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First record of bioluminescence of *Alexandrium fraterculus* (dinoflagellate), in the Uruguayan coast, South Western Atlantic Ocean

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Abstract. In Uruguay *Alexandrium fraterculus* occurs frequently in summer-autumn in low concentrations and occasionally minor blooms, but it was never before associated to bioluminescence. In 2015 an exceptionally large bloom coloured the water reddish during day and glowing blue during night.

Keywords: Uruguay, phytoplankton, dinoflagellates

Resumen: Primer registro de bioluminescencia de *Alexandrium fraterculus* (dinoflagelado) en la costa uruguaya, océano Atlántico Sudoccidental. En Uruguay *Alexandrium fraterculus* ocurre en verano-otoño en bajas concentraciones y ocasionalmente forma floraciones, pero no fue asociado previamente a bioluminiscencia. En 2015 una floración excepcionalmente grande coloreó el agua de rojizo durante el día y brilló en la noche

Palabras clave: Uruguay, fitoplancton, dinoflagelados

Alexandrium fraterculus (Balech) is a cosmopolitan marine, warm water dinoflagellate and its occurrence in the Southwestern Atlantic is associated to the Brazilian Current (Balech 1995). Until 1995 no bloom concentrations were observed (Balech 1995) but it became a regular phenomenon in New Zealand since 1997 with densities frequently higher than 10^3 cells L^{-1} (MacKenzie et al. 2004). In southern Brazil, it has been commonly found in coastal waters in low concentrations (Rörig et al. 1998; Proença et al. 2002) and a notorious bloom was registered in 2004 (Omachi et al. 2007). In Uruguay, during the Harmful Algal Blooms weekly monitoring program, *A. fraterculus* has been found frequently in summer-autumn since 1991 in low concentrations and occasionally a bloom of 4.0 x 10^4 cells L^{-1} was reported (Méndez et al. 1993, Méndez & Brazeiro 1993; Méndez & Ferrari 1995). Although blooms of 7.8 x 10^4 cells L^{-1} were registered in 2009 and a higher density bloom of 1.73 x 10^6 cells L^{-1} in 2013 (unpublished data), it was never before associated to bioluminescence. In March 2015 an exceptionally large bloom was found, colouring the water reddish during day and glowing blue during night.

Bioluminescence is the emission of visible light by living organisms. Predominantly a feature of marine species, bioluminescent species are found in most of the major marine phyla from bacteria to large vertebrates including sharks and angler fish (Haddock et al. 2010), with a diverse array of colours (predominance of blue and green), intensities, and kinetics (Widder 2010). The reasons for the wide distribution of bioluminescence within marine organisms are varied and include strategies for predator avoidance, prey location and attraction and intra-species communication (Haddock et al. 2010). Bioluminescence is reported in members of 18 dinoflagellate genera (Poupin et al. 1999); this group of unicellular organisms contains the only members of the phytoplankton community with the ability to emit visible light. In dinoflagellates, bioluminescence is generally thought to prevent or decrease predation by zooplankton (Haddock et al. 2010) since their light emission reduces grazing by nocturnal predators (Cusick & Widder 2014). The reported bioluminescent species belonging to the *Alexandrium* genera are *A. affine, A. acatenella, A.*

catenella, A. fundyense, A. tamarense, A. monillatum (Poupin et al. 1999; Marcinko et al. 2013a) and *A. ostenfeldii* (Kauko 2013; Le Tortorec et al. 2014) but *A. fraterculus* was not considered bioluminescent before.

Several dinoflagellate characteristics, including bioluminescence, exhibit variability that is under endogenous (internal) circadian control (Kauko 2013; Marcinko et al. 2013b) increasing the number of scintillons (Fritz et al. 1990) showing the bioluminescence just during night. During this bioluminescent bloom *A. fraterculus* showed this behaviour, glowing just during night, bioluminescence was absent during the day even if cells were kept in darkness.

