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FIRST REPORT OF *GYMNODINIUM* SP. (DINOPHYCEAE) BLOOM IN A NEOTROPICAL RIVER OF ARGENTINA

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***Abstract* -** Gualeguaychú river is a freshwater continental located in the Center-Eastern of Argentina corresponding to the Neotropical region. Several uses can be identified such as the conservation of aquatic life, recreation (primary and secondary contact), navigation, recreational and subsistence fishing, and human and animal consumption. From February to April 2018, a bloom of *Gymnodinium sp.* (Dinophyceae) was reported in the low basin of the Gualeguaychú river. The bloom was extended from the Parque Unzué (33º 00' 16.38" S - 58º 29' 21.15" W) to the discharge of the El Cura stream (33º 02' 27.00" S - 50º 20' 29.01" W). This was the first record of a bloom of *Gymnodinium* sp. in that region where potentially toxic cyanobacteria blooms usually have an annual frequency. Consequently, this finding could be interpreted as a global indicator of change. Therefore, it is necessary to advance in systematic monitoring to develop an early warning system in the Gualeguaychú River.

Keywords: *Gualeguaychú river; dinoflagellates; global change; Entre Ríos, freshwater*

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1. INTRODUCTION

Dinoflagellates (Dinophyta) are mostly biflagellate unicellular algae, and they are predominantly found in the surface waters of marine systems (about 90% are marine) with only about 220 species present in freshwater environments (Bellinger & Sigge, 2010). In the most prevalent stage (haploid motile), these organisms can reproduce asexually by binary fission (Krock et al., 2015) and they can be mixotrophic, autotrophic, or heterotrophic (Schnepf & Elbrächter, 1992).

When conditions become limiting for growth, many dinoflagellate species produce temporary or dormant survival stages (Grigorszky et al. 2006 in Rengefors & Kremp, 2018). Many temperate dinoflagellate species undergo a mandatory dormancy period before their resting cysts can germinate (Rengefors & Kremp, 2018). In the last few years, the number of blooms observed by several dinoflagellate species has increased throughout the world (Negri et al., 1992, Krock et al., 2015, Leong et al., 2015, Montero et al., 2017, Baliarsingh et al., 2018, Mulholland et al., 2018, Méndez & Carreto, 2018) with global risks to health and economies (Davidson et al., 2014 in Townhill et al. 2018). A notable feature of harmful algal blooms (HABs) events is that the bloom species are often novel in not having been previously detected, or found to be prolific at the blooming site (Smayda, 1998, Smayda & Reynolds, 2001). Life cycle transitions and their regulation mechanisms play an important role in the bloom dynamics and seasonal succession of planktonic dinoflagellates (Rengefors & Kremp, 2018). The mechanisms of bloom-species selection and the causes of the shifts in phytoplankton community structure favoring flagellate taxa and their blooms are major unresolved HABs issues (Smayda & Reynolds, 2001). HABs of the toxic marine dinoflagellates *Alexandrium tamarense*, *A. catenella*, and *Gymnodinium catenatum*, which cause the neurotoxic paralytic shellfish poisoning (PSP), are a recurrent phenomenon along the Argentinean coast (Carreto et al., 1985, 1998a,b, 2008; Esteves et al., 1992; Santinelli et al., 2002 in Krock et al., 2015). However, the non-toxic blooms are frequently in freshwater and have had less attention because they may be imperceptible (Boltovskoy, 2009) and, in general, they do not represent health risks. Nevertheless, they can represent an important indicator of environmental changes, and the high biomass occurrences can cause negative effects on ecosystems through their sheer quantity, by reducing dissolved oxygen, blocking fish gills, and smothering benthos (Davidson et al., 2014; Anderson et al., 2015; Kudela et al., 2015; Wells et al., 2015; van der Lingen, 2015 in Townhill et al., 2018).

