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The Invasive Cyanobacterium *Cylindrospermopsis raciborskii* in Southern Louisiana

HANS J. RICK, SILKE RICK, SOLEDAD FUENTES, AND JODIE L. NOEL

Since 2002 two areas in southern Louisiana (Caernarvon Breton Sound estuary and Atchafalaya basin region) were studied regarding the occurrence of the invasive and potentially harmful cyanobacterium *Cylindrospermopsis raciborskii*. In the Caernarvon Breton Sound estuary, the species was detected for the first time in Louisiana in July 2002 in Grand Lake during monthly phytoplankton monitoring surveys. The species occurred again in the estuary in summer and fall 2003 and 2004. Three distinct morphotypes (straight, curved, curled) were present, with the curved or curled varieties usually most abundant (> 80%). During summer 2003 the cell numbers in Grand Lake exceeded the critical level of potential toxicity ($> 20 \times 10^6$ cells liter⁻¹). In 2004 the species was a rare sight in the estuary and was not detected any more in 2005. In the Atchafalaya basin region, *C. raciborskii* was frequently detected in fall 2004. The greater Lake Fausse Pointe area was especially a hot spot, with critical level abundances up to 30×10^6 cells liter⁻¹. In both areas *C. raciborskii* occurred only in summer and fall at elevated water temperatures (> 25–33 °C). It was more frequently detected in the southern, less diversion-influenced part of the Caernarvon Breton Sound estuary, whereas it occurred more often in the northern part of the Atchafalaya basin. The species was present in a conductivity range from $\mu\text{S cm}^{-1}$ 700 to 10,000 within the Caernarvon Breton Sound estuary. Samples containing *Cylindrospermopsis* generally were significantly higher in total and cyanobacterial chlorophyll compared to samples without *Cylindrospermopsis*.

INTRODUCTION

Cylindrospermopsis raciborskii (Wołoszyńska) Seenaya et Subba Raju (*Nostocales*, Seenaya and Subba Raju, 1972) is rated one of the most notorious cyanobacterial species (Padisák, 1997; Briand et al., 2004) because of its potential toxicity (Hawkins et al., 1985; Lagos et al. 1999; Humpage et al. 2000), ability to form dense water blooms interfering with several kinds of water use, and invasive behavior at midlatitudes. The species is especially dangerous because in contrast to other harmful cyanobacteria it does not form surface blooms (scum) and cannot be detected by smell, taste, or color of the water (Chiswell et al., 1997; Saker and Griffiths, 2001). Because animals are often not deterred by foul tastes, odors, or surface scums (Saker et al., 1999) they are more prone to cyanobacterial poisonings than humans. Human exposure usually occurs through direct contact, accidental uptake via swallowing or inhaling cells, and uptake of the dissolved toxin via drinking water. Hence, there is a major concern about contamination of drinking and recreational water supplies (Chapman and Schelske, 1997). The major toxin, cylindrospermopsin, a tricyclic guanidine alkaloid, is a potent inhibitor of protein synthesis (Ohtani et al., 1992). It damages a range of organs, especially the liver and kidney,

but also affects the heart, lung, and spleen (Hawkins et al., 1985; Falconer et al., 1999). The toxin can accumulate in tissues of crayfish, shellfish, snails, and daphnids (Saker and Eagelsham, 1999; Nogueira et al., 2004; Saker et al., 2004; White et al., 2006).

Additionally there is growing apprehension about the role of cylindrospermopsin in gastrointestinal and liver cancer with longtime low-level exposure (Humpage et al., 2000; Shen et al., 2002; Falconer, 2005). The “Palm Island Mystery Disease,” which occurred in 1979 in Australia, is an example of a serious human poisoning caused by cylindrospermopsin. In Palm Island, 149 people were hospitalized with symptoms of hepato-enteritis after consuming contaminated drinking water (Byth, 1980; Griffiths and Saker, 2003). Cylindrospermopsin is also believed to be the cause of the gastric ailment known as “Barcoo spews” once prevalent in outback northern and central Australia (Hayman 1992; Chiswell et al., 1997).

At the beginnings of the last century, Wołoszyńska (1912) described *C. raciborskii* as *Anabaena raciborskii*. Because heterocyst location and development proved to be different to *Anabaena*, it was then moved to the genus *Anabaenopsis* (Miller) and finally to *Cylindrospermopsis* (Seenaya and Subba Raju). *Cylindrospermopsis raciborskii* was

considered as typical for the Indo-Malayan flora (Geitler and Ruttner, 1936) but was later classified as pantropical. The compilation of floristic data by Padišák (1997) implies that *C. raciborskii* is not an exclusively tropical or subtropical species, as it shows a small overlap in occurrence with northern circumpolar species. Currently the species is spreading worldwide to midlatitudes (Padišák, 1997; Briand et al., 2004). It was reported from several temperate areas in Europe, such as Hungary (Horecka and Komárek, 1979; Tóth and Padišák 1986, Borics et al., 2000), Austria (Dokulil and Mayer, 1996), France (Couté et al., 1997; Briand et al., 2002), and Germany (Krienitz and Hegewald, 1996; Weidner and Nixdorf, 1997; Fastner et al., 2003). In these areas the phytoplankton in lakes has been studied for a considerable time already; hence it appears that *C. raciborskii* is a new invasive species rather than simply a species that has before been unrecorded. For the Americas, most identifications of *C. raciborskii* occurred in Brazil, which has cyanobacterial monitoring in drinking water reservoirs (Trojan and Garcia, 1989; Falconer, 2005). It was also reported from reservoirs in Venezuela, Nicaragua, Cuba, and Mexico (Komárek, 1984; Padišák, 1997).

The earliest record for North America is probably from Kansas (Wooster Lake), where Prescott and Andrews (1955) recorded the species as *Anabaenopsis seriata*. In 1966, *C. raciborskii* occurred in several small lakes in Minnesota (Hill, 1970). It was reported in Lake Erie in the 1970s (Taft and Taft, 1971). Additionally it was found in Ohio, Michigan, Illinois, Indiana, and Texas, U.S.A. (Padišák 1997; Jones and Sauter, 2005; St. Amand, 2002; Falconer 2005), and recently in Canada (Kling, 2004; Hamilton et al., 2005). Florida's subtropical eutrophic lakes and rivers, which frequently are used as drinking water supplies, often support very large populations of *C. raciborskii* (Chapman and Schelske 1997; Williams et al. 2001; Falconer, 2005). As far as published information can indicate, adjacent states have not been screened for this species yet. Because it is abundant in Cuba; Florida, U.S.A.; Mexico; and subtropical regions elsewhere in the world, it might exist throughout the higher rainfall areas of the southern United States (Falconer, 2005). It is very likely that *C. raciborskii* has been abundant in southcentral temperate water bodies with suitable environmental conditions in central North America longer than the numerous recent records imply (Kling, 2004). More in-depth information about the genus *Cylindrospermopsis* can be found in the compilation by Komárek and Hauer (2004).

