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Research Note

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Proliferación de *Pseudo-nitzschia brasiliana* y *P*. cf. *pseudodelicatissima* (Bacillariophyceae) en el Estero Santa Cruz, norte del Golfo de California, México

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Abstract.- A moderate bloom of *Pseudo-nitzschia* species occurred during collection of phytoplankton in Estero Santa Cruz, the State of Sonora, Mexico, in August 2012. The abundance of *Pseudo-nitzschia* in 2 samples was 84×10^3 and 160×10^6 cells L^{-1}. Results show the presence of 2 species, *P. brasiliana* and *P.* cf. *pseudodelicatissima*, which were responsible for a microalgae bloom. This is the first proliferation of *P. brasiliana* in the Gulf of California. *P.* cf. *pseudodelicatissima* was present in higher densities. A short morphological description is provided, including data from scanning electronic microscopy.

Key words: Diatom blooms, *Pseudo-nitzschia brasiliana*, *P.* cf. *pseudodelicatissima*, oyster farming, Gulf of California, Mexico

Introduction

Microalgae blooms are common natural phenomena along the both coasts of the Gulf of California (Cortés-Altamirano & Núñez-Pasten 1992, Alonso-Rodríguez & Ochoa 2004, Martínez-López *et al*. 2006, 2008; Gárate-Lizárraga *et al*. 2007, 2009). These blooms include dinoflagellates, diatoms, raphidophytes, cyanobacteria, silicoflagellates and ciliates (Cortés-Altamirano & Núñez-Pasten 1992, Gárate-Lizárraga *et al*. 2002, Band-Schmidt *et al*. 2005, Gárate-Lizárraga & Muñetón-Gómez 2008, Gárate-Lizárraga & Muciño-Márquez 2012). Dinoflagellates and diatoms are responsible for most of the blooms in the Gulf of California (Gárate-Lizárraga *et al*. 2001, 2006, Alonso-Rodríguez & Ochoa 2004). Species of diatoms, such as *Chaetoceros curvisetus* Cleve, *C. debilis* Cleve, *C. radicans* F. Schütt, *C. socialis* H.S. Lauder, *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin, *Eucampia zodiacus* Ehrenberg, *Nitzschia sigma* (Kützing) W. Smith, *Pseudo-nitzschia* spp., *Rhizosolenia debyana* H. Peragallo, *Stephanopyxis palmeriana* (Greville) Grunow, and *Thalassiosira* spp. have proliferated in the Gulf of California (Gárate-Lizárraga *et al*. 1990, 2001, 2003, 2006, Cortés-Altamirano & Núnez-Pasten 1992, Gárate-Lizárraga & Muñetón-Gómez 2009, Molina *et al*. 1997).

The potentially toxic diatom genus *Pseudo-nitzschia* is a common component of the microalgal assemblage in the Gulf of California (Gárate-Lizárraga *et al*. 1990, Moreno *et al*. 1996). *Pseudo-nitzschia* blooms in the study region are related to upwelling events (Gárate-Lizárraga *et al*. 2007). At present, 7 species of *Pseudo-nitzschia* have been identified in the Gulf of California: *P. americana* (Hasle) Fryxell, *P. australis* Frenguelli, *P. brasiliana* N. Lundholm, G.R. Hasle & G.A. Fryxell, *P. fraudulenta* (Cleve) Hasle, *P. pseudodelicatissima* (Hasle) Hasle, *P. pungens* (Grunow ex Cleve) Hasle, and *P. subfraudulenta* (G.R. Hasle) G.R. Hasle (Hernández-Becerril 1998, Lundholm *et al*. 2002, Sierra-Beltrán *et al*. 2005, Gárate-Lizárraga *et al*. 2007). Blooms of *Pseudo-nitzschia* have been scarcely reported in the Gulf of California (Sierra-

Beltrán *et al*. 1997, 2005, Cortés-Altamirano & Licea-Durán 2004, Gómez-Aguirre *et al*. 2004, Gárate-Lizárraga *et al*. 2007, Ayala-Rodríguez 2008). This report describes the first proliferation of the potentially toxic diatoms *Pseudo-nitzschia brasiliana* and *P*. cf. *pseudodelicatissima* in the Estero Santa Cruz, Mexico (August 2012), including a brief description of both taxa.