The objective of this work was to describe the first dinoflagellate bloom in a river of the Neotropical region namely Gualeguaychú, in the Center-Eastern of Argentina. Our global hypothesis is that the Gualeguaychu river is experiencing changes within the framework of global change scenarios that can be interpreted as early warning indicators.

2. STUDY AREA

Entre Ríos Province (Center-Eastern of Argentina) has more than 7000 watercourses as rivers, streams, wetlands, and glens. Gualeguaychú basin has a surface of 6690 km², and its main course is the Gualeguaychú river with a length of 250 km. The river runs in the North-South direction and it is parallel to the Uruguay river. The head river is located in Colón at 23 meters above sea level (32° 12' 07" S, 58° 32' 31" W), and the river mouth, in Gualeguaychú at 10 meters above sea level (33° 04' 38" S, 58° 24' 54" W). This river is used for recreation with primary and secondary contact, fishing, aquatic life conservation, small draft navigation, and the potabilization of water sources by permanent and tourist populations. Cardini et al., (2017) and Llorente et al., (2017) mention that the Gualeguaychú river is a particularly nutrient-rich environment, as well as tributary streams to this river in the middle-lower basin (Crettaz-Minaglia et al., 2014; Juárez et al. al., 2016; Gianello et al., 2016; Ávila-Hernández, 2017, Juárez et al., 2018, Ávila-Hernández et al., 2020).

3. METHODS

On February 28th during the austral summer, the presence of green color in the low basin of the Gualeguaychú river was detected. This was observed until austral autumn, April 8th, 2018. The bloom was extended from the Parque Unzué (33° 00' 16.38 "S - 58° 29' 21.15" W) to the outfall El Cura stream (33° 02' 27.00 "S - 58° 20' 29.01" W) (Fig. 1) Water samples were taken every week (n=6) in 5 sites during the day (10:00-15:00 hours) using glass flasks. An aliquot was fixed with formaldehyde 3-5 % (final concentration) (Boltovskoy, 1999), and another aliquot was refrigerated to be observed *in vivo* according to the criteria of Boltovskoy (1999).

In the laboratory, water samples were observed using an optical microscope Olympus® with increases of 40-1000 X, and identified using taxonomic keys (Moestrup & Calado, 2018) according to Boltovskoy (1999). Cell count was carried out in the same microscope using a Neubauer chamber for direct quantification and the results were expressed as cell/mL according to Crettaz-Minaglia et al. (2017). Moreover, *in situ* water parameters were measured: temperature, pH, dissolved oxygen (DO), and electric conductivity (EC) using the following equipment that was previously calibrated (APHA, AWWA, WEF, 2012): pHmeter HI 98127, conductometer HI 98311 and, oximeter YSI model 55. Hydrometric level and rains, temperature, and wind data were registered from Prefectura Naval Argentina (Argentinian Naval Prefecture) and Dirección de Hidráulica de Entre Ríos (Hydraulic Direction of Entre Ríos).

