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# Description of toxigenic species of the genus *Pseudo-nitzschia* in coastal waters of Uruguay: Morphology and distribution

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ABSTRACT

*Pseudo-nitzschia* is a genus of marine diatoms, widely distributed from tropical to polar regions. Over 35 species have been described, some of which produce domoic acid (DA). This neurotoxin is responsible for the intoxication syndrome in humans and marine animals called Amnesic Shellfish Poisoning (ASP). Most of these toxigenic species are an important component of phytoplankton and have been recorded in the Southern Cone of South America (Argentina, Brasil, Chile and Uruguay).

The Dirección Nacional de Recursos Acuáticos (DINARA) conducted a long-term monitoring program along the Uruguayan coast, beginning with the first toxic algal event reported in 1980. Six species of *Pseudo-nitzschia*: *P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. multiseries*, *P. multistriata* and *P. pungens* were reported in Uruguayan waters. Until recently, the only event with detected levels of DA (9.9 μgDA/g), as determined by the HPLC standard method, was caused by *P. multiseries* in December 2001 with values reaching 1200 cells $l^{-1}$. This record represents the first DA report associated with this species in Western Atlantic waters. In February 2009 *P. multistriata* was detected for the first time in Uruguayan waters, with a density of $2 \times 10^6$ cells $l^{-1}$. This report suggests that Uruguay is the southern limit of its biogeographic distribution in the Southwest Atlantic. During this study, analysis by optical and scanning electron microscopy confirmed the presence of four species of *Pseudo-nitzschia* in Uruguayan waters: *P. fraudulenta*, *P. multiseries*, *P. multistriata* and *P. pungens* which are potentially producers of DA. We also include information on the trends in *Pseudo-nitzschia* abundance with water temperature and salinity.


## 1. Introduction

In 1980, as a consequence of the first paralytic shellfish poisoning detection (Davison and Medina, 1982; Davison and Yentsch, 1985), the Dirección Nacional de Recursos Acuáticos (DINARA) established a Monitoring Programme on Harmful Algae and Toxins in Molluscs, along the oceanic coast of Uruguay. However, quantitative reports of *Pseudo-nitzschia* began in 2000, concurrent with the implementation of the official methodology for the analysis of Domoic Acid (Méndez and Ferrari, 2002). The study area (Fig. 1), is under a saline front influence, with high salinity variations due to the interaction between the estuarine water of the Rio de la Plata and the Atlantic Ocean. Furthermore the phytoplankton in this area is strongly influenced by seasonal fluctuations of the Subtropical Convergence between tropical waters during summer and the cold subantartic waters during winter (Castello et al., 1997; Piola et al., 1999, 2000). Salinity in this region is quite variable (0.5–35) and the water temperature is characterized by an annual cycle rising up to 25 °C in the summer and decreasing to 7 °C in the winter (Méndez and Galli, 2008). Although *Pseudo-nitzschia* is common in the region and along the coast of Uruguay, there is a lack of documentation about the environmental conditions favorable for its development. Although many *Pseudo-nitzschia* species are known to produce toxins responsible for Amnesic Shellfish Poisoning (ASP), there is only one report with an incipient level of DA (9.9 μgDA/g) caused by *P. multiseries* in 2001 (Medina et al., 2003), detected through this monitoring program.

According to the literature, several species of *Pseudo-nitzschia* have been recorded for this area: *P. australis* (Lange and Mostajo, 1985), *P. delicatissima* (Machado, 1976; Ferrari, 2009), *P. fraudulenta* (Méndez unpubl.), *P. pungens* (Negri and Inza, 1998), *P. multiseries* (Medina et al., 2003), and *P. multistriata* (Méndez and Ferrario, 2009).

Information on the diversity of toxigenic species able to produce ASP and the period of major risk of their bloom occurrence are important considerations in the DINARA Monitoring Programme. The identification of *Pseudo-nitzschia* at the

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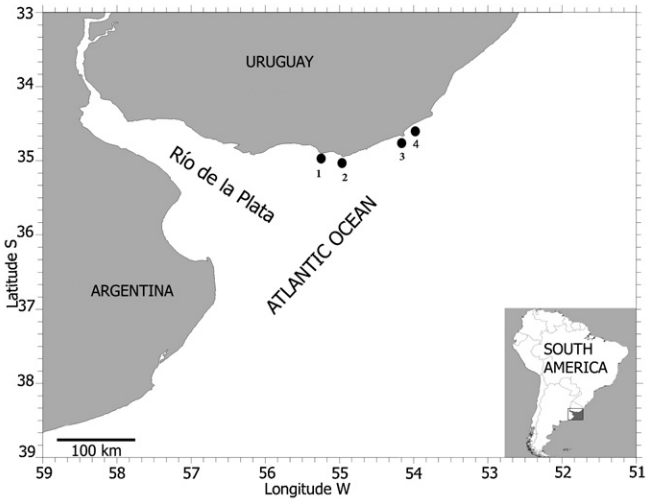


**Fig. 1.** Map of the study area showing the sampling stations. (1) Piriápolis, (2) Punta del Este, (3) La Paloma and (4) Arachania.

species level is not always possible using light microscopy and often requires electron microscopy (EM). The purpose of this study was to confirm the taxonomy of Uruguayan *Pseudo-nitzschia* species using electron microscopy, as well as to describe the environmental conditions during the periods of *Pseudo-nitzschia* spp. blooms.

