Drivers and ecology of Ceratium furcoides invasion of a Brazilian subtropical reservoir and its interaction with the phytoplankton community

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Drivers and ecology of *Ceratium furcoides* invasion of a Brazilian subtropical reservoir and its interaction with the phytoplankton community

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Abstract

We analyzed the phytoplankton interactions during a *Ceratium furcoides* invasion along with the environmental variables contributing to its dominance and blooms in Marrecas, a south-Brazilian reservoir. We monitored Marrecas weekly/monthly (n = 116) from 2014 to 2018. *C. furcoides* avoided summer and preferred spring, which displayed optimal temperatures for the species (15–23 °C), exhibiting in October 2014 the maximum density ever reported (15,865 ind. mL⁻¹). It showed direct correlation with iron, manganese, and turbidity; evidencing dependency of a mixing regime which triggers cyst resuspension (interrupting cysts’ dormancy). *Cryptomonas* spp., *Komma caudata*, *Discostella stelligera* and after 2016 also *Chlamydomonas* sp. were the only species present during *C. furcoides* blooms. *Komma caudata*, *Cryptomonas* sp., and *Aulacoseira alpigena* correlated positively with the invader, as all prefer turbulent waters. *C. furcoides* correlated negatively with Chlorophyta (mainly *Chlamydomonas* spp.) which is vulnerable to mixing, and Bacillariophyta (*Urosolenia eriensis* and *Discostella stelligera*) that prefers clear waters. It also showed a consistent negative correlation with Ochrophyta (*Ochromonas* sp.), Cyanobacteria (*Microcystis*), and *Urosolenia eriensis* suggesting competition for resources. Furthermore, it correlated negatively with species richness. Notwithstanding, based on the decrease of blooms, the phytoplankton community appears to have reached a more stable state (homogeneous species representation). However, further monitoring is required for confirmation.

1. Introduction

Biological invasions are defined as the entry, establishment, spread, and rapid dominance of non-native species in an ecological system (Kernan, 2015). These have commonly been associated with alterations in the structure and functioning of ecosystems, such as changes in the species composition, community dominance, primary productivity, and species diversity which often result in further ecological and economic impacts (Epanchin-Niell and Wilen, 2012; Kernan, 2015). Therefore, managing invasive species is considered necessary for successfully conserving natural and semi-natural landscapes.

Freshwater systems are particularly susceptible to biological invasions, especially by microorganisms, as their establishment is typically unnoticed and favored by water flow (Padišák et al., 2016). Various microalgal species are expanding very fast (Blanco and Ector, 2009; Sukenik et al., 2012), and human-altered systems, e.g., water reservoirs and dams, promote the spreading of these invasive microalgae (Johnson et al., 2008; Kirkwood et al., 2009). Therefore, studying these environments is vital for understanding microalgae invasion.

Around the world, members of *Ceratium* Schrank have invaded continental aquatic systems (Meichtry de Zaburlin et al., 2016) and, in many cases, have produced phytoplanktonic blooms (Matsumura-Tundisi et al., 2010; Van Ginkel et al., 2001). Although Ceratium lacks toxicity, its blooms have been associated with oxygen depletion due to bacterial consumption of collapsed cells harming aerobic aquatic biota (Hart and Wragg, 2009; Matsumura-Tundisi et al., 2010). Furthermore, Ceratium blooms can produce filter clogging and can cause an unpleasant taste and a fetid odor of the hydric resource, affecting the supply of drinking water and water treatment (Matsumura-Tundisi et al., 2010; Van Ginkel et al., 2007).

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2. Materials and methods

2.1. Study area

We conducted the study in the Marrecas reservoir (29°2’S, 50°58’W) within the Taquari-Antas Hydrographic basin (Schneider et al., 2014), in Caxias do Sul city, Rio Grande do Sul, Brazil (Fig. 1). This area is one of the main sub-basins of the Patos Lagoon watershed. It encompasses high fish diversity and is recognized as a freshwater ecoregion draining into the Atlantic Ocean (Abell et al., 2008; Becker et al., 2013).

The Marrecas reservoir was filled in November 2013 to supply 250,000 people with drinking water in Caxias do Sul city (SAMEA, 2015). It has a basin area of 53 km², a flooded area of 2 km², a mean depth of 15 m, a maximum depth of 52 m, and a length of 833.5 m. It has a storage capacity of over 33 Bln L, and an initial flow of 900 L/s of running water (Pionero, 2012; SAMEA, 2015). This mesotrophic reservoir is located in a subtropical biome, characterized by a temperate regional climate that lacks a dry season (Köppen, 1936).

