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Spatial-temporal variation in coiled and straight morphotypes of *Cylindrospermopsis raciborskii* (Wolsz) Seenayya et Subba Raju (Cyanobacteria)[1]

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RESUMO – (Variação espaço-temporal dos morfotipos espiralado e reto de C*ylindrospermopsis raciborskii* (Wolsz) Seenayya et Subba Raju (Cyanobacteria)). O presente estudo reporta o comportamento espacial e temporal dos morfotipos reto e espiralado de *C. raciborskii* em um reservatório da região semi-árida do Brasil bem como os principais fatores responsáveis pela variabilidade. Dois conjuntos de amostras foram coletadas na subsuperfície e próximo ao sedimento, na região central do reservatório, em dois períodos sazonais (seco – janeiro 2005; chuvoso – junho 2005), sendo realizadas amostragens de 20 horas durante horários claros (08hs, 12hs e 16hs) e escuros (20hs, 00hs e 04hs). Medidas de parâmetros abióticos foram realizadas concomitantes as amostragens das variáveis bióticas. Dois morfotipos de *C. raciborskii* foram encontrados no reservatório: reto e espiralado. Não houve diferenças na densidade dos morfotipos reto e espiralado de *C. raciborskii* entre os diferentes horários amostrais em cada período sazonal. Diferenças verticais foram encontradas na distribuição de ambos morfotipos em ambos períodos sazonais, com maiores densidades registradas na subsuperfície. As densidades dos dois morfotipos de *C. raciborskii* foram maiores no período seco, com densidade do morfotipo espiralado na subsuperfície duas vezes maior que o morfotipo reto e o encontrado no período chuvoso. O sucesso ecológico do morfotipo espiralado foi devido à estratificação térmica, enquanto as condições de mistura foram determinantes no sucesso do morfotipo reto.
Palavras-chave: *Cylindrospermopsis raciborskii*; Semi-árido; Variação diária; Variação vertical

ABSTRACT – (Spatial-temporal variation in coiled and straight morphotypes of C*ylindrospermopsis raciborskii* (Wolsz) Seenayya et Subba Raju (Cyanobacteria)). This study reports the spatial and temporal behavior of straight and coiled morphotypes of *C. raciborskii* in a reservoir in Brazil's semi-arid region as well as the main factors responsible for the variability. Two set of samples were collected from the subsurface and bottom in the central region of a reservoir in two seasonal periods (dry – January 2005; rainy – June 2005) over 20-hour sampling periods during daylight (8 am, 12 pm and 4 pm) and dark (8 pm, 12 am and 4 am) hours. Measurements of abiotic parameters were determined concurrently to the sampling of biotic variables. Two *C. raciborskii* morphotypes were found in the reservoir: straight and coiled. There was no difference in density of the straight and coiled *C. raciborskii* morphotypes between the different sampling times for either season. Vertical differences were found in the distribution of both morphotypes in both seasons, with greater densities recorded at the subsurface. Densities of the two *C. raciborskii* morphotypes were greater in the dry season, with the density of the coiled morphotype at the surface two-fold greater than that of the straight morphotype and that found in the rainy season. The ecological success of the coiled morphotype was due to thermal stratification, whereas a mixed condition was determinant in the success of the straight morphotype.
Keywords: *Cylindrospermopsis raciborskii*; Semi-arid; Diel variation; Vertical variation

Introduction

The plankton genus *Cylindrospermopsis* Seenayya et Subba Raju was separated from *Anabaenopsis* Woloszynska due to the different manner of development of the heterocytes (Padisák 1997). The type species *C. raciborskii* (Wolsz) Seenayya et Subba Raju was originally described in 1913 as *Anabaena raciborskii* Woloszynska and is a common pantropical, bloom-forming species (Komárková-Legenerová 1998).

According to traditional morphological criteria, filament shape is a taxonomic characteristic that distinguishes different species. Straight forms are identified as *C. raciborskii* and coiled forms are identified as *C. philippinensis* (Taylor) Komárek and *C. catemaco* Komarková-Legnerová et Tavera (Komárková-Legenerová 1998). However, it is currently believed that *C. raciborskii* alone exhibits an extensive phenotypic plasticity, with straight, sigmoid and coiled filaments that can occur simultaneously (Baker 1996; Wilson *et al.* 2000; Bittencourt-Oliveira & Molica 2003). Blooms formed predominantly by one or both morphotypes are found in nature under similar conditions (Fabbro & Duivenvoorden 1996; Bouvy *et al.* 1999; Saker *et al.* 1999; Briand *et al.* 2002). The ecological conditions under which the species grows and forms blooms appear to be very diverse in tropical and temperate waters (Briand *et al.* 2004). Perennial populations may persist throughout the year in tropical regions (Komárkova *et al.* 1999), where as the occurrence of species is restricted to the summer in temperate regions (Briand *et al.* 2002). Due to the high akinete germination temperatures and the capacity to store phosphorus and fix nitrogen, *C. raciborskii* develops in layers of warm waters with low light intensity and a low concentration of nutrients (Padisák & Reynolds 1998).