## 2. Material and methods

For this study a total of 768 qualitative and quantitative phytoplankton samples from 4 stations along the Uruguayan coast were analyzed between January 2006 and January 2010 (Fig. 1). The samples were fixed with Lugol's and *Pseudo-nitzschia* spp. were counted under inverted microscope Leitz Labovert using the traditional method of Utermöhl (1958), at the National Direction of Aquatic Resources, Uruguay. A total of 14 qualitative samples were washed with distilled water and treated using conventional oxidation methods (Hasle and Fryxell, 1970; Prygiel and Coste, 2000). The material was cleaned of organic matter and mounted on permanent glass slides using Naphrax medium for light microscopy (LM), and onto stub shadowed with gold–paladium for scanning electron microscopy (SEM) according to Ferrario et al. (1995).

Specimens were examined with a phase contrast Leica DM 2500 (LM), equipped with a Leica DFC420 digital camera. For scanning electron microscopy the material was examined with a Jeol JSM-6360 LV at the Museo de La Plata, Argentina. Complementary examinations were carried out by using a transmission electron microscope (TEM) at the National History Museum of Denmark.

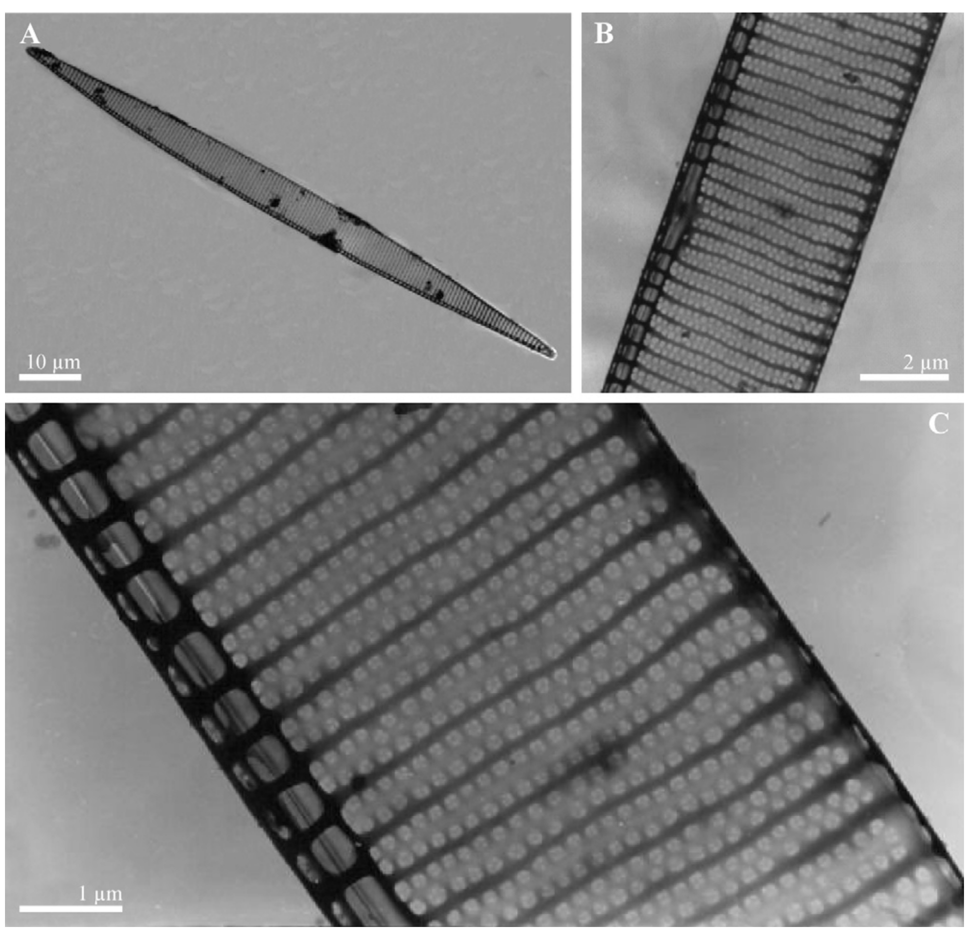


**Fig. 2.** (A–C) *P. fraudulenta*. TEM. (A) General valve view. (B) Part of the valve showing the central interspace. (C) Part of the valve showing biseriate striae. Sample from station 2, September 13, 1992 (Micrograph N. Lundholm).

The surface salinity and temperature were registered with YSI (Mod. 30M), ISI Incorporated, Yellow Springs, OH, USA.

