Short communication

Recent occurrence of *Cylindrospermopsis raciborskii* in Waikato lakes of New Zealand

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Abstract   *Cylindrospermopsis raciborskii* is a toxin-producing species of cyanobacteria that in autumn 2003 was recorded for the first time in three shallow (max. depth ≤5 m) Waikato lakes and a hydro-electric dam on the Waikato River, New Zealand. It formed water blooms at densities >100 000 cells/ml in Lakes Waahi and Whangape. Net rates of population growth >0.2 day^{-1} were recorded for *C. raciborskii* in Lakes Ngaroto, Waahi, and Karapiro, based on comparisons of low numbers (detection of <10 cells/ml) from initial samples and its presence at bloom densities (>15 000 cells/ml) in the subsequent sample "x"–"y" days later. *C. raciborskii* may be well adapted to rapid proliferation in the Waikato lakes, which are eutrophic to hypertrophic, with high light attenuation, and where nitrogen (N) fixation may provide it with a competitive advantage over non-nitrogen fixing algae under N-limited conditions.

Keywords   cyanobacteria; *Cylindrospermopsis raciborskii*; lakes; New Zealand

INTRODUCTION

Cylindrospermopsis raciborskii (Woloszyńska) Seenaya et Subba Raju is a highly adaptable freshwater cyanobacterium that is capable of nitrogen (N) fixation and known to produce the cyanotoxin cylindrospermopsin (CYN) (Hawkins 1997). It was first recorded in tropical and subtropical regions (Fabbro & Duivenvoorden 1996; Chapman & Schelske 1997) but has apparently spread rapidly over the past 10 years to invade temperate regions of Australia, Europe, and North and South America (Padisák 1997). The first confirmed identification of this species in New Zealand was by Wood & Stirling (2003) in Lake Waahi in March 2003. There is considerable morphological variation in *C. raciborskii* (McGregor & Fabbro 2000) and this is one of the reasons for its recent appearance in water bodies where it has been previously unrecorded. A morphologically similar species, *Cylindrospermum minutissimum* Collins, has previously been described in lakes in close proximity to our study lakes (Etheredge & Pridmore 1987). The identification was based upon earlier keys, and the emphasis on the position of the heterocyst being adjacent to the akinete led to material being identified as *C. minutissimum*, while the absence of heterocysts and tapering of trichomes led to identifications of the material as *Raphidiopsis*; both of these forms are described in the New Zealand literature. Correct identification of *Cylindrospermopsis raciborskii* is very difficult, due in part to the large range of morphological variants which have been documented in recent taxonomic investigations (McGregor & Fabbro 2000) and using genetic studies (Saker et al. 1999; Wilson et al. 2000).

   Cylindrospermopsis raciborskii is highly tolerant of a range of salinity and nutrient concentrations and can grow in temperate climates under light and temperature regimes different from those of tropical environments (Briand et al. 2002). These factors make it difficult to predict the distribution and proliferation of this species. High water temperatures (>19°C), however, appear to be a prerequisite for
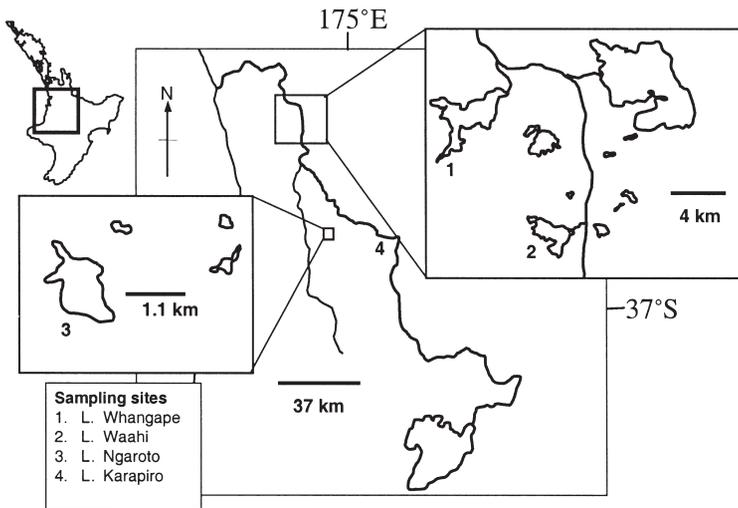
Fig. 1 North Island, New Zealand, expanded to show location of the four lakes sampled, with two areas further expanded. NZMS 265 Series.

development of large populations (Saker & Griffiths 2001; Briand et al. 2002).

