NOTE
BRAZILIAN JOURNAL OF OCEANOGRAPHY, 55(1):57-61, 2007

BLOOM OF *ALEXANDRIUM FRATERCULUS* IN COASTAL WATERS OFF ITAJAÍ, SC, SOUTHERN BRAZIL

Claudia Yuki Omachi, Márcio da Silva Tamanaha & Luís Antônio de Oliveira Proença*

Universidade do Vale do Itajaí
Centro de Ciências Tecnológicas da Terra e do Mar
Laboratório de Estudos sobre Algas Nocivas
(Rua Uruguai, 458, Bloco 19, 88302-202 Itajaí, SC, BRAZIL)

*corresponding author: comachi@gmail.com

Descriptors: Alexandrium, dinoflagellates, bloom, red tide, phycotoxin.
Descritores:

Alexandrium fraterculus (Balech) is a marine dinoflagellate found in warm waters worldwide with its occurrence in the Southwestern Atlantic associated to the Brazilian Current (Balech, 1995). Although no bloom concentrations had been observed until 1995 (Fukuyo *et al.,* 1990; Balech, 1995) it became a phenomenon often observed in New Zealand since 1998 with concentrations frequently higher than an order of 10^3 cells L^{-1} (MacKenzie *et al.,* 2004). In Santa Catarina (SC), a southern Brazilian state, this phytoplankter has been commonly found in coastal waters in low concentrations (Rörig *et al*., 1998; Proença *et al*., 2002). Here we report a bloom of *A. fraterculus* in coastal waters off SC detected in 2004, the first record of this kind in Brazil.

Patches of discoloured water were spotted from the shore in the Cabeçudas beach, Itajaí, SC, on August 19th 2004 and they were sampled in the adjacent beach of Atalaia the day after. These beaches are adjacent to the Itajaí-açu estuary (Fig. 1). Ten days later, discoloured water was spotted again and sampled in the Cabeçudas beach.

Another set of samples from the weekly monitoring programme (WMP), collected between August 12th and September 16th 2004 was also analysed to compare with samples from the blooms. This WMP is run by the UNIVALI for identification of potentially toxic phytoplankton and phycotoxins in Armação do Itapocoroy, Penha, SC (Rörig *et al*., 1998; Proença *et al.,* 1998b) which is about 20 km distant in a straight line from the two bloom sites (Fig. 1).

Samples collected in the blooms were dominated by *A. fraterculus* in chains with up to 60 cells, which were broken in the Lugol´s fixation by the time of counting, one month later. The identification of the species was carried out in the cultured samples. Chains of *A. fraterculus* from both bloom samples were isolated with capillary tubes and incubated in a nutrient enriched seawater medium f/2, 12:12 day:night cycle at 20°C and salinity around 33 to 34. *A. fraterculus* grew well in the culture forming long chains with up to 56 cells (Figs 2a; 2b). The morphology of the main thecal plates (Balech, 1995; MacKenzie *et al.,* 2004) was analysed using a phase contrast Olympus IX50 and a UV epifluorescence Olympus BX40 microscopes on the Calco Fluor stained samples. *A. fraterculus* was identified by examining the apical pore complex, first apical plate and posterior sulcal plate (Balech, 1995; MacKenzie *et al.,* 2004) (Figs 2c; 2d).

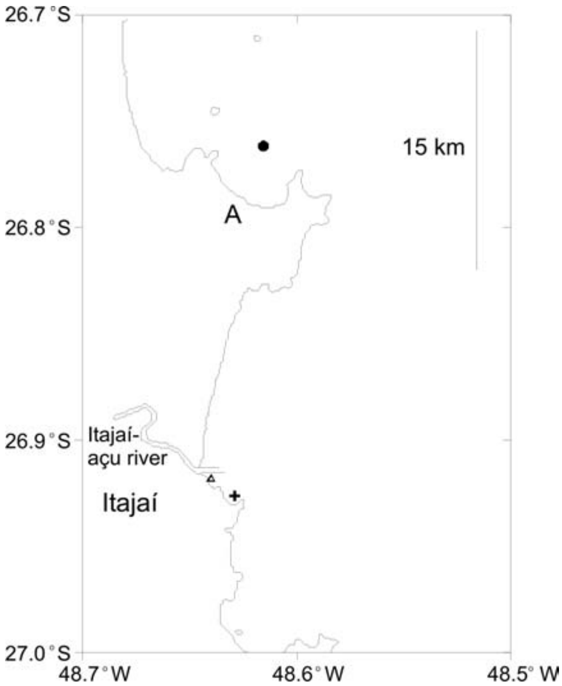

Fig. 1. Bloom sampling sites in Atalaia (triangle) and Cabeçudas (+) beaches, Itajaí - SC and the weekly monitoring site (circle) in Armação do Itapocoroy (A) are indicated.