Here we document the recent records of *C. raciborskii* from coastal Louisiana. Occurrence locations and cell densities are given, and relationships to meteorological, physico-chemical, and planktonic parameters are discussed.

MATERIAL AND METHODS

The Caernarvon Breton Sound estuary was surveyed monthly from Feb. 2002 to Sep. 2004 by sampling a 20-station grid (Fig. 1a, upper left panel). In Oct. 2004, 39 stations were sampled in the Atchafalaya basin region (Fig. 2) along with some additional water bodies to the west (Fig. 3). In 2005 the Caernarvon Breton Sound estuary was surveyed during late Aug. and early Nov.; sampling of the Atchafalaya basin took place in June, Sep., and Oct.

Water samples for total nutrient analysis were collected using a stainless steel bucket poured into acid-washed High Density Polyethylene (HDPE) bottles and stored on ice until being returned to the laboratory. Inorganic nutrients [soluble reactive phosphorus (SRP), ammonium (NH_4^+), silicate (Si), and the sum of nitrate and nitrite (NOX)], total phosphorus (TP), and total nitrogen (TN) samples were analyzed using an automated Lachat Quickchem Autoanalyzer and by manual colorimetric assays (Clesceri et al., 1998). Samples were kept frozen ($< -18^\circ\text{C}$) until final analysis.

Live plankton samples were stored in acid-cleaned polyethylene (PE) bottles and transported to the lab in coolers under ambient water temperature conditions. For subsequent quantification of *C. raciborskii* using the inverted microscope method (Lund et al. 1958; Utermöhl 1958) an aliquot was fixed with neutral formaldehyde to reach a final concentration of 0.4% (Thronsdon, 1978). Because cell walls between individual cells within a trichome are barely visible and there is almost no constriction between the cells (e.g., Hill, 1969; Padišák, 2003), samples were stained with DAPI to visualize cell walls.

Analysis of the phytoplankton community was primarily performed using a pulse amplitude modulation setup (WALZ PHYTOPAM). The four-wavelength excitation method (Schreiber et al., 1995, 1998; Schreiber, 1998) was applied for a quantitative differentiation between three major groups of phytoplankton [1, Cyanobacteria; 2, Chlorophyceae, and 3, heterokontophytes (Chrysophyceae, Xanthophyceae, Eustigmatophyceae, Bacillariophyceae, Raphidophyceae, and Dictyochophyceae), dinoflagellates, and prymnesiophytes]. The method enables quantifying chlorophyll levels within these three groups. In the following, the chlorophyll of

the third group is referred to as “other” chlorophyll.

The quality control program for the PHYTO-PAM measurements included frequent calibration using mono and mixed cultures of diatoms, green algae, and cyanobacteria isolated from the study areas. Chlorophyll values were assessed by the PHYTO-PAM method and for additional cross checking by a U.S. Environmental Protection Agency (EPA)-approved chlorophyll extraction method (Arar and Collins, 1997).

In situ measurements of water temperature and conductivity were performed on each visit directly in the surface layer at each sampling station using a 340i WTW multimeter. Water column light parameters were measured occasionally using a LI-COR LI-185B quantum meter with a spherical quantum sensor SPQ 0602 and a Secchi disc. Meteorological data for Caernarvon Breton Sound estuary were taken from the Louisiana State University (LSU) Agricultural Center’s website (LSU AgCenter Citrus Station: <http://www.lsuagcenter.com/weather/>). The data were used to establish time charts (2002–05) of daily average wind speeds, minimal daily air temperatures (5-d floating mean), and cumulative rainfall for the estuary. Additionally wind, rain, and temperature data from the months before the potential development of *Cylindrospermopsis* (April/May) were compared for the 4 yr of the Caernarvon time series.

Daily discharge volumes of the Caernarvon diversion structure from 2002–05 were obtained through the U.S.G.S. National Water Information System (<http://waterdata.usgs.gov/la/nwis>). The raw data [cubic feet per second (CFS)] were used to calculate monthly averages ($\text{m}^3 \text{s}^{-1}$) for the survey months and at the same time to compare the amount of monthly discharged water to the amount of monthly rainfall for the period April–June in 2002–05. For this purpose the discharge flow ($\text{m}^3 \text{month}^{-1}$) was converted to water height (cm month^{-1}) based on an overall size of the estuary of 656 km^2 .

Statistical analyses [t-tests, analyses of variance (ANOVAs)] were performed using JMP IN 5.1.2 software (SAS Institute). The global positioning system was used to survey all sampling locations.

RESULTS

On 16 July 2002 the potentially harmful cyanobacterium, *C. raciborskii* (Woloszyńska) Seenaya et Subba Raju, was detected for the first time in Louisiana. It was identified in a phytoplankton sample from the Grand Lake area that was collected within monthly phytoplankton surveys

in the Caernarvon Breton Sound estuary (Fig. 1a, midleft panel). The survey grid with station numbers is shown in the upper left panel of Figure 1a. Several morphotypes of *C. raciborskii* were found; it always occurred in straight and curved formations, and, additionally, heavily coiled chains were detected (Fig. 4). Curved and coiled morphotypes were more abundant ($> 80\%$) than straight forms. Cell diameter and cell length were $2\text{--}3 \mu\text{m}$ and $5\text{--}8 \mu\text{m}$, respectively. Cell density for the first record in Grand Lake was $3 \times 10^6 \text{ cells liter}^{-1}$. Since its first occurrence, the species was a frequent encounter in the Caernarvon Breton Sound estuary during summer and fall at elevated temperatures of the surface water ($> 27^\circ \text{C}$) (Fig. 1a,b; Table 1). Highest densities ($\text{max. } 24.5 \times 10^6 \text{ cells liter}^{-1}$) were detected in Grand Lake on 10 July 2003. From the spatial distribution maps it is obvious that the species occurred always in the midsouthern part of the estuary (Fig. 1a,b, St. 7, 8, 9, 16, 17, 22, 23). In 2004 *C. raciborskii* was a more rare sight in this estuary. It was only detected in low abundances ($10,000$ to $50,000 \text{ cells liter}^{-1}$) in two of nine monthly surveys (Fig. 1b; Table 1).

During fall 2004 the species occurred widespread in coastal Louisiana (Fig. 3). *Cylindrospermopsis raciborskii* was relatively abundant in the Atchafalaya basin (Fig. 2; Table 1). Elevated cell densities were detected in the northern part of the basin (up to $5 \times 10^6 \text{ cells liter}^{-1}$) but also in the area of Lake Fausse Pointe (up to $30 \times 10^6 \text{ cells liter}^{-1}$). The species additionally occurred in lower densities ($0.01\text{--}0.16 \times 10^6 \text{ cells liter}^{-1}$) in the vicinity of Lafayette (Lake Martin), Ville Plate (Lake Chicot), and Intracoastal City (Fig. 3). The square marked “LS” in this figure displays a dense *Cylindrospermopsis* bloom, which occurred in the upper Barataria estuary in summer 2003 (Rabalais, 2005). In 2004 the species was detected again in Lake Cataouatche, Salvador, and Allemands (Ren et al., 2005).