In 2012–2013, C. furcoides, was first registered in Maestra and Faxonial reservoirs located in Caxias do Sul, Southern Brazil (Cavalcante et al., 2016). Consequently, the Autonomous Municipal Service of Water and Sewage (SAMEA) initiated a monitoring program in the Caxias do Sul new reservoir (Marrecas), addressing the phytoplankton community and water quality (physical and chemical variables) of the reservoir. From January 2014 to December 2018, technicians from SAMEA performed the sampling on a weekly to monthly basis (depending on the presence of algae blooms) (types of blooms listed in supplementary material) at one monitoring station at the point of water intake of the reservoir. For water quality, the SAMEA technicians collected surface water samples in 500 mL plastic bottles and conducted the analyses at the SAMEA quality control laboratory. The phytoplankton community was analyzed from surface water samples collected in 250 mL glass bottles and preserved with Lugol’s iodine. Phytoplankton quantification followed Utermohl’s method (Utermohl, 1958) with an aliquot of 10 mL in settling chambers analyzed in an inverted microscope (results expressed by individuals mL⁻¹), followed by identification to species level (Guiry and Guiry, 2022). Water temperature (°C), pH, dissolved oxygen (mg L⁻¹), and conductivity (µS cm⁻¹) were measured in the field using a model U-10 Horiba probe, while turbidity employing a turbidimeter HACH 2100 P, CO₂ (mg L⁻¹) was estimated by the titration method with sodium carbonate and total organic matter by the titration method with potassium permanganate. Ammonium (NH₄, mg L⁻¹), total phosphate (PO₄, mg L⁻¹), nitrate (NO₃, mg L⁻¹), total iron (Fe, mg L⁻¹), and total manganese (Mn, mg L⁻¹) were assessed through concentration analyses employing the photometer NOVA 60 Merck with the respective colorimetric test kits (Spectroquant Kits) in the SAMEA quality control laboratory. The measuring range of the kits were: 0.0025–5 mg L⁻¹ for iron, 0.010–10 mg L⁻¹ for manganese, 0.0025–5 mg L⁻¹ for phosphorus, 0.010–3 mg L⁻¹ for ammonium, 0.002–1 mg L⁻¹ for nitrate, and 0.2–20 mg L⁻¹ for nitrite; all refer to limit of quantitation (LOQ). Additionally, since it is known that several meteorological variables affect phytoplankton dynamics (Janatian et al., 2021), we acquired daily meteorological data (daily precipitation, weekly precipitation, air temperature, and wind velocity) for the study period from Caxias do Sul Meteorological Station (INMET- Instituto Nacional de Meteorologia, 2019).

We arranged the phytoplankton dataset by species, including the ones with a > 10% representation (individuals or colonies) in at least one sample (≥10% ind. mL⁻¹). A second dataset was arranged similarly, including the species with > 10% of cell representation (≥10% cells. mL⁻¹). The datasets (meteorological, physical, chemical, and biological variables) were explored using descriptive analyses in RStudio 4.0.2 (RStudio Team, 2016). We employed ANOVA and Kruskal-Wallis tests (if data were not normal) to find temporal patterns (seasonal, inter-annual, and intra-annual), followed by posthoc tests (Tukey test...
or Dunn test, respectively). To address the phytoplankton community, we performed ANOVA/Kruskal-Wallis test on taxon richness, species diversity (Shannon index), and species dominance (Simpson index), all calculated in R (vegan package) (Oksanen et al., 2018).

We performed a non-metric multidimensional scaling (NMDS) (employing the Bray-Curtis dissimilarity index) in R (vegan package) (Oksanen et al., 2018) for analyzing phytoplankton community dynamics and ecological interaction. A series of permutation analyses between the environmental variables and the NMDS results were performed to visualize the effect of the environment on the community (R code in supplementary material). Prior to the NMDS analysis, the data were transformed by log (x + 1). Subsequently, two-way cluster analyses (Sorenson as distance measure) in PC-Ord 6 (McCune and Mefford, 2008) were run on the phytoplankton densities to identify temporal patterns of the species and co-occurrences. All these analyses were supported by correlation analysis based on the Spearman index.

The map of the continent and regions was obtained from GADM (GADM, n.d.), and the Marrecas reservoir map was modified from SAMAE (2015).

**Fig. 1.** Map showing the location of Marrecas reservoir (blue) within Caxias do Sul region (dark green) in Rio Grande do Sul (mid green), Brazil (light green). The map of the continent and regions was obtained from GADM (GADM, n.d.), and the Marrecas reservoir map was modified from SAMAE (2015).

Table 1
Seasonal description of abiotic variables from Marrecas reservoir.