## 3. Results

### 3.1. Species of Pseudo-nitzschia registered and analyzed during this study

#### 3.1.1. Pseudo-nitzschia fraudulenta

The observed specimens of *P. fraudulenta* were characterized by 100 and 6.4 μm valve length and width, respectively. The number of interstriae and fibulae were 14 and 13 in 10 μm, respectively. Each stria had two to three rows of poroids (4–5 in 1 μm). The raphe was interrupted by a larger central interspace. This species was detected at station 2 (September 13, 1993) at temperature 14 °C and salinity 10.8. Samples taken during the bloom were documented under TEM thanks to N. Lundholm cooperation (Fig. 2).

This species has been mentioned as a possible DA source at the Chubut coastal areas, Argentina (Sastre et al., 2007).

#### 3.1.2. Pseudo-nitzschia multiseries

Observed cells of *P. multiseries* were characterized by symmetric frustules, linear to lanceolate in girdle as well as in valve view (Fig. 3A). The length of the valve was 90–140 μm and its width was 3.5–4 μm (Fig. 3B–C). The number of interstriae and fibulae were 12–15 in 10 μm and 13–15 in 10 μm, respectively. Each stria had three rows of poroids, 4–5.5 in 1 μm. The valve shows no central interspace (Fig. 3D).

*P. multiseries* was detected for the first time in 2001 (Station 2 on December 10), with the first DA detection by HPLC in Uruguayan waters (Medina et al., 2003). The environmental conditions at the time of this record were: salinity 25.4 and temperature 20.5 °C. Although this was the first report of DA associated with the presence of *P. multiseries* in the Southwestern Atlantic. *P. multiseries* appeared in other samples studied under SEM from Station 3 (February 25, 2008), at salinity 32.3 and temperature 22 °C, and at Station 2 (January 6, 2009), at salinity 32.5 and temperature 22 °C.

Montoya et al. (2008) reported for the first time the DA production by *P. multiseries* from the Argentine Sea.

#### 3.1.3. Pseudo-nitzschia multistriata

Observed cells of *P. multistriata* overlap for about one-eleventh of the frustule length and the valves were slightly asymmetric, with one side straight and the other convex, tapering rapidly towards rounded ends (Fig. 4A–E). The width of the valves was 2.5–3.3 μm and the length was 52–63 μm. At SEM the valve surface showed 25–28 fibulae in 10 μm and 35–40 striae in 10 μm, each stria was biseriate, rarely with one or three rows of circular small poroids. The poroids number was 11–13 in 1 μm. There is no central interspace between the two central fibulae (Fig. 4F–G).

*P multistriata* was reported only once in the study area, during a bloom in January 2009, at Station 2 (Méndez and Ferrario, 2009). The maximum abundance during the bloom was $2 \times 10^6$ cells $l^{-1}$. For the Southern Cone of South America, other than Uruguay, this species was only documented in Brazil.

#### 3.1.4. Pseudo-nitzschia pungens

The present study confirms the presence of *P. pungens* through SEM images, in samples from station 3 at salinity 32.5 and