Chemical and planktonic parameters from +CLY (containing *Cylindrospermopsis*) and –CYL (without *Cylindrospermopsis*) samples were compared using t-tests. Only data from surveys in which *Cylindrospermopsis* was detected were included in the testing.

A comparison for conductivity based on data from the Atchafalaya (fall 2004) and the Caernarvon Breton Sound estuary (2002–05) is shown in Figure 5a. As expected, no pattern is obvious for the mainly freshwater Atchafalaya samples, whereas for the Caernarvon Breton Sound estuary average conductivities of +CYL samples were lower compared to –CYL samples although this proved not significantly different (Table 2). *Cylindrospermopsis raciborskii* did not use the full

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Fig. 1a, b. Temporal-spatial distribution of *Cylindrospermopsis raciborskii* in the Caernarvon Breton Sound estuary: A grid of 20 to 23 stations (Upper left panel, Fig. 1a) was surveyed monthly from February 2002 to September 2004. Numbers are station numbers, described in Table 1. The species was detected within 11 out of 38 surveys. Black squares with white numbers or markings indicate stations where *Cylindrospermopsis* was present while black numbers show stations where the species was not present during the respective survey. In three surveys are additional stations were sampled. These are marked with crosses for 6/1/2003 and 7/10/2003 or letters for 7/13/2004 survey.

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b

Fig. 1b.

Fig. 2. Spatial distribution of *Cyindrospermopsis raciborskii* in the region of the Atchafalaya basin in fall 2004. Stations south of Interstate 10 (I 10) were surveyed on 10/01/2004, stations north of I 10 on 10/15/2004. White squares indicate stations where *Cyindrospermopsis* was present while black squares show stations with no occurrence of the species. *Cyindrospermopsis* was present in 20 of 38 samples.

range of conductivity in the estuary. It occurred only at conductivities between $700 \mu\text{S cm}^{-1}$ and $10,000 \mu\text{S cm}^{-1}$ (Figure 5b).

Nutrient measurements (NO_3^- , SRP , NH_4^+ , Si , TN , and TP) were conducted only for samples from the Caernarvon Breton Sound time series. Average values of inorganic dissolved nutrients with two of their molar ratios [dissolved inorganic nitrogen (DIN)/ SRP , and Si/DIN] are shown in the Figure 6a,b with standard errors of means (SEM). Whereas no difference between +CYL and -CYL samples was detected for NH_4^+ , SRP , and Si , the average concentration of NO_3^- was significantly lower in the -CYL samples. In turn, DIN/SRP and Si/DIN ratios were influenced in the way that averages for +CYL samples were lower compared to -CYL samples. The datasets, however, proved not significantly different from each other. The situation for total nutrients and their molar ratios is given in Figure 6c. For both TN and TP , averages of

-CYL samples were lower compared to +CYL samples but the finding proved significantly different for TN only. Average TN/TP did not differ between the datasets (Table 3).

Planktonic parameters were analyzed for both the Caernarvon and Atchafalaya datasets (Fig. 7; Tables 2 and 4). In both sets total chlorophyll concentration and cyanobacterial chlorophyll (expressed as percentage of total chlorophyll) were significantly higher in +CYL compared to -CYL samples. Green algal chlorophyll (% of total) was significantly elevated in the Atchafalaya -CYL samples. The Caernarvon data showed the same trend although both sample sets were not significantly different. Conversely, chlorophyll from heterokontophytes, dinoflagellates, and prymnesiophytes was significantly lower in the Caernarvon +CYL samples, a trend that was visible for the Atchafalaya, too.

It is known from other studies that water temperature and water column stability have an important impact on akinete germination and the subsequent development of *Cyindrospermopsis*. For the Caernarvon time series, some proxies (minimal air temperature, cumulative rainfall, average wind speed) describing these parameters are shown in Figure 8. In the shallow lake-like areas of the estuary, the water column stability is strongly influenced by wind and rainfall, whereas the temperature at the water-sediment interface triggers akinete germination. Because water temperature data were not available in high resolution for the time series, the development of the minimal air temperature was used as a proxy. It is assumed that at minimal air temperatures of 18.5°C (Fig. 8, hatched lines) the shallow lakes reach temperatures of $> 22^\circ\text{C}$, allowing *Cyindrospermopsis* akinetes to germinate. Early warming and extensive droughts during spring characterized the years 2002 and 2003, which is in contrast to 2004 and 2005 (Fig. 8).

Since the input of diverted Mississippi River water has an additional influence on the water column stability within the Caernarvon Breton Sound estuary, diversion discharge and rainfall for the months before the potential occurrence of *Cyindrospermopsis* are compared in Figure 9. Rainfall and discharge are displayed in the same dimension to enable a quantitative comparison between both parameters. The amount of water delivered by rain to the estuary is in the same range as the amount discharged by the diversion.

DISCUSSION

Trichome morphology, cell sizes, and cell counts.—It is common to find that *C. raciborskii* exists in different morphotypes (Baker and Fabbro, 1999;

Fig. 3. *Cylindrospermopsis raciborskii* occurrence in southern Louisiana. Triangles show the occurrence in fall 2004 (LC: Lake Chicot, LM: Lake Martin, LFP: Lake Fausse Pointe, AB: Atchafalaya Basin, IC: Intracoastal City). The Caernarvon Breton Sound estuary (CB: circle) was surveyed monthly starting in February 2002. The square (LS) shows the occurrence of the species in the upper Barataria estuary (Rabalais, 2005).

Fig. 4. Different morphotypes (straight, curved, curled) of *Cylindrospermopsis raciborskii* collected in southern Louisiana (Grand Lake, Caernarvon-Breton Sound estuary, 7/10/2003).

TABLE 1. Occurrence of *Cylindrospermopsis raciborskii* in southern Louisiana 2002–04. Sampling dates, locations, positions, and cell numbers per liter. C: Caernarvon Breton Sound estuary; A: Atchafalaya basin area.

