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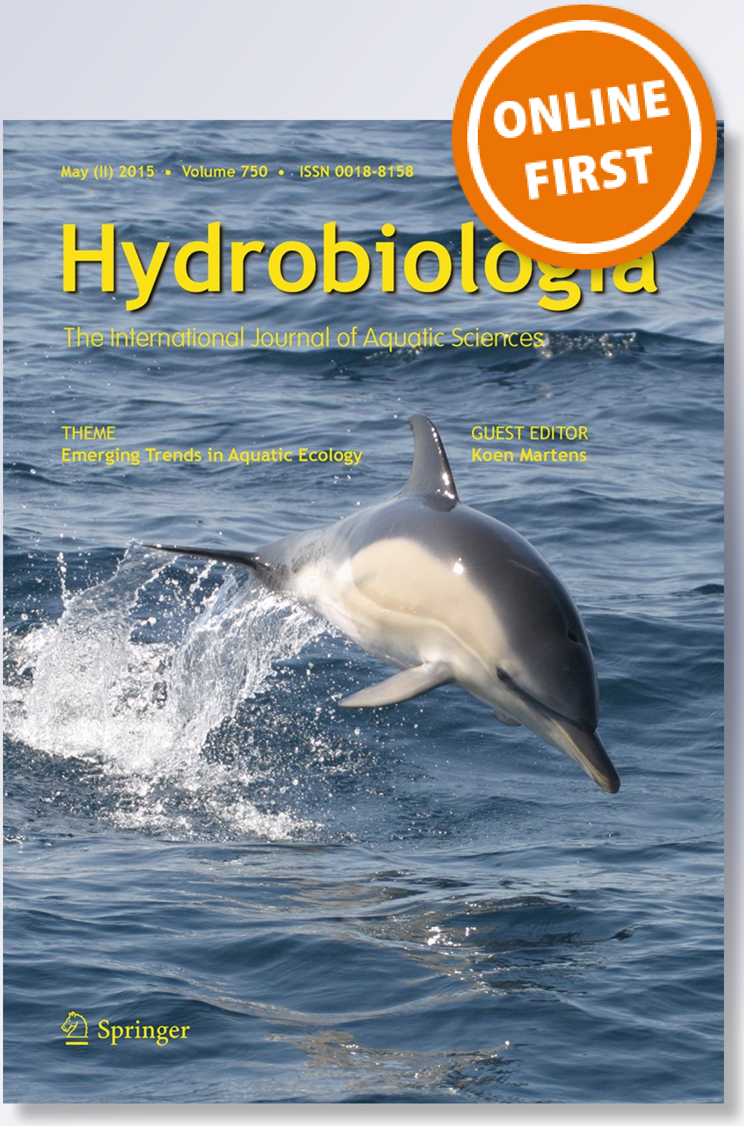

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PHYTOPLANKTON & SPATIAL GRADIENTS

Opinion Paper

Vegetative survival of *Cylindrospermopsis raciborskii* (Cyanobacteria) at low temperature and low light

Martin T. Dokulil

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Abstract Invasive neophytic species such as *Cylindrospermopsis raciborskii* often pose a problem in many regions world-wide. The mechanisms of expansion are not fully understood. Occurrence and survival of the species at unusual ambient conditions are re-analysed from an earlier study to test the ability to survive in the vegetative form at water temperatures below 12°C. Results indicate that vegetative existence at low temperatures and light in the two periods analysed benefited from weather conditions exceeding long-term average climatic records. Winter survival enables the species to re-establish a population when conditions become favourable again. Such metabolic adaptations will allow the species to expand to and thrive in novel environments particularly if weather conditions due to climate warming change.

Keywords Cyanobacteria · Invasive species · Ambient conditions · Occurrence · Climate warming

Introduction

The world-wide expansion of the invasive and potential toxic cyanobacterial species *Cylindrospermopsis raciborskii* can pose a serious problem to surface waters (Padisák, 1997). Among other factors, temperature and light are extensively discussed as main driving variables for the growth and maintenance of the various ecotypes established (Briand et al., 2004; Chonudomkul et al., 2004; Kling, 2009).