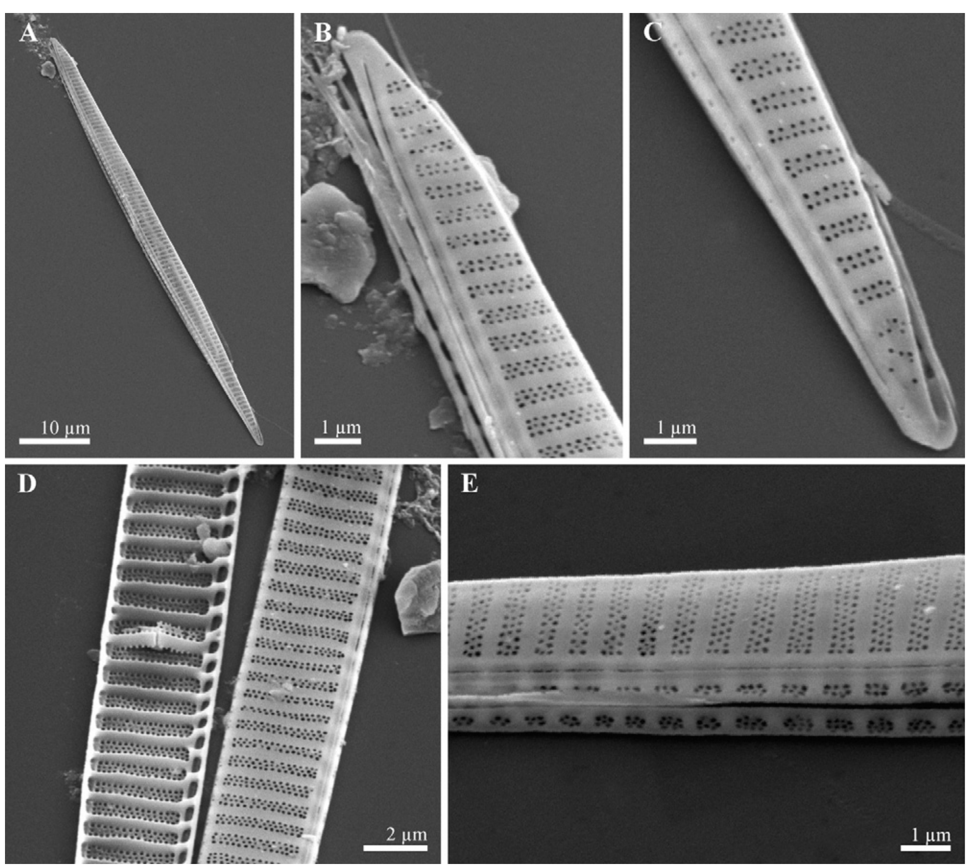


**Fig. 3.** (A–E) *P. multiseries.* SEM. (A) General valve view. (B–C) Valve ends. (D) Central part of the valve. (E) Bi-triseriate striae in the same valve. Sample from station 2, January 6, 2009.

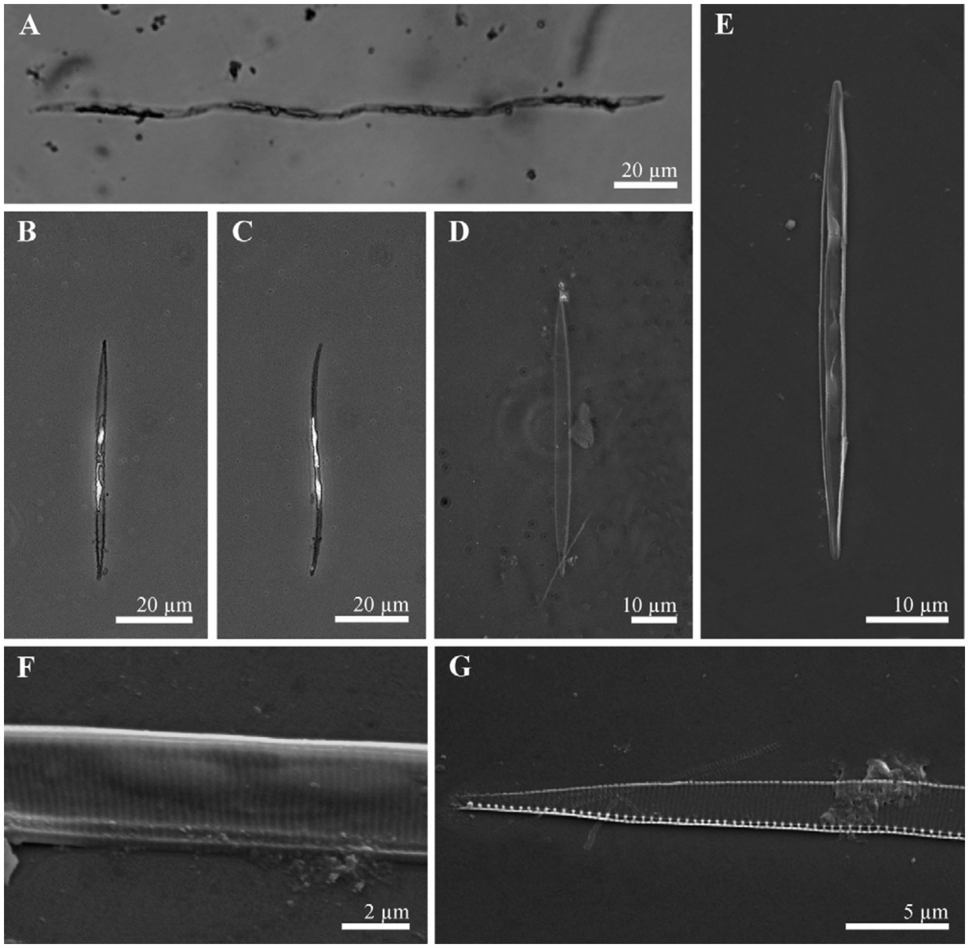


**Fig. 4.** (A–G) *P. multistriata*. (A–D) LM. (A) Chain of cells. (B–C) Living cells, note the chloroplasts. (D) Frustule in valve view. (E–G) SEM. (E) Cell in valve view. (F–G) Central and terminal part of different cells. Sample from station 2, January 9, 2009.