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th></th>
<th>Fall</th>
<th></th>
<th>Winter</th>
<th></th>
<th>Spring</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Max</td>
<td>Min</td>
<td>SD</td>
<td>Mean</td>
<td>Max</td>
<td>Min</td>
<td>SD</td>
</tr>
<tr>
<td>Temp (ºC)</td>
<td>22.34</td>
<td>26.6</td>
<td>19</td>
<td>2.02</td>
<td>15.14</td>
<td>23.2</td>
<td>6.76</td>
<td>4.63</td>
</tr>
<tr>
<td>Temp Max (ºC)</td>
<td>28.28</td>
<td>33.6</td>
<td>25</td>
<td>2.42</td>
<td>20.25</td>
<td>29.6</td>
<td>11.2</td>
<td>4.62</td>
</tr>
<tr>
<td>Temp Min (ºC)</td>
<td>18.06</td>
<td>22.8</td>
<td>14</td>
<td>2.3</td>
<td>12.04</td>
<td>18.2</td>
<td>3.6</td>
<td>4.82</td>
</tr>
<tr>
<td>Rain (mm)</td>
<td>2.6</td>
<td>39.8</td>
<td>&lt; D.L.</td>
<td>8.17</td>
<td>50.6</td>
<td>69.8</td>
<td>&lt; D.L.</td>
<td>14.4</td>
</tr>
<tr>
<td>Rain (mm) (7 days)</td>
<td>47.16</td>
<td>126</td>
<td>6.3</td>
<td>33.2</td>
<td>37.86</td>
<td>131</td>
<td>0.4</td>
<td>36.9</td>
</tr>
<tr>
<td>Wind (m s⁻¹) (2 days)</td>
<td>1.83</td>
<td>2.67</td>
<td>1</td>
<td>0.53</td>
<td>1.56</td>
<td>3.33</td>
<td>0.5</td>
<td>0.61</td>
</tr>
<tr>
<td>WT (ºC)</td>
<td>28.41</td>
<td>26.7</td>
<td>23</td>
<td>0.95</td>
<td>19.25</td>
<td>23.5</td>
<td>14.4</td>
<td>2.91</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>2.77</td>
<td>4.26</td>
<td>1.4</td>
<td>0.72</td>
<td>4.5</td>
<td>18</td>
<td>1.66</td>
<td>3.41</td>
</tr>
<tr>
<td>pH</td>
<td>6.98</td>
<td>7.85</td>
<td>6.2</td>
<td>0.44</td>
<td>6.65</td>
<td>7.9</td>
<td>5.76</td>
<td>0.51</td>
</tr>
<tr>
<td>DO (mg L⁻¹)</td>
<td>6.46</td>
<td>10.4</td>
<td>4.2</td>
<td>1.16</td>
<td>6.27</td>
<td>9</td>
<td>2.2</td>
<td>1.72</td>
</tr>
<tr>
<td>Org (mg L⁻¹)</td>
<td>5.39</td>
<td>8.86</td>
<td>3.8</td>
<td>1.38</td>
<td>4.64</td>
<td>6.6</td>
<td>3.5</td>
<td>0.71</td>
</tr>
<tr>
<td>NH₄ (mg L⁻¹)</td>
<td>0.07</td>
<td>0.25</td>
<td>&lt; D.L.</td>
<td>0.05</td>
<td>0.1</td>
<td>0.21</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>NO₂ (mg L⁻¹)</td>
<td>0.23</td>
<td>0.73</td>
<td>0.1</td>
<td>0.13</td>
<td>0.22</td>
<td>0.63</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>NO₃ (mg L⁻¹)</td>
<td>0.01</td>
<td>0.02</td>
<td>&lt; D.L.</td>
<td>0.01</td>
<td>0.01</td>
<td>0.06</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>PO₄ (mg L⁻¹)</td>
<td>0.3</td>
<td>0.84</td>
<td>0.1</td>
<td>0.19</td>
<td>0.5</td>
<td>1.57</td>
<td>0.17</td>
<td>0.37</td>
</tr>
<tr>
<td>Mn (mg L⁻¹)</td>
<td>0.08</td>
<td>0.19</td>
<td>&lt; D.L.</td>
<td>0.04</td>
<td>0.14</td>
<td>0.45</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>CO₂ (mg L⁻¹)</td>
<td>3.86</td>
<td>7</td>
<td>2</td>
<td>1.37</td>
<td>6.13</td>
<td>16</td>
<td>2</td>
<td>3.22</td>
</tr>
<tr>
<td>Cond. (µS cm⁻¹)</td>
<td>31.21</td>
<td>56.8</td>
<td>26</td>
<td>5.92</td>
<td>36.29</td>
<td>61.6</td>
<td>24.5</td>
<td>8.96</td>
</tr>
</tbody>
</table>

Temp: mean air temperature; Temp Max: maximum air temperature; Temp Min: minimum air temperature; Rain: accumulated precipitation during the day; Rain (7 days): accumulated weekly precipitation; Wind (2 days): average wind velocity from one-day prior sampling and the day of sampling; WT: water temperature; DO: dissolved oxygen; Org: organic matter; NH₄: Ammonium; NO₂: Nitrate; PO₄: Phosphorus; Fe: Iron; Mn: Manganese; CO₂: Carbon dioxide; Cond: conductivity. Detection limits (D.L.) for each parameter are given in material and methods.