Cylindrospermopsis raciborskii has a high growth potential at low irradiance. This gives it an advantage over many other cyanobacteria, which tend to be well adapted to high irradiance (Padisák & Reynolds 1998). It also has a high affinity for phosphorus and can accumulate phosphorus at relatively low ambient concentrations (Isvanovics et al. 2000). Furthermore, heterocysts confer capacity for N fixation when this nutrient is directly limiting growth, and may provide it with a competitive advantage over non-N fixing phytoplankton when there are low ambient concentrations of inorganic nitrogen. These factors may act in isolation or synchronously to contribute to the capacity of *C. raciborskii* to form dense water blooms.

Cylindrospermopsis raciborskii produces the cytotoxic alkaloid cylindrospermopsin (CYN), which has been implicated in many human and animal poisonings (Bourke et al. 1983; Saker et al. 1999). It is also known to be capable of producing paralytic shellfish poisoning (PSP) (Lagos et al. 1999; Neilan et al. 2003). In a review of the cyanotoxin cylindrospermopsin, Griffiths & Saker (2003) describe recent developments in the application of molecular techniques for characterising strains of cyanobacteria that produce this toxin. They also summarise the chemistry and *in vitro* synthesis of the toxin.

Cylindrospermopsis raciborskii may have a detrimental effect on food-webs in lakes, by interfering with carbon transfers. It has been regarded as a poor food source for zooplankton

because of the relative inedibility of the trichomes (Bouvy et al. 2000). Bouvy et al. (2001), however, found that rotifers and copepods were able to shorten trichomes to an edible size which enabled other zooplankton to graze on the shortened trichomes. They also noted that zooplankton species diversity was not affected by blooms of *C. raciborskii*.

In this paper we document the rapid proliferation of *C. raciborskii* in Waikato lakes following the first record of its presence in New Zealand in March 2003. We use *in situ* growth rates (Reynolds 1997) to show the rapid development of populations that occurred in individual lakes and we explore the possible impacts of this species on lake communities and water users.

METHODS

Study sites

Water samples for phytoplankton enumeration were collected from four Waikato lakes (Fig. 1). Lakes Waahi and Whangape are lowland lakes which were once part of an extensive wetland system (Cromarty & Scott 1995). These lakes still support a high diversity of waterfowl and have several threatened species of native birds and fish. The lakes are shallow, highly exposed as a result of vegetation clearance, and hypertrophic (Table 1) (Barnes 2002). Lake Ngaroto is a hypertrophic peat lake, with a largely pastoral catchment (Boswell et al. 1985). These three lakes receive surface and groundwater drainage from their surrounding wetland-catchment systems, but can be expected to have water residence

times of several months (Vant, Environment Waikato pers. comm.) Lake Karapiro is one of eight hydro-electric dams along the Waikato River and is considered eutrophic (Livingston et al. 1986; Vant, Environment Waikato pers. comm.). Based on storage volumes of Lake Karapiro and discharges in the Waikato River (Roper 2002), the mean water residence time is c. 4 days.

Collection of samples and algal enumeration

Water samples were taken from 30 January to 8 May 2003 as part of a regular monitoring programme of Waikato lakes. The lakes are generally monitored fortnightly to monthly during summer and autumn, depending on their level of recreational use, but sampling frequency was increased when high densities of *C. raciborskii* were recorded. The samples were collected from surface waters (depth = 0.2 m). Surface water temperatures were measured in Lakes Waahi and Whangape on each sampling occasion. Phytoplankton were preserved with Lugol's iodine and cell counts were conducted at 100× magnification using the sedimentation technique of Utermöhl (1958). Phytoplankton were identified to species level when possible and *C. raciborskii* was identified using the guide of Baker & Fabbro (2002). For *C. raciborskii* the average number of cells per trichome was calculated for each sample and cell densities were then approximated by multiplying this factor by the number of trichomes counted and adjusting for the sample volume. The maximum sample error with this technique was ±30%. The resolution of this technique was <10 cells/ml for individual species. Net rates of population growth of the phytoplankton between sample dates at individual lakes were calculated using the equation (Reynolds 1997):

$$r_n = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t} \tag{1}$$

where r_n is the net rate of growth (day^{-1}) and N_t and N_0 are cell densities at times t and 0, respectively. Phytoplankton counts were converted to relative abundances on the basis of organism density and then the index of diversity was calculated according to the method of Shannon & Weaver (Washington 1984):

$$H' = -\Sigma p_i \ln(p_i) \tag{2}$$

where H' is the Shannon index of diversity and p_i is the relative abundance of species i:

$$p_i = \frac{N_i}{N_{tot}} \tag{3}$$

where N_i is the cell density of species i and N_{tot} is the total cell density.

