Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms in a shallow pond in France

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Abstract

*Cylindrospermopsis raciborskii*, a potentially toxic blooming cyanobacterium (blue-green alga), responsible for public health problems in Australia, was identified in France in 1994 in a shallow pond south of Paris. A program monitoring the occurrence of *C. raciborskii* in this pond was conducted from July 1998 to October 1999. The phytoplankton assemblages were studied, and limnological parameters (water temperature, dissolved oxygen, pH, conductivity, and dissolved inorganic nutrients) were measured. By multivariate analysis (principal component analysis), we showed that sufficiently high temperatures to allow the germination of akinetes, relatively low nutrient concentrations (soluble reactive phosphorus with a mean concentration of 1 \(\mu\)M and nitrate between 0 and 5 \(\mu\)M, except in February 1999 (21 \(\mu\)M)) and a characteristic high and constant sulfate concentration (8981 ± 471 \(\mu\)M) seemed to be the main factors involved in the proliferation of *C. raciborskii* in the “Francs–Pêcheurs” (FP) pond. In the light of these findings and of bibliographic data, *C. raciborskii* would seem to be characterized by good adaptability, but also by low competitiveness with other phytoplanktonic species in the temperate study area. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Cylindrospermopsis raciborskii*; Cyanobacteria; Environmental factors; Pond; France

1. Introduction

Cyanobacteria or blue-green algae (Cyanophyceae) are highly adaptable prokaryotes able to form water or surface blooms, especially in freshwater ponds or lakes. They were reported to be potentially toxic for the first time by Francis [1], and have been blamed for animal and human poisonings over the past 20 years [2].

*Cylindrospermopsis raciborskii* (Woloszynska) Seeney and Subba Raju [3] has been reported not only in tropical or sub-tropical areas, as first reported, but across a wide range of latitudes [4]. *C. raciborskii* can grow under very varied conditions, and this makes difficult to predict its occurrence and (or) proliferation. However, high temperatures do seem to be essential for it to develop [5]. Perennial populations have only been observed in tropical areas (Australia, [6] and Brazil; [7,8]), whereas in temperate countries, *C. raciborskii* proliferations are limited to warmer periods (Austria, [9] and Hungary, [10]). This species is characterized by its high affinity for phosphorus and high phosphorus-storage capacity [11], which allows it to grow in relatively low levels of phosphate [12,13]. In addition, its preferred nitrogen source is ammonium, and nitrogen deficiency can also be a selective factor for this cyanobacterium [12,5]. Furthermore, the high shade...
tolerance of C. raciborskii also contributes to its proliferation [10]. Its ability to form akinetes when environmental conditions became unfavorable, and to move within the water column as a result of the gas vesicles it contains, also contribute to its ability to adapt to different environments.

Furthermore, the toxic potential of C. raciborskii in both tropical [14,15] and temperate areas [16] makes it particularly important to determine the ecological behavior of the species. In this paper, we describe the occurrence of C. raciborskii blooms in a fish pond near Paris (France). Between July 1998 and October 1999, phytoplankton assemblages from the lake were studied monthly, and the limnological parameters of the lake measured. Principal component analysis (PCA) was performed on environmental and phytoplankton data to study and discuss the ecological conditions of C. raciborskii proliferation in France and in temperate areas more generally.

2. Materials and methods

2.1. Study area and field sampling

The “Francs–Pêcheurs” (FP) pond is a private shallow freshwater pond within the town of Viry–Châtillon (Essonne), 25 km south of Paris. The mean and maximum depths are 2.5 and 7 m, respectively. It is a small former sandpit (15 000 m², 37 000 m³) and has no in- or out-flows.

