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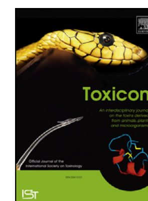

Short communication

Toxic *Trichodesmium* bloom occurrence in the southwestern South Atlantic Ocean

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A B S T R A C T

Harmful *Trichodesmium* blooms have been reported on the continental slope of the southwestern South Atlantic Ocean; we sampled six such blooms. The highest saxitoxin concentration was observed where the number of colonies was proportionally greater relative to the total density of trichomes. *Trichodesmium* blooms are harmful to shrimp larvae and may lead to plankton community mortality. This study is the first record of neurotoxic blooms in the open waters of the South Atlantic.

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The genus cyanobacterium *Trichodesmium* forms extensive blooms on the ocean's surface in tropical and subtropical regions and coastal and oceanic areas with water temperatures greater than 20 °C, strong water column stratification and low nutrient levels (Hood et al., 2004; Fernández et al., 2010).

On the east coast of Brazil, *Trichodesmium* blooms have been reported during spring and summer seasons (Sato et al., 1963; Gianesella-Galvão et al., 1995; Rorig et al., 1998a, 1998b; Naithirithi et al., 2005; Silva, 2005; Siqueira et al., 2006; Carvalho et al., 2008; Proença et al., 2009). These studies describe cyanotoxin production by *Trichodesmium*, which produces allergenic symptoms (Silva, 2005) and neurotoxins (Proença et al., 2009).

Trichodesmium blooms can be harmful for pelagic organisms that eat cells containing toxins but also by large accumulation biomass that clog fish gills and then lead to mortality in fish and shrimp larvae (D'Silva et al., 2012). Nevertheless, Preston et al. (1998) and Negri et al. (2004) stated that shrimp larvae and juvenile oysters did not exhibit poisoning or damage, but they did not consume sufficient nutrients for larval development. *Trichodesmium* is a nutritionally poor source for a predator's metabolism compared with other phytoplankton genera. Silva (2005) reported that, a few days after a major *Trichodesmium* bloom on the southeast coast of Brazil, dead bryozoans covered beaches, extending for kilometers. However, few quantitative data are available on cyanotoxin concentrations associated with *Trichodesmium* blooms in the open sea.

Fourteen records of extensive blooms in the open sea within the study area were sampled by four oceanographic cruises over three consecutive years (2012–2014). In December 2014, six large *Trichodesmium* blooms were observed and sampled in the continental shelf-break area between isobaths at 200 m and 2000 m (Fig. 1). Our aim is to report the first occurrence of large, harmful *Trichodesmium* slicks on the shelf-break between the latitudes 26°S and 28°S in the southwestern South Atlantic Ocean. This study also investigates and quantifies on potential neurotoxins in *Trichodesmium* blooms (see Fig. 2).

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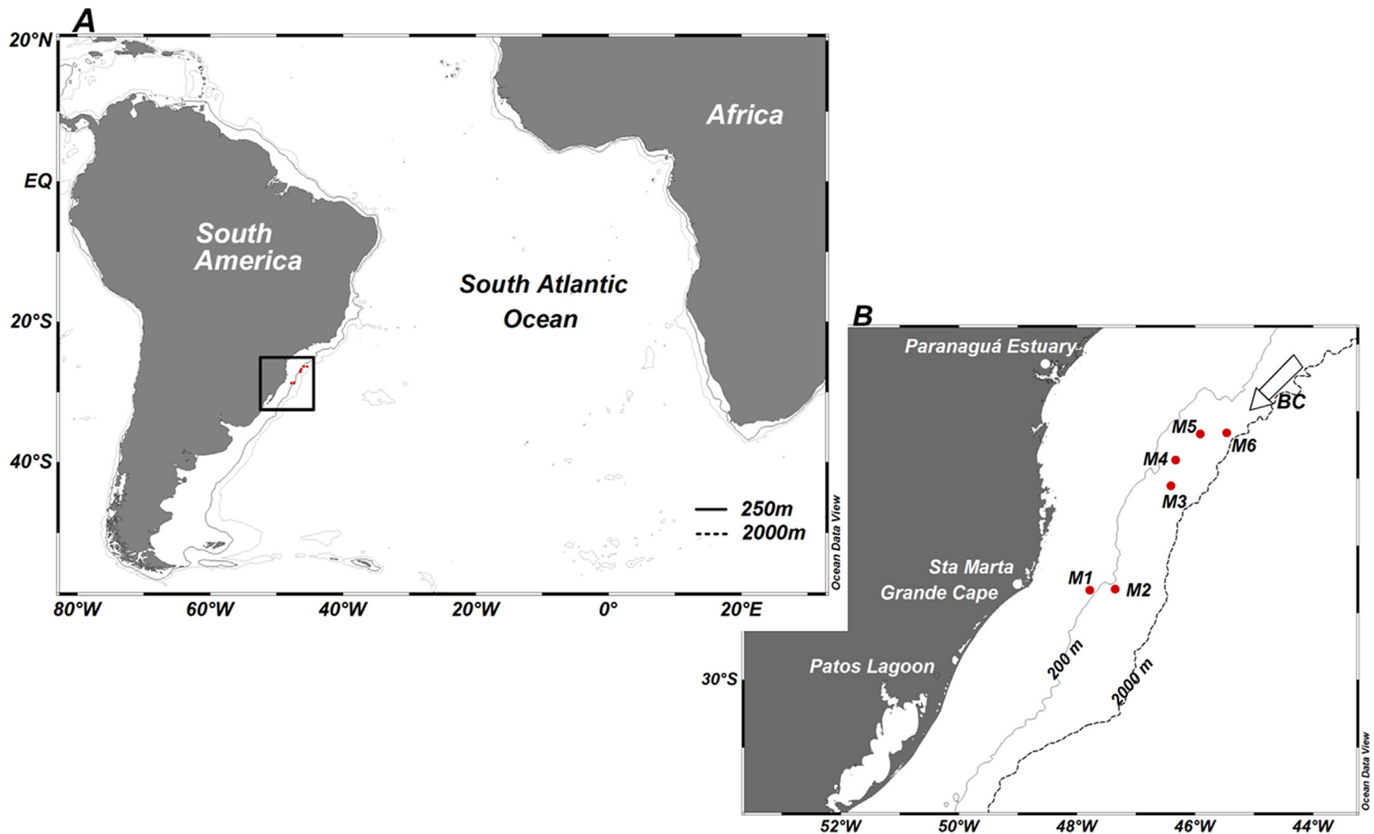