RESULTS AND DISCUSSION

Figure 2 shows cell counts of the total phytoplankton community and of *C. raciborskii* over the sampling period. *C. raciborskii* was first detected in Lake Waahi on 13 March 2003 (Wood & Stirling 2003) and subsequently in Lakes Whangape (10 April 2003), Karapiro (15 April 2003), and Ngaroto (24 April 2003). On 28 March 2003 the highest density of 205 500 cells/ml was recorded in Lake Waahi (Table 2). At this time *C. raciborskii* was not detected in samples from Lakes Ngaroto and Karapiro, and Lake Whangape was not sampled. The first confirmation of *C. raciborskii* in Lake Ngaroto was on 24 April 2003 (Table 2) following its absence from samples on 10 April 2003. Similarly, *C.*

Table 1 Physico-chemical features of the four study lakes. (Secchi, secchi disk depth; TN, total nitrogen; TP, total phosphorus; Chl. *a*, chlorophyll *a*; Max. depth, maximum depth.)
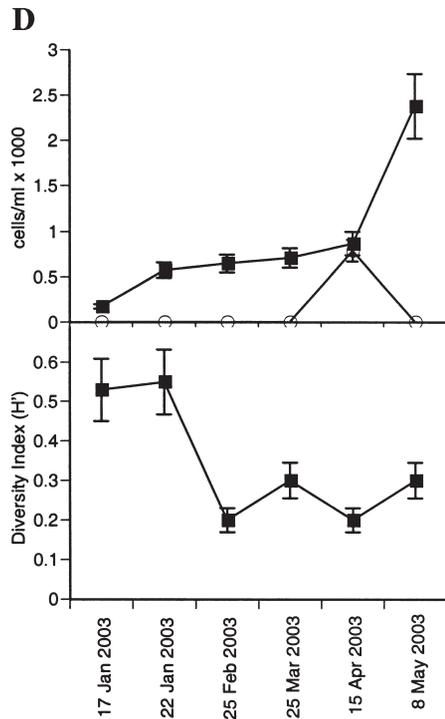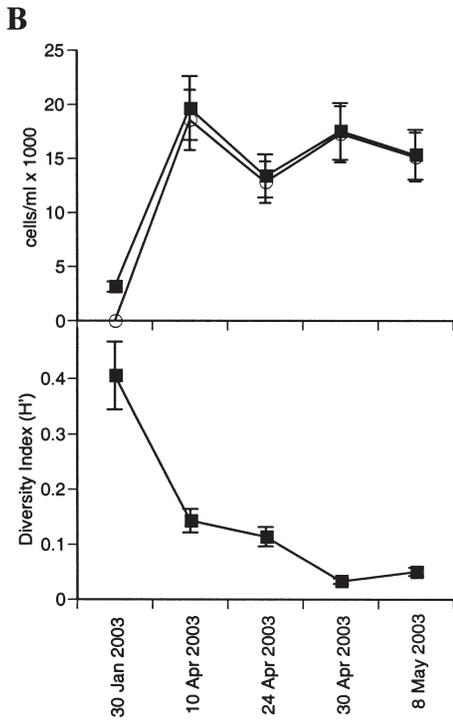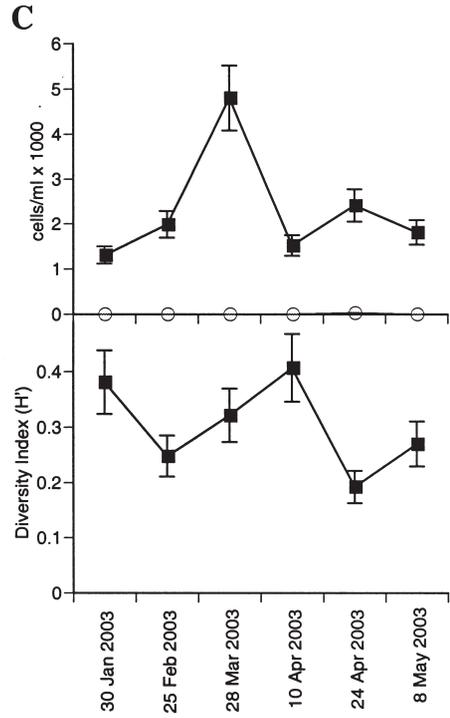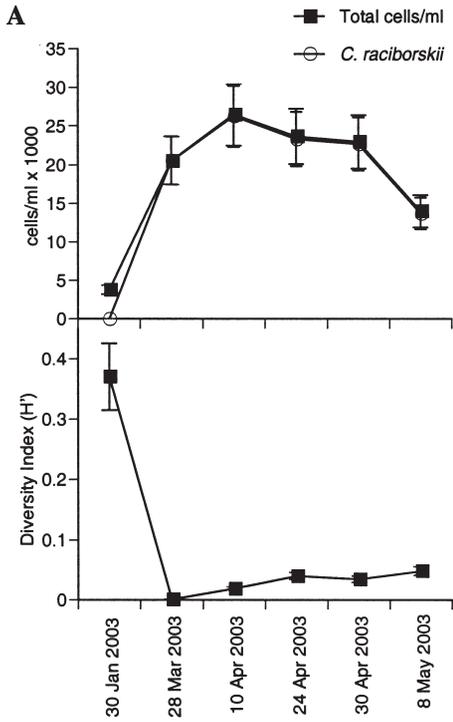
Lake	Secchi (m)	TN[†] (mg/m^3)	TP[†] (mg/m^3)	TN/TP	Chl. *a* (mg/m^3)	Trophic status[‡]	Max. depth (m)	Area (ha)[†]
Waahi	0.41[*]	915	49	18.9	34	Hypertrophic	5.0	522
Whangape	0.5	864	72	11.9	24	Hypertrophic[§]	3.5	1 450
Ngaroto	0.5	360	34	16.9	37	Hypertrophic	4.0	108
Karapiro	2.6	1 900	118	10.6	12	Eutrophic	54.0	860

