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Original Paper

Temporal occurrence of *Ceratium furcoides* (Dinophyceae: Ceratiaceae) during an extreme drought season in Pernambuco state, Northeast Brazil

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Abstract
Ceratium furcoides is an invasive species that has caused ecological imbalance in several reservoirs in Brazil. This study investigates the main factors that may favor the occurrence of *Ceratium furcoides* blooms in a tropical reservoir from the Northeast Brazil, during an extreme drought season. Samples containing phytoplankton were collected monthly from February to September 2017. Quantitative analysis of *C. furcoides* was performed and the cell volume was estimated using geometric formulas. Mean biovolume of *C. furcoides* showed significant differences, ranging from 0.78 $mm^3 L^{-1}$ to 11.29 $mm^3 L^{-1}$ reported in March and September, respectively. Environmental parameters presented low oscillation throughout the study, except the conductivity. Significant relationships among the *C. furcoides* biovolume, water temperature and soluble reactive phosphate were observed. The findings reported here suggest that adverse conditions caused by a drought season did not negatively affect this species.
Key words: bioinvasion, dinoflagellate blooms, drought season, phytoplankton, reservoirs.

Resumo
Ceratium furcoides é uma espécie invasora que tem causado desequilíbrio ecológico em vários reservatórios no Brasil. Este estudo investiga os principais fatores que podem favorecer a ocorrência de florações de *C. furcoides* em um reservatório tropical do Nordeste do Brasil, durante um período de seca extrema. Amostras contendo fitoplâncton foram coletadas mensalmente de fevereiro a setembro de 2017. Análises quantitativas de *C. furcoides* foram realizadas e o volume celular estimado por meio de fórmulas geométricas. O biovolume médio de *C. furcoides* apresentou diferenças significativas, variando de 0,78 mm3 L-1 a 11,29 mm3 L-1 relatado em março e setembro, respectivamente. Os parâmetros ambientais apresentaram baixa oscilação ao longo do estudo, exceto a condutividade. Relações significativas entre o biovolume de *C. furcoides*, temperatura da água e fosfato reativo solúvel foram observadas. Os resultados relatados aqui sugerem que as condições adversas causadas por uma estação de seca não afetam negativamente esta espécie.
Palavras-chave: bioinvasão, florações de dinoflagelados, período de seca, fitoplâncton, reservatórios

Introduction

Invasive species represent serious problems to communities and endemic species, due to their ability of irreversibly changing ecological functioning of ecosystems (Simberloff 1996). Increase in occurrence of biological invasions, associated to climate change, is becoming a prevailing situation worldwide, thus causing serious threats to biodiversity (Vitousek *et al.* 1997; Traveset & Richardson 2014). Success

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of dominance organisms depends on many factors, including their ability to survive under unfavorable conditions and their adaptability to new environments (García-Berthou *et al.* 2005).

Dinoflagellates are a eukaryotic group of microalgae common in both marine and freshwater environments (Saldarriaga & Taylor 2017). Currently, there are about 250–300 freshwater dinoflagellates species known worldwide (Carty & Parrow 2015). *Ceratium* genus is normally found in nutrient-rich waters, especially in phosphate and nitrate, and it was not a common component of freshwater phytoplankton in South America until the 2000s (Lund 1965; Cavalcante *et al.* 2016). Recently, occurrence of this invasive dinoflagellate in South American freshwater ecosystems has caught the attention of the scientific community. *Ceratium* species have asymmetrical cells, and there are only seven species in inland waters (Popovský & Pfiester 1990). In fact, only *Ceratium furcoides* (Levander) Langhans and *Ceratium hirundinella* (O.F.Müller) Dujardin has been recorded in Brazil so far (Santos-Wisniewski *et al.* 2007; Cavalcante *et al.* 2013; Oliveira *et al*. 2019). Several authors reported the occurrence of this genus (Padisák 1985; Wu & Chou 1998; Whittington *et al.* 2000; Oliveira *et al.* 2011; Gil *et al.* 2012; Campanelli *et al.* 2017; Roriz *et al*. 2019). Even in cases of not toxic algae as, *Ceratium* species biomass may be harmful to the fishes and crustaceans due to the high oxygen consumption by bacteria decomposers during bloom decay (Smayda 1997).