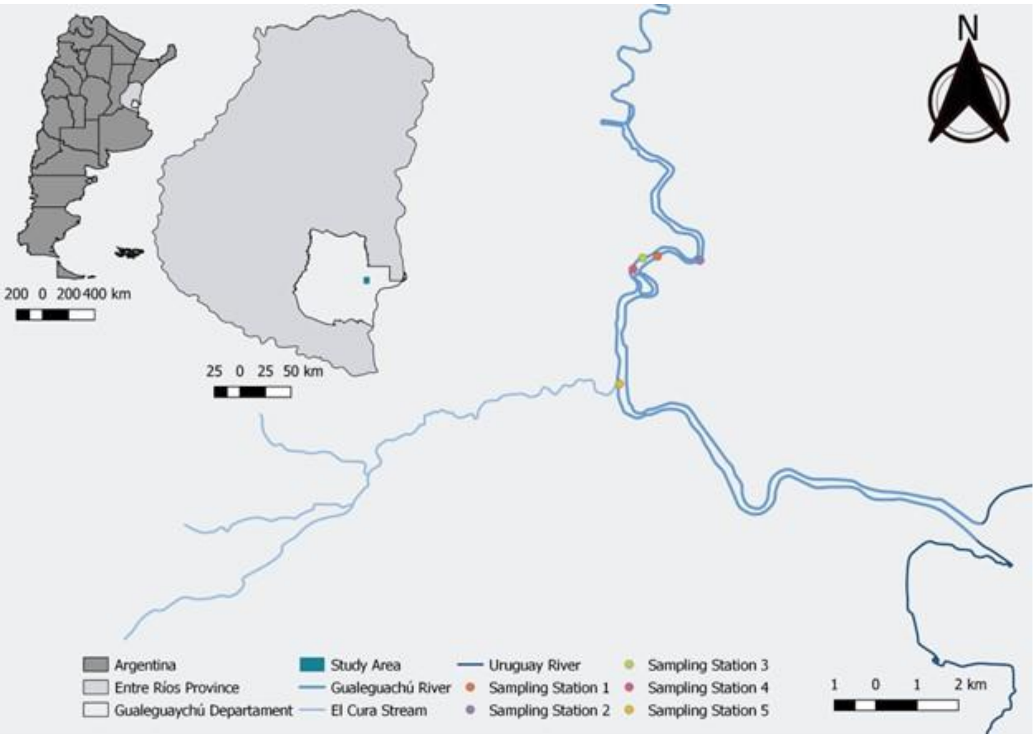


Figure 1. *Study area (QGIS 3.18, 2021). In color circles, the five sampling zones are shown.*

4. RESULTS

Gymnodinium sp. (Dinophyceae) was found and identified in all water samples taken from the Gualeguaychú river. This dinoflagellate was a predominant member and unique in the bloom. An exception was observed in the beach water sampling (33º 00' 59.75 "S - 58º 30' 12.12" W) where it was not observed water green color.

The abundance varied between 1000 and 12000 cells/mL (Fig. 2). High values were found between February 8th and March 13th, and from March 20th, then the low abundances were found until April 8th when they stopped being recorded.

Water parameters did not vary much between the sampling sites and the time while the dinoflagellates were present in the Gualeguaychu river. Water temperature varied between 25.5 - 27.9 °C, pH= 7.80 - 8.23, DO= 7.20 - 8.54 mg/L, and EC= 724 - 777 uS/cm In sampling station 4, where the green color was not observed in the water, the pH and the DO values were lower than in the other sampling stations. In this zone, recreational water use with primary and second contact was observed. Finally, the weather data can be observed in Table 1. In April, the average temperature began to decrease between 0.8 - 3.4°C (maximum= 1.6 - 3.7 °C and, minimum= 3.4 - 6.4°C) continuing to the month of May and, the total rains increased considerably compared to February and March (Table 1). Moreover, the wind speed changed its predominant direction from SSE to N (Table 1). In Figure 3, the hydrometric level data during the study period can be observed. These varied between 1.00 and 2.60 m, being the values lower than alert and evacuation levels.