3. Results

3.1. Meteorological variables

The annual mean temperature during the study period (2014–2018) was 17.5 ºC, and the total annual precipitation ranged between 1972 mm (2017) and 2257 mm (2015), with a mean annual precipitation of 2065 mm (2014–2018) (INMET, 2019). Only temperature showed significant differences between seasons (p < 0.001). No other meteorological variable differed significantly inter-annually, showing regular climate conditions during these five years. Temperatures were characteristic of a subtropical region in the Southern Hemisphere with higher values in spring (Sep-Dec) and summer (Dec-Mar) and lower values in fall (Mar-Jun) and winter (Jun-Sep) (fall and winter with higher peaks in wind velocity). Winter was the rainiest season, followed by fall (Table 1). All seasons differed significantly in temperature (p < 0.001), except comparing fall and winter. The hottest annual temperatures and the highest peaks in weekly precipitation occurred in 2014 and 2015, whereas the coldest temperatures and the highest peaks in wind velocity were observed in 2016 and 2017 (Table 2).
### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Temp (C) Mean</th>
<th>Temp Max (C) Mean</th>
<th>Temp Min (C) Mean</th>
<th>Wind (2 days) Mean</th>
<th>Turbidity (NTU) Mean</th>
<th>DO (mg L⁻¹) Mean</th>
<th>Cond. (µS cm⁻¹) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>18.3</td>
<td>26.6</td>
<td>8.3</td>
<td>4.6</td>
<td>6.19</td>
<td>0.07</td>
<td>36.74</td>
</tr>
<tr>
<td>2015</td>
<td>21.7</td>
<td>31.0</td>
<td>13.6</td>
<td>4.4</td>
<td>5.2</td>
<td>0.03</td>
<td>31.0</td>
</tr>
<tr>
<td>2016</td>
<td>23.1</td>
<td>32.3</td>
<td>16.3</td>
<td>4.6</td>
<td>2.2</td>
<td>0.01</td>
<td>36.4</td>
</tr>
<tr>
<td>2017</td>
<td>23.8</td>
<td>33.6</td>
<td>16.3</td>
<td>4.9</td>
<td>2.0</td>
<td>0.01</td>
<td>32.1</td>
</tr>
<tr>
<td>2018</td>
<td>23.0</td>
<td>34.0</td>
<td>15.7</td>
<td>4.7</td>
<td>1.9</td>
<td>0.01</td>
<td>31.0</td>
</tr>
</tbody>
</table>

#### 3.2. Physical and chemical variables

Seasonally, all physical and chemical variables differed significantly (p < 0.05) except nitrate. Inter-annually, only ammonium (p < 0.0001), iron (p < 0.01), and conductivity (p < 0.0001) differed.

Water was warmer during summer and spring and differed significantly (p < 0.001) between seasons, except comparing fall and spring. Only manganese and carbon dioxide differed significantly, with lower values in warm seasons (p < 0.0001 in summer and p < 0.001 in spring, respectively). Seasonally, organic matter and pH showed significantly higher values in spring (p < 0.001); turbidity, dissolved oxygen, and phosphate were significantly higher in winter and spring (p < 0.001), whereas ammonium, iron, and conductivity showed significantly higher values in winter and fall (p < 0.001) (Table 1). Inter-annually, ammonium was lower in 2015 (p < 0.001), iron in 2017 and 2018 (p < 0.01), and conductivity in 2017 (p < 0.001) (Table 2).

#### 3.3. Phytoplankton community response to Ceratium furcoides’ invasion in Marrecas

We found 108 species belonging to eight different phyla (Bacillariophtya, Chlorophyta, Cryptista, Ochrophyta, Cyanobacteria, Miozoa, Charophyta, Euglenozoa). Of these, only 28 species from six different phyla (Fig. 2) showed a representation ≥ 10% of the total density in at least one sample (ind. mL⁻¹). Throughout the study, the most representative taxa were Miozoa (27%): *Ceratium furcoides* (23%); Cryptista (23%): *Komma caudata* (D.L. Geitler) D.R. Hill (12%) and *Cryptomonas* spp. (10%); Bacillariophtya (20%): *Discostella stelligera* (Cleve & Grunow) Houk & Klee (9%) and *Aulacoseira alpigena* (Grunow) Krammer (4%); Chlorophyta (18%): *Monoraphidium contortum* (Thuret) Komárková-Legnerová (6%). The remaining species showed 29% of the total phytoplankton density, including Ochrophyta (*Gonyostomum* spp. and *Mallomonas* spp.), and Cyanobacteria (*Synechococcus* sp.). Microcystis sp. was the most abundant species when analyzing cell count (cells. mL⁻¹) but was not included in the individuals/colonies analysis (< 10% ind. mL⁻¹).

*C. furcoides* density showed significant differences (p < 0.001) between all pairs of seasons (except between fall and winter) and between some years (e.g., 2014 and 2015 vs. 2016; 2014 vs. 2017, and 2014 and 2015 vs. 2018). *C. furcoides* showed a significant seasonal preference towards spring (p < 0.001) in terms of density, dominance, and frequency of blooms, followed by winter (Fig. 2). Average and maximum density values were highest in 2014 and decreased over the years (Fig. 2). However, its abundance in the community remained higher than 50% in several sampling periods (Fig. 2). *C. furcoides* abundance did not differ by season in 2014 or 2018; but differed between spring and summer in 2015, 2016, and 2017 (p < 0.05); between summer and winter in 2015 (p = 0.03); and between spring and winter in 2016 (p > 0.001).