Sampling was carried out monthly between 2:30 and 4:00 p.m. from July 1998 to October 1999, and weekly from 20 July to 9 September. As no thermic and oxygenation stratifications were observed [17], one sampling station can be taken to represent the entire pond. Water and atmospheric temperatures, pH, conductivity and dissolved oxygen concentration were recorded 0.5 m below the surface of the water using a pH-Meter CG 819 (Schott Geräte), a conductivity meter LF 330/SET (WTW) and an oxygen meter OXI 196 (WTW), respectively. Oxygen concentrations were expressed as the ratio between the concentrations measured and the theoretical solubility of oxygen. Water transparency was measured at the bank using a Secchi disk.

After acetone extraction, chlorophyll a, b and c concentrations were determined spectrophotometrically using the SCOR-UNESCO equations [18]. Phytoplankton samples were harvested from the bank using a plankton net (mesh size: 20 µm). Part of each sample was kept fresh and the rest fixed in 5% formaldehyde. The latter sample was used to identify and count the phytoplankton. A “C. raciborskii trichome unit” was defined to express the results. The value was chosen around the mean value measured, i.e. 70 µm, and counting was done using a “Nikon-optiphot-2” microscope (Nikon, Melville, USA).

2.2. Analysis of nutrients and major ions

Samples were collected in a bottle from the bank zone and analyzed for nutrients (soluble reactive phosphorus, SRP, nitrate and dissolved silica) and major ions (Ca²⁺, K⁺, Mg²⁺, Na⁺, Cl⁻, SO₄²⁻). Water was either filtered on site (soluble fractions) or left unfiltered (total nutrient determinations) and poured into acid-washed polyethylene containers. Analyses were performed according to Greenberg et al. [19].

2.3. Meteorological data

Atmospheric temperatures, the hours of sunshine and rainfall values were obtained for the study period from Météo France.

2.4. Data processing

The relationships between environmental parameters (water temperature, pH, oxygen saturation, rainfall values, hours of sunshine, atmospheric temperature, SRP, NO₃, SiO₂) concentrations of chlorophyll a, b and c and phytoplankton groups (Chlorophyceae, Chrysophyceae, C. raciborskii, other Cyanobacteria, Bacillariophyceae, Dinophyceae, Euglenophyceae) were studied by multivariate analysis. A PCA on the environmental parameters was carried out using the ADE-4 Software Package [20]. Phytoplankton species abundance was treated as a supplementary column in this analysis.

3. Results

3.1. Phytoplankton assemblages

Since July 1998, two blooms of C. raciborskii have occurred in the FP pond. Pale blue-green, straight thin and cylindrical trichomes, bearing terminal drop-shaped heterocysts with pointed ends, were observed (Fig. 1). Trichome length increased during the bloom. The mean value was 119 ± 20 µm (mean ± standard deviation) (n = 20) in September 1998 and August 1999. Heterocysts (length = 5 ± 2 µm, width = 1.75 ± 0.5 µm, n = 20) were observed at one or both ends of the trichomes. Coiled trichomes were never observed. There was little or no constriction at the crosswalls of the vegetative cells (length = 11.5 ± 3.5 µm, width = 1.5 ± 0.3 µm, n = 30) where irregular gas vesicles were noticed. Akinetes (length = 12.5 ± 5.5 µm, width = 3.5 ± 0.4 µm, n = 20) appeared on trichomes and became more numerous (up to three or four per trichome) a few weeks before the cyanobacterium disappeared from the pond (October
1998 and first sample for September 1999). They were generally not contiguous to the heterocytes (Fig. 1A). Akinetes were not encountered in the pond water column between the two *C. raciborskii* bloom periods, but were found to be numerous in sediments in February 1999.

The first trichomes of *C. raciborskii* were identified on 29 July 1998. The cyanobacterium density increased and reached $1.8 \times 10^5$ trichomes per liter (Fig. 2A). However, owing to the monthly sampling frequency, the maximum density may have been missed (between 31 August and 18 October 1998). In November, only a few trichomes were observed. In 1999, the first trichomes appeared earlier, from 22 June (240 trichomes per liter) to 7 September (100 trichomes per liter). Weekly harvesting allowed us to obtain more precise data. The density of *C. raciborskii* increased during July, and reached $2 \times 10^6$ trichomes per liter on 9 August. The cyanobacterium population then fell dramatically and no trichome was observed on 9 September. During both blooms, the water was brown and turbid, but no surface scum was ever noticed. In September 1998 and from 27 July to 17 August 1999, *C. raciborskii* accounted for more than 99% of the total phytoplankton density.