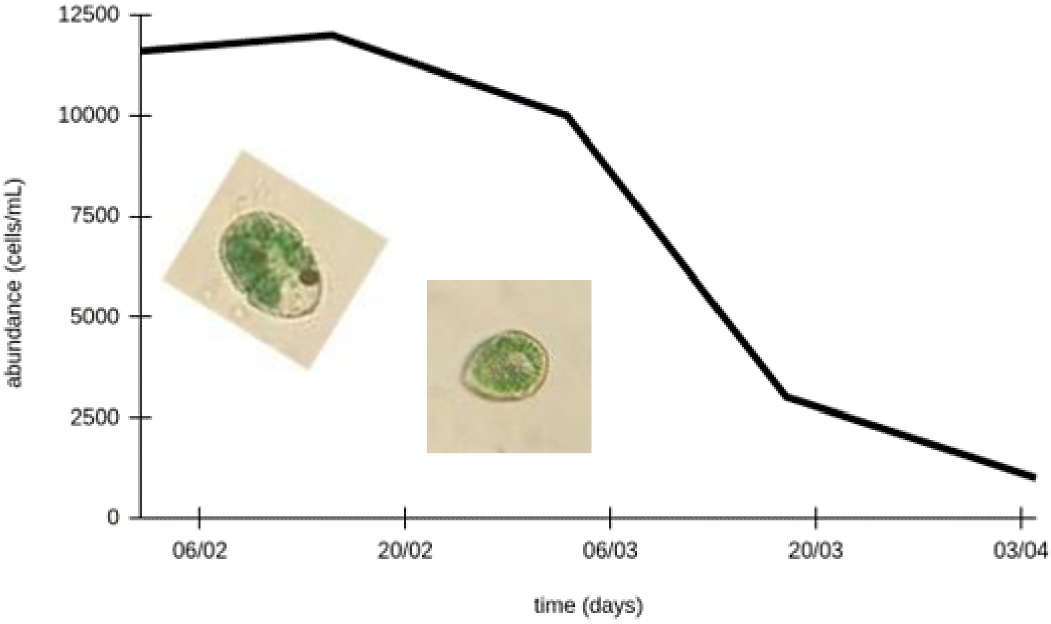
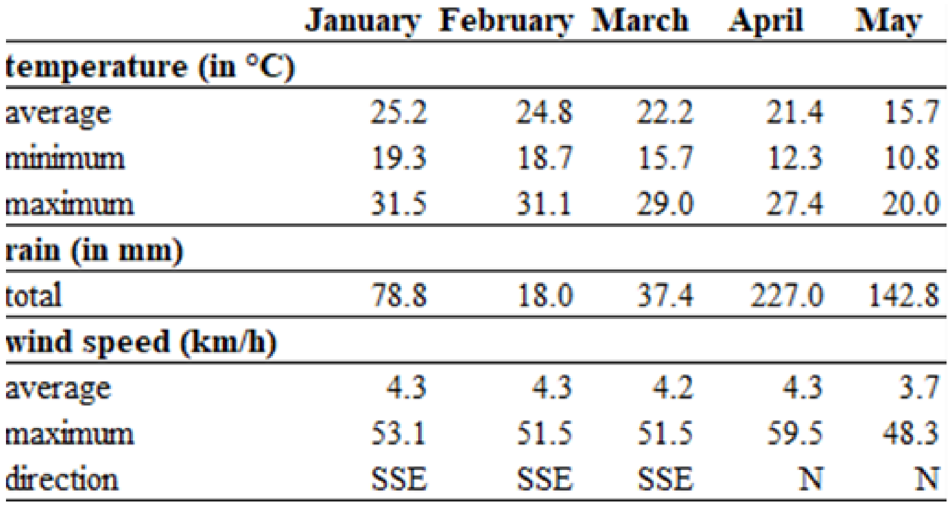


Figure 2. Abundance average of Gymnodinium sp. cells per mL along with the sampling period (from February to April 2018).

Table 1. Environmental conditions during the study period (Dirección de Hidráulica de Entre Ríos).



	January	February	March	April	May
temperature (in °C)					
average	25.2	24.8	22.2	21.4	15.7
minimum	19.3	18.7	15.7	12.3	10.8
maximum	31.5	31.1	29.0	27.4	20.0
rain (in mm)					
total	78.8	18.0	37.4	227.0	142.8
wind speed (km/h)					
average	4.3	4.3	4.2	4.3	3.7
maximum	53.1	51.5	51.5	59.5	48.3
direction	SSE	SSE	SSE	N	N