Materials and methods

The Estero Santa Cruz lies between the delta region of the Rio Colorado and the extensive mudflats of Ensenada Pabellones and Bahía Santa María, Sinaloa, approximately 1,000 km south of the Delta (Meling-López *et al*. 1998, Fleischner & Gates 2009). Estero Santa Cruz is a medium-sized lagoon south of the town of Bahía de Kino. Its 3,622 ha area contains salt marshes, mangroves, and sand/mud flats, as well as permanent channels up to 3 m in depth (Fleischner & Gates 2009). Since the damming of the Río Sonora in 1947, Estero Santa Cruz no longer receives freshwater. The estuary-turned-lagoon is hypersaline (Quevedo-Estrada 2007). This aquatic system is now developing into a region of aquaculture ponds for oyster farming and shrimp cultivation.

As part of a monitoring program, phytoplankton bottle samples were collected on 1 August 2012 at 2 sampling stations in the northern part of the lagoon (Station 1: 28°47'37.88''N, 111°54'43.22''W and Station 2: 28°48'0.35''N, 111°55'1.56''W) where oysters are cultivated (Fig. 1). Phytoplankton surface samples for species identification and counting were collected with plastic flasks. Surface vertical tows were made with a hand net (50 cm in diameter, 20 µm mesh size). A portion of each tow was immediately fixed with Lugol's acid solution. Temperature, salinity, and pH were recorded with a calibrated multi-parameter sampler (HI9828) at each sampling station. Cells were counted in 10 mL sedimentation chambers under an inverted microscope (Axio Observer, Carl Zeiss, Oberkochen, Germany; Hasle 1978). Primary identification was made up to the genus level and further some specimens were examined by scanning electron microscopy (JEOL JSM-5600 SEM, Peabody, Mass.). Samples were prepared for SEM as described in Kaczmarska *et al*. (2005). Preparation and observation was done at the Digital Microscopy Facility, Mount Allison University, Canada. In each sample, specimens identified as *Pseudo-nitzschia* spp. were examined for morphometric characteristics (width and length of the valve, density of striae, fibulae and poroids under SEM).

Results and discussion

Sea surface temperature during the bloom varied from 32.5 to 34°C. Salinity ranged from 34 to 37, and the pH ranged from 7.6-7.7. The species list and abundances are compiled in Table 1. Total phytoplankton abundance at Station 1 was 556 x 10^3 cells L^{-1} and 618 × 10^3 cells L^{-1} at Station 2. Nanoflagellates were numerically the most important group, followed by diatoms and dinoflagellates (Table 1). Among diatoms, abundance of *Pseudo-nitzschia* in the 2 samples was 84 x 10^3 at Station 2 and

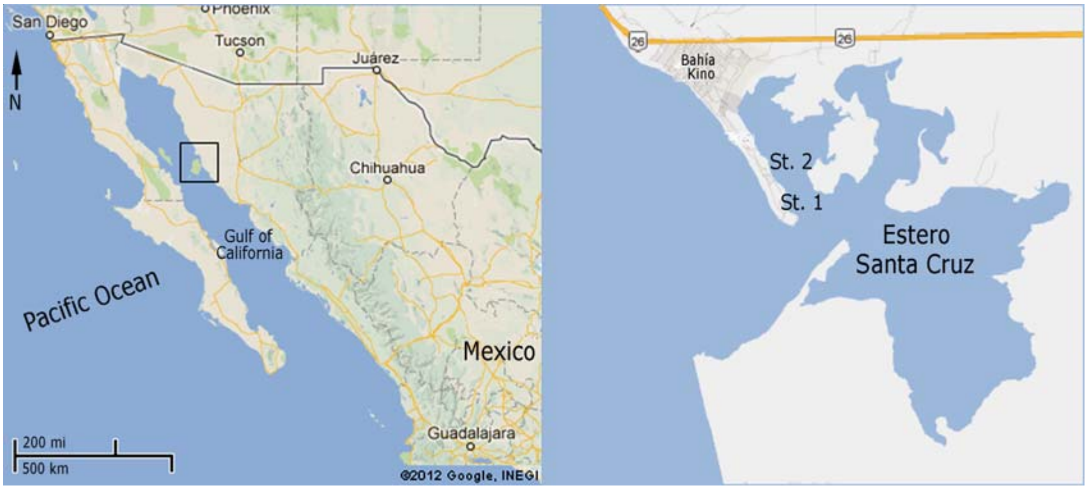

Figure 1. Location of Estero Santa Cruz (~28°48'N, 111°55'W), and sampling sites adjacent to Bahía Kino, Sonora, Mexico in August 2012 / Localización del Estero Santa Cruz (~28°48'N, 111°55'W), y los sitios de muestreos adyacentes a Bahía Kino, Sonora, México en agosto de 2012