Date	Station	N	W	Cell number (L ⁻¹)
16 July 2002	C-16	89.849	29.712	3,350,000
28 Aug. 2002	C-9	89.703	29.696	120,000
28 Aug. 2002	C-18	89.685	29.621	930,000
11 June 2003	C-7	89.789	29.711	10,000
11 June 2003	C-16	89.849	29.712	10,000
23 June 2003	C-16	89.849	29.712	530,000
23 June 2003	C-17	89.772	29.641	50,000
23 June 2003	C-22	89.749	29.671	90,000
23 June 2003	C-23	89.708	29.655	10,000
10 July 2003	C-7	89.789	29.711	1,560,000
10 July 2003	C-15	89.862	29.682	25,000
10 July 2003	C-16	89.849	29.712	24,530,000
10 July 2003	C-17	89.772	29.641	4,720,000
10 July 2003	C-Grand Lake Northwest	89.831	29.722	50,000
10 July 2003	C-Grand Lake South	89.841	29.703	1,070,000
10 July 2003	C-Bay Lori	89.839	29.683	10,000
10 July 2003	C-Oak River	89.817	29.659	40,000
10 July 2003	C-Pointe Fienne Bay	89.77	29.646	10,000
10 July 2003	C-Bay Jack Nevette	89.758	29.636	10,000
10 July 2003	C-Spyder	89.747	29.66	10,000
10 July 2003	C-Lake Faussan North	89.753	29.682	10,000
10 July 2003	C-Alligator Pass	89.817	29.718	10,000
10 July 2003	C-Little Lake South	89.778	29.694	10,000
24 July 2003	C-8	89.708	29.754	30,000
24 July 2003	C-9	89.703	29.696	100,000
24 July 2003	C-11	89.565	29.658	90,000
24 July 2003	C-14	89.91	29.7	1,530,000
24 July 2003	C-15	89.862	29.682	1,060,000
24 July 2003	C-16	89.849	29.712	1,490,000
24 July 2003	C-17	89.772	29.641	650,000
24 July 2003	C-18	89.685	29.621	347,000
24 July 2003	C-22	89.749	29.671	240,000
24 July 2003	C-23	89.708	29.655	580,000
20 Aug. 2003	C-14	89.91	29.7	230,000
20 Aug. 2003	C-15	89.862	29.682	10,000
20 Aug. 2003	C-17	89.772	29.641	116,000
20 Aug. 2003	C-22	89.749	29.671	116,000
24 Sep. 2003	C-5	89.804	29.793	40,000
24 Sep. 2003	C-14	89.91	29.7	7,510,000
24 Sep. 2003	C-15	89.862	29.682	7,360,000
24 Sep. 2003	C-16	89.849	29.712	780,000
22 Oct. 2003	C-7	89.789	29.711	116,000
22 Oct. 2003	C-14	89.91	29.7	10,000
22 Oct. 2003	C-15	89.862	29.682	2,500,000
22 Oct. 2003	C-16	89.849	29.712	30,000
13 July 04	C-Lake Amadee	89.693	29.761	25,000
13 July 04	C-Tanasia Lagoon	89.68	29.773	50,000
03 Aug. 2004	C-8	89.708	29.754	10,000
03 Aug. 2004	C-9	89.703	29.696	40,000
03 Aug. 2004	C-Lake Amadee	89.693	29.761	10,000
04 Sep. 2004	Lake Martin	91.908	30.221	160,000
10 Sep. 2004	Intracoastal City	92.156	29.783	4,000
10 Sep. 2004	Lake Fausse Pointe	91.552	29.936	200,000
29 Sep. 2004	Lake Chicot North Landing	92.286	30.838	10,000
01 Oct. 2004	A-Butte La Rose	91.692	30.281	410,000
01 Oct. 2004	A-Butte La Rose Bay	91.707	30.269	80,000

TABLE 1. continued.

Date	Station	N	W	Cell number (L ⁻¹)
01 Oct. 2004	A-Lake Dauterive	91.622	30.075	10,000
01 Oct. 2004	A-Lake Fausse Park	91.61	30.062	10,000
01 Oct. 2004	A-Lake Fausse Pointe	91.551	29.948	29,840,000
01 Oct. 2004	A-Lake Fausse Pointe	91.552	29.936	24,750,000
01 Oct. 2004	A-Grand Ayoille Cove	91.545	29.923	20,110,000
01 Oct. 2004	A-Chareton	91.513	29.902	30,000
01 Oct. 2004	A-Wilson Landing	91.294	29.722	10,000
15 Oct. 2004	A-Pelba	91.73	30.341	10,000
15 Oct. 2004	A-Atchafalaya near Pelba	91.715	30.36	25,000
15 Oct. 2004	A-Whiskey Bay Boat Launch	91.626	30.369	10,000
15 Oct. 2004	A -Whiskey Bay Pilot Channel	91.636	30.366	30,000
15 Oct. 2004	A-Rama inside Basin	91.512	30.4	25,000
15 Oct. 2004	A-Alabama Bayou 1	91.674	30.41	4,870,000
15 Oct. 2004	A-Alabama Bayou 2	91.672	30.424	1,640,000
15 Oct. 2004	A-Port of Krotz Springs	91.744	30.53	10,000
15 Oct. 2004	A-Dixie Ramp	91.758	30.458	30,000
15 Oct. 2004	A-Atchafalaya Ferry	91.689	30.386	10,000
15 Oct. 2004	A-Lake inside Basin near I 10	91.786	30.344	10,000

Fig. 5. (a) Comparison of conductivity in +CYL (black) and -CYL (white) samples from the Atchafalaya survey in fall 2004 and the Caernarvon Breton Sound time series (2002–2004). Average values are given with standard errors of means (SEM). Numbers at the base of the bars indicate sample sizes. (b) Conductivity scatter plot of +CYL and -CYL samples from the Caernarvon time series.

Komárková et al., 1999; Komárek and Komárková, 2003; Shafik, 2003). To date heavily coiled forms of *C. raciborskii* (Fig. 4, right panel) have not been described for North America. Coiled morphotypes of the species are only occasionally common in Australian populations (Baker and Fabbro, 1999; Komárek and Komárková, 2003). Other species of the genus *Cylindrospermopsis* species like *Cylindrospermopsis acuminato-crispa* (Couté and Bouvy, 2004, Brasil), *Cylindrospermopsis philippinensis* (Komárek and Komárková, 2003, Indonesia, Philippines), *Cylindrospermopsis catemaco* (Komárková-Legnerová and Tavera, 1996, Komárek and Komárková, 2003, southern California, Mexico, Belize), *Cylindrospermopsis helicodea* (Cronberg and Komárek, 2004, central Africa), or *Cylindrospermopsis curvispora* (Cronberg and Komárek, 2004; Komárek and Komárková, 2003, Watanabe, 1995, South Africa, Sri Lanka, Japan) have heavily coiled trichomes only. At the moment it is unclear whether the heavily coiled form is a morphotype of *C. raciborskii* or whether it belongs to another species of the genus. We are in the process of culturing different morphotypes for further evaluation.

Cell widths (2–3 μm) and lengths (5–7 μm) match closely with information from the literature (Hill, 1969; McGregor and Fabbro, 2000; Chonudomkul et al., 2004; Jones and Sauter, 2005). Recorded maximum cell numbers for Grand Lake in the Caernarvon Breton Sound estuary and for the Lake Fausse Pointe area were above the threshold level (20×10^6 cells liter⁻¹) for potential toxicity (McGregor and Fabbro, 2000). Ren Ling even recorded higher maximum cell numbers (up to 34.8×10^6 liter⁻¹) during

TABLE 2. Statistical comparison (t-test) of abiotic and planktonic parameters on samples containing *Cylindrospermopsis raciborskii* (+CYL) and samples without *C. raciborskii* (–CYL) from the Caernarvon Breton Sound estuary (summer–fall 2002–04). α -Levels were generally set at 0.05, and levels for total chlorophyll (Total Chl.) at 0.1. Sample size was 50 for +CYL and 178 for –CYL. “Other” Chl.: chlorophyll from heterokontophytes, dinophytes, and ô prymnesiophytes.

