5-m intervals were determined using a multiparameter probe (H20, Hydrolab, Austin, TX, USA). Photosynthetically active radiation (PAR) was measured at half-meter intervals through the water column using two spherical quantum sensors (SA 193, LI-COR, Lincoln, NE, USA). The mean PAR in the mixed layer (*I*<sub>mix</sub>) was calculated as described by Wiedner et al. (Wiedner et al., 2007).

Aliquots for the determination of the cell number and biovolume were fixed with Lugol’s solution and studied under an inverted microscope according to Utermöhl (Utermöhl, 1958) and Rott (Rott, 1981). Counting and measurement of cell dimensions was carried out at 400× magnification. Length and width of at least 20 vegetative cells, 20 heterocytes and 20 akinetes were measured for each species. The biovolume of each cell type was calculated from abundance and mean cell biovolume. The total biovolume of each species is composed of the sum of the vegetative cells, heterocytes and akinetes.

In Fig. 4 the initiation of the pelagic population and of akinete formation was defined as a minimum proportion of total cell biovolume or akinete biovolume of 5% of the maximum total cell biovolume in the respective year.

**Culture study**

The study was carried out with three *Aphanizomenon gracile* strains (16D11, 30D11, ZIE23AFA), three *Cylindrospermopsis raciborskii* strains (24G7, 19F6, ZIE11CR) isolated from lakes in Northern Germany and three *C. raciborskii* strains from Australia (LJ, AQS) and Africa (CYA507). Detailed information on the origin of the strains is given in Haande et al. (Haande et al., 2008) and Mehnert et al. (Mehnert et al., 2010).

Strains were cultivated in Z8-medium with some modification (Kotai, 1972). It contained additionally 40 mg L<sup>–1</sup> of biotin, vitamin B12 and thiamine hydrochloride. A pH value of 8.0–8.8 was adjusted by addition of NaOH. Erlenmeyer flasks (250 mL) were used as culture vessels and filled with 100 mL of suspension. They were continuously shaken at 80–100 rpm by an orbital shaker (IKA, KS 260 basic, Germany). Cultures were grown in a semi-continuous fashion according to the turbidostat principle as detailed in Mehnert et al. (Mehnert et al., 2010) in growth chambers (BINDER, KBW 400, Germany) with dimmable illumination (OSRAM, Lumilux 18 W/865XT Cool Daylight, Germany) simulating a 12:12 h light:dark photoperiod. Light intensity was measured using an LI-250A light meter (LI-COR, Lincoln, NE, USA) equipped with a microspherical quantum sensor (US-SQS/L Walz, Effeltrich, Germany).

The cultures were grown at eight different light intensities ranging from 20 to 330 μmol photons m<sup>–2</sup> s<sup>–1</sup> at 15 and 20°C. Samples for microscopic analysis were taken at each combination of light and temperature when cultures reached steady state and were fixed with Lugol’s solution. In each sample the width of 30 filaments and the length and width of 30 heterocytes and 30 akinetes were measured (OLYMPUS BX51, 400× magnification, cellP<sup>D</sup> software, Olympus Soft Imaging Solutions GmbH, Münster). For biovolume determination the length of ~100 filaments was measured, and the number of akinetes and heterocytes in the same transects of the phytoplankton counting chamber (Hydro-Bios, Kiel) was determined using an inverted microscope (Leitz, Laborvert FS, 100× magnification, TSO-VID-Mess-HY software).

**Table I: Morphometric and trophic parameters of the investigated lakes**

<table>
<thead>
<tr>
<th>Lake</th>
<th>Maximum depth (m)</th>
<th>Mean depth (m)</th>
<th>Area (km&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Volume (m&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Chl <em>a</em> (μg L&lt;sup&gt;–1&lt;/sup&gt;)</th>
<th>TP (μg L&lt;sup&gt;–1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melansee</td>
<td>2.4</td>
<td>1.6</td>
<td>0.12</td>
<td>0.17 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>70</td>
<td>44</td>
</tr>
<tr>
<td>Langer See</td>
<td>3.8</td>
<td>2.1</td>
<td>1.55</td>
<td>3.27 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>102</td>
<td>66</td>
</tr>
<tr>
<td>Petersdorfer See</td>
<td>4.0</td>
<td>2.3</td>
<td>0.23</td>
<td>0.53 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>76</td>
<td>42</td>
</tr>
</tbody>
</table>

Concentrations of chlorophyll *a* (Chl *a*) and total phosphorus (TP) are given as vegetation means (June–November) for the years 2006–2008.
The total biovolume is given for the culture study. The field study only the percentage of akinete biovolume on vegetative cells was not determined. Hence, in contrast to (Utermöhl, 1958) and Rott (Rott, 1981). The number of C. raciborskii produced, in more than half of the cases, a similar or higher amount of akinetes per year compared with A. gracile. The ratio of akinetes to total cells produced within 1 year was always higher for C. raciborskii in comparison with A. gracile (Fig. 5B). The analysis of the pooled data set revealed that the proportion of akinetes (on the total cell biovolume, %BVAK and on the total cell number, %CNAK, respectively) was significantly higher in C. raciborskii (%BVAK = 15%; %CNAK = 9%) compared with A. gracile (%BVAK = 8%; %CNAK = 2%) (\(z = -2.910, P = 0.004; z = -4.384, P < 0.001\)).