Reservoirs are important aquatic bodies used to drinking water supply to regions that are affected by long drought seasons. The dynamics of the communities that inhabit these aquatic bodies, may be directly linked with drought events (Lacerda *et al.* 2018; Crossetti *et al*. 2019). The invasion and dominance by *C. furcoides* in a tropical semiarid reservoir (located at the state of Pernambuco, Northeastern Brazil) was reported by Oliveira *et al*. (2019). In addition, these authors showed the nine species occurrence, in addition to *Ceratium*, ecological indices and multivariate relationships with environmental parameters. However, it could be observed that the other species biomass present at the same reservoir was not significant when compared to *Ceratium* biomass. In this sense, the goal of the present study was to examine the same data set reported in Oliveira *et al.* (2019) using univariate analyses focusing on how extreme drought season can affect the occurrence of this invasive species.

Material and Methods

Study area

The present study was carried out at the Cachoeira II reservoir (07°58'12"S, 38°19'52"W) (Fig. 1) located in the state of Pernambuco, Northeast Brazil. This reservoir has a 21,031,000 m³ of water capacity, but due to a long period of drought before and during this study, the reservoirs were at low levels. According to the Departamento Nacional de Obras Contra as Secas (DNOCS,

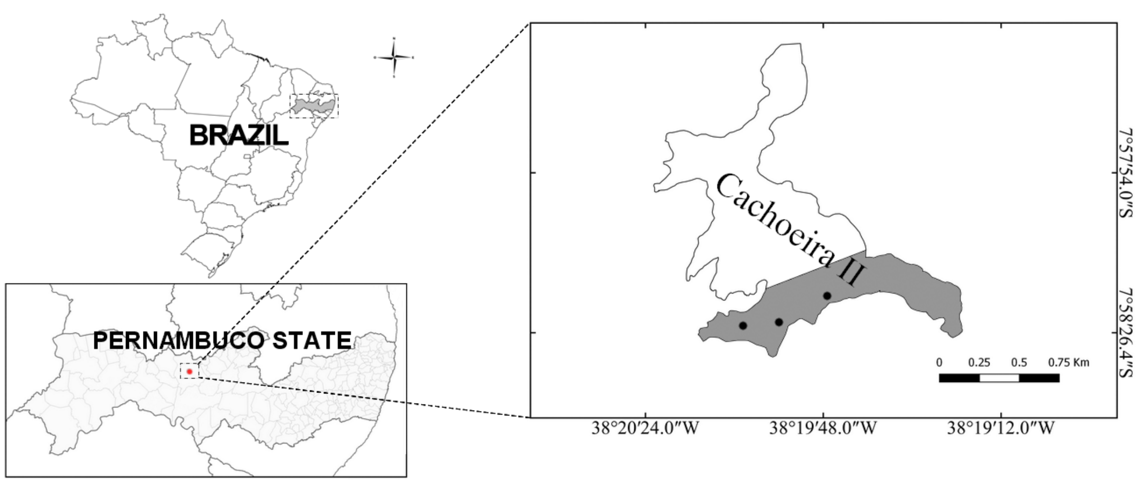

Figure 1 – Map of study area, showing the municipalities in Pernambuco state, Brazil, and the Cachoeira II reservoir located in Serra Talhada city (highlight point). The reservoir area during the study is represented by the gray color.

Brazilian Department of Constructions Against Droughts), since January 2017 this reservoir is at less than 1% of its maximum capacity.

Sampling procedures

Samples containing phytoplankton were collected, between February and September 2017, in three different points, monthly during daytime (approx. at 10 a.m.). In October 2017, this reservoir was completely dry, making it impossible to continue sampling for this study. Vertical sampling (with bottles) were carried out at an average depth of 0.5m.

