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Bloom of the Yessotoxin producing dinoflagellate *Protoceratium reticulatum* (Dinophyceae) in Northern Chile

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ABSTRACT

In summer 2007, a dinoflagellate preliminarily identified as *Protoceratium reticulatum* bloomed in Bahía Mejillones, northern Chile. Phytoplankton samples were analyzed in detail by light and scanning electron microscopy revealing the presence of resting cyst and motile cells of *P. reticulatum*. Oceanographic and phytoplankton data suggest that the bloom was initiated offshore by motile cells and germinated cysts during an upwelling pulse. These cells were advected into the bay when upwelling relaxed and grew without any relevant competitor. Phytoplankton net samples were found to contain yessotoxin as the only toxin in an estimated proportion of 0.2 and 0.4 pg cell^{-1}, thus confirming that *P. reticulatum* is a source of yessotoxin in northern Chilean waters and consequently that it poses a risk for human health and mollusk exploitation in the area.

1. Introduction

Harmful Algal Blooms (HABs) are phenomena produced by the proliferation and occasional dominance of particular species of toxic or harmful algae. The outbreaks of these species have become a serious threat to aquaculture and resource exploitation in coastal upwelling systems and other coastal regions (Crespo and Figueiras, 2007; Kudela et al., 2005; Trainer et al., 2010). These impacts range from mortalities of wild and farmed shellfish or fish to illness and death of humans and marine mammals due to potent toxins (Anderson, 2007; Blanco, 2001; Hallegraeff, 1993; Landsberg, 2002).

The coastal upwelling system of Chile constitutes a large part of the Humboldt Current System and is considered one of the most productive fishery regions in the world oceans (Sherman et al., 1993; Thiel et al., 2007). Due to its high productivity, this upwelling area is susceptible to HABs (Pitcher and Pillar, 2010; Trainer et al., 2010).

Some of the most important types of HABs have been detected in this upwelling area (Trainer et al., 2010). A number of toxins are involved in them, including domoic acid, saxitoxin and their analogs, pectenotoxins, azaspiracids, spirolides and yessotoxins (Álvarez et al., 2009a, 2009b, 2010; Blanco et al., 2007; Krock et al., 2009; López-Rivera et al., 2009).

In the northern Chilean coast, one of the most important upwelling centers is located in the Mejillones Peninsula, Antofagasta (23°S) (D'Aubarede, 1967; Marín et al., 1993, 2003). In this area, the plankton community is usually dominated by diatoms throughout the year, but dinoflagellates or ciliates can also bloom, especially during summer (Avaria and Muñoz, 1983, 1985; Avaria et al., 1982; Marín and Olivares, 1999). The most important bloom-forming species detected to date were the ciliate *Myrionecta rubra* (Marín et al., 1993) and the dinoflagellate *Prorocentrum micans* (Rodríguez et al., 1991). Recently, a bloom of the dinoflagellate *Alexandrium tamarense* has been reported and was associated with the presence of paralytic shellfish toxins in two bivalves, the scallop *Argopecten purpuratus* and the mussel *Semimytilus algosus* (Álvarez et al., 2009b).

Protoceratium reticulatum (Claparéde and Lachmann) Bütschli is a species present in Northern Chile known to have the capability of producing HABs in different areas around the world. This organism has been associated with a mass mortality of marine fauna in South Africa (Grindley and Nel, 1970; Horstman, 1981). Since these reports, the presence of HABs produced by *P. reticulatum* has been observed in New Zealand (MacKenzie et al., 1998), Canada (Cassis, 2005), Norway (Aasen et al., 2005) and Japan (Koike et al., 2006).

Satake et al. (1997), using isolates obtained in New Zealand waters identified *P. reticulatum* as the first yessotoxin producer. Yessotoxin (YTX) is a marine polyether compound isolated in 1986 from the scallop *Patinopecten yessoensis* in Japan by Murata et al. (1987). Numerous analogs and derivatives have been described (reviewed by Paz et al. (2008)). These toxins have been included within the

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Diarrhetic Shellfish Poisoning (DSP) group because they yield positive results in the traditional mouse bioassay for DSP toxins (Aune et al., 2002; Terao et al., 1990). Nevertheless, recent evidence suggests that YTXs should be excluded from the DSP group because these compounds do not produce diarrhea or inhibit protein phosphatase activity (reviewed by Paz et al. (2008)). YTXs have been shown to have cardiotoxic effects in mice (Aune et al., 2002; Terao et al., 1990) and to be potent cytotoxins (Pérez-Gómez et al., 2006). These effects prompted the European Authorities to establish a maximum permitted level in shellfish of 1 mg YTX equivalents kg^{-1} (EFSA, 2009).

After the first report in New Zealand waters, this group of compounds has been recorded in *P. reticulatum* from different areas around the world, including Japan (Eiki et al., 2005), Italy (Ciminiello et al., 2003), Canada and UK (Stobo et al., 2003), Norway (Samdal et al., 2004), North America and Spain (Paz et al., 2007). *P. reticulatum* is not the only YTX producer and currently, *Lingulodinium polyedrum* (Stein) Dodge (Paz et al., 2004) and *Gonyaulax spinifera* (Claparede and Lachmann) Diesing (Rhodes et al., 2006; Riccardi et al., 2009) have also been reported to produce these toxins.

