Protoceratium reticulatum bloom in NW Iberia mid-shelf waters

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Abstract

The yessotoxin (YTX)-producing dinoflagellate *Protoceratium reticulatum* is a cosmopolitan species occasionally observed in Portuguese coastal waters. In September 2019, and for the first time, a bloom was detected during a cruise carried out offshore F. Foz, Portugal (latitude 40º13´ N). We sampled a cross shelf section three times in one week, and the results revealed the bloom was already present at mid-shelf in stratified warm waters and was separated from the coast by coastal upwelling waters. Wind data, sea surface temperature (SST) and chlorophyll *a* (Chl *a*) satellite images indicated that two days prior to the cruise there was a short but strong upwelling event and that, in the leeward side of an upwelling plume rooted at cape Mondego, there was a large patch of Chl *a*. The *P. reticulatum* bloom coincided with the northern side of this patch. It was distributed above the pycnocline, in waters with temperatures from 14º to 17ºC, reaching maxima of 2,250 cells L^{-1} at the surface (17 ºC). During the upwelling relaxation conditions observed until the end of the cruise, the bloom approached the coast although being observed in low numbers at the most coastal station. Cysts of *P. reticulatum* were observed in the water column, mainly at the end of the cruise, suggesting encystment was occurring. *P. reticulatum* co-occurred within a dense mixture of diatoms and dinoflagellates, in particular other HAB species such as a *Dinophysis acuta* (22.4 x 10^3 cells L^{-1}) and *Pseudo-nitzschia seriata* group (137 x 10^3 cells L^{-1}).

Keywords: Protoceratium reticulatum, NE Atlantic, Iberian upwelling system, encystment

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Introduction

Protoceratium reticulatum (Claparède & Lachmann) Bütschli 1885, is a cosmopolitan dinoflagellate with a life cycle that includes planktonic and benthic cyst stages. Strains and isolates of *P. reticulatum* from different regions of the world showed the species comprises at least three ribotypes corresponding to cold, moderate and warm ecotypes, with optimum growth respectivelfy at 15 °C, 20 °C and from 20 to 25 °C (Wang *et al*., 2019). The species is a yessotoxin-YTX producer (Satake *et al*., 1997) and blooms have been associated with shellfish diseases and mass mortalities worldwide (Cassis, 2005; King *et al*., 2021).

On the west coast of Portugal *P. reticulatum* vegetative cells have been reported in plankton samples since the 80´s, although seldom observed (Moita and Vilarinho, 1999). The species was also reported more recently in south Iberian Atlantic coastal waters (Paz *et al*., 2007; Santos *et al*., 2021). Despite the few references on the presence of this species in the water column, historical cyst records in sediment cores indicate that *P. reticulatum* has been a common species in Atlantic Iberian phytoplankton assemblages in the last two centuries (Amorim and Dale, 2006; Ribeiro *et al*., 2016; García-Moreiras *et al*., 2019) with a distribution center around 39°51.0′ N, 9°17.0′ W, close to major discontinuities in bottom topography (Nazaré Canyon) and coastal morphology (Estremadura Promontory) (Ribeiro *et al*., 2016). Similar results were obtained in studies based on surface sediments along the W Atlantic Iberian coast. In 1996, a cyst survey covering inshore stations along the coast showed a maximum relative abundance of *P. reticulatum* (24%) at 39°21.1' N (Amorim, 2002). This was also reported by Sprangers *et al*. (2004) from box core surface sediments taken offshore NW Iberia where this species reached the highest relative abundances (> 50%) at the latitude of the Nazaré Canyon (39°35.4' N). In 2019, a surface cyst survey covering the shelf between 40°07' N and 40°49' N (F. Foz and Aveiro) revealed the species maxima were located at mid-shelf, in muddy sediments, reaching 25% of total cysts at 40°12' N (García-Moreiras *et al*., 2021).

This paper describes the oceanographic conditions associated with the first *P. reticulatum* bloom detected in Portuguese shelf waters during the last 40 years of studies.