Environmental variables

Water temperature, pH and conductivity parameters were measured *in situ*, at the same point of the plankton sampling, with a multiparameter probe (HI 9829 model, Hanna Instruments Lda., Portugal). Water samples were collected from the lake surface for chemical analysis. Nitrate (NO_3), nitrite (NO_2) and ammonia (NH_4) levels were evaluated by spectrometry with wavelengths ranging from 420 to 630nm, using Alpha's colorimetric test kits. Soluble reactive phosphate (SRP) analyses were performed, following the ascorbic acid method (APHA 2012). Rainfall data were obtained from Serra Talhada-A350 meteorological station (OMM: 81912 at ca. 7 km from reservoir), available from the National Institute of Meteorology website (INMET 2018).

Rainfall data showed that 2017 was an atypical year for Serra Talhada city (Fig. 2). Highest recorded rainfall was 125.0 mm on July 2017. In August and September 2017, the same volumes (2.4 mm) were recorded, the lowest values recorded in this study. These data are opposite to the historical average (last 30 years) for this region, when rainfall highest occurred between January and March.

Data analysis

Samples for quantitative phytoplankton analysis were taken and were immediately fixed with formaldehyde (4%). The species was identified according to Popovský & Pfiester (1990). Quantitative analyses were conducted on three water samples from different collection points, with an optical microscope binocular (model BA300, Motic®, China), and a Sedgwick-Rafter (ind mL^{-1}), the species were counted at 400x of magnification. After the sample was added to the chamber, it was left resting for 15 min for a complete sedimentation of the specimens.

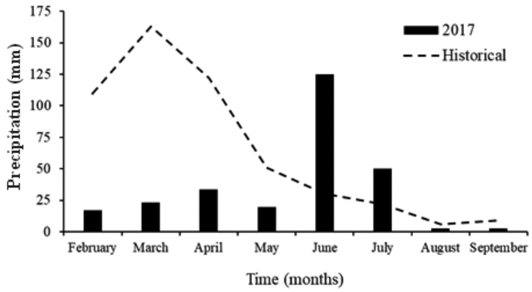

Figure 2 – Monthly and historical (last 30 years) rainfall (mm) at the Serra Talhada-A350 meteorological station (OMM: 81912) at ca. 7 km from Cachoeira II reservoir.

For cell volume (μm^3) calculation, twenty specimens of *C. furcoides*, randomly selected, were measured. The cell volume was estimated using geometric formulas adapted from Cavalcante *et al.* (2018), where the cell has the shape: ellipsoid + 2 cones + cylinder (Fig. 3) and its volume is determined by formula:

$$V = \frac{\pi}{4} \cdot a_2 \cdot b_2^2 + \frac{\pi}{12} \cdot a_3 \cdot b_3^2 + \frac{\pi}{12} a_4 \cdot b_4^2 + \frac{\pi}{6} \cdot a_1 \cdot b_1 \cdot b_2$$

Monthly biovolume of *C. furcoides* was estimated by multiplying cell density (obtained in the Sedgwick-Rafter chamber) by the mean cell volume.

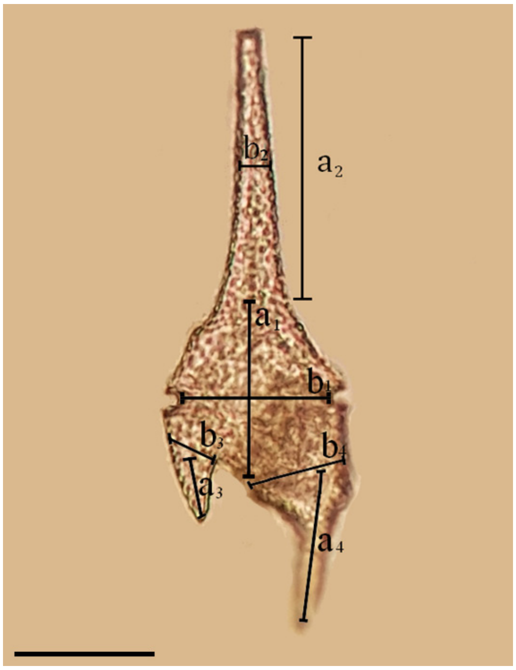

Figure 3 – Dimensions evaluated for each *Ceratium furcoides* cell. a = length, b = width. Scale bar: 40 µm

In the present study, we arbitrarily assumed bloom cells densities above 100 cells mL^{-1}, because from this threshold had a distinct ecological importance at the Cachoeira II reservoir on the same studied period (Oliveira *et al*. 2019).