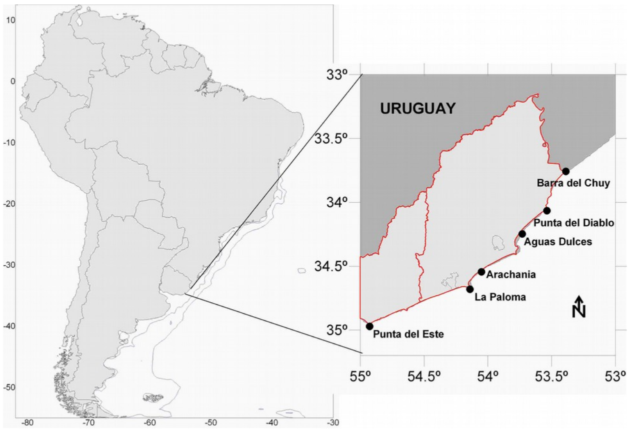

Figure 1. Map showing the sampled stations (black dots).

Hence, since the phytoplankton sampling in the monitoring program is during day light, it could be possible that previous *A. fraterculus* blooms were bioluminescent and they were not detected.

Patches of discoloured water were noticed from the shore in La Paloma beach on March 16th 2015; few days later the water was spotted with discoloured patches in Punta del Este (Fig. 1, Fig. 2 A).

The bloom was recorded throughout the month with different intensities in several coastal sites. During the night it was observed bioluminescence in all these sites (Fig.2 B-C).

Samples collected with a plastic bucket in the blooms were dominated by *A. fraterculus* (Fig.3) in chains of variable length, some samples were taken alive and other fixed with Lugol. Cells of *A. fraterculus* were counted in an inverted microscope Olympus IM following Utermöhl (1958) at a final magnification of 100 x (Andersen & Throndsen 2003). Abundance of *A. fraterculus* was as high as 4.7 x 10^7 cells. L^{-1}, 2.8 x 10^5 cells L^{-1} and 3.6 x 10^5 cells L^{-1} in La Paloma, Arachania and Punta del Este, respectively on March 16th.

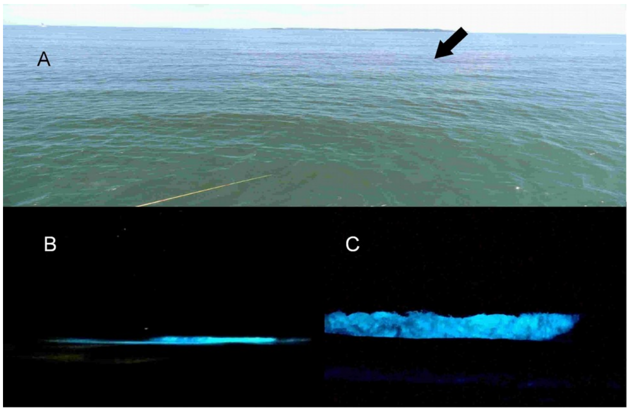

Figure 2. Photographs of *Alexandrium fraterculus* bloom observed during day and night. A) Punta del Este, black arrow shows water discoloration; B) Night photograph showing the bioluminescence in Aguas Dulces; C) Night photograph showing the bioluminescence in La Paloma.

The maximum abundance on March 23th was 2.6 x 10^7 cells L^{-1} in Aguas Dulces and 3.69 x 10^5 cells L^{-1} in Barra del Chuy on April 6th (Fig. 4) a potential evidence of an eastward transport of the bloom (Fig. 1).