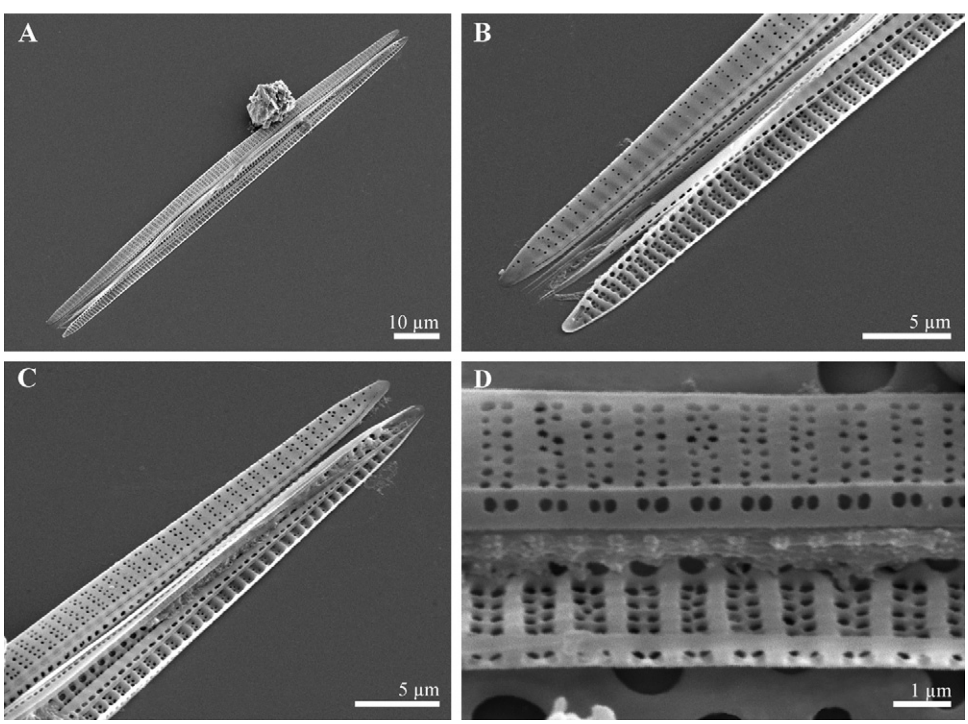


**Fig. 5.** (A–D) *P. pungens*. SEM. (A) General valve view. (B–C) External and internal valve view of the ends. (D) Central part of the valve in external and internal view. Note the mantle in both valves. Sample from station 3, November 11, 2008.

temperature 18 °C. Observed specimens of *P. pungens* were characterized by symmetric frustules, linear to lanceolate in valve view. The length of the valve was 90 μm and width 2.8 μm (Fig. 5A). The number of interstriae and fibulae were 12 in 10 μm. Each stria had two rows of poroids, 3–4 in 1 μm. The central zone of the valve shows no central interspace (Fig. 5D).

*P. pungens* was previously cited for this region by Hasle (1972), just outside the common fishing zone between Uruguay and Argentina. Using SEM, Negri and Inza (1998) reported *P. pungens* as a common species in Uruguayan and Argentinean shelf waters in samples taken a few miles off the Uruguayan coastal stations (October 29, 1993). The cited authors reported *P. pungens* at salinity between 10.4 and 28.1 and temperature 16–17.2 °C. Samples taken from two miles off station 2 on 10th September 2000, showed the presence of *P. pungens* under SEM (Ferrari unpub.). The conditions during this event were salinity 32.3 and temperature 14 °C. Other observation of this species was in December 2003, at salinity 29.6 and temperature 18.3 (Ferrari, 2009).

This species have been reported as possible DA source at Chubut, Argentina (Sastre et al., 2007), and Brazil (Proença unpub.).

### 3.2. Further information of other species of Pseudo-nitzschia previously registered in Uruguay

#### 3.2.1. Pseudo-nitzschia australis

Although this potentially toxic species was not found during this study, it has been previously reported in Uruguayan waters by other authors (Lange and Mostajo, 1985; Negri and Inza, 1998). In the Southern Cone of South America *P. australis* had been reported for Argentinean shelf waters, usually abundant in late autumn and winter (Ferrario et al., 1999, 2002; Negri et al., 2004; Almandoz et al., 2007), Chile (Rivera, 1985; Lembeye, 2008). For Brazilian waters, it was found southwards along Rio Grande do Sul State (Odebrecht et al., 2001; Fernandes and Brandini, 2010). *P. australis* was reported as a producer of DA in Argentina and Chile (Negri et al., 2004; Alvarez et al., 2009).

#### 3.2.2. Pseudo-nitzschia delicatissima

*P. delicatissima* has been reported for Uruguayan waters (Machado, 1976; Ferrari, 2009), and its determination was based on LM observations. Since they were not found in the material analyzed for the present study, we consider that further analysis under electron microscope is necessary to confirm its presence in Uruguayan coastal waters. Until now this species has not been reported as a DA source in the Southern Cone of South America.

## 4. Discussion and conclusion

We review six potentially toxigenic members of the *Pseudo-nitzschia* genus reported from Uruguayan coastal waters. Four of these (*P. fraudulenta*, *P. multiseries*, *P. multistriata* and *P. pungens*) were identified in Uruguayan waters in this study, and their species designation was confirmed by light and electron microscopy. Complementary information about the environmental conditions during the occurrence of *Pseudo-nitzschia* are included.

*P. australis* was previously reported for Uruguayan waters, but was not found during this study. This species is morphologicaly similar to *P. seriata*. The interspecific differences include by the striae ultrastructure which is biseriate in *P. australis* and more than two in *P. seriata*.