	Conductivity	Cyanobact. Chl.	Green algal Chl.	“Other” Chl.	Total Chl.	% Cyanobacteria of total	% Greens of total	% “Other” of total
Significant difference	No	Yes	No	No	Yes	Yes	No	Yes
t-ratio	–1.935	2.727	–0.313	0.022	2.151	2.481	–0.668	–3.672
+CYL samples								
mean	3,196	18.6	7.1	8.5	34.1	49.9	25.1	25.0
SD	2,189	16.9	7.4	7.6	18.2	27.9	25.0	13
–CYL samples								
mean	4,254	11.5	7.6	8.5	27.6	38.8	27.8	33.4
SD	5,907	13.3	15.1	7.1	21.6	28.1	25.7	18.1

a bloom in Lake Cataouatche, Barataria estuary, LA, in summer 2003 (Rabalais, 2005).

Temperature.—Both in the Caernarvon Breton Sound estuary as well in the Atchafalaya basin area *C. raciborskii* was only detected in summer and fall at surface temperatures ranging from 25 C to 33 C. It is already well known that the occurrence of *C. raciborskii* is heavily affected by water temperature (Branco and Senna, 1994; Souza et al., 1998; McGregor and Fabbro, 2000). In the tropics the species is often found year round in the plankton (Bouvy et al., 1999; Briand et al., 2004), but in temperate and subtropical regions its occurrence is restricted to warm summer months (Briand et al. 2002; Saker et al., 2003). In the field, the species seems to prefer surface water temperatures exceeding 23 C (Padisák, 1997; McGregor and Fabbro, 2000), whereas temperatures around 30 C were found to be optimal for the growth of the species in cultures (Shafik et al., 2001; Briand et al., 2004).

Light.—Because of their low latitude location, the Caernarvon Breton Sound estuary as well as the Atchafalaya basin experience relatively high solar radiation. Water surface light measurements during summer and fall at no cloud cover–midday ranged from 2,500 to 2,800 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. Due to high plankton densities, vertical light profiles revealed often relatively high turbidities in the areas of *C. raciborskii* occurrence. Vertical attenuation coefficients (k_d) were in the range of 3.8–2.3 accompanied with relatively low Secchi disc transparencies (0.2–0.6 m). These numbers show that almost 90% of the surface light is already attenuated at depths of 0.6 to 1.0 m, respectively.

It is well know that *C. raciborskii* has a high tolerance for low light levels (Padisák and Reynolds, 1998; Briand et al. 2002). Shafik et al. (2001) calculated a light tolerance for the species as low as 26 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ in Lake Balaton (Hungary). In shallow turbid Brazilian lakes the species even survived at 0.5 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ (Bouvy et al., 2000). On the other hand, *C. raciborskii* is able to tolerate relatively high light levels up to 500 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ (Briand et al., 2004). Secchi disc readings of 30 cm are common in *C. raciborskii* blooms (Mayer et al., 1997; Padisák, 1997). For Florida blooms, Chapman and Schelske (1997) reported mean Secchi depths less than 1.0 m, and for blooms in Europe, Secchi depths sometimes decreased to 0.07 m (Briand et al., 2002).

Conductivity.—In the Caernarvon Breton Sound estuary, *C. raciborskii* was detected within conductivities ranging from 700 to 9,000 $\mu\text{S cm}^{-1}$ (up to 4.8 PSU, Fig. 5b). Tolerance to elevated conductivities was already compiled by Padisák (1997) as the species occurred twice at salinity levels of 1.5–2 PSU in the North Caspian Sea, at conductivities above 4,000 $\mu\text{S cm}^{-1}$ in Lake Alexandria, and above 2,000 $\mu\text{S cm}^{-1}$ in Venezuela. Laboratory experiments by Moisaner et al. (2002) showed that 2 g liter^{–1} NaCl at least slowed growth of the species, whereas the upper limit for salinity tolerance was reached at 4 g liter^{–1} NaCl, which is in good accordance with the highest salinities (4.8 PSU) where the species could be detected in the Caernarvon Breton Sound estuary.

Water column stability.—In the shallow (max. 2.5–3 m) lake-like water bodies of the Caernarvon Breton Sound estuary, the stability of the

Fig. 6. Comparison of nutrient parameters of +CYL (black) and -CYL (white) mixed layer water samples from the Caernarvon Breton Sound time series (2002–2004). Average values are given with standard errors of means (SEM). Numbers at the base of the bars indicate the sample sizes. (*) indicates significant differences between datasets. (a) NO_x, SRP, and DIN/SRP. (b) NH₄⁺, Si, and Si/DIN. (c) TN, TP, and TN/TP.

water column is mainly affected by the activity of the diversion structure, wind, and rainfall. During the time series *C. raciborskii* only occurred in areas that were not directly influenced by the diverted waters and hence had high water residence times (Fig. 10). The species could only be detected once within the major eastern pathway of the diverted water in Sep. 2003 (Fig. 10, squared dot), when the diversion discharge was relatively low (17.3% of maximal capacity). A prolonged water residence time is often a necessary prerequisite for *C. raciborskii*

dominance (Branco and Senna, 1994; Bouvy et al., 1999, 2003a; Bormans et al., 2004).

The flow of the diverted water has quadruple negative effects on the success of *C. raciborskii*. First, water column stability, which often is regarded as a necessary prerequisite for its occurrence (e.g., Saker and Griffiths, 2001), is disturbed and the water residence time reduced. Second, during times of high flow, the species' akinetes resting in the upper layers of the sediment might be flushed out of the estuary. Third, the diverted water dilutes and flushes the entire planktonic community, and fourth, it introduces high nutrient concentrations into the estuary, depriving *C. raciborskii* of its competitive advantage of using low nutrient levels most efficiently (Bouvy et al., 2000; Istvánovics et al., 2000; Shafik et al. 2001; Briand et al. 2002).

Rainfall and wind speed are additional factors influencing water column stability in these shallow lakes. In contrast to the regional effects of diverted water, rainfall and wind affect the estuary more evenly. The amount of water delivered by rain to the estuary (656 km²) is at least in the same range as the amount discharged by the diversion (Fig. 9).

Overall the Caernarvon Breton Sound data support the view that *C. raciborskii* favors relatively stable water column conditions (Komárek and Kling, 1991; Lake Victoria, Uganda; Harris and Baxter, 1996; North Pine Dam, Australia; Saker and Griffiths, 2001; Lake Julius, Australia; Berger et al., 2006; Lake Guiers, Senegal). Several *Cylindrospermopsis* blooms in Australia and South America occurred in years of low rainfall and increased drawdown of the water column (Bouvy et al., 1999, 2003a; Saker and Griffiths, 2001). In fact, we have experienced a similar situation in early summer 2006 in the Lake Fausse Pointe area (Louisiana), when *C. raciborskii* bloomed (up to 88×10^6 cells liter⁻¹) after an extremely dry and warm spring with low water levels.