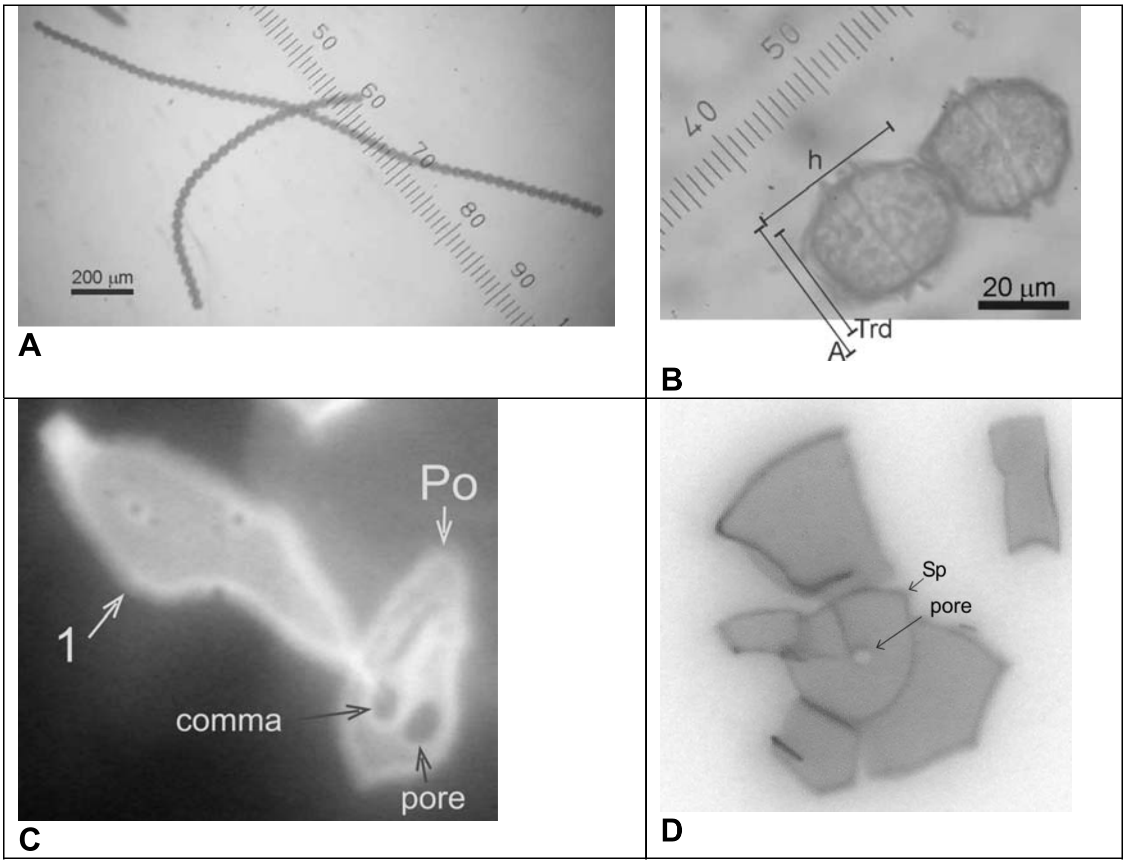

Fig. 2. *A. fraterculus* cells from the culture: a) in chains, b) cells in details (400x): A: width, Trd: transdiameter and h: height (Balech, 1995), c) apical pore complex (Po) with characteristic comma and pore, first apical plate (1) and d) posterior sulcal plate (Sp) with pore, between other plates. Light microscope: a and b. Epifluorescence: c and d.