The proportion of akinetes (%BVAK) increased towards the end of the growth season when the vegetative cells declined faster than the akinetes (Figs 1–3C). This coincided with a decrease in the water temperature and with decreasing light availability (Figs 1–3D). In both species a significant negative correlation was found between akinete proportion (%BVAK and %CNAK) and water temperature and between akinete proportion and underwater light supply (Table II). The akinete proportion (%BVAK) of C. raciborskii rose clearly at temperatures < 20°C, whilst that of A. gracile did not increase until the temperature fell < 15°C (Fig. 6A). In contrast, there was no great difference between the two species in the light-dependent onset of their akinete proportion (Fig. 6B). The akinete portion of both species increased at light intensities < 100 μmol photons m\(^{-2}\) s\(^{-1}\). The net growth rate of both populations was also negatively correlated with akinete proportion (%BVAK and %CNAK), but weaker than the temperature and the light intensity (Table II). Akinete formation was at its highest, when the growth of the population ceased (Fig. 6C).

Culture study
Two of the tropical C. raciborskii strains (AQS, CYA507) formed no akinetes under all the light and temperature conditions tested, while the third strain (IJ) formed a low percentage of akinetes (3.3%) only at 20°C and 20 μmol photons m\(^{-2}\) s\(^{-1}\) (data not shown). The akinete proportion (%BVAK) of all three A. gracile strains was clearly higher at 15°C in contrast to 20°C (\(P \leq 0.05\)), whereas the strains of C. raciborskii isolated from German lakes showed no clear pattern (Fig. 7). Cylindropermopsis raciborskii strain ZIE11CR had a higher akinete proportion at 15°C in comparison with 20°C, the opposite was true for strain 24G7; strain 19F6 produced hardly any akinetes at all. The C. raciborskii strains produced a significantly higher akinete proportion at 20°C in comparison with the A. gracile strains (\(T = 2.528, P = 0.015\)), while it was
vice versa at 15°C ($T = -3.332$, $P = 0.002$). The effect of light intensity on the akinete proportion differed between the two species and between 15 and 20°C (Fig. 7). At 20°C the akinete proportion of the C. raciborskii strains 24G7 and ZIE11CR increased with increasing light intensity and did not decline until the highest light intensity of 300 μmol photons m$^{-2}$ s$^{-1}$, whereas at 15°C the akinete proportion showed no clear trend with light intensity. In the case of A. gracile at 15°C the akinete proportion of two strains decreased with increasing light intensity, while the third strain (30D11) behaved in the opposite way. At 20°C the akinete proportion of A. gracile strains was too low to see any trend with light.

The relation between growth rate and akinete proportion was inconsistent. For example, the two C. raciborskii strains 24G7 and ZIE11CR had an increased akinete proportion with increasing growth rates at 20°C, but not at 15°C (Fig. 7).

**DISCUSSION**

The results of the field study in the three North German lakes show that C. raciborskii and A. gracile differed in their population dynamics and akinete formation: the vegetative period of the pelagic population of C. raciborskii was on average 64 days shorter than that of A. gracile. However, the calculation of the annual pelagic akinete production revealed that C. raciborskii produced, in more than half of the cases, at least a similar amount of akinetes per year as A. gracile. Based on the analysis of the pooled data set of all lakes we found that the proportion of akinetes on total biomass was higher in C. raciborskii than in A. gracile. Thus, the invasive species is obviously able to compensate for the disadvantage of its shorter growth period by forming akinetes at a significantly higher percentage of total biomass or cell number in contrast to the native species A. gracile. This confirms the results of Padišák and Istvánovics (Padišák and Istvánovics, 1997) who found that C. raciborskii produces a much higher amount of akinetes per unit biomass than other Nostocales in Lake Balaton. Considering that C. raciborskii populations in the tropics produce hardly any akinetes (Saker and Griffiths, 2001; Everson et al., 2011), it is likely that during the poleward spread of C. raciborskii those subpopulations were selected that were able to form akinetes. By the selection akinetes could have served by itself as a dispersal unit or only those filaments that formed akinetes were able to hibernate and

![Fig. 1. Seasonal patterns of C. raciborskii and A. gracile total cells and akinetes in the pelagic zone of Lake Petersdorfer See from 2006 to 2008.](https://academic.oup.com/plankt/article-abstract/36/2/378/1503627)
Fig. 2. Seasonal patterns of *C. raciborskii* and *A. gracile* total cells and akinetes in the pelagic zone of Lake Melangsee from 2006 to 2008. For further details see Fig. 1.

Fig. 3. Seasonal patterns of *C. raciborskii* and *A. gracile* total cells and akinetes in the pelagic zone of Lake Langer See from 2006 to 2008. For further details see Fig. 1.
to establish a new population (Sukenik et al., 2012). Although only three temperate and three tropical isolates were analysed in the present study, it has been confirmed that all temperate isolates formed akinetes in contrast to the tropical isolates.