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to test monthly differences of biovolume with a *post hoc* Tukey's test to compare means (Zar 2013). Linear regressions (data log transformed) was performed to identify the influence of the environmental variables on biovolume of *C. furcoides*. Statistical analyses were performed at 5% significance level on Statistics version 6.0 (Statsoft Inc., USA).

Results

Environmental variables are showed in Table 1. The highest and lowest water temperature values were measured in April and July 2017 (26.78 and 22.03 ºC, respectively). The gradual reduction in the water temperature observed between March and July 2017 is related to the increase in the reservoir volume caused by the rains. Even though there was a variation of more than 4 ºC, no significant differences were observed (p = 0.496). The pH recorded was 8.08 ± 0.38 with no significant differences throughout the study (p = 0.489). Variations in NH_4 (p = 0.417), NO_2 (p = 0.58), NO_3 (p = 0. 154), and SRP (p = 0.327) did not significant differences during the study months. Only conductivity data presented statistical differences ($p < 0.001$) in this study.

Ceratium furcoides was recorded in all months of this study; in February at an average density of 150 ± 32 cells mL^{-1} fallowed by an 80% bloom-decay in March (Fig. 4). The highest densities were reported in September (435 ± 31 cells mL^{-1}), August (400 ± 28 cells mL^{-1}) and June (370 ± 32 cells mL^{-1}). As the biovolumes for the 20 cells counted each month did not present significant differences (p = 0.674) among the months of the study, the monthly biovolume showed similarity with cell density. As with cell density, higher biovolumes was reported in September (11.29 mm^3 L^{-1}) and August (10.38 ± 0.70 mm^3 L^{-1}), followed by 9.60 ± 1.09 mm^3 L^{-1} reported in June.

Direct and inverse relations among abiotic and biovolume suggested some preferences during this drought season. The determination coefficient in linear regression between *C. furcoides* and water temperature (R^2 = -0.738; p = 0.008) was the most significant in this study. In addition, correlations with SRP (R^2 = 0.707; p = 0.009) was also significant. However, while the correlation with SRP was positive, with water temperature it was negative (Fig. 5).

Discussion

Several factors are associated with the dispersion of phytoplankton species and may positively contribute to invasion success. These are: independent wind mediated dispersion, which enables spore transportation and high contamination of water bodies through anthropogenic actions. In general, any de-structuring of the physical and

Table 1 – Range, mean and standard deviation (*n = 24*) of environmental variables of Cachoeira II reservoirs, during *Ceratium furcoides* occurrence in 2017.

	Max	Min	Mean	SD
Temperature (ºC)	28.33	22.03	25.17	2.69
pH	8.61	7.57	8.08	0.38
Conductivity (µS cm^{-1})	601	299	374.12	116.94
NH_4 (mg L^{-1})	0.57	0.2	0.31	0.11
NO_3 (mg L^{-1})	0.41	0.02	0.18	0.13
NO_2 (mg L^{-1})	0.05	0.01	0.02	0.01
SRP (mg L^{-1})	1.24	0.40	0.78	0.27
TDS (ppm)	301	150	187.75	58.21

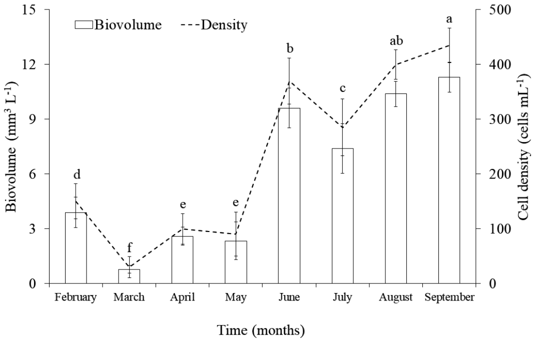

Figure 4 – Biovolume (mm^3 L^{-1}) and cell density (cells mL^{-1}) of *Ceratium furcoides* during February-September 2017 in Cachoeira II reservoir, Brazil. Data presented in mean ± standard deviation. Equivalent letters indicate statistical equality ($p < 0.05$) using one-way ANOVA (Kruskal-Wallis test) followed by the Tukey's test.

chemical environment of inland water bodies can lead to changes in the natural dynamics of their biological communities (Nogueira *et al.* 2010). Here, rainfall data may explain the variations of the environmental variables.