Numerous observations in the field and experimental results on isolated strains concluded that optimal temperature range for *C. raciborskii* is between 20 and 35°C (Briand et al., 2004). It has been shown in several cases, however, that *C. raciborskii* can adapt to lower temperatures, thus increasing its temperature tolerance range (Chonudomkul et al., 2004; Sinha et al., 2012). The taxon proliferated between 15 and 18°C in an urban oxbow lake in Vienna, Austria (Dokulil & Mayer, 1996) and dominated the phytoplankton at 11–12°C in an artificial subtropical lake in Uruguay (Fabre et al., 2010). It is commonly believed that *C. raciborskii* does not survive vegetatively at low temperatures. These observations might largely depend on the detection limits applied. Persistence and tolerance of strains to low ambient temperatures result in competitive advantage enabling the species to colonize waters in temperate or colder regions of the globe (Varkonyi et al., 2000). Surviving filaments of the species may sometimes contribute much less than

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10% to total phytoplankton biovolume (e.g. Kokociński et al., 2010, 2012; Messineo et al., 2010). Such low densities are often neglected or not reported.

Parts of an older data set (Dokulil & Mayer, 1996) are re-analysed here to test the hypothesis that *C. raciborskii* is able to survive in the vegetative form at temperatures below 12°C. Since the time of observation, the species has been exterminated by restoration of the lake already described several times (e.g. Dokulil & Teubner, 2003; Dokulil et al., 2011).

Methods

The location and the methods used have been published earlier in Dokulil & Mayer (1996). In brief, the shallow urban oxbow lake 'Alte Donau' (area 1.6 km^2, maximum depth 6.8 m) was sampled at fortnightly intervals from April to September and monthly from October to March starting from April 1993 until now. Because water column is usually well mixed, integrated samples were taken with an acrylic-glass tube from five stations to compensate longitudinal variability of the two basins in 1993 and 1994. All data presented here are averages of the five stations.

Temperature and oxygen profiles were recorded using a submersible profiler (Hydrolog, Grabner). The depth of the euphotic zone corresponding to 1% surface light intensity was estimated from measurements of underwater photon flux density applying a 4π underwater quantum sensor (LI-COR, USA). Standard analytical techniques were used for all chemical quantities (Mackereth et al., 1978).

Biovolume of the phytoplankton species was estimated by counting at least 600 cells on an inverted microscope and size measurements on an image analysis system using geometric approximations (Lund et al., 1958, Hillebrand et al., 1999). All blue-green algal filaments and at least 20 individuals of the other species were sized from each sample.

The seasonality graph and all statistics were made with SigmaPlot 12 (SystaSoftware, Inc.). All data were tested for normality by the Kolmogorov–Smirnov test. Biovolumes were log transformed prior to statistical evaluation. Surface and contour plots were gridded with power 2 and smoothing factor 0.5 in Grapher 9 (Golden Software, Inc.).

Results

The seasonal cycle of total phytoplankton biovolume (TB) and the cyanobacterial contribution, almost entirely equivalent to *Cylindrospermopsis raciborskii,* are presented together with average water temperature (WT) and mean euphotic depth (z_{eu}) from May 1993 to May 1995 in Fig. 1. Biovolumes peaked in the second half of both years coinciding with minimum transparency and declining water temperatures. Considerable part of total biomass was represented by *C. raciborskii* (mean 38%, range 0–85%) when temperatures were at or below 12°C (Fig. 1). These periods were characterised by mean water temperature of 6.5 ± 3.7°C and improved transparency with z_{eu} extending far beyond the mean depth of 2.3 m (mean 3.4 m, range 1.3–4.7 m). Air and water temperature during both winters were well above the respective long-term average of 1.06 and 1.55°C. During winter 1993/1994 (December–February), air temperature averaged 2.57°C and mean water temperature was 2.37°C, while temperatures were 2.93 and 2.81°C, respectively, in the following winter 1994/1995. Overall, air temperature explains over 60% of the variability of water temperature both annually and in winter. Ice cover was only observed in January 1995 when average air temperature was 0.4°C. The lake was ice covered, however, in the preceding winter (1992/1993) and in the two following winters (1995/1996 and 1996/1997) when air temperatures were 0.37, −1.93 and −0.47°C, respectively. The sunshine duration was 83 and 113 h in January and February 1994 which was significantly higher than the long-term sunshine duration of about 41 and 67 h, respectively. Sunshine hours were 67 h in January 1995 and 96 h in February. Both periods analysed here had similar total phosphorus concentrations of 49 ± 7.2 and 50 ± 29 μg P l^{-1}. Variability was much larger in the second period with concentrations larger than 80 μg P l^{-1} in November and December 1994. Concentrations dropped to less than 30 μg P l^{-1} in January under ice and increased thereafter. Total nitrogen concentrations were 1.36 ± 0.43 and 1.91 ± 0.99 mg N l^{-1} for the respective periods with similar variation as described above for phosphorus in the 2nd period. The mean N:P ratios were 27 and 38 for the two periods in question.

