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Rhizosolenia setigera *bloom in tropical coastal lagoon*

FIRST RECORD OF *RHIZOSOLENIA SETIGERA* (COSCINODISCOPHYCEAE, BACILLARIOPHYTA) BLOOM IN A TROPICAL URBAN COASTAL LAGOON AND EFFECTS ON THE ZOOPLANKTON COMMUNITY

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Abstract: Interactions with the sea are frequently important in coastal lagoons surrounded by urban areas, but besides improving water quality the connection with the sea can bring marine organisms to the lagoonal ecosystem. We present here an unprecedentedly high density (up to 7.5 x 10^6 cells L^{-1}) of the marine diatom *Rhizosolenia setigera* (Bacillariophyceae, Centrales) for four weeks in a Brazilian urban coastal lagoon, which is normally dominated by picocyanobacteria. *Rhizosolenia setigera* formed mats around the marginal area of the lagoon and affected the zooplankton community. As *R. setigera* is included among marine diatoms considered harmful and can synthesize highly branched isoprenoid alkenes (HBI), cell pellets were harvested during the bloom for analysis, but these compounds were not detected. Our findings highlight that even a short-term bloom of a marine diatom can render consequences upon the resident zooplankton community, possibly with cascading effects on other components of the local trophic web.

Keywords: phytoplankton; *Euplotes woodruffi;* eutrophic lagoon; Rhizosoleniaceae; Lagoa Rodrigo de Freitas.

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Coastal lagoons rank among the most productive ecosystems on Earth, providing a wide range of ecosystem services and resources, but are critical habitats of environmental changes at the transition between saline and freshwater domains (Kennish & Paerl, 2010). Regarding coastal lagoons separated from the sea by a sandbar, a common tool to improve the water quality is by the connection with the sea through artificial openings of the sandbar or by the construction and maintenance of artificial channels (Mayjor et al., 2023). The increase in water exchanges between the two systems provides the export of the lagoon's nutrients, decrease the level of the lagoon during heavy rainfall, and even promote the entrance of marine organisms enhancing fish activities in the lagoon.

In southeast Brazil, lies the Rodrigo de Freitas Lagoon (RFL) (22º 58' S, 43º 12' W), an urban coastal system situated within a major metropolitan area in Rio de Janeiro city. At the beginning of the 20th century, the development of large banks of Characeae impairing boat sport activities and events of fish kill resulted in the construction of an artificial channel to maintain a permanent connection between the lagoon and the sea.

In the present study, we reported a high abundance of a marine diatom in RFL detected during a seasonal sampling campaign conducted to characterize the lagoon's biological communities. The identified diatom, *Rhizosolenia setigera* Brightwell 1858 (Bacillariophyceae, Centrales) has not been previously registered in RFL by previous studies (e.g., Domingos et al., 2012; Soares et al., 2012), and as far as we know, this is the first report of *R. setigera* bloom in a tropical coastal lagoon. *R. setigera* can produce bloom events in oceans, and this diatom is known to synthesize polyunsaturated alkenes that may be noxious to aquatic organisms (Esenkulova et al., 2021). Furthermore, *Rhizosolenia* spp. have morphological structures, such as spines and setae, that can clog fish gills, causing mechanical irritation, puncturing surface gill lamella leading to secondary infection (Anderson et al., 2021; Esenkulova et al., 2021). *Rhizosolenia* species when ingested by bivalve shellfish can impart intensely persistent bitter taste rendering them unsuitable for human consumption. Therefore, our target was to follow the diatom bloom in RFL

and to conduct a chemical analysis to search for the presence of HBI during the bloom. Moreover, due to the possible direct effect of a phytoplankton bloom on the zooplankton community, sampling was conducted to detect qualitative short-term effects on this community.

The RFL has a surface area of 2.5 km^2, and a mean depth of 4 m. The lagoon is connected to the sea through the artificial channel Jardim de Alah (Figure 1), which is managed by the municipality of Rio de Janeiro city through a floodgate. Currently, the lagoon is daily monitored by the municipality through a multiparameter probe that acquires temperature and dissolved oxygen data. In addition, phytoplankton samples are weekly collected for the evaluation of the dominance of phytoplanktonic groups (PMRJ, 2023).