In our previous study, *Ceratium furcoides* even at a low density (about 100 cells mL^{-1}) was caused an apparent ecological imbalance in the Cachoeira II reservoir. In February at an average density of 150 ± 32 cells mL^{-1} was able to result Shannon and Simpson indices equal to zero. In March, when the *C. furcoides* population was reduced on 80%, these same indices increased until June (one of the highest reported densities, 370 ± 32 cells mL^{-1}). In the months following the bloom reported in June, diversity and richness indices fell gradually to zero (in August) once again, expressing a new dominance (Oliveira *et al*. 2019).

Ceratium furcoides had its first report in 2007 in Minas Gerais, Brazil (Santos-Wisniewski *et al.* 2007), and was later recorded for the first time in a Brazilian semiarid region, in 2011 (Oliveira *et al.* 2011). Despite the scarce records, the species is in continuous expansion in Brazil, to the north and south of this country (Oliveira & Oliveira 2018). According to Meichtry de Zaburlín *et al.* (2016) and Cassol *et al.* (2014), the *Ceratium* invasion and appropriation in new areas can be linked to climate change and reservoir constructions, since it eventually develops better in lentic environments.

Ceratium furcoides was commonly found in cold waters; Cavalcante *et al.* (2016) found that temperatures of 15 to 27 ºC are considered optimal for the development of *C. furcoides* in reservoirs in Southern Brazil. Also, in Brazil at

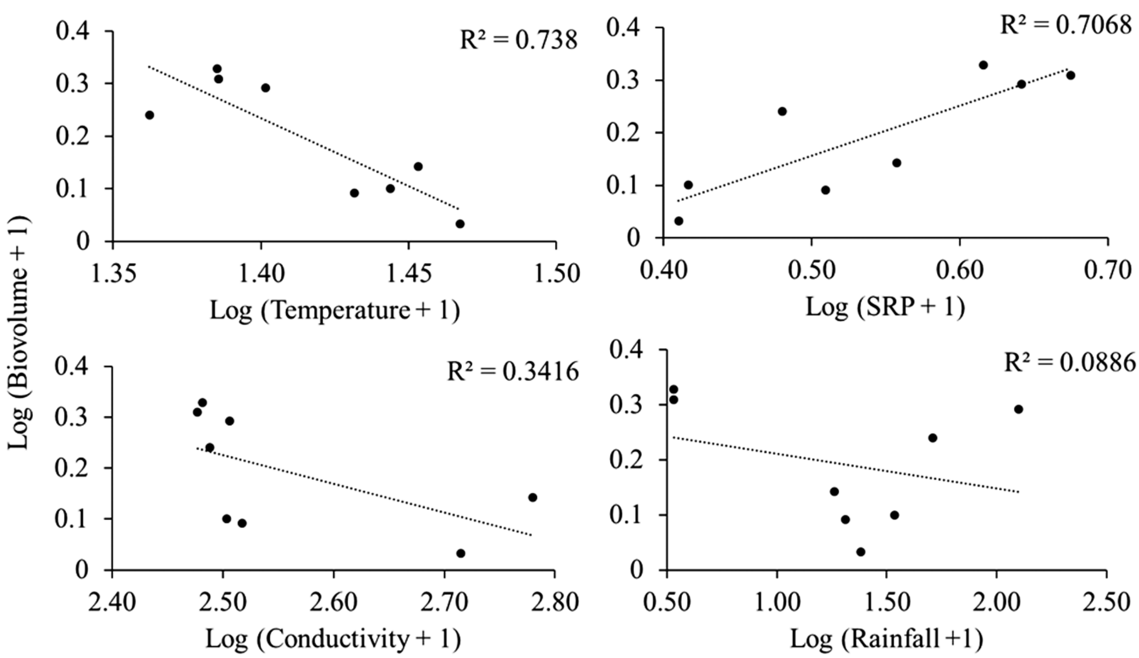

Figure 5 – Linear regression between *Ceratium furcoides* biovolume and the environmental variables. Data normalized by Log + 1. Temp = temperature; Cond = conductivity; Rain = rainfall; Biov = biovolume.