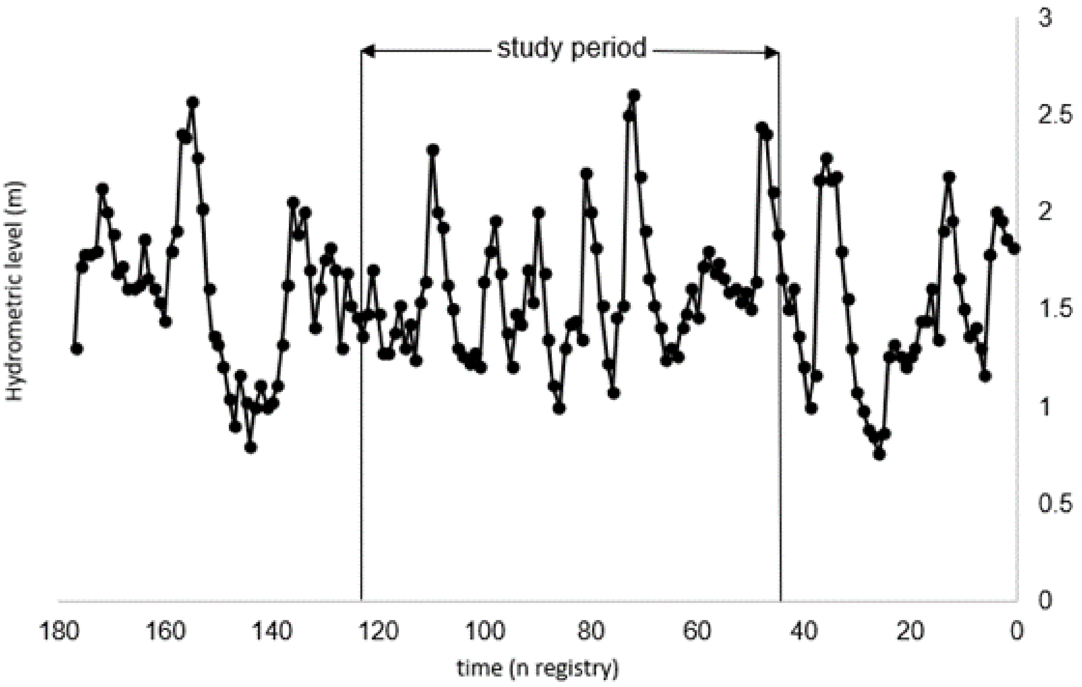


Figure 3. Hydrometric level of the Gualeguaychú river between 1st February and 30th April 2018. The time axis contained the registry every 12 hours. Data were obtained from Prefectura Naval Argentina. Alert level = 3.5 m, Evacuation level = 3.8 m.

5. DISCUSSION

This is the first record and field observation of *Gymnodinium* sp. bloom in the Uruguay river basin particularly in the Gualeguaychú river. In the Uruguay river basin, potentially toxic cyanobacteria blooms have been reported in the Salto Grande reservoir (Crettaz-Minaglia & Bordet, 2013), Nuevo Berlín, Fray Bentos and, Las Cañas (Ferrari et al., 2011). Moreover, during dinoflagellate bloom in the Gualeguaychú river, cyanobacterial blooms were reported in the Uruguay river through *Programa de Vigilancia de Playas de la Comisión Administradora del Río Uruguay* (Beaches Monitoring Program of the Administrative Commission of the Uruguay River, CARU). O'Farrel & Izaguirre (2004) mention that these areas have higher pollution downstream from the cities and industrial centers, and the heavy metals and agricultural chemicals of the organophosphate and organochlorine groups were not found at elevated levels and were not considered problems for the lower stretch. Llorente et al. (2017) mention that in the Gualeguaychú river mouth, the water quality is compromised for the conservation of aquatic life (basic use according to CARU, 1986). The same authors reported phosphates values between 0.001-1.68 mg P-PO_4^{-3}/L with an average of the 0.136 mg P-PO_4^{-3}/L and nitrates values between 0.001-0.810 mg NO^{-3}/L with an average of 0.265 mg NO^{-3}/L. Other studies have been conducted in tributaries water bodies of the Gualeguaychú river in areas where the main activities are agriculture and livestock (Crettaz-Minaglia et al., 2014; Juarez et al., 2016; Gianello et al., 2016 and Juárez et al., 2018) and, in an urban stream that receives discharges from the city's wastewater treatment plant (Ávila-Hernández, 2017 and Ávila-Hernández et al., 2020). In these studies, relatively high nutrient values have been recorded, being classified as eutrophic to hypereutrophic and it was observed that the study area could be impacted by diverse anthropic activities that alter the water quality of the streams affecting, consequently, the quality of the Gualeguaychu river. Sampling station 3 of the study carried out by Ávila-Hernández (2017) and Ávila-Hernández et al. (2020), coincides with sampling station 5 of this study. On this site, phosphates values were recorded ranging from 0.32 to 1.73 mgP-PO_4^{-3}/L; total phosphorus between 1.04 to 4.93 mgP/L, those of COD ranged between 21 and 111 mgO_2/L. The pH, OD, CE, and SDT values were similar to those registered in all the sites of this study.