Fig. 1. Map of the South Atlantic Ocean. The black square in (A) shows the sampled area in the southwest of the South Atlantic Ocean (B). A map of the sampled area; the bloom locations are indicated with red dots. The black arrow indicates the direction and location of the Brazil Current (BC) flow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The surface water temperature and salinity were measured using the profiler sensor CTD (SeaBird CTD/Carrousel 911 + system®). At each *Trichodesmium* slick, we collected a water sample 15 m deep using a Van-Door bottle for inorganic dissolved-nutrient analyses, including analyses for phosphate ($PO^{-3}{}_4$), nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4). The sample was filtered through a cellulose acetate filter (0.45 μm), and the filtrate was immediately frozen in a freezer for later analyses in the laboratory. The wind speed was obtained from the Advanced Scatterometer (ASCAT) aboard the Meteorological Operational Polar Satellite (MetOp-A) with the spatial resolution 0.25°. The data were acquired from the ftp://ftp.ifremer.fr/ifremer/cersat/products/gridded/mwf-ascat/data/daily/Netcdf/site of the Laboratoire D'Oceanographie Spatiale, the French Research Institute for Exploitation of the Sea.

Samples from the *Trichodesmium* bloom surface were preserved in a 4% formalin solution in dark glass bottles. To count the colonies and trichomes, we added 1 mL of acetic acid to collapse the gas vesicles in the *Trichodesmium* cells, which facilitated sedimentation (adapted from Cronberg et al., 2004).

The trichomes and colony counts were performed in the settling chamber using the Utermöhl method (1958) with a 100x increase by an inverted microscope. The trichomes and colonies were counted in half or full chambers. For the total trichome count, the number of free trichomes was added to the number of colonies and converted into the trichome equivalent per liter using the value 200 trichomes col^{-1} (Carpenter, 1983). For conversion to cells it was assumed, on the basis of previous measurements, that there were 120 cells per trichome (Carpenter and Romans, 1991).

The *Trichodesmium* bloom samples were collected at the surface water using a bucket and were filtered using glass fiber filters (0.7 μm) GF/F (Whatman®) at volumes ranging from 60 to 470 mL until the filter was clogged. To remove the sea salt, the filters were washed with distilled water and transported in liquid nitrogen to the laboratory and then maintained in an ultrafreezer at −80 °C until they were analyzed. The presence of cyanotoxins was investigated using high-performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection (Rourke et al., 2008).

For the total saxitoxin analyses, variants of Gonyautoxin standards (GTX-1, GTX-2, GTX-3, GTX-4 and GTX-5) and three saxitoxins (Neosaxitoxin (NeoSTX), Decarbamoylsaxtoxin, and DcSTX) were used in addition to the saxitoxin (STX) standard. All standard analyses were done in triplicate. Control experiments (mobile phase and 0.05 N hydrochloric acid) were also performed in triplicates. Further, the chlorophyll-a pigments and their degradation products we analyzed by HLPC following the method described in Mendes et al. (2007).

A *Trichodesmium* bloom sample was collected using a bucket, concentrated in a 50 μm plankton net and washed with distilled water to remove the sea salt. In the laboratory, the sample was lyophilized to dry in a lyophilizer (Edwards Micromodulyo). The lyophilized powder was used to test for toxicity in a 3.5-mg mL^{-1} seawater solution. The equivalent saxitoxin content in the lyophilized powder was determined using an extract solution composed of 76 mg ml-1 at 0.05 N HCL. The toxicity experiments were performed using post-larvae (45 days old) of the white shrimp *Lithopenaeus vannamei*. The experiments were performed over 96 h at the concentrations of 0; 0.22; 0.44; 0.88; 1.75; 3.50 mg mL^{-1} of seawater and were performed in triplicate with a minimum of 5

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