Over the years, taxon richness, diversity (Shannon index), and dominance (Simpson index) were greater in 2018 than in 2014 (p < 0.05). The season with significantly lower species richness, dominance, and diversity (p < 0.05) was spring, while summer showed the highest, followed by fall and winter (Table 3). This seasonal pattern was observed yearly, except in 2017, when winter was the lowest, followed by spring. Cyanobacteria emerged in 2016. It avoided summer and increased its abundance during fall and winter (and spring only in 2018), always avoiding the periods with *C. furcoides* dominance. Chlorophyta showed all its peaks in summer, but in 2015 showed its peaks also in late spring. Bacillariophyta showed its peaks in summer and fall (the highest in 2015), avoiding *C. furcoides* peaks. Cryptista appeared to be more dominant during winter and spring and occasionally in fall, but during 2015 and 2016 it also showed high dominance in summer. Ochrophyta preferred summer and fall, avoided spring, and seemed to avoid *C. furcoides* peaks (Fig. 2). *C. furcoides* co-occurred principally with *Cryptomonas* spp. (all years), *Komma caudata* (all years), and *Discostella stelligera* (2015 vs. 2018).
number of colonies above 10% ind. mL$^{-1}$ bacteria was under-represented, with only **Korshikoviella monas** way dendrogram, Fig. 3), it still co-occurred in spring with **Aulacoseira alpigena**, **Discostella stelligera**-(all years), **Monoraphidium tortile** was clustered independently in 2016 (in the two-year dendrogram, Fig. 3). Some of the phytoplankton species showed relatively high densities, principally during the periods when **C. furcoides** showed low densities, including **Korshikoviella** sp. (2014), **Urosoienia eriensis** (H.L.Smith) Round & R.M. Crawford (2014), **Eutetramorus sp.** (2015), **Monoraphidium tortile** (West & G.S.West) Komárková-Legnerová (2015), **Aulacoseira alpigena** (2016), **Ankistrodesmus arcuatus** Korshikov (2016, 2017), and **Monoraphidium minutum** (Nägeli) Komárková-Legnerová (2018) (Fig. 3). Since we focused on individuals/colonies in our study, the abundance of Cyanobacteria was under-represented, with only **Synechococcus** showing a number of colonies above 10% ind. mL$^{-1}$. When integrating cell numbers (instead of number of colonies) in the analysis, a few other species showed >10% representation. Of these, the **Microcystis** genus (**M. aeruginosa** (Kützing) Kützing, **M. protocystis** Crow, **M. sp.**) was the most representative. **Microcystis** showed occasional peaks (fall 2014 and summer 2017) in periods with low **C. furcoides** densities.

Annually, **C. furcoides** correlated negatively with most groups, species richness, and many species; and correlated positively with **Cryptista and Komma caudata** during the last two years (Table 4). Seasonally, **C. furcoides** correlated positively with **Aulacoseira alpigena**, **Nitzschia sp.**, and **Cryptomonas sp.**. The cell densities analysis (10% cells. mL$^{-1}$) showed that **Microcystis sp.** correlated negatively (rs = −0.43, p = 0.03) with **C. furcoides** in 2018. Furthermore, the number of cells of the cyanobacteria skyrocketed (> 2000 until 97,461 cells. mL$^{-1}$) soon after, probably representing an "ecological release" effect. These two species correlated negatively also in fall (rs = −0.45, p = 0.02) and spring (rs = −0.30, p = 0.08), while in winter, the invader correlated negatively with **Pseudanabaena mucicola** (rs = −0.33, p = 0.06). 3.4. Phytoplankton and environmental variables

The two-dimensional NMDS solution was acceptable (stress 0.17). The ordination showed the species (20 out of 28) and environmental variables (9 out of 21) that correlated significantly to the NMDS: first and second axis (p < 0.01 and p < 0.05) (more info in supplementary material). Only 9 out of 21 environmental variables were plotted: year ($r^2 = 0.67$), turbidity ($r^2 = 0.22$), manganese ($r^2 = 0.21$), iron ($r^2 = 0.19$), organic matter ($r^2 = 0.13$), NH4 ($r^2 = 0.10$), pH ($r^2 = 0.09$), CO2 ($r^2 = 0.071$), and minimum temperature ($r^2 = 0.07$). The annual variability was important in the contribution of the species to the community. This was especially the case in species such as **Chroomonas sp.**, **Discostella sp.**, **Aulacoseira spp.**, **Komma caudata**, **Nitzschia sp.**, **Aulacoseira alpigena**, and **Eutetramorus sp.**, all of which increased in abundance over the years studied. The NMDS displayed **C. furcoides** separated from the rest of the phytoplankton community and showed a general positive

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**Table 3**

Species richness, diversity, and dominance index in temporal scale.