Common inhabitants of shallow temperate waters were also present in the phytoplankton (Table 1). Among the Cyanobacteria, *Planktothrix agardhii* was often observed, but always below $10^5$ trichomes per liter. It formed small blue mats in April 1999. *Fragilaria* sp. (Bacillariophyceae) was continuously present during the study, and replaced *C. raciborskii* as the dominant species from November 1998 ($1.2 \times 10^5$ cells per liter) to March 1999 ($2.4 \times 10^5$ cells per liter) (Fig. 2A). Its density peaked in January 1999, with $3.3 \times 10^7$ cells per
The Chrysophyceae Mallomonas sp. was only identified in February and March 1999 with densities of $1.7 \times 10^5$ and $1.2 \times 10^5$ cells per liter, respectively. Two other taxa proliferated to $10^3$ cells per liter: Euglena sp. in December 1998 ($4.9 \times 10^3$ cells per liter) and Peridinium sp. in September 1999 ($1.8 \times 10^3$ cells per liter).

Chlorophyll $a$ peaks were observed in September 1998 ($81.6 \mu g L^{-1}$, first bloom of C. raciborskii) (Fig. 2B), December 1999 ($51.0 \mu g L^{-1}$, Fragilaria sp. and Euglena sp.), March 1999 ($31.1 \mu g L^{-1}$, Fragilaria sp. and Mallomonas sp.) and at the beginning of August 1999 ($152.4 \mu g L^{-1}$, second bloom of C. raciborskii). Chlorophyll $b$ values were always very low ($<3.6 \mu g L^{-1}$) except in October 1999 ($8.8 \mu g L^{-1}$), and they generally coincided with the presence of Euglenophyceae species (Fig. 2B). Chlorophyll $c$ was detected from November 1998 to June 1999, peaking in December 1998 ($12.2 \mu g L^{-1}$), corresponding to the presence of chlorophyll $c$ containing algal groups, i.e. the Bacillariophyceae Fragilaria sp. (Fig. 2B), in February and March, the Chrysophyceae Mallomonas sp. was detected.
3.2. Physico-chemical parameters

During the blooms of *C. raciborskii*, water transparency did not exceed 0.4 m until 2 September 1999, but thereafter soon increased again and reached a value in excess of 1 m on 7 September. The minimum Secchi depth reached 0.25 m on 9 August 1999. Water temperatures ranged between 17°C (September 1998) and 25.7°C (maximum reached on 20 July 1999) (Fig. 2C). The pond did not freeze in the winter of 1998–1999 although it had done so in previous years.

There were high pH values (>8) from January to September 1999, with maxima at 9.2 and 9.4 on 25 August and 2 September 1999, respectively. This corresponded to the end of *C. raciborskii* bloom (Fig. 2C).

Oxygen concentrations were high (>10 mg L\(^{-1}\)) from January to April and during August 1999 (Fig. 2C). Two considerable falls in oxygen concentration were recorded in October 1998 (1.3 mg/L) and September 1999 (0.5 mg/L, only 6% of oxygen saturation). These dramatic decreases in the dissolved oxygen concentration observed at the end of the two *C. raciborskii* blooms can be attributed to bacterial degradation of the phytoplankton. This was also responsible for catastrophic fish deaths observed in the FP pond on 9 September 1999.

Nitrate was only detected from October to December 1998 and in February, September and October 1999 (Fig. 3). The concentration only exceeded 5 \(\mu\)M in February 1999 (21 \(\mu\)M). SRP concentrations ranged between 0.1 (October 1998) and 4 \(\mu\)M (May 1999), with an average concentration around 1 \(\mu\)M (Fig. 3).