relatively low temperatures (19 ± 3 ºC) the *C. furcoides* occurrences at the Garças reservoir, São Paulo state was reported (Crossetti *et al*. 2019). However, some reports showed its adaptation to tropical environments: *e.g.* on the São Francisco River, occurrences *C. furcoides* occurrences were reported at an average temperature of 29.9 °C (Silva *et al*. 2018). Here, with higher temperatures (from 21 to 29 °C), the establishment this specie at the Cachoeira II reservoir was verified. Although the temperature was not limiting factor, in the linear regression, the preference for lower temperatures was proven, corroborating with previous studies (Silva *et al*. 2012; Jati *et al*. 2013). High *Ceratium* biovolumes were recorded as ordinary events in annual phytoplankton fluctuations of many temperate waterbodies. In subtropical environments, *Ceratium* spp. were reported at higher densities, as *C. hirundinella* in Argentina (Silveiro *et al*. 2009) with 5,634 cells mL^{-1} and *C. furcoides* in Southern Brazil with 2,819 cells mL^{-1} (Cavalcante *et al*. 2016). The differences in temperature could justify the low density in the present study, when compared to those previously mentioned. However, up to the temperature range reported in this study, it cannot consider temperature as a limiting environmental variable.

The affinity of *Ceratium* for high phosphate concentrations in the Faxinal reservoir was also showed by Cavalcante *et al*. (2016). According to these authors, *Ceratium* blooms occurred when the concentrations were higher than 0.05 mg L^{-1}. In fact, the phosphate concentrations presented by them were significantly lower than those reported at the Cachoeira II reservoir. The results of the present study, besides corroborating with the direct relationship between *Ceratium* and SRP, show that this species was able to withstand high concentrations (higher than 0.80 mg L^{-1}). The phosphorus is an important macronutrient for phytoplankton growth and its assimilation is to produce phospholipids, ATP and nucleic acids (Ji *et al*. 2013). However, high concentrations can cause cell damage and even inhibit growth (Li *et al*. 2018); here, *Ceratium* cells proved to be resistant to this nutrient. Other studies suggest positive correlations between *Ceratium* and nitrogen compounds (NH_4, NO_2 and/or NO_3; Gil *et al.* 2012; Nishimura *et al*. 2015), however, this was not a behavior found in the present study.

High *Ceratium* biovolumes are recorded as ordinary events in annual phytoplankton fluctuations of many temperate waterbodies. In subtropical environments, *Ceratium* spp. were reported at higher densities, as *C. furcoides* in São Francisco River with 5,600 cells mL^{-1} (Silva *et al.* 2018) and *C. furcoides* in southern Brazil with 2,819 cells mL^{-1} (Cavalcante *et al.* 2016). The differences in temperature could justify the low density in the present study, when compared to those previously mentioned. However, we cannot consider temperature as a limiting environmental variable.

The results of the present study showed that *C. furcoides* was adapted to the environmental conditions in the Cachoeira II reservoir. In fact, corroborating Cavalcante *et al.* (2016), the highest biovolumes of *C. furcoides* were recorded in spring-summer. Biovolume recorded in the present study, although relatively lower than the densities recorded in Southern Brazil, can pose a great risk to local biodiversity.

Our results showed that the adverse environmental conditions, caused by extreme drought, did not affect the *Ceratium* population in Cachoeira II reservoir. Individuals of *Ceratium furcoides* were recorded during all months of the study - February to September 2017. Higher biovolume were positively related to SRP and temperature, showed by the direct and inverse relationship in the linear regression. An apparent adaptation of *C. furcoides* to Cachoeira II reservoir was evident, corroborating with reports in the worldwide. Further studies may be performed to evaluate strategies to reduce the population of this invasive dinoflagellate.

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