Material and Methods

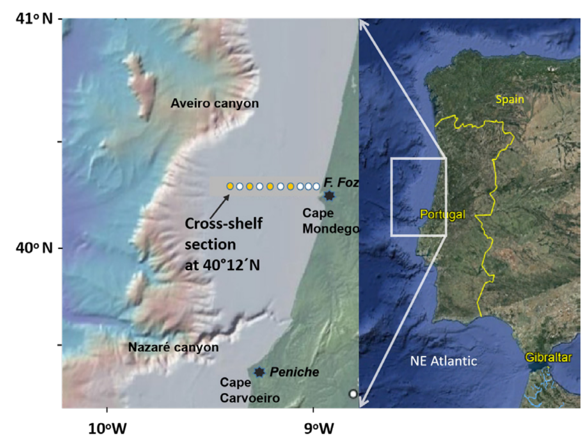

Fig. 1. Map with the location of sampling stations: phytoplankton and nutrients (white circles); CTD casts (yellow and white circles).

Study area

The NW coast of Portugal is part of the north Atlantic Iberian upwelling system, where seasonal upwelling is induced by northerly winds from late spring to early Autumn

(Fiuza *et al.*, 1982). The study was conducted where the NW Portuguese shelf is wider, located between two submarine canyons, and where stratification conditions are enhanced during summer (Fig. 1). Under upwelling conditions, that area can be limited by two major upwelling filaments, rooted at F. Foz (at Cape Mondego) and Aveiro, between which, there is a recurrent lower average summer frontal probability (Relvas *et al.*, 2007). This means that it can be described as a mid/outer shelf stratified pool surrounded by nutrient upwelled enriched waters, that could favour the development of populations of autotrophic dinoflagellates.

Data and methods

Data are from a cruise onboard of the NRP Auriga that covered a cross shelf section, at 40º12´ N, three times during the same week (12, 14 and 19 Sept 2019) (Fig. 1). Water column temperature and salinity were profiled with a CTD Ocean Seven 320Plus Woce, Idronaut, coupled with a Seapoint nephelometer. Water samples were collected with Niskin bottles at 5 m, 10 m, 20 m, 30 m, 50 m and 75 m (or near bottom in shallower areas). Phytoplankton water samples were preserved with hexamethylenetetramine buffered formalin and the cells were counted in 50 mL subsamples by the Utermöhl technique (Hasle, 1978). Different cells isolated from water samples were analyzed under a Scanning Electron Microscope (SEM). The identification of *P. reticulatum* was based on the morphologic characteristics of cells isolated from the cruise and observed under SEM (*e.g.* epitheca with six pre-cingular plates) as on the presence of cysts with a characteristic archeopyle and typical hooked processes.

Water samples for nutrient determination were preserved at temperatures below -20 °C and the analyses were performed within 1 month after sampling. Nutrients (nitrate plus nitrite - NO_x, ammonia - NH_4, reactive phosphorus - PO_4, reactive silica - SiO_2) were analysed by UV/vis spectroscopy and using a Skalar SANplus Segmented Flow autoanalyser (see Borges *et al.*, 2019).

To provide information on the regional and large-scale patterns before the surveys, synoptic maps of satellite-derived SST and Chl *a* were produced. The SST data, retrieved from the Sea and Land Surface Temperature Radiometer (SLSTR) at ~1 km resolution, were obtained from EUMETSAT. The Chl *a* data at 300 m resolution were provided by the PML Remote Sensing Group / Natural Environment Research Council (NERC) Earth Observation Data Acquisition and Analysis Service (NEODAAS).

Results and Discussion

Figure 2 shows that three upwelling favourable wind events (black arrows) occurred since the 30th August until the beginning of the cruise, led to a decrease of about 2.5º C (red arrow) in SST. After the first cross shelf section coverage, on Sept 12th, upwelling relaxation conditions prevailed up to the end of the cruise. Those upwelling vs. downwelling conditions are also visible on the satellite SST images (Fig. 3). The spatial distribution of the upwelling filament was complex and quite parallel in NE-SW direction as highlighted by the dashed line on the left top image (SST on Sept 4th.). The bottom images in figure 3 show that on the leeward side of those filaments (highlighted by the dashed lines), there is a progressive increase in chl *a* that reached concentrations higher than 5 mg m^{-3}.

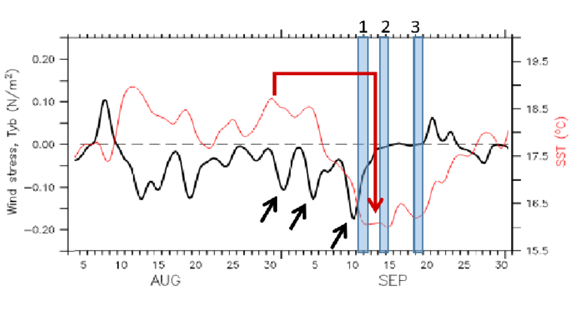

Fig. 2. Daily meridional wind stress anomaly (black) and SeaSurface Temperature (red) during August and September 2019. Blue columns indicate sampling dates.

