Harmful Algae 63 (2017) 45–55

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

# Species occurrence of the potentially toxigenic diatom genus *Pseudo-nitzschia* and the associated neurotoxin domoic acid in the Argentine Sea

Gastón O. Almandoz[a,b,*], Elena Fabro[a,b], Martha Ferrario[a,b], Urban Tillmann[c], Allan Cembella[c], Bernd Krock[c]

[a] División Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Paseo del Bosque s/n (B1900FWA), La Plata, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, 1033 Buenos Aires, Argentina
[c] Alfred Wegener Institut-Helmholtz Zentrum für Polar- und Meeresforschung, Ökologishe Chemie, Am Handelshafen 12, 27570 Bremerhaven, Germany

ARTICLE INFO

*Article history:*
Received 28 October 2016
Received in revised form 17 January 2017
Accepted 17 January 2017
Available online xxx

*Keywords:*
Amnesic shellfish poisoning
South-western Atlantic
*Pseudo-nitzschia* diversity
*P. australis*
*P. brasiliana*
Domoic acid

ABSTRACT

The marine diatom genus *Pseudo-nitzschia*, the major known producer of the neurotoxin domoic acid (DA) responsible for the amnesic shellfish poisoning (ASP) syndrome in humans and marine mammals, is globally distributed. The genus presents high species richness in the Argentine Sea and DA has been frequently detected in the last few years in plankton and shellfish samples, but the species identity of the producers remains unclear. In the present work, the distribution and abundance of *Pseudo-nitzschia* species and DA were determined from samples collected on two oceanographic cruises carried out through the Argentine Sea (~39–47°S) during summer and spring 2013. Phytoplankton composition was analysed by light and electron microscopy while DA was determined by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). The genus *Pseudo-nitzschia* was recorded in 71 and 86% of samples collected in summer and spring, respectively, whereas DA was detected in only 42 and 21% of samples, respectively. Microscopic analyses revealed at least five potentially toxic species (*P. australis*, *P. brasiliana*, *P. fraudulenta*, *P. pungens*, *P. turgidula*), plus putatively non-toxigenic *P. dolorosa*, *P. lineola*, *P. turgiduloides* and unidentified specimens of the *P. pseudodelicatissima* complex. The species *P. australis* showed the highest correlation with DA occurrence ($r = 0.55$; $p < 0.05$), suggesting its importance as a major DA producer in the Argentine Sea. In the northern area and during summer, DA was associated with the presence of *P. brasiliana*, a species recorded for the first time in the Argentine Sea. By contrast, high concentrations of *P. fraudulenta*, *P. pungens* and *P. turgidula* did not correspond with DA occurrence. This study represents the first successful attempt to link toxigenicity with *Pseudo-nitzschia* diversity and cell abundance in field plankton populations in the south-western Atlantic.



## 1. Introduction

The marine diatom genus *Pseudo-nitzschia* H. Peragallo is distributed worldwide and often represents an important, even dominant component of phytoplankton assemblages (Lelong et al., 2012). The genus now comprises 48 recognized species (Teng et al., 2014, 2015, 2016; Percopo et al., 2016), most of them described within the last two decades. Among these species, at least 23 have been mentioned as potential producers of domoic acid (DA) (Teng et al., 2015, 2016), a secondary amine neurotoxin. This neurotoxin can be accumulated throughout the marine food web, causing amnesic shellfish poisoning (ASP), a neurological syndrome with even lethal effects on sea birds, marine mammals and humans in extreme cases (Fire and Van Dolah, 2012).

The existence of toxigenic and non-toxigenic strains of the same species has been recurrently mentioned for several *Pseudo-nitzschia* species (e.g. Rhodes et al., 1996; Villac et al., 1993; Thessen et al., 2009; Sahraoui et al., 2011). Environmental factors, such as ambient nutrient concentrations, temperature, salinity, irradiance, photoperiod and pH, have been known to influence and under some circumstances to induce DA production in laboratory culture experiments (Lelong et al., 2012; and references therein). In addition, it has been recently observed that DA toxin content

* Corresponding author at: División Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n (B1900FWA), La Plata, Argentina.
*E-mail address:* galmandoz@fcnym.unlp.edu.ar (G.O. Almandoz).

http://dx.doi.org/10.1016/j.hal.2017.01.007
1568-9883/

increased when cells of *Pseudo-nitzschia* species were exposed to copepod grazing (Tammilehto et al., 2015; HarÐardóttir et al., 2015). In the field, however, is difficult to provide strong evidence for the association of environmental conditions with DA production by *Pseudo-nitzschia* species (Marchetti et al., 2004; Schnetzer et al., 2007; Guannel et al., 2015).