In more recent studies, specimens from Beagle Channel, Argentina analyzed by SEM (Almandoz et al., 2008) show that the characteristics of the interstriae, fibulae and absence of interspace would correspond to both *P. australis* and *P. seriata*. However they note that the ultrastructure of the striae have characteristics intermediate between these two species, thus raising the possibility that *P. seriata* may not be restricted to the Northern Hemisphere.

It is also important to mention that the first DA event reported in Argentina was associated with a *P. australis* bloom in 2000 (Negri

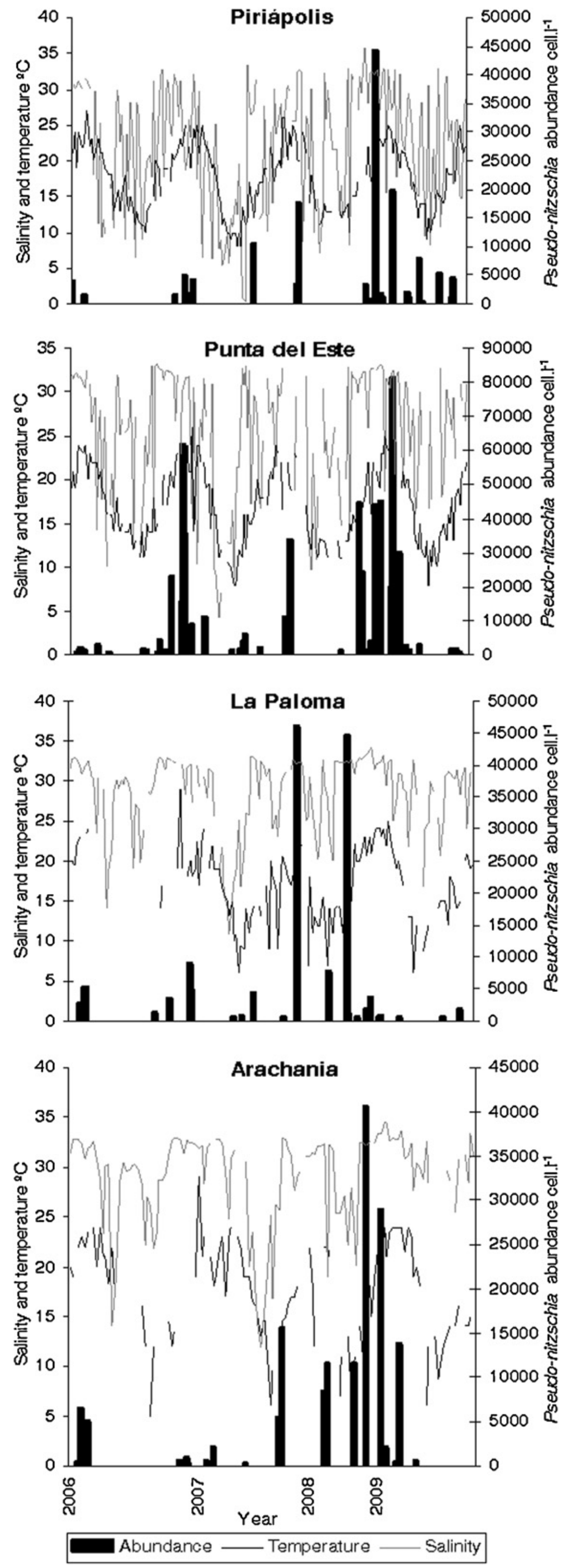


**Fig. 6.** Temporal series of *Pseudo-nitzschia* spp. abundance, salinity and temperature at the four sampling stations.

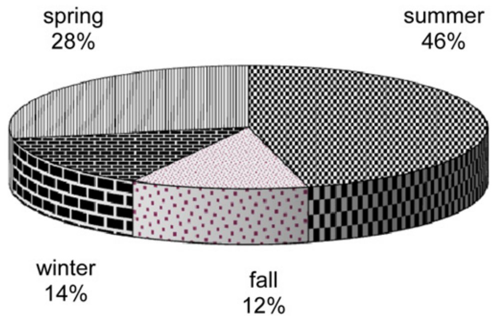


**Fig. 7.** Seasonal frequency of *Pseudo-nitzschia* spp. blooms reported in Uruguayan waters from January 2006 to January 2010.

et al., 2004). This study reported DA in plankton, mussels and anchovy stomachs, and for this reason, small fishes are potential ASP vectors from phytoplankton to humans.

Reports of *P. delicatissima* for the Southern Cone may not be reliable because the material was analyzed only with LM, except for Chile where Rivera (1985) analyzed samples using EM and reports the species between 18°17′ and 42°07′ latitude S. However Lundholm et al. (2006) attributed the great variability of *P. delicatissima* found by Rivera (1985) to a mixture in their samples of the species *P. delicatissima*, *P. decipiens* and *P. dolorosa*, the latter two being recently named species (Lundholm et al., 2006).