In Chile, a country with over 4000 km of coastline, the presence of YTX has been reported in two very distant locations: in the Chonos Archipelago and Bahía Arica. That is, from latitudes as different as 43 and 18 ºS, respectively. In the Chonos Archipelago, YTX was detected in *Mytilus chilensis* (Yasumoto and Takizawa, 1997) and, some years later, cysts and motile cells of *P. reticulatum* were also found (Seguel and Sfeir, 2003, 2005), pointing (but not providing unequivocal evidence) to this species as the YTX producer. Recently, YTX has also been detected in Bahía Arica (18°38′S, 70°21′W), where it was associated with the presence of cysts of *P. reticulatum* (Krock et al., 2009). It is not known if YTX is present in intermediate latitudes or whether *P. reticulatum* in the area produces the toxin, and if so, if toxic *P. reticulatum* can bloom in Chilean waters producing HABs or if it is only a minor co-occurring species in phytoplankton blooms.

The aim of this study was to describe, from a taxonomic, oceanographic and toxicological point of view, the first *P. reticulatum* bloom detected in one of the most important upwelling centers of the area, which is located in the Mejillones Península, Antofagasta.

2. Material and methods

2.1. Study area

Bahía Mejillones (23º10′ S, 70º45′ W) is located in northern Chile in the central region of the coastal Humboldt Current upwelling system. The bay is open to the North and closed to the South by the Mejillones Península (Fig. 1). It comprises an area of about 285 km^2, with a maximum depth of 120 m (Cerda et al., 2010). In this area, upwelling occurs through the year with a maximum and minimum intensity in October and January, respectively (Marín and Olivares, 1999; Navea and Miranda, 1980; Rodríguez et al., 1991). The annual cycle is characterized by a cold mixing period in winter, followed by a stratification period during spring and summer, with mean sea surface temperatures of 13.5 °C and 22 °C, respectively (Escribano, 1998). Dissolved oxygen concentrations in the water column are low with values near 5 mg L^{-1} at the surface and 0.1 mg L^{-1} at depths greater than 50 m (Apablaza and Palma, 2006; Morales et al., 1996; Rodríguez et al., 1986).

2.2. Environmental variables

Oceanographic data were collected from January 28th to March 9th, 2007 at one station (23°5′ 24″S; 70° 28′ 44″ W). Temperature and dissolved oxygen were measured with a YSI 550A oxygen meter at 1, 2, 5, 10 and 15 m depth. In addition, water temperature was recorded every hour throughout the sampling period with a StowAway Tidbit underwater Temp Data Logger with sensors located at 5, 10 and 15 m.

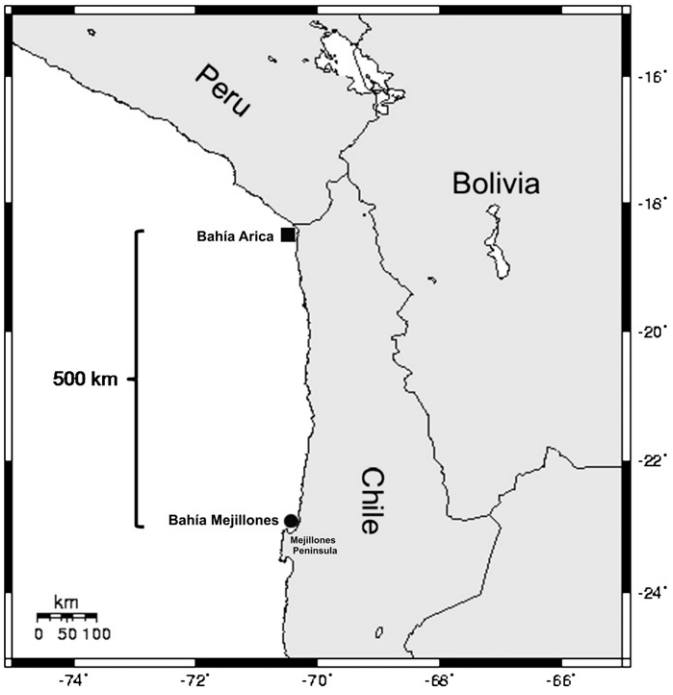

Fig. 1. Location of the sampling station at Bahía Mejillones (circle). Bahía Arica (square) represents a site on the northern Chilean coast where yessotoxin was previously detected.

2.3. Satellite-derived sea surface temperature (SST)

Satellite measurements of sea-surface temperature (SST) were obtained daily from February 5th to March 9th, 2007 by the Advanced Very High Resolution Radiometer (AVHRR) onboard the NOAA satellites, which have a resolution of 0.1 °C and 1.1 km. Normalization and calibration of AVHRR data have been made by CTD temperature measurements obtained *in situ* in the first 5 m of water column (Pettiagiani et al., 1992).

Data recorded during the sampling period were processed by Remote Sensing and Geographic Information System Laboratory of the Pontificia Universidad Católica de Valparaíso, Chile. Processed data were used to create four weekly images using the RECLASS module of the GIS IDRISI software (Silva et al., 2003) at the Geographic Information System Laboratory of the Instituto de Fomento Pesquero, Chile.

2.4. Biological samples and phytoplankton quantification

Phytoplankton samples were collected once a week from January 28th to March 9th, 2007, by means of vertical net hauls (20 μm mesh) and a 15 m hose, in order to obtain integrated samples of the entire water column. The samples were collected at the same station as the oceanographic data. Two aliquots were preserved – one with formaldehyde 4% (net hauls) and another with lugol's iodine (hose) – for taxonomic and quantitative analyses, respectively. Phytoplankton composition and abundance of *P. reticulatum* motile cells and cysts were quantified using the Utermöhl method, described by Hasle (1978), using 20-mL sedimentation chambers with an Olympus IX71 inverted microscope at 400× magnification.

2.5. Protoceratium reticulatum taxonomic analyses

For identification of *P. reticulatum*, some cells were placed on microscope slides with a drop of sodium hypochlorite to separate the thecal plates.