<table>
<thead>
<tr>
<th>Year</th>
<th># spp mean</th>
<th>sd</th>
<th>Shannon Index mean</th>
<th>sd</th>
<th>Simpson Index mean</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>6.53</td>
<td>1.68</td>
<td>1.08</td>
<td>0.49</td>
<td>0.50</td>
<td>0.22</td>
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<tr>
<td>2015</td>
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<td>2.10</td>
<td>1.17</td>
<td>0.40</td>
<td>0.56</td>
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</tr>
<tr>
<td>2016</td>
<td>7.04</td>
<td>2.26</td>
<td>1.14</td>
<td>0.44</td>
<td>0.54</td>
<td>0.21</td>
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<tr>
<td>2017</td>
<td>7.23</td>
<td>1.24</td>
<td>1.32</td>
<td>0.31</td>
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<tr>
<td>2018</td>
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<td>1.74</td>
<td>1.46</td>
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<td>Spring</td>
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<td>0.52</td>
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</tr>
<tr>
<td>Summer</td>
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<td>1.26</td>
<td>0.43</td>
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<tr>
<td>Winter</td>
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<td>1.73</td>
<td>1.44</td>
<td>0.39</td>
<td>0.67</td>
<td>0.14</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Dynamics of the phytoplankton community: densities in ind. mL$^{-1}$ (a) and percentage (b). Legend: S: summer, F: fall, W: winter, & SP: spring.
Fig. 3. Two-way dendrogram based on the phytoplankton density in 2014, 2015, 2016, 2017, and 2018. Darker-colored squares represent higher relative abundances per species per sampling occasion.
relationship between the invader and turbidity, organic matter, iron, manganese, and pH, all of which appeared to be associated with spring and winter. The invader showed the opposite relationship with *Mallo*monas spp., *Urosolenia eriensis*, *Monoraphidium contortum*, *Sphaerocystis schroeteri* Chodat, among others, which were more associated with temperature and CO$_2$ (Fig. 4).

Regarding the environmental variables (meteorological, physical, and chemical), *C. furcoides* density generally correlated positively, especially with organic matter (both seasonally and inter-annually) and turbidity. In 2017, both the highest correlations and correlations with the most variables were observed. Only temperatures, NH$_4$, CO$_2$, and conductivity correlated negatively with the invader (Table 5).

### 4. Discussion

#### 4.1. Ceratium furcoides in Southern Brazil and its invasion of Marrecas reservoir

Subtropical systems in South America have shown favorable conditions for the invasion by *C. furcoides*, especially in Southern Brazil (Cavalcante et al., 2016). In this context, *C. furcoides* has displayed intensive population growth, quickly reaching high abundance after colonizing several reservoirs, e.g., Faxinal, Maestra, and based on our results, Marrecas. This prevalence provides further evidence of the invasive nature of this dinoflagellate (Cavalcante et al., 2016, 2013).
C. furcoides density during our study (mean: 744.2 ind. mL$^{-1}$, max. peak: 15,865 ind. mL$^{-1}$, and 35 occurrences over 500 ind. mL$^{-1}$) was considerably high when compared with other reservoirs in South America, such as the Yacyreta reservoir in Argentina (max. peak: 15 ind. mL$^{-1}$) (Meichtry de Zaburlin et al., 2014), Furnas reservoir in Brazil (max. peak: 29 ind. mL$^{-1}$) (Silva et al., 2012), Riogrande II reservoir in Colombia (max. peak: 41 ind. mL$^{-1}$) (Bustamante-Gil et al., 2012), Maestra and Faxinal (max. peak: 2680 and 2819 ind. mL$^{-1}$) (Cavalcante et al., 2016), Xinço reservoir in north-central Brazil (max. peak: 5600 ind. mL$^{-1}$) and in a shallow urban lake in Southern Brazil (max. peak: 10,170 ind. mL$^{-1}$) (Silva et al., 2019). When compared with the bloom of 21,455 ind. mL$^{-1}$ at the Billing reservoir in São Paulo, Brazil (Matsumura-Tundisi et al., 2010) which is the highest density reported in the literature, our 744 ind. mL$^{-1}$ reported mean and even our maximum density in Marrecas (15,865 ind. mL$^{-1}$) seem relatively small. However, according to a Brazilian study, the peak observed in Billings reservoir was a typographical mistake as the C. furcoides population graph did not exceed the 25 ind. mL$^{-1}$ (Cavalcante et al., 2016). If so, the peak reported in our study during the spring of 2014 would be considered the highest register of C. furcoides density until now. Such a high density might be associated with the filling of the new reservoir.

4.2. Temporal variability of Ceratium furcoides abundance and dominance due to climatic conditions

In this study, C. furcoides abundance and dominance showed clear temporal variability, peaking in spring and decreasing over the years. Generally, during the first months after a reservoir filling (in our case November until December 2013), the concentration of nutrients and the water turbidity increase considerably due to the resuspension process and the death of terrestrial vegetation at the bottom. This process of trophic upsurge leaves light as a limiting factor (Geraldes and Boavida, 1999). In Marrecas, although the nitrate and phosphate were not higher in the first year, there was a high concentration of CO$_2$, organic matter, iron, Mn: manganese; WT: water temperature; CO$_2$: carbon dioxide; DO: dissolved oxygen; NH$_4$: Ammonium; PO$_4$: Phosphate; Cond: conductivity; Rain7: weekly precipitation; Tmx: daily maximum temperature; Tned: daily mean temperature; wind2: mean wind velocity; pH.

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In our study, the invader preferred spring, probably responding to spring temperatures that correspond to the species’ optimum (Heaney et al., 1988; Pollingher, 1988). Other factors, such as the mixing regime and the availability of nutrients, might also be contributing to its seasonality since spring showed higher values of turbidity, manganese, iron, dissolved oxygen, nitrates, phosphates, and organic matter, and lower values of conductivity, CO₂, and ammonium. These variables even correlated with the invader density.