On July 14, 2021 - herein considered as the 1st week, phytoplankton samplings at two points (LRF1 and LRF2; Figure 1) detected a high abundance of a marine diatom in the RFL. In the 2nd week, phytoplankton samples were taken at seven points in the marginal area of the lagoon (LRF1-LRF7; Fig. 1) and zooplankton at one of them (LRF6). From the third to the 5th week, phytoplankton, as well as zooplankton samples, were collected only at LRF6, which was considered representative of general conditions of the lagoon since it is far from both the marine entrance and the input of the main river. All phytoplankton samples (500 mL of subsurface water) for quantitative analyses were collected in triplicate and were immediately fixed (Lugol's solution). Qualitative zooplankton samplings were conducted by surface trawls with a 68-µm mesh net; one-half of the sample was fixed (neutralized formalin, 4%) and the other was kept alive for observation under the microscope on the same day. The plankton analyses were conducted under an Olympus BX-51 microscope and specimens were measured and photographed with Toup View 3.7 image software. The dominant diatom was enumerated in random fields, using the settling technique under an inverted microscope Olympus CK-X41. During plankton samplings, temperature (°C) and salinity were measured using a portable thermosalinometer (Hanna, HI98319). Superficial water (5.5 L) was harvested in the 2nd week for alkenes determination on a PerkinElmer GC-MS (Clarus 680). Interpretation of GC mass

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spectrum was conducted using the database of the National Institute of Standards and Technology (NIST).