The chlorophyll *a* concentration (Chl *a*) was analysed by drowning around 80 mL of the water sample onto GF/F Whatman (bloom) or GF50-A Schleicher & Schuell (WMP) glass fibre filters and extracting in 90 % acetone for 24 h at -18°C. It was analysed in a Shimadzu LC 10 HPLC system fitted with a SPDM-10A diode array detector (1 nm resolution) (Proença, 1997) and a 3.5 cm length Shimadzu Shim-pack FLC-ODS column. Chl *a* in the Atalaia beach was the highest reaching 19.0 mg m^{-3} whilst in the Cabeçudas beach it was 4.6 mg m^{-3} (Fig. 3a). Chl *a* from the WMP varied between 1.0 and 2.8 mg m^{-3} with the highest on August 26th (Fig. 3a).

Around 300 mL of the blooms and WMP water samples were preserved in 2 % Lugol and cells of *A. fraterculus* were counted in an inverted microscope Olympus IX50 following Utermöhl (1958) at a final magnification of 100 x (Andersen & Throndsen, 2003). Abundance of *A. fraterculus* was as high as 7.0 x 10^5 cells L^{-1} and 8.8 x 10^4 cells L^{-1} for the Atalaia and Cabeçudas beaches, respectively (Fig. 3b). No cell of *A. fraterculus* was found in the sample from the WMP in August 12th, but they reached 7.0 x 10^3 cells L^{-1} a week later and to the maximum of 1.4 x 10^5 cells L^{-1} on August 26th, coincident with the highest Chl *a* concentration (Fig. 3). On the fifth week after the first detection, no cell of *A. fraterculus* was found in the sample of WMP (Fig. 3b). The difference between the Chl *a* concentration and the cell counting for August 12th and September 16th was because Chl *a* accounted for all the phytoplankton species present in the water sample while the cell counting was carried out for *A. fraterculus* only.

The cell size was determined at a final magnification of 400 x, measuring width (A), transdiameter (Trd) and height (h) (Fig. 2b) following Balech (1995). In general, the cells were wider than long, with variable size and smaller than those described in the literature (Table 1) as more elongated (Balech, 1995; MacKenzie *et al.,* 2004).

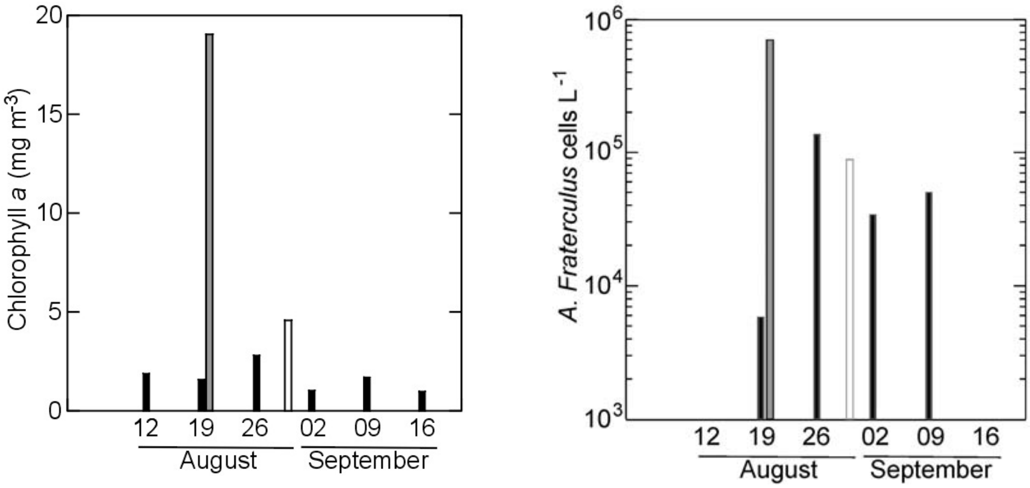

Fig. 3. a) Chl *a* and b) cell counting of *Alexandrium fraterculus* for weekly monitoring programme (black) superimposed with samplings during the bloom events in the Atalaia (gray bar, August 20th) and Cabeçudas (open bar, August 30th) beaches. No cell of *A. fraterculus* was found on the August 12th and September 16th 2004.

Table 1. Comparison of width (A), transdiameter (Trd) and height (h) of cells of *Alexandrium fraterculus* from different sources.

A (µm)	Trd (µm)	h (µm)	Reference
-	28.0 to 43.0	28.0 to 43.0	Fukuyo *et al*. (1990)
29.0 to 47.0	26.0 to 37.5	32.0 to 49.5	Balech (1995)
30.0 to 34.0	-	32.0 to 35.0	MacKenzie *et al.* (2004)
27.5 to 42.5	22.5 to 32.5	25.0 to 40.0	Present work

Phycotoxin mice assays turned negative for paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) (Proença *et al*., 2002) for all the samples, confirming previous studies suggesting that *A. fraterculus* is not linked to PSP toxins (Noguchi *et al*., 1985; Proença *et al.,* 2002; MacKenzie *et al*., 2004). Although non toxic, this species belongs to the toxic genus *Alexandrium* (Balech, 1995).