In the Northern Hemisphere, spring is characterized by an important change in the phytoplankton community that follows the strong winter characterized by extreme cold, surface ice, and snow (Forström et al., 2007). Therefore, C. furcoides may remain with this tendency (increasing in spring) during its invasion, even in subtropical environments with different climatic conditions. This preference for spring by C. furcoides has been reported in Faxinal (Cavalcante et al., 2016), in an urban lake in Porto Alegre, southern Brazil (Silva et al., 2019), and in Rio Grande II (Bustamante-Gil et al., 2012). In Uruguay, C. furcoides appears to respond positively to stronger winds, shorter water columns, and lower temperatures, preferring winter and spring (Bordet et al., 2017).

Nevertheless, most previous studies have shown C. furcoides peaks in other seasons, including winter (Silva et al., 2012) and fall (Almanza et al., 2016; Wu and Chou, 1998). This was also observed in our study, although less frequently. Even though our results indicate summer as the least-preferred season for the invader, this was its favorite season in Maestra (only 20 km from Marrecas). Despite its location, Maestra differs greatly from Marrecas and Faxinal as it is a eutrophic-hypertrophic water body (Cavalcante et al., 2016). Therefore, the trophic state might be an important factor in dinoflagellate dynamics. However, other drivers, such as location and climate, must also play an important role in this dynamic since most C. furcoides blooms in Europe happen in summer (Hickel, 1988; Napiórkowska-Krzebietke et al., 2017; Nóges et al., 2011).

C. furcoides correlated positively with turbidity with all its blooms (> 2500 ind. mL⁻¹) occurring with turbidity above 10 NTU. As Marrecas is considered a mesotrophic reservoir, the turbidity observed in the study was exceptionally low. Thus, it could be either biogenic (a consequence of the phytoplankton abundance) or abiotic (due to abiotic elements like nutrients and metals). In this case, it was probably (at least partially) abiotic as metals such as manganese and iron (normally abundant in the hypolimnion or deeper layer (Becker et al., 2008a)) correlated positively with turbidity and the invader. This could be explained based on the mixing regime, as in Cavalcante et al. (2016). During the mixing period, the sediments from the bottom (including metals) are resuspended to the epilimnion, increasing their levels on the surface and, therefore, the turbidity (Becker et al., 2008b).

This relationship between the invader and the mixing regime is likely associated with its life cycle since C. furcoides can produce cysts (resistant dormant propagules) (Hickel, 1988) that then sink to the bottom where they persist. During the mixing period, if there are cysts, these are resuspended to the epilimnion, and the high levels of light and temperature in this upper layer interrupt their dormancy and lead to a process of excystment (Hickel, 1988). This potential link between turbulence and C. furcoides highlights the influence of hydraulic dynamics as one of the primary factors determining the structural and temporal changes in phytoplanktonic communities characteristic of tropical high-mountain reservoirs (Moréia et al., 2015).

Winter corresponds to the mixing period in nearby reservoirs (Becker et al., 2009; Cavalcante et al., 2015), and winter was also when the resuspension was higher in Marrecas. However, winter was only the second most preferred season by the invader. It is possible that sometimes the microorganisms need more time (e.g., one season) to respond to the effect of the mixing regime, based on the time required for the excystment (Hickel, 1988). However, during the quantification, few cysts were detected (personal comment of Patricia Buffon), but these might have been floating in other depths. Temperature, as explained before, must also affect the invader as all its peaks happened with water temperatures between its optimum (15 – 23 °C) (Heaney et al., 1988; Pollingher, 1988; Van Ginkel et al., 2001). Winter in Marrecas often showed temperatures below these values and therefore it might not have been warm enough for the population to grow optimally. The species also avoided the high temperatures of summer (mean of 25 °C), corresponding to the C. furcoides population decline (Pollingher and Hickel, 1991).

C. furcoides density correlated positively with organic matter, pH, and dissolved oxygen. However, it is still not clear if these conditions (high organic matter, well-oxygenated, and neutral to slightly basic waters) favor the development of the species (as it has been observed for other dinoflagellates (Popovsky and Pfister, 1990)) or if these conditions are a result of the presence of the species since it exhibits high density and biomass (organic matter) and produces oxygen as a by-product of photosynthesis (decreasing CO₂) which increases pH (Silva et al., 2018). Despite the reasons behind it, it is crucial to continue monitoring these variables along with the invader, as species of this genus have been associated with massive fish killings due to oxygen depletion following their blooms (Nicholls et al., 1980).

4.3. Ecological interactions between Ceratium furcoides and the phytoplankton community

Biological invasions can modify a community’s species composition by adding new members (facilitation), removing others (local extinctions), or affecting the community’s dominance and the abundance of local species (Gurevitch and Padilla, 2004; Mooney and Cleland, 2001). Understanding these ecological interactions between the invaders and the native species is key for assessing other ecological impacts on the system (Crosetti et al., 2018; Silva et al., 2019). In our study, K. caudata, C. cryptomonas, Discostella stelligera, and Chaetoceros spp. were the only taxa present during C. furcoides peaks. All of them were observed in more than 90% of the samples, so their occurrence might be explained based on their endurance (Reynolds et al., 2002) rather than as a positive response to C. furcoides dominance.