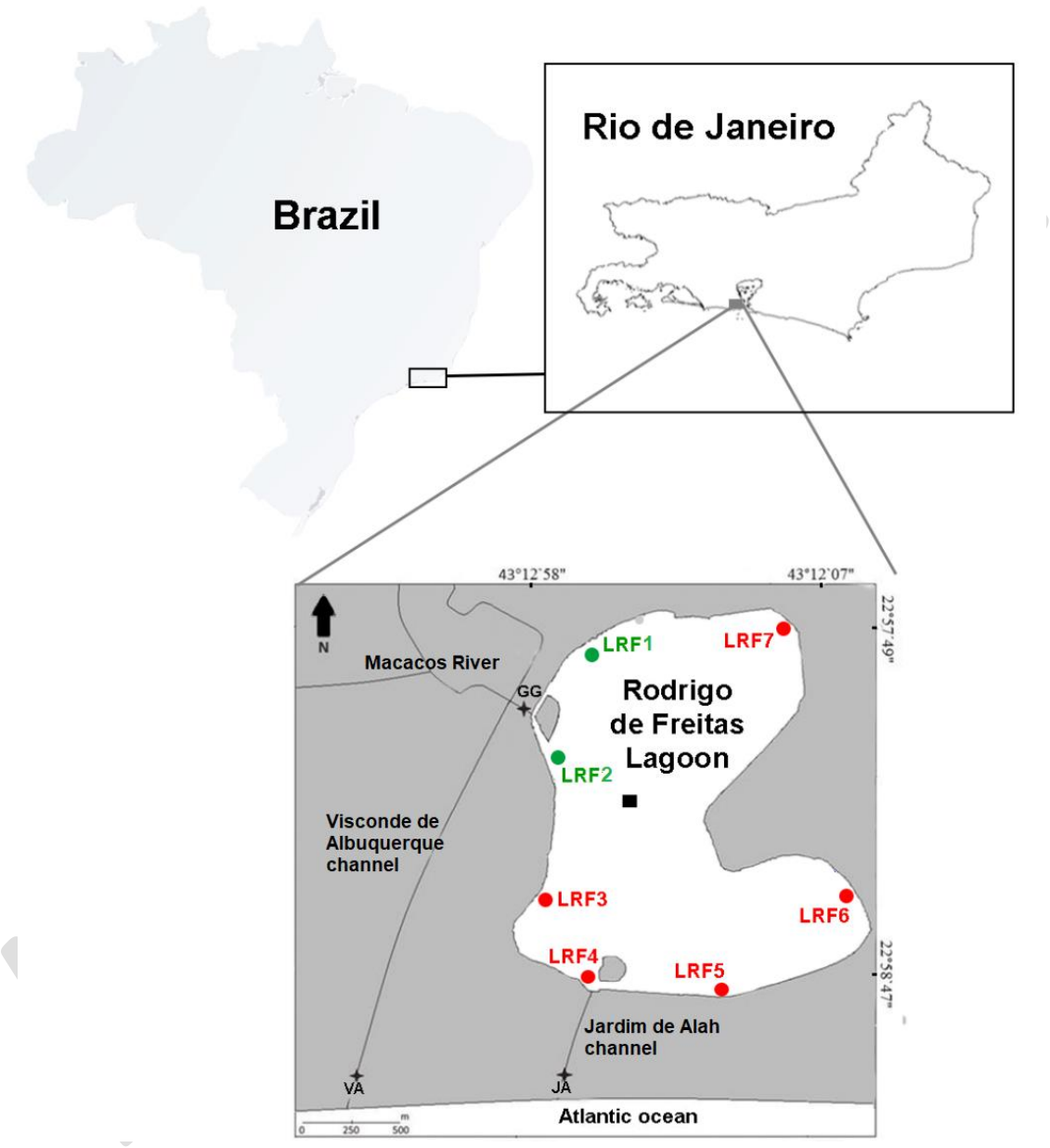

Figure 1. Location of the Rodrigo de Freitas Lagoon, Rio de Janeiro city, Southeastern Brazil. Sampling stations (LRF1-LRF7) and green color distinguish 1st week (●) sampling. The monitoring station of the municipal government is indicated by a square (■), and the location of artificial floodgates: GG General Garzon, VA Visconde de Albuquerque, and JA Jardim de Alah.

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Over the weeks of plankton observation, the interaction between the RFL and the sea remained constant. Water temperature and salinity of the lagoon varied from 21.6-23.5 °C and 8.5-16.15, respectively, and mean values of 22.91 ± 0.93 °C and 14.78 ± 1.93. The dominant diatom was identified as *Rhizosolenia setigera* (Figure 2) in accordance with Brightwell (1858), Sunesen & Sar (2007), Tabassum & Safullah (2011), and Yun et al. (2011). The species is characterized by solitary cells, cylindrical and straight with sharp ends. Valves conical overly elongated valvar axis, culminating in a fine and long central process, being uniformly straight with the base slightly thickened to a short distance. The process has a needle shape. Intercalary bands of the frustule were not visible in our analyses. Cells have numerous small ellipsoidal chloroplasts. Measures of organisms from LRF: length without processes: 257 - 434 µm; process length: 68 - 120 µm; diameter 6.5 - 12 µm.

Despite the two decades of phytoplankton monitoring in RFL, dominance by the marine diatom *Rhizosolenia setigera* had never been recorded before. Firstly, it should be noted that the entry of *R. setigera* into the lagoon should not be an isolated event, as this species is ubiquitous in the oceans, including coastal regions and bays (Stefanidou et al., 2020; Esenkulova et al., 2021; Lemos et al., 2022). In a historical study of phytoplankton in Guanabara Bay (Rio de Janeiro, Brazil), *R. setigera* was reported as being widespread within this system (being detected in 59% of the studied sampling sites; Villac & Tenenbaum, 2010).

In the 1st week of sampling, a high density of *R. setigera* (4.5 x 10^6 cells L^{-1}) was found, and the shift from the usual dominance of picocyanobacteria to a marine diatom was tracked by the monitoring program of the municipality (PMRJ, 2023). In 2nd week, *R. setigera* attained an average abundance of 6.5 x 10^6 cells L^{-1} and formed mats that could be seen at various points of the lagoon. Moreover, the watercolor of RFL dramatically changed exhibiting a darkish-brown appearance. The formation of aggregates (aggregation of cells) and mats is described as a common feature of this diatom bloom in oligotrophic ocean (Yao et al., 2021). The mats formation by *Rhizosolenia* spp. was suggested as an adaptation for anti-grazing in an environment dominated

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by small micro-grazers, and as a strategy to perform extensive migration in the sea to acquire nitrate at depth and return to the surface to photosynthesis (Singler & Villareal, 2005).