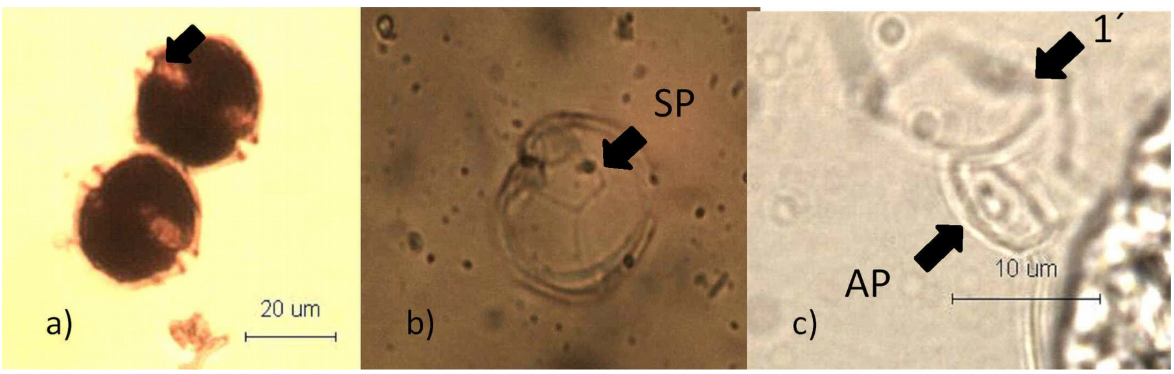

Figure 3. Optical micrographies of *Alexandrium fraterculus*. A) two cells chain, black arrow shows the typical horseshoe-shaped nucleus. B) Hypoteca with sulcal posterior plate (SP) C) Apical pore (AP) and first apical plate (1´).

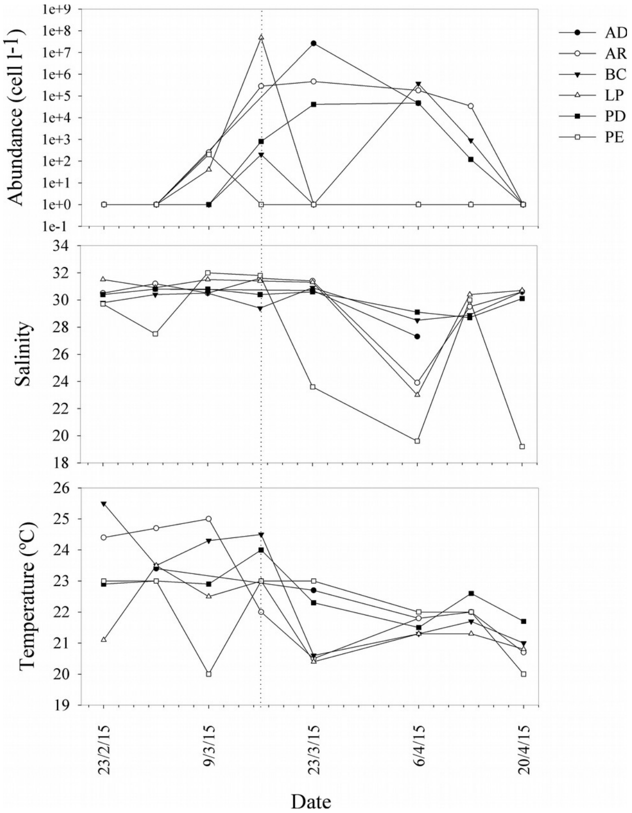

Figure 4. A) Abundance of *Alexanrium fraterculus* (cells L^{-1},) in each sample site. B) Salinity for the period of the bloom C) temperature (ºC) for the period of the bloom. LP (La Paloma), AR (Arachania), AD (Aguas Dulces), PD (Punta del Diablo), BC (Barra del Chuy) and PE (Punta del Este). Dashed line shows the start of the bloom.

Also different states of the bloom (accumulation of reserves, encystment) were detected through time (Fig. 5). One month later a new lower intensity bloom was detected, reaching 5.19 x 10^5 cells L^{-1} and 1.25 x 10^5 cells L^{-1} at Arachania and Barra del Chuy respectively on June 1st.

The bloom coincided with a sudden drop in temperature and salinity (Fig. 4). This feature is common to other dinoflagellate which blooms at the starting of the autumn when an increase in Río de la Plata discharge promotes high stability of the water column (Martínez & Méndez 2013).