Over the last twenty years, the frequent occurrence of *C. raciborskii* has been reported in bodies of water in the center-west (Branco & Senna 1994; 1996), southern (Komárková *et al.* 1999), southeastern (Souza *et al.* 1998) and northeastern (Bouvy *et al.* 2000; 2001; Chellapa & Costa 2003; Costa *et al.* 2006; Panosso *et al.* 2007; Chellappa *et al.* 2008; Dantas *et al.* 2008) regions of Brazil. According to Bittencourt-Oliveira & Molica (2003), the northeastern region of the country – more specifically the state of Pernambuco – has extensive records of the presence of *C. raciborskii* and is the only region with records of the coiled morphotype thus far.

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The present study reports the spatial and temporal behavior of the straight and coiled morphotypes of *C. raciborskii* in a reservoir in a semi-arid region of Brazil as well as the main factors responsible for the variability.

Material and methods

The study was carried out at the Mundau Reservoir (08°56'47" S and 36°29'33" W) in the state of Pernambuco (northeastern Brazil), which is located 716 m above sea level. The reservoir is intended for the public water supply and was constructed to accumulate 1,968,600 m^3. It currently receives part of the domestic drainage from the city of Garanhuns (Moura *et al.* 2007b).

Two set of samples were collected from the subsurface and bottom (8 m) in the central region of the reservoir in two periods: the dry season (January 2005) and the rainy season (June 2005). Sampling was carried out over 20-hour periods during daylight (8 am, 12 pm and 4 pm) and dark (8 pm, 12 am and 4 am) hours. One hundred mL aliquots of water were collected and preserved in an acetic Lugol's solution for the identification and determination of phytoplankton density (ind.L^{-1}) using an inverted microscope and following the Utermöhl method (Utermöhl 1958). Trichomes of *C. raciborskii* were photographed using a light microscope (Nikon E200, Melville, NY, 120 USA) equipped with a video camera system (Samsung SCC833, Tokyo, Japan) and employing the Imagelab software program (Softium, Brazil). Data on density were used to determine the dominance of *C. raciborskii,* based on the criteria described by Lobo & Leighton (1986). The species was considered *abundant* when the value calculated was greater than the mean value of the community and *dominant* when this value surpassed 50% of the total phytoplankton density.

Concurrently to the samplings of algae, the following abiotic variables were determined *in situ*: water temperature and dissolved oxygen using an oximeter (Schott Glaswerke Mainz, handylab OX1); electrical conductivity using a conductivimeter (Schott Glaswerke Mainz, handylab LF1); turbidity using a turbidimeter (Hanna Instruments, HI 93703); and pH using a potentiometer (Digimed, DMPH-2). Water transparency was determined using a Secchi disc (25 cm in diameter) and underwater light (µmol.m^{-2}.s^{-1}) was determined using a light sensor (LICOR mod. 250).

Water from two depths was collected using van Dorn bottle and aliquots were placed in 300 mL polyethylene flasks for the analysis of dissolved and total nutrients. The determination of concentrations of total nitrogen (µmol. TN.L^{-1}), nitrite (µmol.N-NO_2.L^{-1}) and nitrate (µmol.N-NO_3.L^{-1}) followed the procedures described in Valderrama (1981), Mackereth *et al.* (1978) and Golterman *et al.* (1971), respectively. Total phosphorus (µmol.TP.L^{-1}) and total dissolved phosphorus (µmol.TDP.L^{-1}) were determined following Valderrama (1981). Orthophosphate (µmol.P-PO_4.L^{-1}) was determined following Strickland & Parsons (1965).

For the trophic characterization of the ecosystems, we employed the Carlson Trophic State Index, adapted by Toledo Jr. *et al.* (1983) for tropical regions. Calculations were based on total phosphorus content, for which ultra-oligotrophic (≤ 20), oligotrophic (21 – 40), mesotrophic (41 – 50), eutrophic (51 – 60) and hypertrophic (≥61) conditions were then determined (Kratzer & Brezonik 1981).

Analysis of variance (ANOVA) was used with a 5% level of significance to determine the degree of temporal variation (time of day and season) and spatial variation between depths. Pearson's correlation analysis (*r)* was performed for the straight and coiled morphotypes in relation to environmental variables using the Statistica 2004 software program (StatSoft, Inc., Tulsa, OK, USA).