Filtered seawater samples frozen at -18°C were analysed a month later for nitrate, nitrite, ammonium and phosphate (Strickland & Parsons, 1972). Unfortunately samples collected for silica analysis were lost during the laboratory procedures. Nitrate, nitrite and phosphate (Fig. 4) reached their minimum value a week after the maximum cell concentration and then increased seven times (nitrate) or triplicated (nitrite) by September 16th, when no cell of *A. fraterculus* was found. Nitrate was the main nitrogen form corresponding to more than 60 % of the total dissolved nitrogen (tDN), except on September 2nd, when ammonium accounted for about 94 % of the tDN, but *A. fraterculus* was still densely present in the following week. Ammonium can become dominant when the system becomes anoxic, when algal cells die with subsequent release of ammonium or due to the bacterial activity (Spencer, 1975). Phosphate was always lower than 0.06 µM with a slight decrease on September 2nd. No phosphate measurement was available for September 16th when other nutrients presented maximum values (Fig. 4). The N:P ratio was always bigger (28:1) than the Redfield ratio of 16:1 (Redfield, 1963) with a peak of 93:1 on September 2nd.

Although the interval between the samplings in the WMP was relatively large, i.e. one week, this pattern in the nutrients may be explained by the uptake of nitrate, nitrite and phosphate for the growth of the phytoplankton until the biomass maximum on August 26th; then the subsequent release of ammonium due to the start of the decay of the phytoplankton a week after and when no cell of *A. fraterculus* was detected, three weeks later, the nutrients were high again.

None of the water characteristics analysed (data not shown) could be correlated to the bloom concentrations. Only small changes in salinity (33.9 to 34.2) and depth of Secchi disk (2.5 and 3.2 m) were detected during this period. The temperature increased from 19°C to 21°C between August 26th and September 2nd. These values differ from those when *A. fraterculus* was found in low concentrations (between 20 and 705 cell L^{-1}) during the same WMP in 1997 (Rörig *et al.,* 1998). At that time, water was warmer (21 to 25°C), saltier (33 to 37) and clearer (Secchi disk between 3.3 and 5.0 m) (Rörig *et al.,* 1998).

During August and early September 2004, when *A. fraterculus* was found in high concentrations, the wind was intense, blowing from N, NNE and SSW. In the morning of September 13th, the wind strengthened, its direction changed to ENE and this condition lasted for 36 hours. On September 16th, no cell of *A. fraterculus* was found. These changes in wind conditions may have contributed for the bloom to decay. Similar conditions were observed during the decay of a *Mesodinium rubrum* bloom in the same area in 2001 (Proença, 2004).

Although the bloom lasted for more than four weeks, the weekly sampling resolution during the WMP might not have covered in detail all of its dynamic behaviour. Additionally, other factors like grazing, bacterial activity and environmental factors of the water column should be assessed in a better time resolution if the purpose is to follow the development and decay of a bloom and its controlling factors.

The proximity in space and time between the samplings makes us believe that these three patches of *A. fraterculus* were part of one bigger bloom event, spanning for two to four weeks in an area covering at least 20 km along the coast of the cities of Itajaí and Penha.

The presence of toxic phytoplankton (Rörig *et al.,* 1998; Proença *et al.,* 2001; Schmitt & Proença, 2000), cases of human intoxication (Zenebon & Pregnolatto, 1992) probably due to mussel ingestion in SC (Proença *et al.,* 1998a, b) and blooms, like the present one, might be a warning that the region may face an outbreak of harmful phytoplankton species in high concentrations which may result in financial losses and social harm for the region.

Acknowledgements

The authors would like to thank Sergey Araújo for the wind data measured at CTTMar / UNIVALI and to Valdenir Manoel Inês for his help during the bloom samplings. Comments from two anonymous reviewers led to substantial improvements to the manuscript. C.Y.O. was funded by the Brazilian Government under the CNPq contract 150028/2004-0.