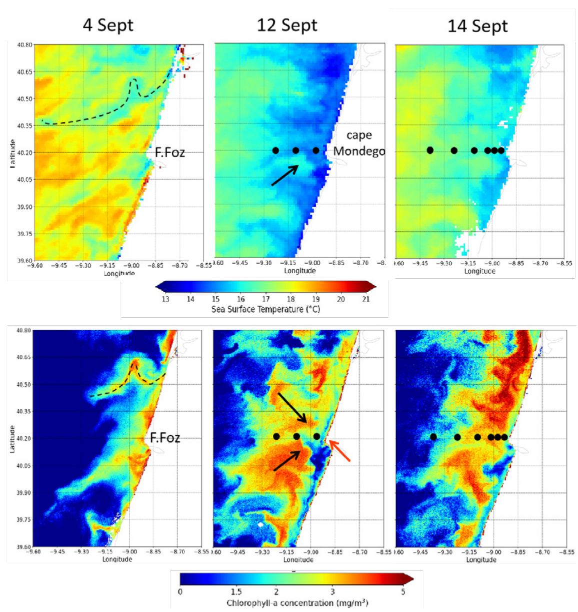

Fig. 3. Maps of satellite SST (ºC) (top) and Chl *a* concentration (mg m^{-3}) (bottom), one week before the beginning of the cruise (12th Sept 2019) and two days after (adapted from Nunes, 2021).

On Sept 12th, the sampling section was very close to the cape Mondego filament. To the south of the filament, a pool of warmer water can be observed (black arrow) and the filament seems to interrupt the Chl *a* patch. Another important feature to retain from the images on Sept, 12th and 14th is that, the main chlorophyll patches are displaced from the coast (red arrow) by the colder coastal upwelled waters. The vertical distribution of water density (σ_T) (Fig. 4A) followed the temperature (see Sept 14th on Fig. 4B), due to a very small variation in salinity (35.75 to 35.9). The water column was stratified with upwelling of colder waters close to the coast on the first two days. On the third day there is clear upwelling relaxation. The distribution of NO_x and PO_4 shows the water column was not nutrient depleted, even at midshelf which is not a common feature at this time of the year above the pycnocline (Moita, 2001).

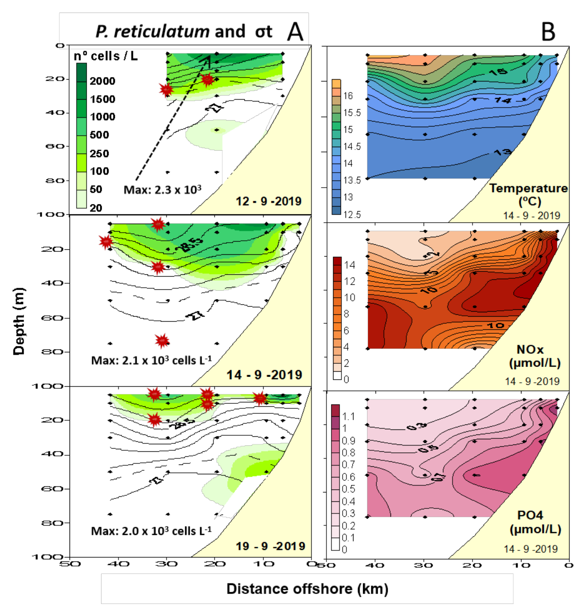

Fig. 4. Cross-shelf distribution of: (A) *P. reticulatum* (green) underlying density anomaly - σ□ (isolines) on Sept 12, 14 and 19th; Red stars indicate *P. reticulatum* cysts presence in the water; (B) Temp., NO_x and PO_4 on Sept 14th.

The *P. reticulatum* bloom was already established at mid-shelf (Fig. 4A) on Sept 12th, in waters with temperatures from 14 ºC to 17 ºC, and was separated from the coast by colder upwelled waters more visible on Sept 14th. With the upwelling relaxation, the bloom approached the coast never exceeding 200 cells L^{-1} at the innermost station. All the observed cysts (red stars in Fig. 4A) had cell contents and were distributed above the picnocline matching the distribution of the planktonic stages. This suggests that cysts originated from encystment in the water column and not by cyst resuspension from sediments.