The literature gives also reports contradicting the species' dependence on water column stability, especially regarding the rain effect. In Mir Alam Lake, India, the species "multiplied profusely in late summer and throughout the rainy season" (Seenayya and Zafar, 1979). Similar findings are reported from Lake Kariba, Zimbabwe (Ramberg, 1987). Branco and Senna (1994) found *C. raciborskii* dominant in Paranoa Reservoir, Brazil, after rainy periods diluting nutrient levels.

In Lake Griffin (FL) it dominated shallow, well-mixed waters (Dobberfuhl, 2003). Antenucci et al. (2005), who found *C. raciborskii* dominant before and after artificial destratification of the North Pine Dam reservoir (Australia), suggested

TABLE 3. Statistical comparison (t-test) of nutrient parameters on samples containing *Cylindrospermopsis raciborskii* (+CYL) and samples without *C. raciborskii* (–CYL) from the Caernarvon Breton Sound estuary (summer–fall 2002–04). α -Levels were generally set at 0.05, and levels for NOX, DIN/SRP, and TN at 0.1. Sample size was generally 29 for +CYL and in most cases 178 for –CYL. Exceptions: sample size was 107 for (–CYL) TN and (–CYL) TN/TP, it was 110 for (–CYL) TP.

	SRP	NO ₂ [–] + NO ₃ [–]	NH ₄ ⁺	Si	DIN/SRP	Si/DIN	TN	TP	TN/TP
Significant difference	No	Yes	No	No	No	No	Yes	No	No
t-ratio	–1.202	–4.37	0.071	–0.685	–2.732	–1.753	2.077	1.436	0.078
	+CYL samples								
mean	1.5	1.1	4.6	47.2	3.5	29.5	87.1	4.3	21.9
SD	0.9	1.0	14.1	22.9	7.4	29.3	23.2	1.5	7.8
	–CYL samples								
mean	1.8	8.5	4.4	50.6	9.9	53.5	76.5	3.9	21.7
SD	0.9	20.1	10.2	30.3	23.0	151.0	27.9	1.4	12.7

the ability of the species to compete for phosphorus triggered the dominance during stratification, whereas after destratification its superior light utilization capability was the determining factor.

Nutrients.—Because of the influence of the diverted, nutrient-rich Mississippi water, the Caernarvon Breton Sound estuary is classified as eutrophic (Lane et al., 1999, 2004). Despite low diversion discharge at times of *C. raciborskii*

occurrence (average 6.6% of maximum capacity) dissolved concentrations of Si, NH₄⁺, SRP, and overall TP and TN levels were still elevated (Table 3). In contrast, NOX concentrations were relatively low forcing the molar ratio of DIN/SRP to levels way below the Redfield ratio of 16 and implementing a possible nitrogen limitation to the phytoplankton community.

A statistical comparison of nine nutrient parameters for +CYL and –CYL samples re-

Fig. 7. Comparison of chlorophyll parameters of +CYL (black) and –CYL (white) mixed layer water samples from the Atchafalaya surveys in fall 2004 and the Caernarvon Breton Sound time series (2002–2004). Average values are given with standard errors of means (SEM). Numbers at the base of the bars indicate the sample sizes. Sample sizes in (b), (c), and (d) are identical to (a). (*) indicates a significant difference between datasets. (a) total chlorophyll, (b) cyanobacterial chlorophyll, (c) green algal (chlorophyceae) chlorophyll, and (d) heterokontophyte, dinophyte, and prymnesiophyte chlorophyll.

TABLE 4. Statistical comparison (t-test) of abiotic and planktonic parameters of samples containing *Cylindrospermopsis raciborskii* (+CYL) and samples without *C. raciborskii* (–CYL) from the Atchafalaya basin area (Oct. 2004). α -Levels were generally set at 0.05, levels for total chlorophyll (Total Chl.) at 0.1. Sample size was 20 for (+CYL) and 18 for (–CYL). “Other” Chl.: chlorophyll from heterokontophytes, dinophytes, and prymnesiophytes.

	Conductivity	Cyanobacterial Chl.	Green algal Chl.	“Other” Chl.	Total Chl.	% Cyanobacteria of total	% Green of total	% “Other” of total
significant difference	No	Yes	No	No	Yes	Yes	Yes	No
t-ratio	0.353	2.701	–1.447	1.699	2.086	3.459	–2.199	–0.483
+CYL samples								
mean	272	25.4	1.534	21.132	47.98	48.033	5.424	46.967
SD	57	30.1	3.34	28.376	56.173	16.516	12.036	16.017
–CYL samples								
mean	265	7.0	4.4	10.2	21.4	29.7	21.0	49.6
SD	62	5.2	7.6	4.6	8.6	16.1	29.0	17.4

vealed significant differences only for dissolved NOX and TN levels (Fig. 6; Table 3). Samples containing *Cylindrospermopsis* displayed higher TN and lower NOX levels compared to –CYL samples. SRP and TP concentrations showed the same pattern, but differences were not significant. Consequently, the average DIN/SRP ratio of +CYL samples was lower compared to –CYL samples.

On the first glance these findings seem contradictory but they can be explained by the species’ ecology. Bouvy et al. (1999) state that *C. raciborskii* is possibly less dependent on nutrients

than other cyanobacteria and is often found at low DIN/SRP ratios. In subtropical and temperate regions the species is known to bloom relatively late in season when the water is warm and most of the nutrients have already been used by other phytoplankton species. Its capability to use even extremely low nutrient concentrations most efficiently (Branco and Senna, 1994; Istvánovics et al., 2000; Shafik et al., 2001; Briand et al., 2002), coupled with the tolerance for low light, enables the species to thrive in systems characterized by high plankton densities and low dissolved nutrient concentrations. This exactly

Fig. 8. Meteorological data (daily average wind speed, minimal air temperature, cumulative rainfall) for the Caernarvon Breton sound estuary (LSU-AG Center, Citrus station 2002–2004). Minimal air temperature are given as 5-day floating average.

Fig. 9. Comparison on monthly diversion discharge (gray scale bars with SD) and rain (hatched bars) for the Caernarvon Breton Sound estuary. Height of the diversion discharge was calculated from USGG discharge data and a total area of the estuary of 656.4 km².

describes the situation in the lake-like structures within the Caernarvon Breton Sound estuary in summer. Dense, often nitrogen-limited phytoplankton communities dominated in areas where *C. raciborskii* was most abundant. Because the species is diazotrophic, it compensates for low DIN concentrations by fixing molecular nitrogen

(Presing et al., 1996; Ryan et al., 2003). Laboratory data showed that at a fixed SRP supply, the biomass in cultures developed similarly, irrespective of the amount and portioning of available nitrogen forms. Added ammonium and nitrate was fully taken up by *C. raciborskii*, and the remaining nitrogen demand was met by N₂-fixation. It was suggested that the nitrogen fixation system stopped and was then activated again depending on the nitrogen content of the cells (Strober et al., 2003).