Results

Both straight and coiled morphotypes of *C. raciborskii* were found in the Mundaú reservoir. Few trichomes had heterocytes and akinetes (Fig. 1a-b).

Total density of the other plankton algae (without *C. raciborskii*) ranged from 14.4 x 10^6 at 8 pm on the bottom in the rainy season to 68.7 x 10^6 ind. L^{-1} at 8 am at the subsurface in the dry season. *C. raciborskii* density ranged from 13.1 x 10^6 at 4 pm at the bottom in the rainy season to 113.6 x 10^6 ind. L^{-1} at 8 am at the subsurface in the dry season, thereby demonstrating the considerable participation of the species in the phytoplankton community. The density of *C. raciborskii* was greater than the other algae at the subsurface at all times of the day and in both seasons, demonstrating dominance. Despite the high density of the species at the bottom, it was less than the density of other algae in both seasons at nearly all times of day, except at 12 am in the rainy season, when the density of the species was slightly greater. At the bottom, *C. raciborskii* was classified as abundant at all times of the day, except at 12 am in the rainy season, when it was classified as dominant (Fig. 2).

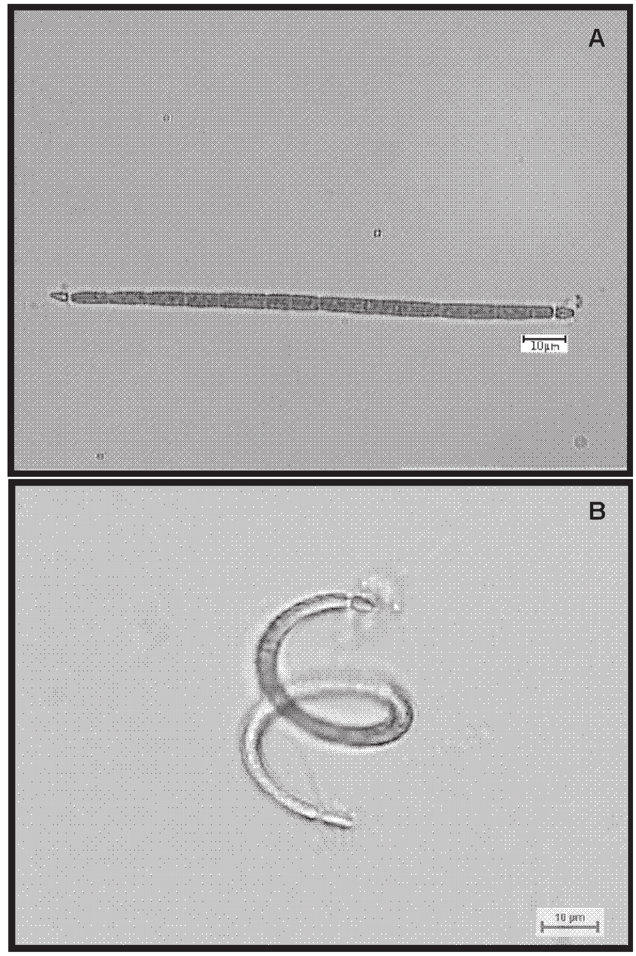

Figure 1. Morphological features of *Cylindrospermopsis raciborskii*. (a) Straight morphotype, with hetrocytes in the terminal position of the trichome occurring at both ends; (b) Coiled morphotype, with heterocytes in the terminal position of the trichome occurring at both ends.

Figure 3a-d displays the variation in straight and coiled morphotypes at the different sampling times in both seasons. There were no significant differences in density between morphotypes. There was no significant difference in the density of the coiled morphotype at different times of the day in the dry season (F=1.057, $p > 0.05$) or the rainy season (F=0.305, p>0.05). For the straight morphotype, these values were F=0.904 ($p > 0.05$) and F=0.253 ($p > 0.05$) for the dry and rainy season, respectively.