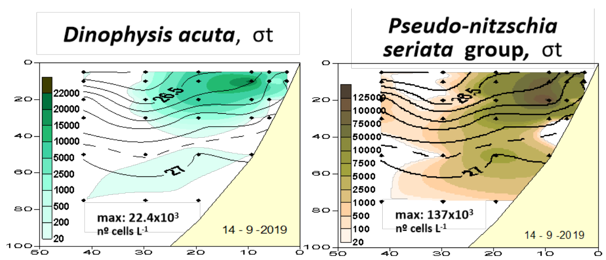

Fig. 5. Cross-shelf distribution of *D. acuta* and *P. seriata* group underlying density anomaly (σT).

Protoceratium reticulatum reached its maximum (2.3 x 10^3 cells L^{-1}) on Sept 12th, at the surface (5 m depth), while other HAB accompanying species such as *D. acuta* and *P. seriata* group had their maxima on Sept 14th and were observed below *P. reticulatum* in the water column. *Dinophysis acuta* reached one of the highest maxima for the region, at lower depths than usually reported (10 m instead of the pycnocline). Despite the bloom approaching the coast on Sept 19th, the HAB monitoring program did not detect *P. reticulatum* cells nor yessotoxins in shellfish, which may reflect the low species concentrations observed at the innermost stations (https://www.ipma.pt/pt/bivalves/fito/index.jsp).

This is the first reported *P. reticulatum* bloom on the Portuguese coast, with concentrations close to those described to cause yessotoxin shellfish contamination in Norway (Aasen *et al*., 2004). Very little is known on *P. reticulatum* in Iberian waters. Work is now ongoing to investigate its ribotype and yessotoxin profile. This work also describes, for the first time in the study area, at the end of summer and after important upwelling events, that the presence of stratified waters not nutrient depleted at mid-shelf were able to support dense phytoplankton blooms at the surface (> 5 mg m^{-3} Chl *a*). These blooms were characterized by a mixture of diatoms with autotrophic and heterotrophic dinoflagellates.

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References

Aasen, J., Samdal, I.A., Miles, C.O., Dahld, E., *et al*., (2005). Toxicon 45, 265-272.

Amorim, A. (2002). Ph. D Thesis, University of Lisbon, Lisbon. Portugal. 161 pp.

Amorim, A. and Dale, B. (2006). Afr. J. Mar. Sci. 28, 193-197.

Borges, C., Palma, C., da Silva, C.B. (2019). Anal. Chem. 91, 5698-5705.

Cassis, D. (2005). M.S. Thesis, The Faculty of Graduates Studies (Oceanography). The University of British Columbia, Vancouver, p. 73.

Fiúza, A., Macedo, M.E., Guerreiro, M.R. (1982). Oceanol. Acta 5, 31-40.

García-Moreiras, I., Delgado, C., Martínez-Carreño, N., García-Gil, S., Muñoz Sobrino, C. (2019). Glob. Planet. Change 176, 100-122.

Hasle, G.R. (1978). *In*: Sournia A (ed). Phytoplankton Manual. VI. Monographs on Oceanic Methodology. UNESCO, Paris, pp. 191-196.

Moita, M.T. and Vilarinho, M.G. (1999). Portugaliae Acta Biol., Sér. B, Sist. 18, 5-50.

Moita, M.T. (2001). Ph. D Thesis, University of Lisbon, Lisbon. Portugal. 272 pp.

Nunes, P. (2021). MSc Thesis, University of Lisbon, Lisbon. Portugal, 53 pp.

Paz, B., Riobó, P., Ramilo, I., Franco, J.M. (2007). Toxicon 50, 1-17.

Relvas, P., Barton, E.D., Dubert, J., Oliveira, P.B., *et al*., (2007). Prog. Oceanogr. 74, 149-173.

Ribeiro, S., Amorim, A., Abrantes, F., Ellegaard, M. (2016). The Holocene 26, 874-89

Santos, M., Moita, M.T., Oliveira, P.B., Amorim, A. (2021). J. Sea Res. 167, 101982.

Satake, M., MacKenzie, L., Yasumoto, T. (1997). Nat. Toxins 5, 164-167.

Sprangers, M., Dammers, N., Brinkhuis, H., van Weering, T.C.E., Lotter, A.F. (2004). Rev. Palaeobot. Palynol. 128, 97-106.

Wang, N., Mertens, K.N., Krock, B., Luo, Z., *et al*., (2019). Harmful Algae 88, 101610.