The dissolved silica concentration increased from July to November 1998 (up to 169 \(\mu\)M), and then fell rapidly to a very low level until May 1999 (Fig. 3). The
concentration then rose again to reach 292 µM in October 1999.

The main characteristics of the water chemistry were constant high concentrations of sulfate ($\text{SO}_4^{2-}$, 8981 ± 471 µM, $\gg \text{Cl}^-$) and calcium ($\text{Ca}^{2+}$, 6486 ± 286 µM, $\gg \text{Mg}^{2+} > \text{Na}^+ > \text{K}^+$), a consequence of the gypsic nature of the bedrock. This was confirmed by the high conductivity found (from 1344 in July 1998 to 1872 µS cm$^{-1}$ in October 1999, data not shown).

3.3. Meteorological data

Atmospheric temperatures and hours of sunshine were highest in August 1998, and from May to July 1999 (Fig. 4). The highest rainfall figures were recorded during three spells: 10–20 October 1998, 1–10 April 1999 and 1–10 September 1999 (Fig. 4).

3.4. PCA analysis

The two major axes, 1 and 2, accounted for 40.7% and 20.8% of the total variance, respectively. We therefore focused our analysis on these two axes. Projection of the samples in the first plane (axis 1/axis 2) showed a seasonal distribution of these samples (Fig. 5A), except for September 1998, that seemed to be more related to spring-summer conditions rather than to autumnal conditions.

With regard to the environmental parameters, dissolved silica, insolation and percent of saturation make the greatest contributions to the first axis, whereas
climatic parameters (water and atmosphere temperatures, hours of sunshine) and chlorophyll \( a \) and \( c \) content contributed mainly to the second axis (Fig. 5B).

When the phytoplankton groups were projected onto this plane as supplementary variables (Fig. 5B) \( C. \) raciborskii was clearly opposed to other phytoplankton groups, including other Cyanobacteria, on these two axes. The Bacillariophyceae, Chrysophyceae and Euglenophyceae occurred together on the two axes. The positions of the Chlorophyceae and Dinophyceae species is more difficult to analyze, due to their very low densities during this study. They could not be clearly linked to any parameters.

4. Discussion

The presence of \( C. \) raciborskii in the FP pond in 1998 and 1999 is consistent with its first appearance there in 1994 [17], and indicates that the organism seems to be
well established in this pond. France is one of the more northerly countries in its geographic distribution, together with Austria, Germany and Hungary [4]. However, whereas C. raciborskii populations can persist all year round in warm waters, its presence is limited to shorter periods at northern latitudes.

PCA analysis showed that the occurrence of C. raciborskii blooms depended mainly on climatic parameters: water and air temperatures, and hours of sunshine (Fig. 5B). This confirms the observations of Padišák [4] showing that a water temperature ranging from 22°C to 23.5°C is a key factor for the germination of akinetes. Such temperatures were reached in July 1998 and at the end of June 1999. After this first critical step in the development of C. raciborskii, warm temperatures are also needed during cell multiplication [9,13]. A sharp fall in water temperatures (from 23°C to 14°C) probably kept the maximum density of C. raciborskii low in 1998 (1.8×10^5 trichomes per liter, September 1998). In contrast, a prolonged period of warm temperatures in 1999 (between 20.8°C and 25.7°C in July/August 1999) was favorable to the major proliferation observed during this period (2.0×10^6 trichomes per liter).