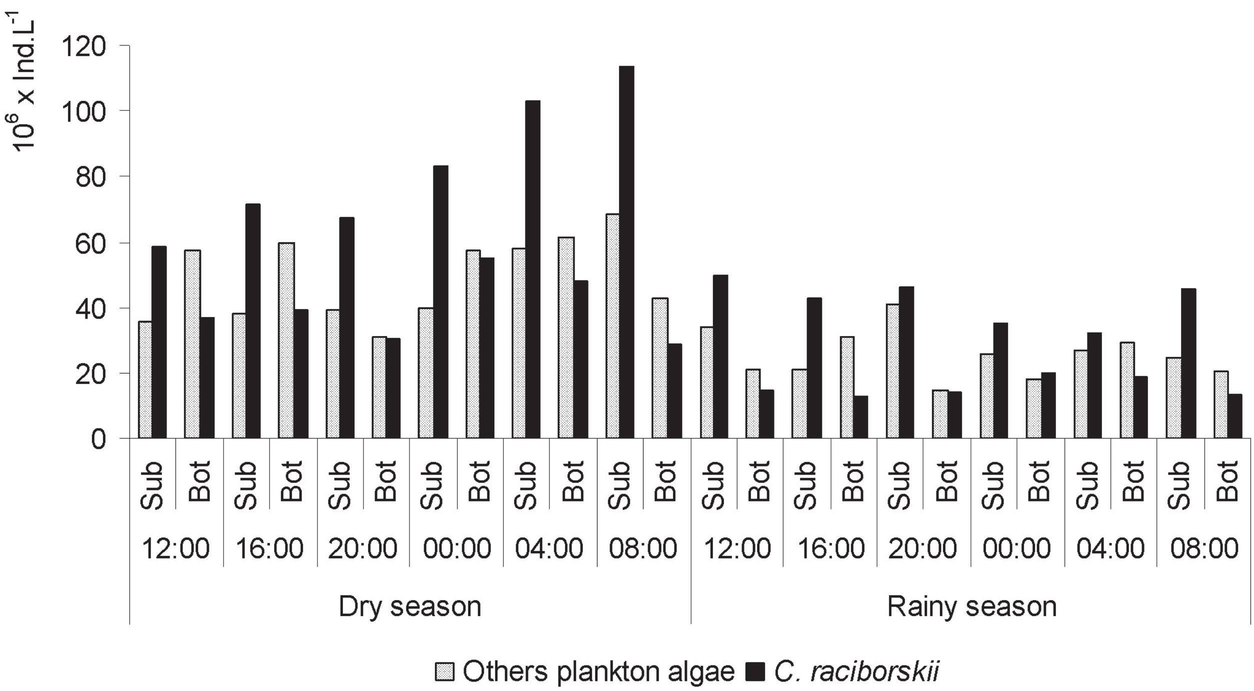

Figure 2. Total density of phytoplankton (without *C. raciborskii*) and density of *C. raciborskii* at subsurface and bottom of Mundaú reservoir at different times of the day in the dry and rainy seasons. Sub: subsurface; Bot: Bottom.

Vertical differences were found in the distribution of both morphotypes in both seasonal periods, with greater densities recorded at the subsurface (Fig. 3a-d). Density of the two *C. raciborskii* morphotypes was higher in the dry season, with the density of the coiled morphotype at the surface two-fold greater than that of the straight morphotype in this season. In the rainy season, there was an accentuated reduction in the density of the coiled morphotype, with lower values in comparison to the straight morphotype (Fig. 3a-d).

Figures 4a-l and 5a-l displays the physico-chemical variation between times of the day and depths in the dry and rainy seasons. No significant differences ($p > 0.05$) were found in the abiotic variables between sampling times in the dry season. In the rainy season, significant differences ($p < 0.05$) occurred in water temperature, pH and total dissolved phosphorus.

Vertical differences ($p < 0.05$) were found in the dry season for temperature, dissolved oxygen, pH, turbidity, total nitrogen, total phosphorus and total dissolved phosphorus. In the rainy season, all variables (except total phosphorus) exhibited vertical differences.

There were significant differences between seasons ($p < 0.01$) regarding water temperature, dissolved oxygen, electrical conductivity, turbidity, pH, total nitrogen, nitrate, nitrite, total dissolved phosphorus and orthophosphate. There were no statistically significant differences between seasons ($p > 0.05$) regarding light intensity and total phosphorus, although higher light intensity values were observed at the subsurface in the dry season.

Based on physical-chemical characteristics, Mundaú reservoir is classified as a hypertrophic tropical system. The correlation analysis with the pooled data of both seasons revealed that the straight and coiled morphotypes of *C. raciborskii* had a positive correlation with water temperature and pH as well as a negative correlation with turbidity, total nitrogen, nitrate, nitrite and total phosphorus. The coiled morphotype was also positively correlated with light intensity and orthophosphate as well as negatively correlated with electrical conductivity (Tab. 1).

In the dry season, significant correlations were found between the *C. raciborskii* morphotypes and environmental variables in the reservoir. The straight morphotype had a positive correlation with turbidity and pH and a negative correlation with total phosphorus, whereas the coiled morphotype had a positive correlation with water temperature, turbidity and pH and a negative correlation with total nitrogen, total phosphorus and total dissolved phosphorus (Tab. 1).

In the rainy season, significant correlations were also found between the *C. raciborskii* morphotypes and environmental variables in the reservoir. Both morphotypes had positive correlations with light intensity, water temperature, dissolved oxygen, electrical conductivity and pH, whereas the two morphotypes had negative correlations with turbidity, total nitrogen, nitrate, nitrite and total phosphorus (Tab. 1).