Average total biovolume was significantly related to water temperature and highly correlated with z_{eu} for

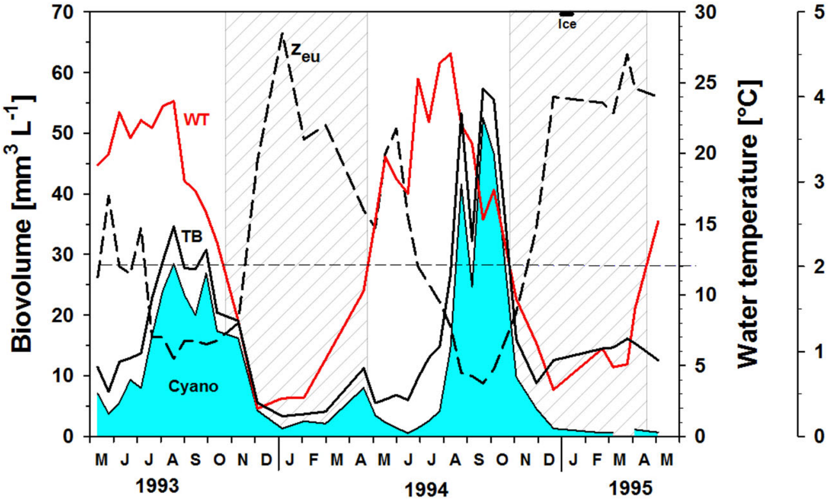

Fig. 1 Seasonal cycle of total phytoplankton biovolume (TB), cyanobacterial contribution (Cyano), water temperature (WT) and euphotic zone depth (z_{eu}) from May 1993–April 1995. All values are averaged from five sampling stations. The *horizontal dashed line* indicates 12°C and the *vertical hatched area* delineates the periods with water temperatures below 12°C. The period of ice cover is indicated by a *thick black bar*

the 2 years (Table 1). Biovolume of *Cylindrospermopsis* was not significantly related to temperature below 12°C but correlation to euphotic zone was highly significant ($r^2 = 0.59$).

The relative biovolume contribution of *C. raciborskii* was related to water temperature below 12°C and euphotic zone in Fig. 2. Contributions of *C. raciborskii* to total biovolume of over 50% were linked to temperatures of 8–13°C and euphotic zone depths of 1–4 m. At temperatures below 8°C biovolume contribution remained above 30%. Lower contributions were associated with deeper euphotic depth.

Discussion

Reynolds (1994) suggested that large overwintering populations are one reason why cyanobacteria with low specific growth rates become dominant in summer phytoplankton communities. In this study, high biovolume contributions of *C. raciborskii*, often much larger than 50%, survived periods of water temperature below 12°C in the urban oxbow lake Alte Donau. Vegetative survival of the population was enabled by warmer than usual winter conditions, minimal ice cover, higher sunshine duration and decent nutrient availability. As indicated by the N:P ratios, a surplus of nitrogen was present making energy consumption through N-fixation unnecessary as demonstrated experimentally (Schuh, 1997). The population was able to inoculate the phytoplankton assemblage in the following spring. Bittencourt-Oliveira et al. (2011) reported better development of the coiled morphotype under conditions of de-stratification, low light intensity and milder temperatures, characteristic for the winter months in Brazil. Vegetative winter survival of *C. raciborskii* was also observed in Australia at water temperatures around