In the 3rd week, the average abundance of *R. setigera* was 7.5 x 10^6 cells L^{-1} and in the 4th week decreased to 1.1 x 10^6 cells L^{-1} whilst the relative abundance of other marine phytoplankton taxa increased. During the 5th week, *R. setigera* was rarely observed within the phytoplankton community.

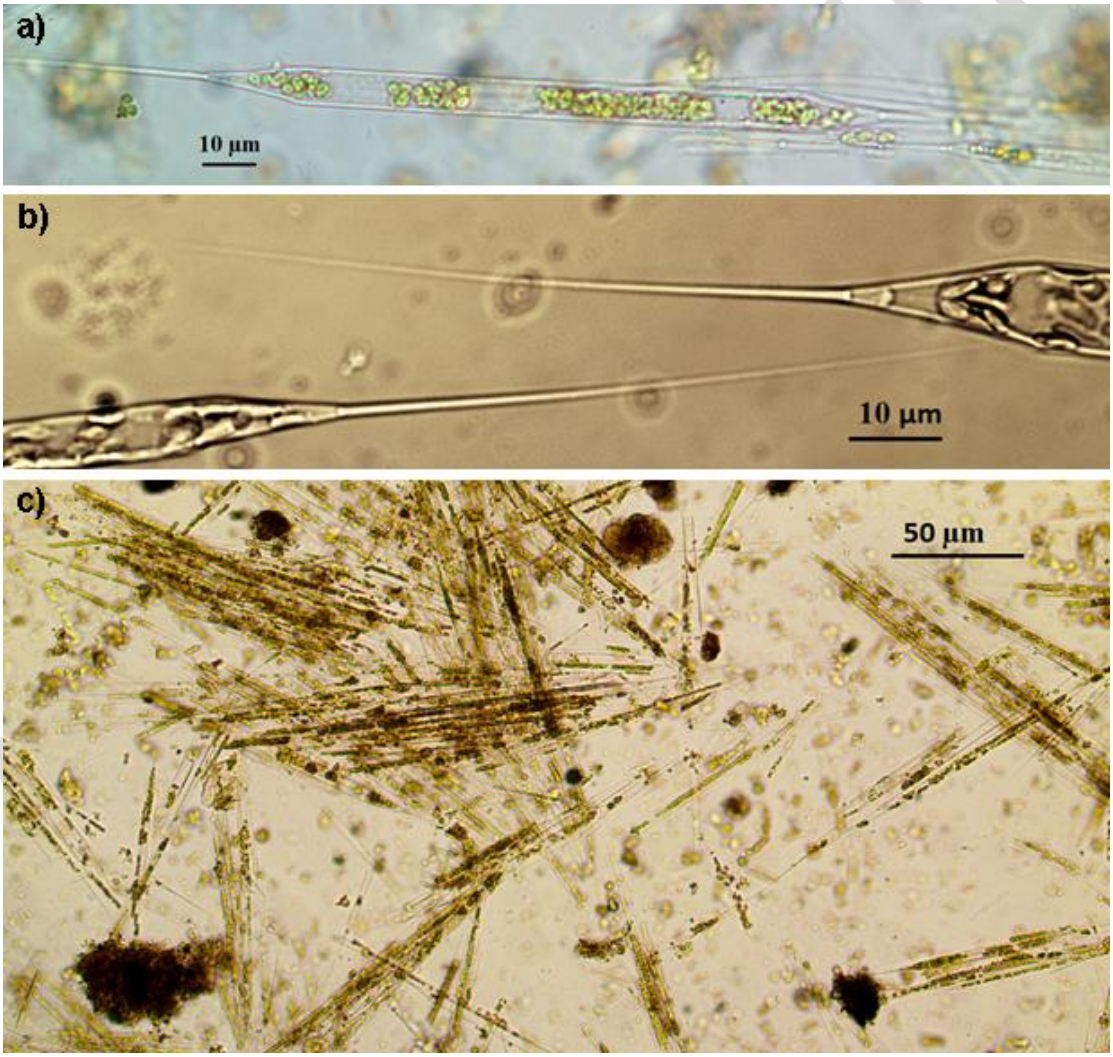

Figure 2. Cells isolated from plankton samples collected at Rodrigo de Freitas Lagoon, Rio de Janeiro, Brazil; a) Live specimens of the diatom *Rhizosolenia setigera*; b) Detail of the "long thin needle" design of *R. setigera* cells in a fixed sample; and c) Mats of *R. setigera*.

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In the present study, HBI biosynthesized by the harvested strain of *R. setigera* was not detected. This result may be related to characteristics of RFL strain, to the limitation of the analytical method, or both. However, in occurrences of the bloom of *Rhizosolenia* in RFL, the possible production of HBI might be analyzed, as the presence in other components of the aquatic food web such as mollusks and fish since it can bring damaging repercussions to the already stressed aquatic ecosystem.