Discussion

C. raciborskii density was high in both seasons. This occurrence was associated to high temperatures, which were always above 22°C in the reservoir, as well as alkaline

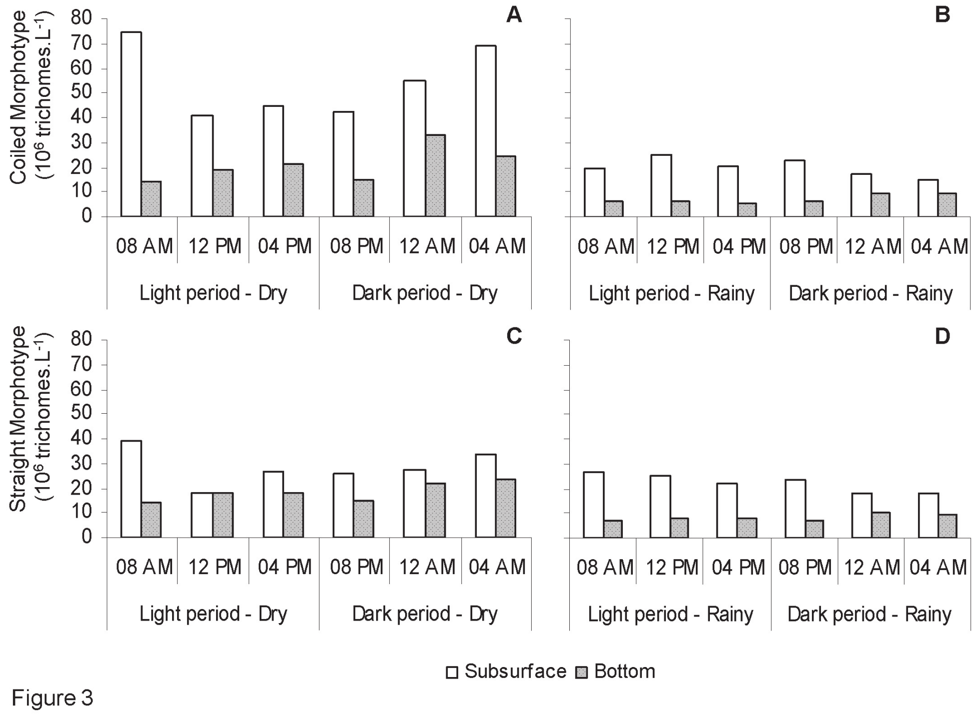

Figure 3. Variation in *C. raciborskii* density at the subsurface and bottom at different times of the day in the Mundaú reservoir (PE, Brazil). A. Coiled morphotype during light and dark hours in the dry season; B. Coiled morphotype light and dark hours in the rainy season; C. Straight morphotype light and dark hours in the dry season; D. Straight morphotype light and dark hours in the rainy season.