The fact that in Brazilian reservoirs, even at low nitrate and nitrite concentrations, *C. raciborskii* trichomes often do not develop heterocysts and hence do not fix N₂ was attributed to its preferential uptake of ammonium (Presing et al., 1996; Bouvy et al., 1999; Briand et al., 2002). In any case the individuals from the Caernarvon population regularly displayed heterocysts despite the often relatively high ammonia levels (Table 3).

Cylindrospermopsis raciborskii has the important competitive advantage of being extremely efficient at taking up and storing phosphorus (Shafik et al., 2001). Compared to other cyanobacteria, its SRP uptake is four times and SRP affinity is even an order of magnitude higher. Blooms are sustained at SRP levels as low as 1–

Fig. 10. Map of the Caernarvon Breton Sound estuary with stations sampled in the 2002–2004 time series (x). Hatched arrows show major paths of diverted Mississippi water. The diverted water does not affect the dash-dotted area to the Northeast. *Cylindrospermopsis raciborskii* was detected in 2002 or 2003 in the area surrounded by the solid line and at one station in Lake Leary (black dot). In 2004 the species occurred only in low numbers in the areas marked by dotted lines.

2 $\mu\text{g-P liter}^{-1}$ (Istvánovics et al., 2000). Average SRP concentrations during surveys when *C. raciborskii* was detected in the Caernarvon Breton Sound estuary were 25 to 50 times higher. No difference was detected between stations featuring high abundances of *C. raciborskii* (average 51 ± 5) and stations that did not (average $53 \pm 28 \mu\text{g SRP liter}^{-1}$). Hence, in this estuary *C. raciborskii* could not take advantage of its competitiveness regarding phosphorus utilization. In Florida lakes, Dobberfuhl (2003) saw negative correlations between *C. raciborskii* abundance and dissolved total phosphorus (DTP: SRP + DOP, dissolved organic phosphorus): *C. raciborskii* declined at DTP $> 20 \mu\text{g liter}^{-1}$ whereas *Microcystis* gained maximum densities at $80 \mu\text{g liter}^{-1}$. The Chl *a*/DTP ratio (by weight) is considered a valuable parameter assessing plankton blooms with respect to the occurrence of *C. raciborskii* (Dobberfuhl, 2003). In Florida lakes, blooms without *C. raciborskii* had low ratios (> 0.5), whereas Chl *a*/DTP increased to almost two as the species got more abundant.

For the Caernarvon Breton Sound the findings were comparable. Because DOP data were not available we used the Chl *a*/SRP instead of the Chl *a*/DTP ratio. The Chl *a*/SRP ratios (by weight) were elevated in samples with high *C. raciborskii* abundance (average: 0.98 ± 0.55) compared to samples with low or no abundance of the species (average: 0.68 ± 0.81) although the difference was not statistically significant.

Planktonic parameters.—For both of the survey areas (Atchafalaya, Caernarvon), +CYL samples showed significantly higher total chlorophyll and cyanobacterial chlorophyll levels compared to -CYL samples. Considering the ability of the species to cope with low light and low nutrient situations (Branco and Senna, 1994; Shafik et al., 2001), these results are in line with expectations. Despite its relatively low chlorophyll content per biomass unit (Dokulil and Mayer, 1996; McGregor and Fabbro, 2000), *C. raciborskii* is known to occur frequently or even dominate in high-chlorophyll environments (Mayer et al., 1997; Bouvy et al., 1999, 2003a, b; Borics et al., 2000; Jones and Sauter, 2005). Often the species occurs together with other bloom-forming cyanobacterial genera, such as *Oscillatoria*, *Microcystis*, *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, and *Raphidiopsis* (Seenayya and Zafar, 1979; Ramberg, 1987; Hawkins and Griffiths, 1993; Domitrovic et al., 1998; Chellappa and Costa, 2003). In 2003 in the Grand Lake area it was mainly associated with high densities of the potentially saxitoxin-producing cyanobacterium

Anabaena circinalis (Baker and Humpage, 1994; Bowling, 1994).

Souza et al. (1998) describe shifts from *C. raciborskii*-dominated to green algal (*Scenedesmus* sp.) dominated communities in Billings reservoir, Brazil. In African lakes, Ramberg (1987) and Berger et al. (2006) monitored seasonal dominance shifts from *C. raciborskii* toward diatoms. High abundance of *C. raciborskii* is frequently associated with reduced species richness of the phytoplankton community (Branco and Senna, 1996; Borics et al., 2000; Dobberfuhl, 2003). The later findings are in line with our results of lower percentages of both green algal and other chlorophyll (from heterokontophytes, dinoflagellates, and prymnesiophytes) in samples where *C. raciborskii* was detected.

Interannual variability.—The spatial and annual temporal distribution of *C. raciborskii* in the Caernarvon Breton Sound estuary could be attributed to an interplay of temperature, water column stability, and nutrient availability within the estuary. Differences in the species' abundance during the years 2002–05 (Fig. 1a,b; Table 1) remain to be explained. In 2002 and 2003 *C. raciborskii* was very abundant in parts of the estuary, which were not primarily affected by the diverted water (Fig. 10). In 2003, the year of highest abundance, the species occurred already in June, whereas it appeared in 2002 and 2004 1 mo later. Rabalais (2005) and Ren et al. (2005) also reported high abundances of the species from the upper Barataria estuary (Lake Cat-aouatche, Salvador, and des Allemands) in June 2003.

Compared to 2003, in 2004 and 2005 extensively more water was diverted thru the estuary before the potential biomass development of *C. raciborskii*, exposing the species to less favorable conditions. Thirteen times the amount of 2003 was discharged in the period from April to May in 2004 and even 30 times the amount of 2003 was discharged in the period from April to May in 2005. In 2004 the species was detected first in low abundances ($25\text{--}50 \times 10^3 \text{ cells liter}^{-1}$) in an area not affected by the diverted waters (13 July, Lake Amadee, Tanasia Lagoon; Fig. 1b, mid right panel). Then later in Aug., during a period of reduced diversion discharge (3.6% of maximum capacity) it appeared in relatively low numbers ($10\text{--}40 \times 10^3 \text{ cells liter}^{-1}$) in the southeastern part of the estuary. Finally, during two surveys in summer and fall 2005 and monthly campaigns in 2006 (April–Aug.) *C. raciborskii* could no longer be recorded in the estuary.

Ephemeral occurrence of the species in subtropical and temperate regions is documented in

the literature (Bouvy et al., 1999; Hamilton et al., 2005; Jones and Sauter, 2005) and in most cases is explained by climatic variability. Since the species is not able to survive the lowered water temperature during winters suspended in the water column, it relies on resting stages, so called akinetes, which are able to survive harsh environmental conditions in the upper sediment. The existence of akinetes in the sediment is a prerequisite for the following thoughts.