In the lakes studied akinete formation of both species started mostly later than the first appearance of vegetative cells in the pelagic zone. In both species the proportion of akinetes on total biovolume and cell number, respectively, increased towards the end of the growth season.

Table II: Correlation statistics between the akinete proportion on total cell number (%CN\textsubscript{AK}) and biovolume (%BV\textsubscript{AK}) of C. raciborskii and A. gracile and the water temperature, the mean photosynthetically active radiation in the mixed water column (I\textsubscript{mix}) and the net growth rate of the population

<table>
<thead>
<tr>
<th></th>
<th>C. raciborskii</th>
<th>A. gracile</th>
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<tbody>
<tr>
<td></td>
<td>%BV\textsubscript{AK}</td>
<td>%CN\textsubscript{AK}</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>( \rho = -0.612 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>I\textsubscript{mix} (( \mu \text{mol photons m}^{-2} \text{s}^{-1} ))</td>
<td>( \rho = -0.580 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Net growth rate (day(^{-1}))</td>
<td>( \rho = -0.420 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

Data from three lakes and 3 years were pooled. \( \rho \), correlation coefficient; \( P \), significance.
reflecting the stronger decrease of vegetative cells compared with akinetes. In lakes of the northern hemisphere both light and temperature decrease between June and November, and we found a negative correlation between akinete proportion and light and temperature as well as the population net growth rate. Since light and temperature as potentially limiting factors of population growth cannot be treated as independent variables in the field study we conducted culture experiments to study the impact of light, temperature and growth on akinete formation.

Light limitation is considered to be a main trigger of akinete formation by many authors (Kaplan-Levy et al., 2010). In our culture experiments we found no consistent pattern between light intensity and akinete formation of the different strains studied. The assumption that light limitation induces akinete formation was mainly derived from dense batch cultures that develop many akinetes during the late exponential growth phase. Fay et al. (Fay et al., 1984) demonstrated that a decreased light penetration in a dense culture leads to enhanced akinete differentiation, whereas decreased illumination in a dilute culture suspension diminishes akinete differentiation. Our cultures were grown semi-continuously which avoids increased self-shading. Hence, we agree with Fay et al. (Fay et al., 1984), who concluded that the limitation of photochemically utilizable radiation and not the reduction of white light comprising full spectral range of radiation causes akinete formation.

In the culture experiment we found that the akinete proportion of the _A. gracile_ strains was significantly enhanced at 15°C in contrast to 20°C. Such a clear pattern was not found for the _C. raciborskii_ strains. The akinete proportion of the _C. raciborskii_ strains already increased at temperatures of 20°C. This was also observed for _C. raciborskii_ populations in our field study and in Lake Kinneret when water temperatures decreased <25°C (Alster et al., 2010). The temperature that stimulates akinete development in a Mediterranean isolate of _Aphanizomenon ovalisporum_ ranges between 20 and...
25°C (Cirés et al., 2013), while subarctic and temperate Anabaena strains develop maximum akinete proportions between 10 and 15°C (Li et al., 1997). Consequently, there is no common temperature, at which akinete development was induced in the species investigated.

The present study and those of Cirés et al. (Cirés et al., 2013) and Li et al. (Li et al., 1997) demonstrate that akinete formation is induced at temperatures lower than optimal growth temperatures, but higher than minimal temperatures required for growth. Decreased cell division has been suggested as a possible signal for akinete formation (Adams and Duggan, 1999). We could not find a consistent relation between growth rate and akinete formation in our culture study, where akinete proportion was enhanced in strains growing at suboptimal low rates, but also in strains growing at optimal high rates. This indicates that low energy alone does not mediate akinete formation.

Similarly, Argueta and Summers (Argueta and Summers, 2005) found that the ATP level did not vary between a Nostoc punctiforme mutant strain with inducible akinete formation and a wild type strain of N. punctiforme. They concluded that a signal other than energy level may trigger akinete formation. The biochemical processes that translate an external signal like light intensity and temperature into an internal signal that triggers the differentiation of a vegetative cell into an akinete are still unknown. Sukenik et al. (Sukenik et al., 2013) detailed which molecules and proteins could be involved in such a signal cascade, for instance reactive oxygen species or α-ketoglutarate like during heterocyst differentiation or universal stress proteins, whose synthesis is stimulated in Escherichia coli by stationary phase, starvation and other stress conditions.

Summarizing the results of this study we have shown that populations of the invasive species C. raciborskii can compensate for a shorter growing season by a higher akinete production per unit biomass compared with the native Nostocales species, A. gracile. Therefore, we conclude that the present C. raciborskii populations have a similar potential for an akinete-based life cycle performance as A. gracile populations have in temperate lakes. However, the successful establishment of populations in the following year is not only dependent on akinete production but also on other processes influencing the survival of akinetes. These include loss processes such as grazing, infection and burial in the sediment, the maturation and germination of akinetes as well as the subsequent development of young filaments in the open water.

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