Table 1. Abundance of microalgae species recorded in the Estero Santa Cruz, Bahía Kino, Sonora during the proliferation of *Pseudo-nitzschia* spp. in August 2012 / Abundancia de las especies de microalgas registradas en el Estero Santa Cruz, Bahía Kino, Sonora durante la proliferación de *Pseudo-nitzschia* spp. en agosto de 2012.

Species composition	Sampling station (cells L^{-1})	
	1	2
Diatoms		
Bacteriastrum sp.	100	100
Chaetoceros spp.	29300	30700
Chaetoceros peruvianus Brightwell	1600	1000
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin	22700	27000
Eucampia zodiacus Ehrenberg	100	0
Guinardia striata (Stolterfoth) Hasle in Hasle & Syvertsen	6000	8200
Pseudo-nitzschia brasiliana N. Lundholm, G.R.Hasle & G.A.Fryxell	68200	33700
Pseudo-nitzschia cf. *pseudodelicatissima* (Hasle) Hasle	92700	50400
Navicula spp.	600	0
Rhizosolenia setigera Brightwell	27300	16000
Rhizosolenia spp.	500	600
Centric unidentified diatoms	3600	4700
Pennate unidentified diatoms	6200	8600
Total abundance of diatoms	258900	181000
Dinoflagellates		
Tripos furca (Ehrenberg) F. Gómez,	0	100
Prorocentrum micans Ehrenberg	200	100
Prorocentrum minimum (Pavillard) J.Schiller	100	0
Peridinium quinquecorne Abé	0	300
Protoperidinium spp.	400	300
Naked dinoflagellates >20 um	700	0
Naked dinoflagellates <20 um	1100	1600
Total abundance of dinoflagellates	2500	2400
Nanoflagellates	295200	435000
Total phytoplankton abundance	556600	618400

160 x 10^3 cells L^{-1} at Station 1, respectively. Scanning electronic microscopy showed 2 species: *P. brasiliana,* with abundances varying in the 2 sampling stations from 33 x 10^3 to 68 x 10^3 cells L^{-1}, respectively, and *P.* cf. *pseudodelicatissima,* with abundances varying in the 2 sampling stations from 50 × 10^3 to 92 × 10^3 cells L^{-1}, respectively.

Pseudo-nitzschia brasiliana N. Lundholm, G.R. Hasle & G.A. Fryxell. SEM examination showed that this species has linear frustules in girdle view, rectangular shape in valve view, with widely rounded ends (Fig. 2 a-b) and lacks a central nodule (Fig. 2 c). *P. brasiliana* was mainly observed as single cells or forming chains of 2 or 3 cells. Morphological measurements are: 36.4-40.1 µm long, 2.07-2.25 µm wide; 23-26 striae in 10 µm, and 22-26 fibulae in 10 µm, 7-9 poroids in 1 µm. The species morphometric data coincided with the original description and literature (Lundholm *et al*. 2002, Quijano-Scheggia *et al*. 2011, Sahraoui *et al*. 2011, and Parsons *et al*. 2012). *P. brasiliana* was first described in Brazil by Lundholm *et al*. (2002) and has also been reported in other warm water regions, such as the Gulf of Panama, Gulf of Mexico, central Mexican coast along the Pacific; Brazil, Indonesia, Malaysia, Thailand, South Korea, Tunisia, and Vietnam (Lundholm *et al.* 2002, Villac *et al.* 2005, Lim *et al.* 2010, Quijano-Scheggia *et al*. 2011, Sahraoui *et al*. 2011).