The second environmental factor involved in the proliferation of C. raciborskii was the level of nutrients in the FP pond. A lack of nitrate is considered to be the main reason for the proliferation of a heterocytic species such as C. raciborskii. However, low percentages of heterocysts were reported in trichomes from some reservoirs with low nitrate concentrations [7,13] and were attributed to the preference of C. raciborskii for ammonium as a nitrogen source. In the FP pond, low nitrate concentrations were always observed in summer and no value was available for ammonium. However, trichomes nearly always had heterocytes (data not shown), and this probably means that there was a low level of ammonium in the FP pond water. Thus, as recorded in the “Peri lagoon” [8] and in lake Balaton, [13], proliferation of C. raciborskii in the FP pond occurred when concentrations of phosphorus and nitrogen were low. In the same way, in the eutrophic Paranôa reservoir, where Microcystis aeruginosa is the dominant species, Branco and Senna [12] noticed that proliferation and dominance of C. raciborskii occurred only during the rainy season, when the nutrients had been diluted. As mentioned by Bouvy et al. [21], eukaryotic algal groups and other Cyanobacteria (Fig 5B), seem to be more dependent on high nutrient levels than C. raciborskii.

The third environmental factor involved in the proliferation of C. raciborskii was the low transparency of the water in the pond during the blooms, which gives C. raciborskii an indirect competitive advantage over other heterocystous Cyanobacteria. Indeed, Padišák and Reynolds [10] propose a specific assemblage for C. raciborskii (Sn), based on its shade tolerance, which is different from that of other heterocystous Cyanobacteria (H). During the bloom in 1999, water transparency was very low. The photic zone estimated by the use of the Secchi disk [22] decreased to 0.675 m, in the same range as in the Paranoa reservoir [12], only one half that in eight other tropical Brazilian reservoirs dominated by Cylindrospermopsis spp. [23], and four times lower than that in the Fitzroy River [6].

Finally, the constant high salinity, and especially the high concentration of sulfate, also seem to be important factors in accounting for the low phytoplankton diversity and the proliferation of C. raciborskii, since other freshwater phytoplanktonic species present in the pond are less tolerant of such high salinity. Indeed, C. raciborskii is known to grow in a wide range of salinity levels [4], and its dominance in the Ingaçeira reservoir also coincided with the highest conductivity values [7].

In other respects, C. raciborskii did not seem to be a good competitor in other ponds. A perennial Planktothrix agardhii bloom occurred in a water body located near (<25 m) the FP pond, in which C. raciborskii was never observed [24]. This water body was characterized as having higher SRP levels and lower salinity, allowing the growth of P. agardhii. Furthermore, despite no C. raciborskii bloom having occurred in the summers of 2000 and 2001 (personal data) due to insufficient summer temperatures, no other phytoplankton species proliferated during this period in the FP pond. This means that C. raciborskii did not outcompete other phytoplankton species but that this species was the only one able to proliferate in the FP pond during warm summers such as 1994, 1998 and 1999.

5. Conclusions

The optimum conditions for the growth of C. raciborskii in the FP pond seemed to be close to those observed in tropical areas, and the main limiting factor for the proliferation of C. raciborskii in this pond was the water temperature during akinetes germination and cell division. However, a global analysis of these results suggests that C. raciborskii has good adaptability, but poor ability to compete with microalgae in temperate areas. This adaptability was demonstrated by the fact that C. raciborskii, which is described as a tropical Cyanobacteria, is able to grow in temperate climates under different light and temperature conditions. In addition, C. raciborskii seems to be very tolerant of salinity and nutrient concentrations. These features allow C. raciborskii to colonize new biotopes when the environmental conditions become favorable. However, C. raciborskii appears to be a poor competitor in temperate areas. So far, this species has only been reported in a few water bodies in temperate areas [4].
the FP pond, due to particular environmental conditions (high salinity), *C. raciborskii* has no competitor in summer, as demonstrated by the fact that no other microalgae or Cyanobacteria blooms were observed in summer, even when *C. raciborskii* did not proliferate. Furthermore, *C. raciborskii* was never observed in the lake near the FP pond, where competition with *P. agardhii* occurred.

A broader investigation is being developed to confirm these hypotheses about the adaptability and competitiveness of *C. raciborskii* on several strains from tropical and temperate locations. Together with genetic studies performed to determine phylogenetic relationships between all these strains, the relative growth rates under various light, temperature and competitive conditions will be compared.

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