Cylindrospermopsis raciborskii akinetes are only able to germinate in a relatively narrow, but high temperature range (22–25 °C). Favorable environmental conditions following akinete germination, such as a relatively undisturbed water column, low winds, and low rainfall, can foster mass development of the species (Padisák, 1997, 2003; Moore et al., 2003, 2004, 2005).

Comparing *C. raciborskii* high-abundance (2002, 2003) and the low- or nonabundance years (2004, 2005), temperature development and weather factors influencing water column stability seem worthy of consideration. The development of important influencing parameters (cumulative rainfall, average daily wind speed, minimal daily air temperature) for these years is shown in Figure 8. We choose the development of the minimal daily air temperature (5-d floating mean) as a proxy because time series data of water temperature at the sediment water interface (important for akinete germination) were not available. It is assumed that at a minimal daily air temperature of 18.5 °C (shown as a hatched line parallel to the time axis) the heating of the water column is sufficient to reach the critical temperature for akinete germination (22–25 °C).

The cumulative rainfall for the first 5 mo in the years of high *C. raciborskii* occurrence was 50% or even less compared to years of low and no occurrence (2002: 29.7, 2003: 24.9, 2004: 61.9, 2005: 69.8 cm). Average daily rain during periods of potential akinete germination (April–June) was also significantly lower in 2002 and 2003 compared to 2004. In 2005 the average was higher again, but because of a pronounced variability it did not differ significantly from the 2002 and 2003 values (Fig. 11a; Table 5).

This underlines the hypothesis that rainfall (rain deficit, lack of water renewal) is an important parameter influencing the occurrence of the species (Harris and Baxter, 1996; Domitrovic et al., 1998; Bouvy et al., 1999, 2003a; Saker and Griffiths, 2001). Additionally differences in timing and duration of spring droughts over the years became obvious. The severe 2002 drought started 3 March and lasted for 76 days, with a total rainfall of 5.4 cm. In 2003 there were

65 days of drought beginning on 17 March, yielding 4.3 cm of rain. In 2004 the dry period started very early (25 Feb.) and lasted for only 42 days, yielding 1.6 cm, whereas in 2005 we recorded an exceptionally late 53-d drought (7 April–29 May) with 6.5 cm rainfall.

The average minimal air temperature for April was significantly higher in 2002, and for May was significantly higher in 2003 compared to the respective months of the other years within the time series (Fig. 11b; Table 5). In 2002 and 2003, the critical temperature for *C. raciborskii* akinete germination was probably reached in the first half of the dry season, enabling the akinetes to germinate and giving the species a favorable environment for subsequent biomass build-up. In the low abundance year 2004 this critical temperature probably did not occur in dry season at all, and it was only reached late in the dry period 2005, exposing the species to less favorable conditions for akinete germination.

In 2003, the year of highest *C. raciborskii* abundance, average winds during the time of possible akinete germination were significantly lower compared to years of low or nonoccurrence of the species (Fig. 11a; Table 5). The fact that April and May 2003 were much calmer compared to 2002, 2004, and 2005 is pronounced by the frequency of low wind days (< 6.5 km h⁻¹). Almost half of the time showed low winds in 2003 whereas this percentage ranged from below 10% to 30% in the other years (Fig. 11a).

The fact that the species did not occur in the estuary in 2006 might be a side effect of Hurricane Katrina hitting the area directly at the end of Aug. 2005. The estuary was completely flushed with salt water, and lake sediments were eroded to a huge extent. Akinetes resting in the upper sediment layers were mixed into the water column and flushed out or at least exposed to elevated salinities that presumably killed them. Since the diversion structure was closed completely after Katrina until mid-Jan. 2006 (Chuck Villarrubia, pers. comm.), remaining akinetes were exposed to high salinity conditions at least for 4.5 mo. From mid-Jan. to mid-June 2006 the diversion structure was run at relatively high discharge (50% more compared to the period 2001–05), which also might not have been beneficial for potentially surviving akinetes.

CONCLUSIONS

The occurrence of *C. raciborskii* in the Caernarvon Breton Sound estuary is influenced by an interplay of water temperatures and timing of

Fig. 11. Oneway ANOVA based on meteorological data from the Caernarvon Breton Sound time series (2002–2005). (a) Average daily wind speed and weekly cumulative rain for April–May is displayed along with SEM error bars. White squares show the percentage of low-wind days ($< 6.5 \text{ km h}^{-1}$) in April and May. (b) Average daily minimal air temperatures with SEM error bars for April and May 2002–2005. Bars not connected by the same letter are significantly different.

water column disturbances, indicated by diversion discharge, rainfall, and wind speed. If the critical temperature for enabling akinetes to germinate is reached during times of low disturbance and this calm situation continues for two or three additional weeks, these conditions favor a rapid development of *C. raciborskii*. In addition to the meteorological conditions, the flushing effect by diverted waters seems to be of crucial importance. During the 2-mo time period before the mass development of the species, only a small amount of Mississippi water (ca. 6–8% compared to 2002 and 2004) was diverted through the estuary in 2003, the year we

recorded highest biomasses and widespread spatial abundance of *Cylindrospermopsis*. In 2005 when the species was no longer observed, flushing during April to May was almost 30 times higher compared to 2003. These results give some implications for managing the water quality in the Caernarvon Breton Sound estuary. If the water level of the Mississippi River permits it, the estuary should be flushed thoroughly during April and May. Additionally, it would be beneficial to change the hydrology in the estuary in such a way that the Spanish Lake–Grand Lake area receives more of the diverted water. This would enhance

TABLE 5. One-way ANOVA of abiotic parameters (average daily wind, weekly cumulative rain, min. daily temperature) for April–May 2002–05, Caernarvon Breton Sound estuary. Years/months not connected by the same letter are significant different ($\alpha = 0.05$). Wind ($n = 244$) and rain data ($n = 35$) were pooled for both months [df (degrees of freedom) = 3]; temperature data ($n = 244$) were analyzed for each month (df = 7).

	Daily wind	Weekly cumulative rain	Daily minimum temperature	
F-ratio	3.5346	2.2194	17.7836	
Prob. > F	0.0155	0.1056	0.0001	
Dimension	[km h ⁻¹]	[cm]	[C]	
Year/month	2002 (A)	2002 (B)	April 2002 (C)	May 2002 (B)
Mean	10.67	0.47	17.96	20.3
SD	3.62	1.08	3.6	3.93
Year	2003 (B)	2003 (B)	April 2003 (D)	May 2003 (A)
Mean	8.29	1.0	15.89	22.62
SD	5.73	1.68	4.62	2.55
Year	2004 (A)	2004 (A)	April 2004 (D)	May 2004 (B)
Mean	10.16	4.48	15.33	20.22
SD	3.35	4.85	3.64	3.49
Year	2005 (A, B)	2005 (A, B)	April 2005 (B)	May 2005 (B, C)
Mean	9.54	2.26	20.22	19.19
SD	3.92	4.69	3.49	3.04

the flushing of akinetes in these “seeding” areas for *Cylindrospermopsis* and could also be used as an active management tool to control potentially developing blooms of the species.

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