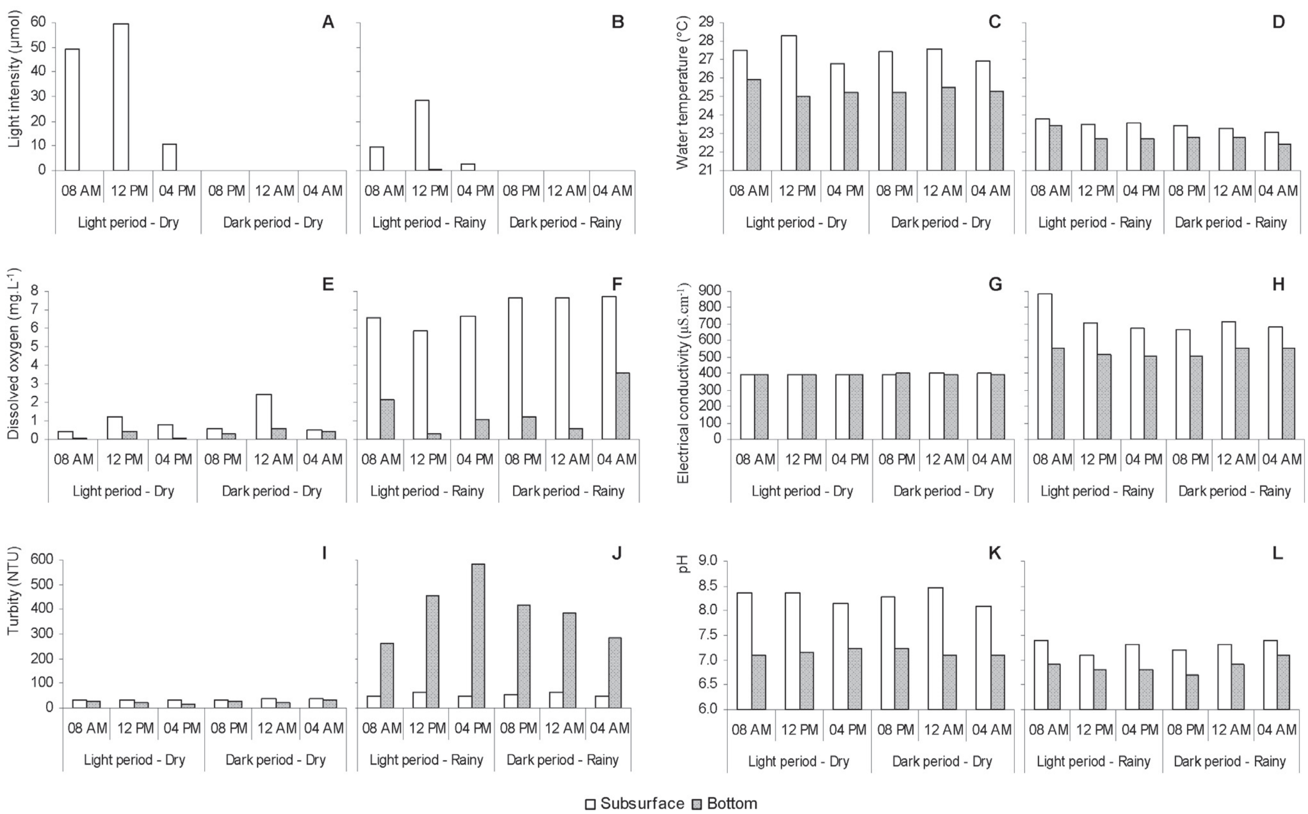

Figure 4. Physiochemical variation at subsurface and bottom in the Mundaú reservoir in the dry and rainy seasons. a. Light intensity in the dry season; b. Light intensity in the rainy season; c. Water temperature in the dry season; d. Water temperature in the rainy season; e. Dissolved oxygen in the dry season; f. Dissolved oxygen in the rainy season; g. Electrical conductivity in the dry season; h. Electrical conductivity in the rainy season; i. Turbidity in the dry season; j. Turbidity in the rainy season; k. pH in the dry season; l. pH in the rainy season.