Table 1 Regression statistics for log transformed *C. raciborskii* biovolume (Cr) for all data (All) and data with water temperature <12°C according to $y = ax + b$

Variables	*a* value	*b* value	r^2	*F*	*P*	Signif	*N*
All: WT/Cr	0.031	0.266	0.160	6.645	0.0141	*	38
All: z_{eu}/Cr	−0.432	1.722	0.773	119,244	<0.0001	***	38
<12°C: WT/Cr	0.009	0.310	0.005	0.054	0.819	n.s.	14
<12°C: z_{eu}/Cr	−8.709	31.455	0.586	49.570	<0.0001	***	14

WT water temperature as °C, z_{eu} euphotic zone in metres, *Signif* 95% significance level, *n.s.* not significant

* Significant, *** highly significant

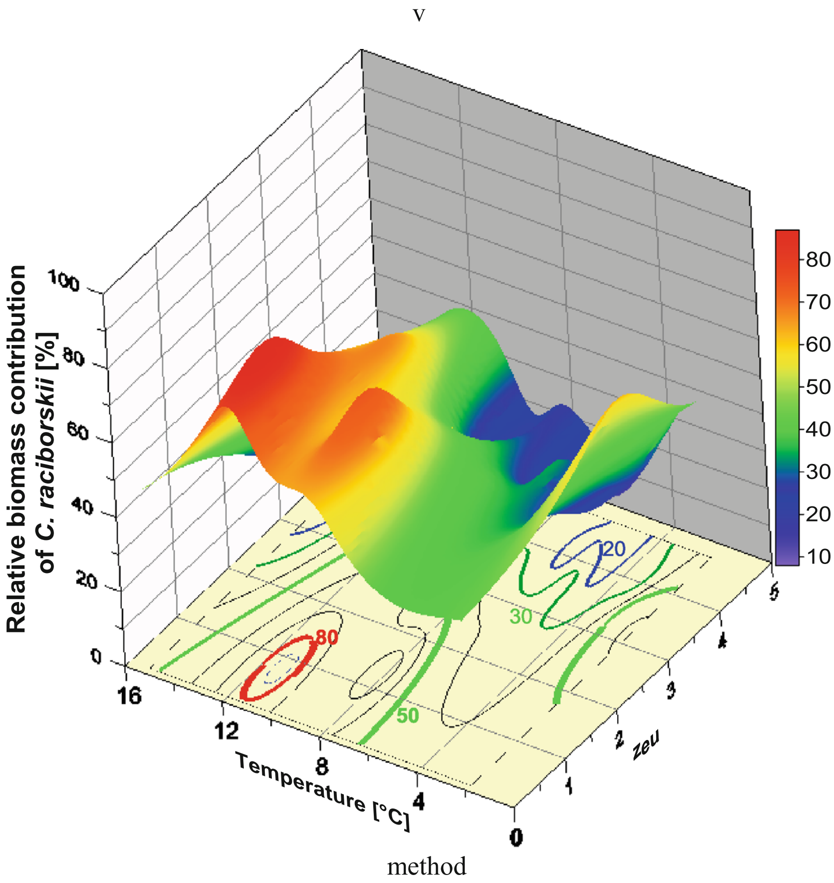

Fig. 2 Surface and contour plot of relative biomass contribution of *Cylindrospermopsis raciborskii* in relation to water temperature and euphotic zone depth (z_{eu}). The contour plot visualizes the relation in two dimensions

16°C (Everson et al., 2011) and in New Zealand at temperatures below 12°C (Wood et al., 2014). For the temperature zone, however, most authors believe that the species can only survive in winter in the form of akinetes (e.g. Mehnert et al., 2010). These authors also compared growth rates of several native and invasive Nostocalean species in microcosm experiments. They concluded that the invasive species, including *C. raciborskii,* grew faster at temperatures above 10°C which will promote the development of Nostocalean species in general and invasive species in particular.