[*] Irwin (1981).
[†] Environment Waikato unpubl. data (1995–2003).
[‡] Barnes (2002).
[§] Burns & Rutherford (1998).

◄ **Fig. 2** Temporal distribution of Shannon-Weiner diversity index and cell counts of the total phytoplankton community, and *Cylindrospermopsis raciborskii* in the four study lakes. **A**, Lake Waahi; **B**, Lake Whangape; **C**, Lake Ngaroto; **D**, Lake Karapiro.

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raciborskii was not detected in samples from Lake Karapiro on 28 March 2003 but attained 7 900 cells/ml on 24 April 2003 (Table 2).

Net rates of population growth (r_n) for *C. raciborskii* were calculated for the four lakes (Table 2) using Equation 1. Values of r_n represent a mean value between sampling intervals, which varied in frequency from daily to approximately monthly. We chose to use maximum values of r_n selected from successive sampling within individual lakes to support our assertion that *C. raciborskii* populations grew rapidly following their first detection. Maximum values of r_n were consistently high across all lakes, and varied from 0.17 to 0.26 day^{-1}. In Lake Karapiro r_n was 0.26 day^{-1}, but this value must be interpreted cautiously as the low water residence time (c. 4 days) suggests that populations may have been influenced by "seeding" from upstream impoundments of the Waikato River. Water residence times in several of these impoundments are substantially longer than those in Lake Karapiro (Roper 2002). By contrast, inflows to Lakes Waahi, Ngaroto, and Whangape are small, suggesting that population expansion could have been supported by recruitment, rapid germination events, or growth. There may have been different population densities within the depth profile and further investigations will need to take the mixing and variability in sampling into account.

Saker & Griffiths (2000) found that *C. raciborskii* from isolates in culture had a maximum growth rate (divisions/day) of 0.9–1.2 day^{-1} at its optimum temperature (>25°C), but a maximum growth rate of only 0.3–0.5 day^{-1} at 20°C. For our sampling days, measured surface water temperatures were 16.1–20.4°C in Lake Waahi and 16.0–20.8°C in Lake Whangape. Our *in situ* values of r_n suggest that *C. raciborskii* populations may have increased for at least some of the period between sampling intervals, at rates close to maximum values observed under laboratory culture conditions.