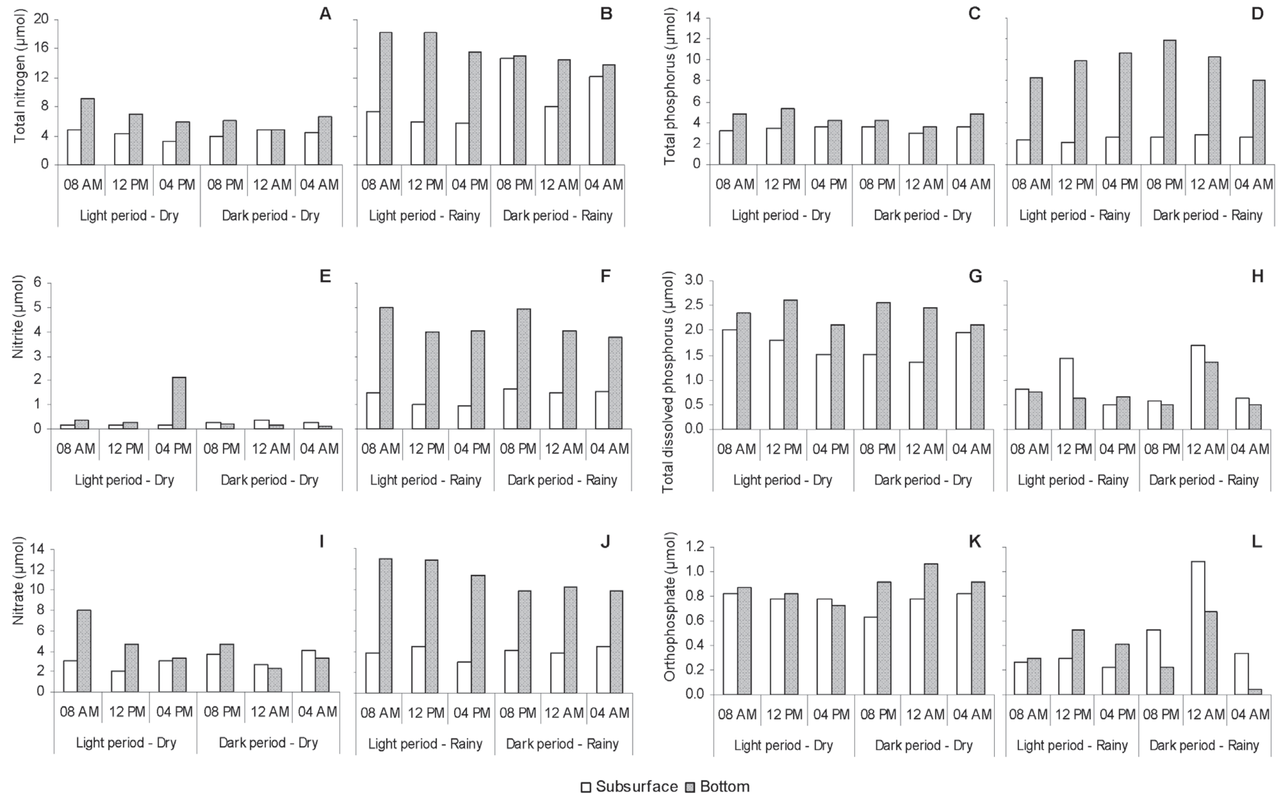

Figure 5

Figure 5. Physiochemical variation at subsurface and bottom in the Mundaú reservoir in the dry and rainy seasons. a. Total nitrogen in the dry season; b. Total nitrogen in the rainy season; c. Nitrate in the dry season; d. Nitrate in the rainy season; e. Nitrite in the dry season; f. Nitrite in the rainy season; g. Total phosphorus in the dry season; h. Total phosphorus in the rainy season; i. Total dissolved phosphorus in the dry season; j. Total dissolved phosphorus in the rainy season; k. Orthophosphate in the dry season; l. Orthophosphate in the rainy season.

pH and the hypertrophy of the system. The environmental conditions in the Mundaú reservoir are the same reported by other researchers as favorable to blooms of this species in other ecosystems in northeastern Brazil (Bouvy *et al.* 2000; Chellapa & Costa 2003; Moura *et al.* 2007b; Chellappa *et al.* 2008; Von Sperling *et al.* 2008). Regardless of geographic location, these populations grow and form blooms only in warm waters with temperatures above 25°C (Padisák 1997; Saker & Griffiths 2001) or in hypolimnetic waters with temperatures above 23°C (McGregor & Fabbro 2000).

The greatest densities of two morphotypes of *C. raciborskii* in the dry season occurred at a mean temperature of 27.4°C. Blooms recorded in the rainy season occurred at a mean temperature of 23.5°C, which is a reflection of the mixture condition evidenced for the period, as stated by Moura *et al.* (2007a). Although thermal stratification influences the occurrence of high densities of *C. raciborskii* (Bouvy *et al.* 2000), de-stratification or a mixture condition can be an advantage to the species in tropical environments, especially considering the fact that this taxon is pre-adapted to low-light conditions (Shafik *et al.* 2001).

The population dynamics of *C. raciborskii* is dependent upon the presence of particular environmental conditions, which mainly include warm, stratified surface waters, low light and alkaline pH (Padisák 1997; McGregor & Fabbro 2000; Briand *et al.* 2004). Although *C. raciborskii* is less dependent upon nutrients than other cyanobacteria (Bouvy *et al.* 2000), an affinity for phosphorus is suggested (Isvánovics *et al.* 2000).

The role of nutrients in the development of *C. raciborskii* was not relevant. The greatest densities occurred in the period with less nitrogen content, which was limited throughout the entire study. Furthermore, in environments with reactive soluble phosphorus concentrations greater than 10 $\mu g.L^{-1}$, the development of cyanobacteria is regulated by physical factors (Saker *et al.* 1999). *C. raciborskii* blooms do not depend upon the presence of nitrogen, as the species is able to fix atmospheric nitrogen through heterocytes. This appears contradictory, as few trichomes with heterocytes were found in the reservoir. However, studies have found that *C. raciborskii* appears not to be dependent upon the fixation of nitrogen (e.g. Griffiths & Saker 2003) and that few heterocytes are found even at lower concentrations of this element (Pressing *et al.* 1996). This is especially due to its greater affinity for ammonium than other nitrogenated elements (Bouvy *et al.* 1999; Briand *et al.* 2002).

Concerning the different morphotypes of *C. raciborskii*, there was no evident variation in density throughout the daily sampling times. The stable behavior of the morphotypes occurred due to environmental stability at the different times of the day in each season. This finding corroborates that

Table 1. Correlation values of environmental variables and densities of different *C. raciborskii* morphotypes in the Mundaú reservoir between seasonal periods. Values in bold type = significant differences.