Discostella stelligera and Urosolenia eriensis correlated negatively with the invader; additionally, these Bacillariophyta preferred summer and fall (least preferred by the invader) and avoided its peaks. It is possible that C. furcoides outcompeted these diatoms, as U. eriensis decreased considerably after showing strong negative correlations with the invader, as in Almanza et al. (2016). However, Urosolenia can migrate vertically (McKay et al., 2000), so it may not have been collected in the last years due to its movement lower in the water column. Other members of Bacillariophyta (Aulacoseira alpigena and Nitzschia sp.) correlated positively with the invader in winter and fall. Consequently, interspecific competition explains only partially the interaction of diatoms and the invader. The higher densities of the Bacillariophyta in summer and fall are likely based on its preference for clear waters (Reynolds et al., 2002) as turbidity decreases in these seasons. Furthermore, the Bacillariophyta decrease in spring might be due to the vulnerability of these taxa to pH increase and CO₂ decrease (Reynolds et al., 2002), conditions met in this season.

After C. furcoides, Chlorophyta was responsible for the largest algae blooms in Marrecas. These generally occurred in summer, but the largest was in late spring 2015 after the peak of the dinoflagellate. Chlorophyta is a common component of the freshwater phytoplankton in Brazilian subtropical lakes and usually co-occurs with Ceratium blooms (Casol, 2014; Hackbart et al., 2015). However, in Marrecas, the Chlorophyta group seemed to avoid C. furcoides blooms and correlated negatively with it. In general, chlorophytes are opportunistic organisms that proliferate rapidly and depend on high levels of nutrients and light intensity (Olrík, 1994). The negative interaction of Chlorophyta with C. furcoides might result from competition for phosphate, as this nutrient was low in fall and summer. Chlorophyta’s decrease in winter and spring might be due to the mixing, as this group is highly susceptible (Reynolds et al.,
The decrease in *C. furcoides* blooms could also be related to abiotic factors such as weaker or shorter mixing periods, triggering low cyst resuspension during the last few years of our study. Nevertheless, this would not explain the other groups’ bloom decreases. Finally, it could also be associated with an increase in herbivory (top-down control) due to the arrival of new taxa over time and the increase of zooplankton (e.g., copepods and cladocerans) and predators (e.g., other dinoflagellates species) effective in reducing *Ceratium* blooms (Nielsen, 1991; Olsen et al., 2002). Future research must focus on the relation of *Ceratium* with the zooplankton community and other dinoflagellate species.

4.6. Limitations of our study

Our study analyzed superficial samples from a specific point in the reservoir for over five years, limiting it to a temporal rather than spatial focus. Future research must include a spatial approach (e.g., scan the water column) as *C. furcoides* can migrate in the water column. Furthermore, field studies such as ours necessarily work with many uncontrolled conditions. In this way, even if numerous environmental variables are measured, much uncertainty remains (unlike in laboratory studies), limiting our findings’ scope. However, only field studies address real conditions (not possible in the lab), making them critical for hypothesis building. Despite our study’s limitations, it analyzes ecological field data on a new environment during a biological invasion, conditions rarely studied despite their importance in ecology. Furthermore, this research is relevant to society as it addresses a water reservoir supplying the second most populated city in Rio Grande do Sul and has implications for the management of other tropical and subtropical reservoirs.

5. Conclusion

Marrecas presented ideal conditions for the establishment of the invader *Ceratium furcoides*. This dinoflagellate exhibited seasonality, avoiding summer and peaking in spring. Spring displayed optimal temperatures for the species (15–23 °C) and during 2014 it showed the maximum density ever reliably reported in the literature (15,865 ind. mL⁻¹), probably responding to the trophic upsurge from the filling of the new reservoir (Nov. 2013). *C. furcoides* correlated positively with iron, manganese, and turbidity probably responding to the mixing period which resuspends its cysts (interrupting cyst dormancy).

*Ceratium furcoides* correlated positively with *Komma caudata*, *Cryptomonas* sp., and *Aulacoseira algigena*, since all prefer turbulent waters. It correlated negatively with Chlorophyta (mainly *Chlamydomonas* spp.) vulnerable to mixing, and Bacillariophyta (*Urosolenia eriensis* and *Distotella stelligera*) that prefers clear waters. It also showed a consistent negative correlation with Ochrophyta (*Malomona* spp.) and to a lesser degree with Cyanobacteria (*Microcystis*), suggesting competition. Another potential consequence of the invader might be biodiversity loss. Notwithstanding, based on the decrease of blooms, the phytoplankton community appears to have reached a more stable state (with a more homogeneous representation of the species). However, further monitoring and research integrating zooplankton is required to confirm this claim and clarify further planktonic interactions in aquatic systems.

CRedit authorship contribution statement

Oscar Alberto Rojas Castillo: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. Leonel Pereira: Writing – review & editing, Supervision. Patricia Buffon: Methodology, Resources, Data curation. Luciana de Souza Cardoso: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Investigation, Writing – review & editing, Funding acquisition, Project administration, Supervision.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data is in supplementary material (rest of the dataset will be shared on request).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.limno.2023.126080.

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