Macrophyte populations in Lakes Waahi, Ngaroto, and Whangape have decreased, and largely disappeared, over the past one to two decades (Boswell et al. 1985; Barnes 2002). This change can be expected to increase exposure of bottom sediments to the erosive effects of wind, leading to sediment resuspension and reduced light availability (Hamilton & Mitchell 1996; Scheffer 1998). These conditions may favour growth of *C. raciborskii*, with its capacity to grow well at low irradiance (Padisák & Reynolds 1998). These three lakes appear to provide ideal conditions for the proliferation of *C. raciborskii*, with high concentrations of nutrients and low water clarity (Table 1). Other shallow lakes in New Zealand which have changed to an "alternative stable state" (Scheffer 1998) signalled by nutrient enrichment, high turbidity, and loss of macrophytes (Hamilton & Mitchell 1997), could also be expected to be susceptible to blooms of *C. raciborskii*, depending on water temperature. *C. raciborskii* does not appear to be favoured by a specific thermal stratification regime (Antenucci et al. 2002) whereas bloom formation in many other species of cyanobacteria (e.g., *Microcystis* sp. and *Anabaena* sp.) may be closely linked to the presence of thermal stratification (Reynolds 1998; Oliver & Ganf 2000).

The ability of *C. raciborskii* to fix N may also be advantageous if its geographical range in New Zealand is extended, as phytoplankton in many lakes of the Central Volcanic Plateau of the North Island have previously been found to be N-limited (White et al. 1985).

Cylindrospermopsis raciborskii can form water blooms which may be nearly monospecific, because of its ability to fix N and subsist under different stratification regimes and at low irradiance. On 10

Table 2 Net rates of growth and cell counts of *Cylindrospermopsis raciborskii*.

Lake	Net rate of growth (day^{-1})	Time period	Counts cells/ml	
			28 Mar 2003	24 Apr 2003
Waahi	0.21	30 Jan – 28 Mar 2003	*	128 000
Whangape	0.17	30 Jan – 10 Apr 2003	205 000	233 000
Ngaroto	0.24	10 Apr – 24 Apr 2003	<10	372.0
Karapiro	0.26	25 Mar – 15 Apr 2003	<10	7 900

* Not sampled on this occasion.

April 2003, *C. raciborskii* formed an almost monospecific bloom in Lake Waahi comprising >262 700 cells/ml (Fig. 2A), with only one other species recorded, the diatom *Aulacoseira granulata*, observed at a density of 1900 cells/ml. As the bloom decreased, species diversity increased with the appearance of other species at densities <1000 cells/ml, including *Peridinium* sp., *Trachelmonas volvocina*, and *Scenedesmus* sp. The change in species diversity in lakes before and after the invasion of *C. raciborskii* was evaluated with the Shannon index, H'. In Lakes Waahi and Whangape, H' decreased from 0.4 to 0.05 between 30 January and 8 May 2003 (Fig. 2A,B). This decrease demonstrates the trend of reduced species diversity and nearly monospecific blooms of *C. raciborskii* in these two lakes in early May. The change in species diversity was very small in Lakes Karapiro and Ngaroto with the introduction of *C. raciborskii*. Lake Ngaroto (Fig. 2C) had large blooms of *Microcystis aeruginosa* (>10 000 cells/ml) during the summer and *C. raciborskii* did not reach very high densities; species diversity was little affected by the introduction of *C. raciborskii*. In Lake Karapiro, low water residence times may have prevented *C. raciborskii* from reaching densities c. 10 000 cells/ml that might have affected the diversity (Fig. 2D).

Cylindrospermopsis raciborskii may have comparable physiological characteristics to Reynolds' (1997) "Association S" group comprising solitary filamentous Oscillatoriales. This group may dominate the plankton of near-continuously mixed, exposed, turbid systems which are often nutrient-enriched; consistent with the shallow Waikato lakes in this study. Association S species are also highly adapted to low irradiance, as is *C. raciborskii*. *C. raciborskii* has been categorised as "Association H" by Reynolds (1997), however, corresponding to phytoplankton growth adapted to high light levels.

Cylindrospermopsis raciborskii appears to have high dispersal capabilities in view of its rapid establishment in temperate lakes worldwide. Padisák (1998) proposed likely mechanisms of dispersal of akinetes by migratory birds or vegetative forms with the importation of tropical fish. *C. raciborskii* has expanded its geographical range rapidly overseas and more recently in the Waikato region of New Zealand. The high rates of growth in the Waikato lakes, coupled with its apparent rapid dispersion, could have serious implications for lakes in New Zealand. Wood & Stirling (2003) detected the presence of the cyanotoxins CYN and deoxy-cylindrospermopsin (do-CYN) in samples from Lake Waahi. These factors collectively suggest that the proliferation of *C. raciborskii* in individual lakes and its geographical dispersion in lakes with high recreational or user values should be closely monitored.

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