The chlorophyll *a* concentration (Chl *a*) of the bloom was analysed by filtering 200 mL of the water through GF/C Whatman glass fibre filters and extracting pigments with 90 % acetone (Parsons et al. 1984). Chl *a* reached 47.8 mg m^{-3} in Aguas Dulces on March 23th, unfortunately in La Paloma beach on March 16th when there was the highest abundance of *A. fraterculus* Chl *a* was not measured.

It is worth to point out that during the extent of the blooms phycotoxin mice assays turned negative for Paralytic Shellfish Poisoning (PSP) and Diarrheic Shellfish Poisoning (DSP) for all the samples, even with that vast cell density. These results are opposed to the consideration of the first bloom registered in Uruguayan waters, when it co-occurred with the toxic dinoflagellate *Gymnodinium catenatum* (Méndez et al. 1993) and confirmed that this species of the *Alexandrium* genera is not linked to PSP toxins as stated by other authors (Proença et al. 2002; MacKenzie et al. 2004; Omachi et al. 2007). However, during these blooms bathing in the sea and eye exposure caused eye irritation as in other phytoplankton species blooms (Pilotto et al. 1997). Both the cell abundance and the chlorophyll *a* concentration found in this occasion were higher than those found in the Brazilian bloom (Omachi et al. 2007).

A. fraterculus could be used in ecotoxicity assays as other bioluminescent microalgae due to the diminishing of light emission when exposed to toxic pollution (Sanchez-Fernandin 2015), this feature

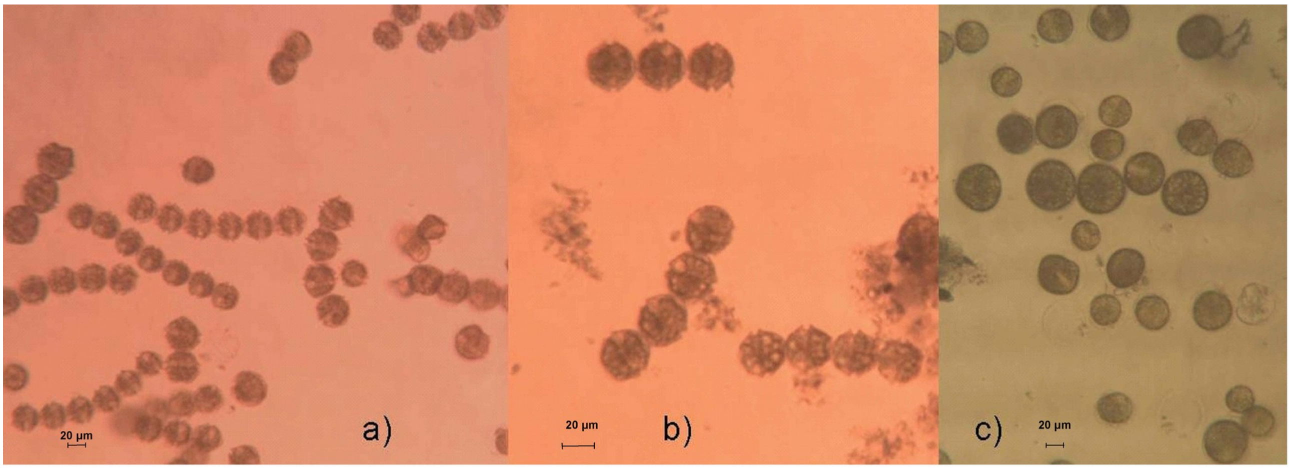

Figure 5. Optical micrographies of *Alexandrium fraterculus* during different stages of the bloom a) initial b) accumulation of reserves c) encystment.

could be useful in the actual context when the coastal zones are constantly exposed to pollutants and it is needed to test their toxicity .

The proximity between the sampling sites makes it possible to think that these patches of *A. fraterculus* were part of one bigger bloom event, covering at least 150 km along the Uruguayan coast, spanning for four weeks in the area. Blooms, like the present one, appear to be more frequent in Uruguay and might be a warning that the region may confront outbreaks of harmful phytoplankton species in high concentrations which may lead to financial losses and social harm for the region.

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