Even though seven freshwater species of Dinophyceae have been reported to form blooms in Argentina, 2 of these have a unique report in this country (Boltovskoy, 2009). This is the first report of a bloom of dinoflagellates in the Uruguay river basin. It can be possible due to the blooms can be not detected or the dinoflagellate dispersion thought cysts have occurred in the study period. They are transported by seeding within the sediment layer (Bravo & Figueroa, 2014).

The conditions that favor its development are not clearly established, generating different opinions among authors (Boltovskoy, 2009). Dinoflagellates can occupy a variety of ecological niches, but they possess some general traits that make them particularly successful under specific habitat conditions (Reynolds et al. 2002). Freshwater varies immensely across the range of temperature, salinity, pH, nutrients, and watercolor (colored organic matter) and different species have different distributions and biogeography shaped by environmental or historical factors, or both (Rengefors & Kremp, 2018). In temperate freshwater systems, these factors change throughout the seasonal cycle (Rengefors & Kremp, 2018). According to the same authors, the temperature is a major environmental gradient determining phytoplankton distribution as it varies significantly in time (during the seasonal cycle) and space (across latitudes and at different altitudes). In temperate freshwater habitats, specific temperature adaptations with defined optima along the seasonal temperature gradient are a major driver of seasonal succession patterns (Rengefors, 1998). In this study, it was observed the dinoflagellates were dominant during summer (Pollingher & Serruya 1976 in Rengefors & Kremp, 2018) and that the bloom disappeared coinciding with decreased temperatures and increased rains. The onset of suboptimal temperatures is a common factor in promoting cyst formation (Grigorszky et al. 2006, Flaim et al. 2010). Rengefors & Anderson (1998) showed that this seasonal pattern could be explained by lifecycle events (cyst germination) governed by temperature and endogenous factors. Indeed, during dinoflagellate evolution, the need to adapt to fluctuating environments and/or to seasonality is thought to have driven the development of this life cycle stage (Bravo & Figueroa, 2014).

The genus *Gymnodinium* is defined as Type I by Smayda & Reynolds (2001) and it is characterized by small size and a high growth rate that can thrive in high nutrient concentrations (eutrophic and hypereutrophic watercourses) and turbulence. This is equivalent to the r-strategist according to Margalef's model (Margalef, year) and C-strategist according to Reynolds's model (Reynolds, 2002) (Smayda & Reynolds, 2001). Due to its colonizer character, it is possible that the Gualeguaychú River is in a period of dynamic change, possibly in a scenario of global change and current strong anthropogenic pressures. According to van der Lingen et al. (2015), the incidence of HABs caused by dinoflagellates may be increasing as a consequence of climate change. Therefore, it is essential to continue monitoring in order to generate early warnings due to the river's importance to the local and tourist population.

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