Pseudo-nitzschia cf. *pseudodelicatissima* (Hasle) Hasle was also identified. This species was found in both

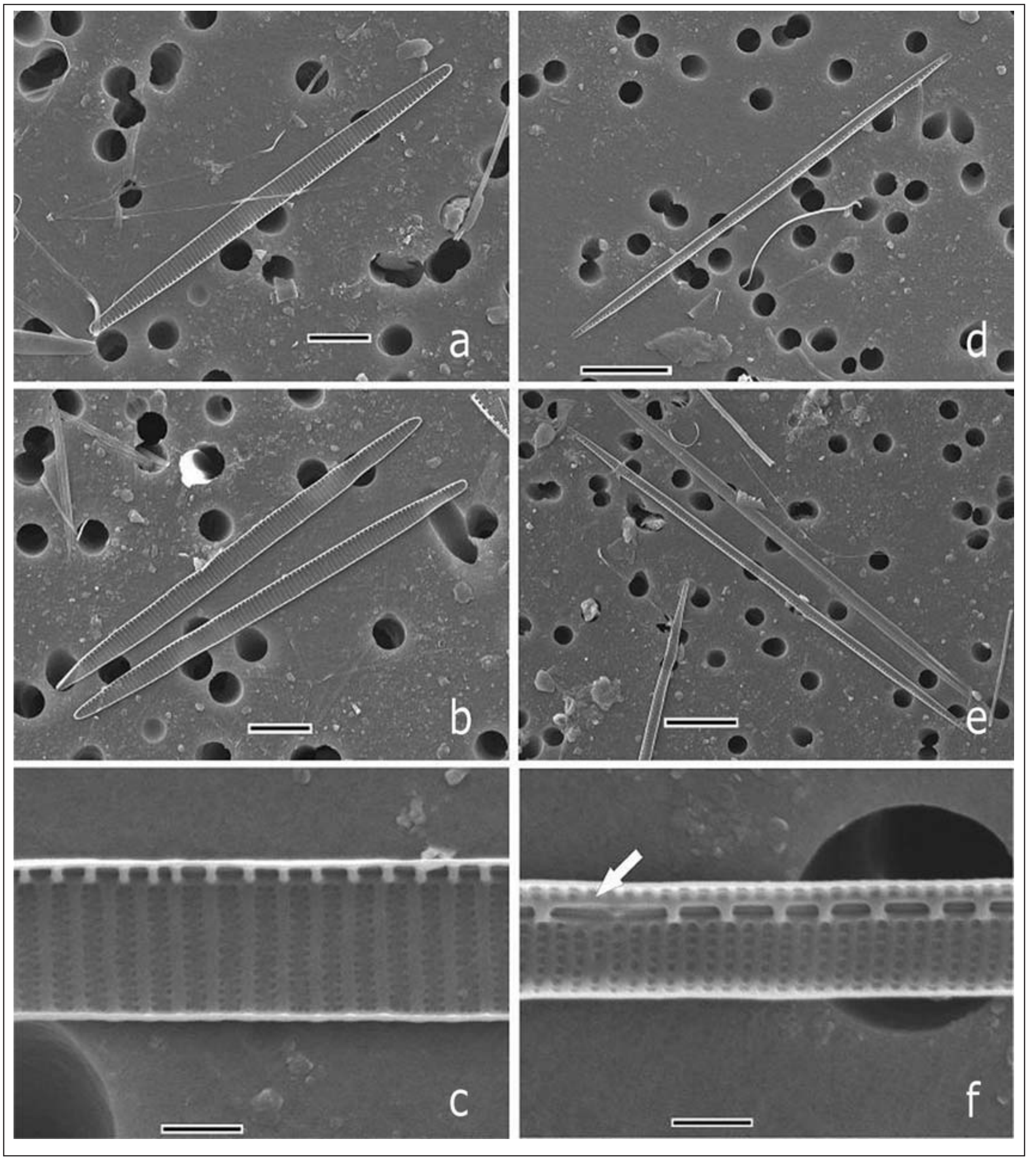

Figure 2. Scanning electron micrographs of *Pseudo-nitzschia* spp. collected in the Estero Santa Cruz, Bahía Kino, State of Sonora, Mexico. (a-b) valves of *P. brasiliana*, scale bar = 5 µm; (c) central part of the valve of *P. brasiliana* showing absence of central interspace, scale bar = 1 µm; (d-e) valves of *P.* cf. *pseudodelicatissima*, scale bar = 10 µm; (f) *P.* cf. *pseudodelicatissima* central interspace, scale bar = 1 µm / Imágenes de microscopio electrónico de *Pseudo-nitzschia* spp. colectadas en el Estero Santa Cruz, Bahía Kino, Sonora, México. (a-b) *P. brasiliana*; escala = 5 µm; (c) parte central de la valva de *P. brasiliana* mostrando la ausencia de interespacio central, escala de la barra = 1 µm; (d-e) *P.* cf. *pseudodelicatissima*, escala de la barra = 10 µm; (f) *P.* cf. *pseudodelicatissima* mostrando el interespacio central, escala = 1 µm.