In the second week with a high density of *R. setigera*, the zooplankton was mainly comprised of cirriped nauplii and by the prevalence of a ciliate identified as *Euplotes woodruffi* Gaw, 1939 (Hypotrichiid, Euplotidae) (Figure 3) in both preserved and live samples. The identification of *E. woodruffii* was based on basic ciliatology methods, and macronuclei features in accordance with Curds (1975), Song & Bradbury (1997), and Tirjaková et al. (2015). The body size of *E. woodruffi in vivo* was 105 – 155 μm long and about 72 – 110 μm wide. Body shape is broadly obovate, dorsoventrally flattened with the right margin being more convex than that of the left. The adoral zone of the membranellas occupies more than half of the body length. The macronucleus is either T- or Y-shaped.

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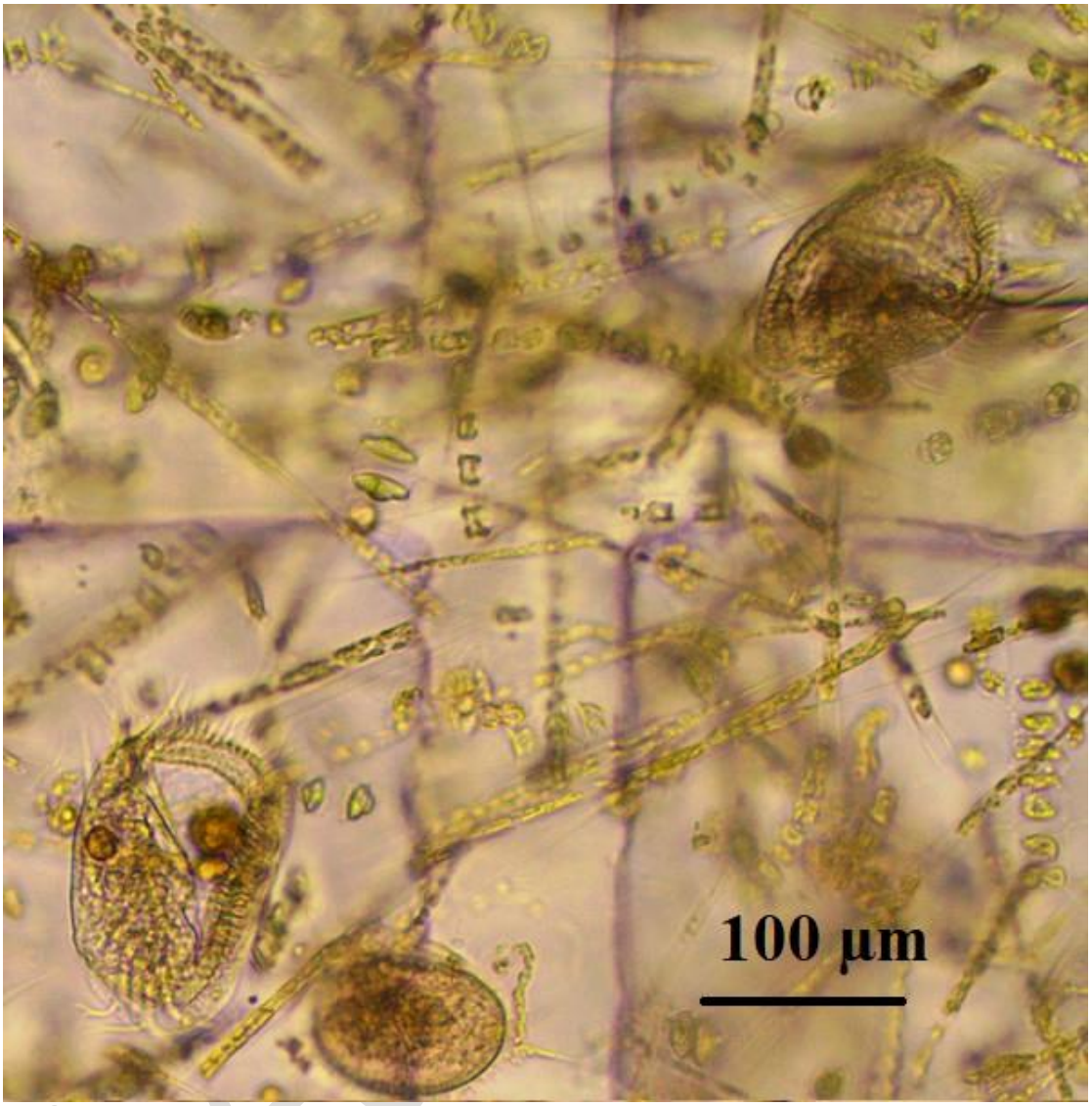