References

Andersen, P. & Throndsen, J. 2003. Estimating cell numbers. In: Hallegraeff, G. M.; Anderson, D. M. & Cembella, A. D. (eds). Manual on harmful marine microalgae. Unesco Publiching, Paris, p. 99-129.

Balech, E. 1995. The genus *Alexandrium* Halim (Dinoglagellata). Cork: Sherkin Island Marine Station.

Fukuyo, Y.; Takano, H.; Chihara, M. & Matsuoka, K. 1990. Red tide organisms in Japan – an illustrated taxonomic guide. Tokyo: Uchida Rokakuho. 430p.

MacKenzie, L.; Salas, M.; Adamson, J. & Beuzenberg, V. 2004. The dinoglagellate genus Alexandrium (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. Harmful Algae. 3:71-92.

Noguchi, T.; Maruyama, J.; Ikeda, T.; Fukuyo, Y. & Hashimoto, K. 1985. *Protogonyaulax fractercula* as a nontoxic plankton. Bull. Japan. Soc. Sci. Fish., 51(8):1373.

Proença, L. A. O. 1997. Separação de pigmentos fotossintéticos do fitoplâncton por meio de cromatografia líquida de alta eficiência (CLAE). Notas Téc. FACIMAR, 1:23-31.

Proença, L. A. O. 2004. A red water caused by *Mesodinium rubrum* on the coast of Santa Catarina, southern Brazil. Braz. J. Oceanogr., 52(2):153-161.

Proença, L. A. O.; Lagos, N.; Rörig, L. R.; Silva, M. & Guimarães, S. 1999. Occurrence of paralytic shellfish poisoning – PSP in southern Brazilian waters. Ciência e Cultura, 51(1): 16-21.

Proença, L. A.; Rörig, L. R.; Barreiros, M. A. & Lagos, N. 1998a. A possible case of diarrhetic shellfish poisoning in Santa Catarina, southern Brazil. In: Congresso Latino-Americano de Ficologia, 4. Caxambú-MG. Anais 2:259-263.

Proença, L. A. O.; Schmitt, F.; Costa, T. & Rörig, L. R. 1998b. Just a diarrhea? Evidences of diarrhetic shellfish poisoning in Santa Catarina, Brazil. Ciência e Cultura, 50(6):458-462.

Proença, L. A. O.; Tamanaha, M. S. & Souza, N. P. 2001. The toxic dinoflagellate *Gymnodinium catenatum* GRAHAM in Southern Brazilian waters: occurrence, pigments and toxins. Atlântica, 23:9-65.

Proença, L. A. O.; Tamanaha, M. S. & Resgalla Jr., C. 2002. Toxicity of the aqueous extract of *Alexandrium fraterculus* Balech. In: International Conference on Harmful Algae, 10. St Petersburg, EUA.

Redfield, A. C.; Ketchum, B. H. & Richards, F. A. 1963. The influence of organisms on the composition of sea water. In: Hill, M.N. (ed.) The Sea. New York: Interscience. v. 2:26-77.

Rörig, L. R.; Guimarães, S. C. P.; Lugli, D. O.; Proença, L. A. O.; Manzoni, G. C. & Marenzi, A. C. 1998. Monitorização de microalgas planctônicas potencialmente tóxicas na área de maricultura da enseada de Armação do Itapocoroy-Penha-SC. Notas Téc. FACIMAR, 2:71-79.

Schmitt, F. & Proença, L. A. O. 2000. Ocorrência de dinoflagelados do gênero *Dinophysis* (Ehrenberg, 1839) na enseada de Cabeçudas (verão e outono de 1999). Notas Téc. FACIMAR, 4:49-59.

Spencer, C. P. 1975. The micronutrient elements. Riley, J. P. & Skirrow, G. eds. Chemical Oceanography. London: Academic Press. 1087p.

Strickland, J. D. H. & Parsons, T. R. 1972. A practical handbook of seawater analysis. 2^{ed}. Ottawa: Fisheries Research Board of Canada.

Utermöhl, H. 1958. Zur vervollkomnung der quantitativen phytoplankton metodik. Mitt. Int. Ver. Limnol., 9:1-38.

Zenebon, O. & Pregnolatto, N. P. 1992. Memórias técnico-científicas da divisão de Bromatologia e Química. São Paulo: Instituto Adolfo Lutz: 100 anos do laboratório de Saúde Pública. Edição Comemorativa. p. 173-198.

(Manuscript received 04 November 2005; revised 23 June 2006; accepted 21 December 2006)