Since the first report of *P. fraudulenta* as a producer of DA (Rhodes et al., 1998), it has not been re-registered as a producer of DA. Sastre et al. (2007) mentioned DA levels in samples with a dominance of *P. fraudulenta* from waters of Chubut, Argentina, but they could not associate that species specifically with toxin production since it was found along with *P. pungens*, both potential DA-producing species.

*P. multiseries* (=*P. pungens var. multiseries*) was the first diatom species documented as a producer of DA (Bates et al., 1989), the neurotoxin having originally been isolated from the red macroalga *Chondria armata* (Takemoto and Daigo, 1958). DA caused intoxication for the first time in Canada in 1987 causing the death of three people and illness of over 100 (Bates et al., 1989). To our knowledge, DA events produced by *P. multiseries* in the Southern Cone of South America have never been associated with human intoxication.

The number of poroids in *P. multistriata* was the same as the material analyzed from the Atlantic coast of France (Nezan et al., 2007), the Gulf of Naples, Italy (Sarno and Dahlmann, 2000) and Peter the Great Bay of Russia (Orlova et al., 2008). Although this differs from the original description (Takano, 1993) of 5–6 poroids per 1 μm, Larsen and Nguyen-Ngoc (2004) noted that this number is probably a lapse, since the Takano indication of 5–6 poroids is in conflict with his published photographs. *P. multistriata* has been detected in many other locations throughout the world: Japan (Takano, 1993), New Zealand (Rhodes et al., 2000), Italy (Sarno and Dahlmann, 2000), Vietnam (Larsen and Nguyen-Ngoc, 2004), Spain (Quijano-Scheggia et al., 2005) and France (Nezan et al., 2007). In February 2009 *P. multistriata* was detected for the first time in Uruguayan waters (Méndez and Ferrario, 2009). This is the second report of this species for South America since *P. multistriata* had been previously found only in Brazil (Villac et al., 2002; Villac and Noronha, 2008a,b). As there are no reports of this species in higher latitudes, our work suggests that Uruguay is the southern limit of its biogeographic distribution in the Southwest Atlantic.

Toxin levels detected in Uruguay during *P. multiseries* blooms were below the regulatory level. However, the confirmation of its presence during several summer periods, when mussel consumption increases in the coastal zone, highlights the importance and need for permanent phytoplankton monitoring in this area.

Our results indicate that *Pseudo-nitzschia* is a common genus in Uruguayan waters. Of the six species discussed here, four (*P. australis*, *P. fraudulenta*, *P. multiseries*, and *P. pungens*) have been mentioned as possible DA source in the Southern Cone, however there have been no human intoxication reports.

The temporal series of *Pseudo-nitzschia* spp. abundance, salinity and temperature at the four sampling stations are shown in Fig. 6. Blooms have been detected more frequently at Punta del Este than the rest of the coastal stations. However during 2008 and 2009 high densities have been detected all along the study area (Fig. 6).

During the present study *Pseudo-nitzschia* spp. was detected in 116 samples. We observed 74% of its occurrence between spring and summer (Fig. 7). Most abundant blooms of *Pseudo-nitzschia* were observed in association with high salinity and temperature (Fig. 8A–B). There are four documented species of *Pseudo-nitzschia* in Uruguayan waters (*P. multiseries*, *P. multistriata*, *P. pungens* and *P. delicatissima*) detected at water temperature >17 °C, and two species (*P. fraudulenta* and *P. australis*) at water temperature <17 °C (Table 1 and Fig. 9).

Hydrographic conditions show that although salinity in the study area is highly variable, *Pseudo-nitzschia* is rarely present in the phytoplankton community at salinities lower than 17. The most abundant *Pseudo-nitzschia* spp. blooms ($>20 \times 10^3$ cells $l^{-1}$) occurred at salinities higher than 30 (Fig. 8A). According to