	Dry season				Rainy season				Two seasonal periods			
	straight		coiled		straight		coiled		straight		coiled	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Underwater light (μmol.m^{-2}.s^{-1})	0.277	0.383	0.432	0.161	0.577	0.049	0.592	0.042	0.385	0.063	0.473	0.019
Dissolved oxygen (mg.L-1)	0.228	0.476	0.406	0.190	0.854	0.000	0.851	0.000	0.660	0.000	0.828	0.000
Water temperature (°C)	0.528	0.077	0.741	0.006	0.797	0.002	0.750	0.005	0.088	0.683	-0.206	0.333
Electrical conductivity (μS.cm-1)	0.442	0.150	0.501	0.097	0.907	0.000	0.799	0.002	-0.099	0.643	-0.410	0.047
Turbity (NTU)	0.597	0.040	0.700	0.011	-0.867	0.000	-0.871	0.000	-0.714	0.000	-0.543	0.006
pH	0.652	0.022	0.820	0.001	0.815	0.001	0.750	0.005	0.749	0.000	0.884	0.000
Total nitrogen (μmol)	-0.561	0.058	-0.661	0.019	-0.818	0.001	-0.800	0.002	-0.765	0.000	-0.706	0.000
Nitrate (μmol)	-0.415	0.179	-0.473	0.120	-0.922	0.000	-0.915	0.000	-0.792	0.000	-0.643	0.001
Nitrite (μmol)	-0.252	0.429	-0.254	0.425	-0.938	0.000	-0.930	0.000	-0.770	0.000	-0.659	0.000
Total phosphorus (μmol)	-0.603	0.038	-0.764	0.004	-0.924	0.000	-0.913	0.000	-0.766	0.000	-0.545	0.006
Total dissolved phosphorus (μmol)	-0.483	0.112	-0.591	0.043	0.242	0.449	0.295	0.351	0.354	0.089	0.388	0.061
Orthophosphate (μmol)	-0.169	0.600	-0.259	0.416	0.035	0.914	0.084	0.794	0.350	0.093	0.430	0.036

described by McGregor & Fabbro (2000), who found that tropical reservoirs with the same characteristics observed in the present study are propitious to the development of large *C. racibosrskii* populations. The stability in the Mundaú reservoir was therefore evidenced by the limited variation in density of the two morphotypes throughout the different times of the day.

The behavior of the straight and coiled morphotypes exhibited seasonal differences. Vertical differences were found in the distribution of both morphotypes in both seasonal periods, with greater densities recorded at the subsurface. In the dry season, the density of the coiled morphotype was much higher than that of the straight morphotype. This certainly occurred due to the higher temperature in this season and the evident vertical variation in this variable. In the rainy season, there was an accentuated reduction in the density of the coiled morphotype, with values below those for the density of the straight morphotype. This reduction was certainly due to the homogeneity of the water column and the reduction in light intensity values in this season.

Little is known regarding the ecological advantages and disadvantages of the different morphotypes of a species. Specifically for *C. raciborskii*, environmental factors that contribute toward the formation of the two morphotypes are related to resistance to sinking (Padisák *et al.* 2003) and predation (Fabbro & Duivenvoorden 1996). The coiled form has less resistance to sinking in comparison to the straight form (Padisák *et al.* 2003), whereas Fabbro & Duivenvoorden (1996) found that the coiled form is less susceptible to predation than the straight form. According to Padisák *et al.* (2003), water density is one of the most important factors related to resistance to sinking and is influenced by thermal gradients. Such gradients may favor forms that are less resistant to sinking, such as the coiled filaments of *C. raciborskii*. The thermal stratification of the water in the Mundaú reservoir (Moura *et al.* 2007b; Dantas *et al.* 2008) certainly contributed toward the greater density of the coiled morphtype in the dry season in comparison to the rainy season, when the reservoir was de-stratified.

Among zooplankton organisms, rotifers are only dominant during and immediately following a *C. raciborskii* bloom (Griffiths & Saker 2003). In Brazil, rotifers and copepods may actually be able to cut up and shorten filaments into edible sizes for other zooplankton. Rotifer and copepod densities increased with *C. raciborskii* blooms and decreased following the bloom, whereas cladoceran density increased after blooms. Analyzing the zooplankton community in the Mandaú reservoir during the same period in which the present study was carried out, Dantas *et al.* (in press) found that Rotifera was the dominant group throughout the study, followed by Copepoda and a small contribution from Cladocera. This reveals that predation is not a determinant factor in the density of *C. raciborskii* morphotypes, as there were not enough predators for this species in the reservoir during the study period.

Conclusion

The difference in behavior of the straight and coiled *C. raciborskii* morphotypes between seasons was due to the significant environmental differences between the two periods. Water temperature, pH and phosphorus concentration were greater in the dry period, whereas electrical conductivity, turbidity and nitrogen concentrations were greater in the rainy season. These variables explain the greater densities of these morphotypes in the dry season, whereas thermal behavior explains the differences in density between morphotypes. The ecological success of the coiled morphotype was due to thermal stratification, whereas the mixture condition was determinant in the success of the straight morphotype.

Acknowledgments

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