samples, but it was more abundant in sample 1 (Table 1). Examination by SEM showed that this species has completely linear and symmetrical valves, terminating at rounded apices (Figs. 2 d-e). *P*. cf. *pseudodelicatissima* was observed as single cells or forming chains of 2 or 3 cells. A central, larger interspace, corresponding to 4-6 striae, was observed (Fig. 2 f). *P*. cf. *pseudodelicatissima* cells are 55.6-82.3 µm long, 1.40-1.61 µm wide; 41-42 striae in 10 µm, 6-7 poroids in 1 µm, and 15-18 fibulae in 10 µm. Cells are linear in valve view, tapering part near the tips very short. Their morphometric characteristics and measurements are consistent and within the range of *P*.

pseudodelicatissima (Hasle & Syvertsen, 1997, Lundholm *et al*. 2002, Hong-Chang *et al*. 2012, Parsons *et al*. 2012).

At least 4 different species have been described in the literature in the *P. pseudodelicatissima/cuspidata* complex: *P. pseudodelicatissima*, *P. cuspidata* (Hasle) Hasle, *P. calliantha* Lundholm, Moestrup & Hasle, and *P. caciantha* Lundholm, Moestrup & Hasle (Lundholm *et al*. 2003). The *pseudodelicatissima/cuspidata* complex is distinguished by differences in the structure of the poroid hymens and girdle bands (Lundholm *et al.* 2003), separating *P. pseudodelicatissima* and *P. cuspidata* from *P. calliantha*. The hymen in *P. cuspidata* and *P. pseudodelicatissima* is similar and could be distinguished between these 2 species using transmission electron microscopy. Although *P. pseudodelicatissima* resembles *P. cuspidata*, our specimens are most likely not *P. cuspidata* because the valves are clearly linear and not lanceolate. They are distinguished by cell width, which is relatively thin in *P. pseudodelicatissima* (Lundholm *et al.* 2003).

In previous reports, toxicity of *P. brasiliana* in several strains from by Brazil, Spain, and Malaysia were negative (Lundholm *et al*. 2003, Quijano-Scheggia *et al*. 2010); however, *P. brasiliana* from Bizerte Lagoon, Tunisia produces domoic acid (Sahraoui *et al*. 2011). *Pseudo-nitzschia pseudodelicatissima* can produce domoic acid, a causative agent of amnesic shellfish poisoning, notably some strains isolated from the Gulf of Mexico and Greece (Pan *et al*. 2001, Moschandreou *et al*. 2010). However, domoic acid has not been detected in strains of *P. pseudodelicatissima* from Gafahna, Portugal, Denmark Strait (northwest of Iceland), and Bizerte Lagoon, Tunisia (Lundholm *et al*. 2003, Sahraoui *et al*. 2011).

Given the occurrence of the potentially toxigenic diatoms *P. brasiliana* and *P.* cf. *pseudodelicatissima* at moderate cell densities in the Estero Santa Cruz, it appears that toxic events may occur in the future. Here we report the first proliferation of *P. brasiliana* in the Gulf of California. The first bloom of *P. pseudodelicatissima* was reported in Mazatlán, Sinaloa (8 July 2004). The death of ten brown pelicans (*Pelecanus occidentalis californicus* Ridgway), as well as several fish species were partly attributed to the *Pseudo-nitzschia* bloom (Sierra-Beltrán *et al*. 2005). In this study, we did not isolate either species of *Pseudo-nitzschia* to perform toxicity tests. A bloom of the 2 potentially toxic species of *Pseudo-nitzschia* in this lagoon system warrants a systematic and continuous monitoring program by health authorities, because commercial shellfish aquaculture sites are located there.

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