Proliferation of *Pseudo-nitzschia brasiliana* and *P.* cf. *pseudodelicatissima* (Bacillariophyceae) in the Estero Santa Cruz, northern Gulf of California, Mexico

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 84x10³ and 160x10⁶ 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) Reimmann & 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 & Núñez-Pasten 1992, Gárate-Lizárraga & Muñetón-Gómez 2009, Molina et al. 1997).

The potentially toxic diatom genus *Pseudo-nitzschia* is a common component of the microagal 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 x 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...
160 x 10^3 cells L^{-1} at Station 1, respectively. Scanning electronic microscopy showed 2 species: *P. brasiliiana*, 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 x 10^3 to 92 x 10^3 cells L^{-1}, respectively.

*Pseudo-nitzschia brasiliiana* 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. brasiliiana* 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. brasiliiana* 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

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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}) |
|--------------------------------------------|---------------------------------|
|                                            | Station 1 | Station 2 |
| **Diatoms**                                |           |           |
| *Bacillariastrium* sp.                     | 100       | 100       |
| *Chaetoceros* sp.                          | 29300     | 30700     |
| *Chaetoceros perovicus* Brightwell         | 1600      | 1000      |
| *Cylindrotheca closterium* (Elmenberg) Reimann & J.C.Lawson | 22700 | 27000 |
| *Eucampia zodiaca* Ehrenberg               | 100       | 0         |
| *Gonioglena striata* (Stolterfoth) Hasle & Syvertsen | 6000 | 8200 |
| *Pseudo-nitzschia brasiliiana* Lundholm, G.R.Hasle & G.A.Fryxell | 68200 | 33700 |
| *Pseudo-nitzschia cf. pseudodelicatissima* (Hasle) Hasle | 92700 | 50400 |
| *Navicula* sp.                             | 600       | 0         |
| *Rhizosolenia setigera* Brightwell         | 27300     | 16000     |
| *Rhizosolenia* sp.                         | 500       | 600       |
| Centric unidentified diatoms               | 3600      | 4700      |
| Pennate unidentified diatoms               | 6200      | 8600      |
| Total abundance of diatoms                 | 258900    | 181000    |
| **Dinoflagellates**                        |           |           |
| *Tripos furca* (Elmenberg) F. Gómez,       | 0         | 100       |
| *Proorocentrum micans* Ehrenberg           | 200       | 100       |
| *Proorocentrum minimum* (Pavillard) J.Schiller | 100    | 0         |
| *Peridinium quinquecorne* Abé               | 0         | 300       |
| *Protoperidinium* sp.                      | 400       | 300       |
| Naked dinoflagellates >20 μm               | 700       | 0         |
| Naked dinoflagellates <20 μm               | 1100      | 1600      |
| Total abundance of dinoflagellates         | 2500      | 2400      |
| **Nanoflagellates**                        |           |           |
| 295200                                     | 43500     |
| Total phytoplankton abundance              | 556600    | 618400    |
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. brasiliiana in several strains from by Brazil, Spain, and Malaysia were negative (Lundholm et al. 2003, Quijano-Scheggia et al. 2010); however, P. brasiliiana 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. brasiliiana 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. brasiliiana 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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