Under similar winter conditions as in this analysis, perennial survival was reported for *Planktothrix agardhii* by Schreurs (1992). The species constituted 50% or more of the annual phytoplankton biomass in shallow European lakes when winters were mild with little ice and snow cover. Winter survival was observed in *Planktothrix rubescens* also which is not explicitly mentioned in Dokulil & Teubner (2012). Since *Planktothrix* spp. produce no akinetes, survival depends entirely on vegetative filaments which can survive more than 10 weeks in cold and dark winter conditions (Holland & Walsby, 2008).

Several studies have demonstrated that *C. raciborskii* proliferates at higher or increasing temperatures. Some authors suggest, therefore, that climate warming might influence occurrence, geographical spreading and invasion (Padisák, 1997; Briand et al., 2004; Wiedner et al., 2007; Sinha et al., 2012), while others prefer the wide tolerance spectrum of the species to explain its potential to survive and thrive in novel environments (Rzymski & Poniedziałek, 2014). The first identification in Java (Woodszynska, 1912) and the affinity to warm temperatures usually encountered and classified the species initially as ‘tropical’ to ‘subtropical’. As a consequence, low water temperatures were expected to be the main barrier for expansion into northern or southern latitudes. Since *C. raciborskii* started to grow at 17°C in German waters, Mischke (2003) discussed gradual adaptation to lower temperatures to explain an increasing tendency for the replacement of other cyanobacteria. The temperature requirement observed by Mischke (2003) corresponds to findings by Dokulil & Mayer (1996) and Dokulil & Teubner (2000).

Formation of akinetes in *C. raciborskii* has been reported as a form of perennation (Padisák, 1997; Padisák & Istvánovics, 1997). Vegetative perennation and survival in natural populations remained unclear (Fabbro & Duivenvoorden, 1996). Survival might depend on ecotypes of *C. raciborskii* as Piccini et al. (2011) reported. Isolates tolerated temperatures of 14°C, and no akinetes were formed.

Similarly, Dvořák & Hašler (2007) observed high morphological variability in *C. raciborskii* at about 14°C, and Bonilla et al. (2012) reported that the species succeeds at temperatures as low as 11°C. Analysing the occurrence of the species at the north-eastern limit of the geographical range, Kokociński and Soininen (2012) concluded that higher temperatures facilitate the initiation of growth early in the season but growth might continue at relatively low temperature once populations are well developed. In an earlier study by Kokociński et al. (2010), *C. raciborskii* was present all year round but in very small biovolumes when water temperatures were low. These results correspond to the survival of *C. raciborskii* at temperatures below 12°C reported here, which suggests that vegetative existence is possible and might be important to re-establish a population when water temperatures rise again. The contrasting results regarding water temperature are most likely associated with the occurrence of genetically and ecophysiologically different ecotypes of *C. raciborskii*, resulting in a phenotypic plasticity which can explain the ongoing expansion andsuggest future predominance under climate warming.

At present, all available information indicate that *C. raciborskii* is a taxon highly adaptable and in the process of radiation, as morphotypes, ecotypes and life-cycle stages suggest (e.g. Moustaka-Gouni et al., 2009). Clones of high physiological tolerance can acclimate or adapt to lower temperatures thus enabling the expansion to novel environments. Global warming supports the invasive potential of the species through temperature increase and perhaps the associated ecological changes.

Conclusion

Vegetative appearance and survival of *C. raciborskii* at low temperatures and low light intensities seem to be an important feature of the taxon complex to expand, inoculate and establish populations into new environments and climate regions. Increasing incidences of weather conditions exceeding long-term averages due to climate change might promote proliferation of cyanobacteria in the near future. Metabolic information at the lower end of environmental variables, however, is too incomplete at present to make definite predictions. Observations and experiments shall pay more attention to low occurrences of much less than 10% at unusual ambient conditions particularly in winters of mid- or high latitudes. In a changing world, detection of species invasion and survival at low level becomes increasingly essential to act before severe manifestations, e.g. as surface blooms become apparent.

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