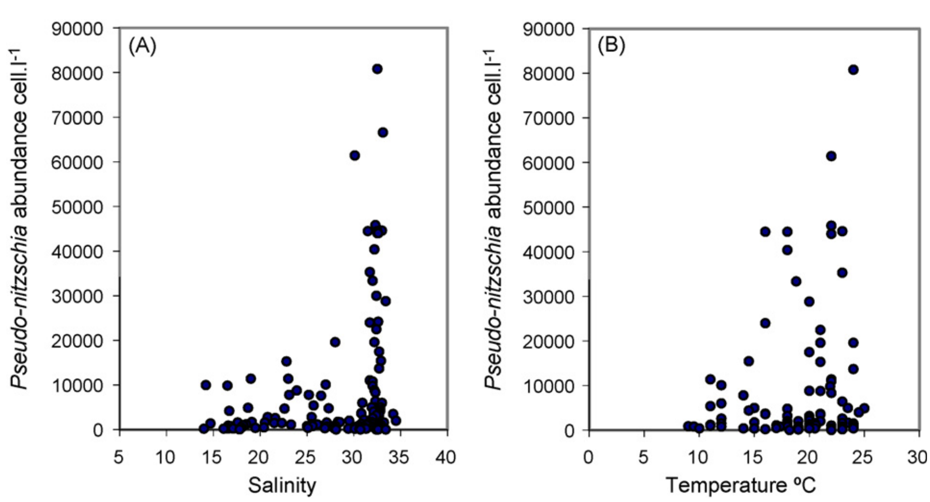


**Fig. 8.** Relationship of *Pseudo-nitzschia* spp. abundance versus salinity (A) and versus temperature (B).

**Table 1**
Records of the toxigenic *Pseudo-nitzschia* species for the study area and environmental conditions.

| Species | Latitude S | Longitude W | Salinity | Temperature, °C | Month | References with SEM images descriptions | Other references |
|---|---|---|---|---|---|---|---|
| *P. multistriata* | 34°55′ | 54°55′ | 32.5–34.4 | 19–23 | Jan | Méndez and Ferrario (2009) | |
| *P. multiseries* | 34°55′ | 54°55′ | 25.4 | 20.5 | Dec | Present study | |
| *P. pungens* | 34°50′ | 54°10′ | 32.5 | 18 | Nov | Present study | |
| | 35°27′–35°57 | 55°08′–55°56′ | 10.4–28.1 | 16–17.1 | Oct | | Negri and Inza (1998) |
| | 35°00′ | 53°30′ | 29.6 | 18.3 | Dec | | Ferrari (2009) |
| *P. fraudulenta* | 34°55′ | 54°55′ | 10.8 | 14 | Sept | Present study | |
| | 34°40′ | 52°20′ | 28.2 | 16.3 | Dec | | Ferrari (2009) |
| *P. australis* | 36°24′ | 55°37′ | 30.9 | 14.2 | Oct | | Negri and Inza (1998) |
| | 34°00′–35°05′ | 51°50′–52°45′ | n/d | n/d | – | | Lange and Mostajo (1985) |
| *P. delicatissima* | 35°05′–36°50′ | 52°43′–54°51′ | n/d | n/d | – | | Machado (1976) |
| | 38°10′ | 52°00′ | 35 | 17 | Dec | | Ferrari (2009) |

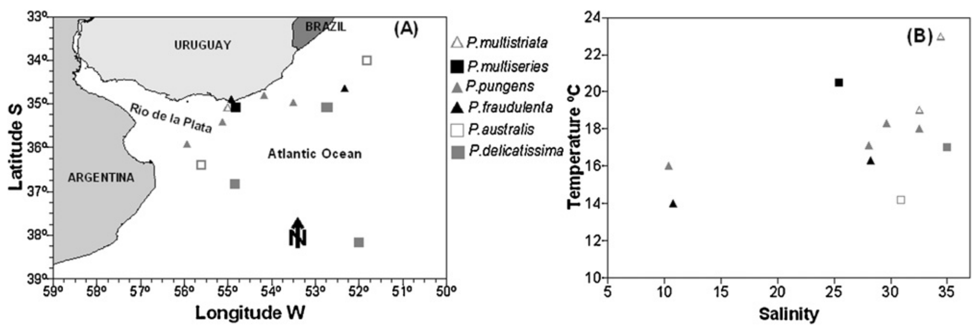


**Fig. 9.** (A) Map of *Pseudo-nitzschia* species and (B) associated temperature and salinity according to Table 1.

previous studies (Méndez and Galli, 2008) this condition is coincident with dry periods in the Rio de la Plata basin.

Although *Pseudo-nitzschia* spp. is not always present in the coastal phytoplankton, it can develop blooms of great magnitude ($>30 \times 10^3$ cells $l^{-1}$) during certain periods of the year. 74% of the *Pseudo-nitzschia* spp. reports occur during spring and summer. Most of the reports of *Pseudo-nitzschia* spp higher than $5 \times 10^3$ cells $l^{-1}$ (71%) were detected with water temperatures greater than 17 °C (Fig. 8B) – this temperature is typically observed between October and April in the coastal area, a period that coincides with high consumption of fisheries products by the local population and tourists, which increases the potential impact of a toxic bloom (Fig. 9).

Our study of harmful algal blooms in the coastal waters of Uruguay shows the presence of several *Pseudo-nitzschia* species able to produce DA. These toxigenic species were documented at different periods throughout the year. However, the most worrisome looms were produced by *P. multistriata* and *P. multiseries* during summer, under high salinity conditions in Uruguayan waters. Both species have been reported throughout the world as potential producers of DA.

It is well known that during summer, the warm coastal current from Brazil affects Uruguayan waters. Thus, the recent report of a DA event at the coast of Santa Catarina (Brazil), during a *P. cf. calliantha* bloom in January 2009 (Proenca et al., 2011), illustrates the risk that other toxic species may be advected to the coastal waters of Uruguay. The presence of these potentially toxic species in Uruguayan waters and their high abundance both argue that there is a need to continue with the concurrent monitoring of phytoplankton in the water column and toxicity in mussels, complementary with public education, to decrease the risk and potential effects of these phenomena in the region.

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