Figure 3. The ciliate *Euplotes woodruffi* among marine diatoms from Rodrigo de Freitas Lagoon.

In the 3rd week, with the presence of mats of *R. setigera* in RFL, the zooplankton continued to be mostly comprised of the ciliate *E. woodruffi* and Cirripedia nauplii. When the abundance of *R. setigera* decreased and the dominance of picocyanobacteria increased in the 4th week, the zooplankton community was comprised by cirriped nauplii, ciliates (most tintinnids, and few *Euplotes*), polychaete and bivalve larvae, the rotifer *Synchaeta* cf. *littoralis*, ostracods, and marine calanoid copepods (*Acartia* spp.). In the 5th week of sampling, when *R. setigera* was rarely

observed, the zooplankton community was characterized by the presence of tintinnid ciliates, marine copepods, nauplii of cirripeds, ostracods, and larvae of polychaetes and mollusks.

Thereby, *R. setigera* high density affected deeply the zooplankton community. Under mostly mesohaline conditions in the RFL, the zooplankton is comprised of micro-grazers such as rotifers and copepod nauplii, euryhaline copepods, and meroplanktonic organisms (Souza et al 2011). Nevertheless, a prevalence of a single species of ciliate in the zooplankton community was yet to be reported for RFL. The dominant *E. woodruffi* is a euryhaline ciliate and a highly likely cosmopolitan distributed species (Tirjaková et al. 2015). *Euplotes woodruffi* had already been collected from the Jacarepiá lagoon, a brackish water environment (RJ, Brazil) (Senra et al., 2016), and among 60 different morphospecies of ciliates from the Maricá-Guarapina lagoon complex (Nunes, 2019). Nunes (pers. Comm.) had already registered this species in the RFL.

Our study shows that the high density of the marine diatom *R. setigera* in the phytoplankton community impaired the water quality of RFL through changes in the water colour, and mats formations and affected the local zooplankton. Our findings highlight the importance of the qualitative monitoring of the lagoon's plankton community. Despite its short duration (~ four weeks), this blooming event is likely to bring to bear repercussions on the other trophic links of the local aquatic chain, thus constituting yet another source of stress upon this multi-impacted urban lagoon.

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DECLARATIONS

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Conflict of interest

None.

Data availability

All data used during the study appear in the submitted article and are available.

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