Toxigenic *Pseudo-nitzschia* species are typically found in coastal waters, whereas open-ocean strains are usually found to be non-toxigenic or producers of only very low DA cell concentrations (Marchetti et al., 2008; Trick et al., 2010). Nevertheless, it is unclear if this tendency reflects genetic distinctiveness ("ecotypes") or is largely a function of ambient environmental influences on toxin production. From a global perspective, most toxic DA events have been associated with the occurrence of *Pseudo-nitzschia australis* Frenguelli, which together with *P. multiseries* (Hasle) Hasle and *P. seriata* (Cleve) Peragallo present the highest per cell DA concentrations measured in laboratory cultures (Trainer et al., 2012; and references therein). In any case, there is a great inter- and intraspecific variability in DA cell quotas measured among *Pseudo-nitzschia* strains isolated into culture from different regions of the world (Trainer et al., 2012). In addition, highly variable cell quotas are typically found among natural *Pseudo-nitzschia* populations (Schnetzer et al., 2007; Thessen and Stoecker, 2008), but some of this apparent variability may be attributable to DA estimates based upon total cell densities of *Pseudo-nitzschia*, which usually includes several co-occurring species.

Several previous studies have focused on the occurrence of *Pseudo-nitzschia* species in coastal and shelf waters from the Argentine Sea. These taxonomic surveys, conducted exclusively by means of light and electron microscopy analyses, have revealed a high species richness at the morphological level (Negri and Inza, 1998; Ferrario et al., 1999, 2002; Sastre et al., 2001; Almandoz et al., 2007; Sunesen et al., 2009). Similarly, DA has been frequently detected in phytoplankton samples from different zones of the Argentine Sea in the last few years (Negri et al., 2004; Sastre et al., 2007; Cadaillón, 2012; Krock et al., 2015). Indeed, *Pseudo-nitzschia* blooms have been suggested as potentially responsible for calf mortalities of southern right whales along the Valdés Península (D'Agostino et al., 2015; Wilson et al., 2015). During these episodes, DA production was mainly associated with elevated cell densities of *P. australis* (Negri et al., 2004) or *P. fraudulenta* (Cleve) Hasle (Sastre et al., 2007), but the co-occurrence of several other *Pseudo-nitzschia* species (Cadaillón, 2012; Krock et al., 2015), makes it difficult to unequivocally identify the responsible taxa. Likewise, analyses of DA toxin production of local strains of *P. pungens* (Grunow ex Cleve) Hasle (Sar et al., 2006) and *P. multiseries* (Montoya et al., 2008) revealed either negative or positive results, respectively, while DA production for other species found in the Argentic Sea has not been yet evaluated. All this indicate that DA production by *Pseudo-nitzschia* species in Argentine waters and the south-western Atlantic remains poorly understood.

In the present work, the distribution and abundance of *Pseudo-nitzschia* species and DA was analysed from two sampling cruises carried out through the Argentine Sea (~39–47°S), covering coastal, shelf and shelf-break waters during summer and spring 2013. The main goal was to reveal the identity of major DA producers in the field, by associating abundance and distribution of *Pseudo-nitzschia* species and DA.

## 2. Material and methods

### 2.1. Plankton and toxin sampling

Water samples were collected during two oceanographic expeditions in the Argentine Sea (Fig. 1). The first expedition was conducted in late austral summer on the *R/V Bernardo Houssay* from 11 to 22 March 2013, with 24 sampling stations located between ≈39 and 43°S. This cruise was divided into two legs K1 and K2, which comprised 8 and 16 sampling stations, respectively. The second expedition was carried out in austral spring aboard the *R/V Puerto Deseado* from 26 October to 9 November 2013, with 43 sampling stations located between ≈39 and 47°S. At each station, temperature and salinity were measured by CTD (conductivity-temperature-depth) profiler, except from leg K2 of Expedition 2, for which only temperature data were obtained with a multiparameter probe.

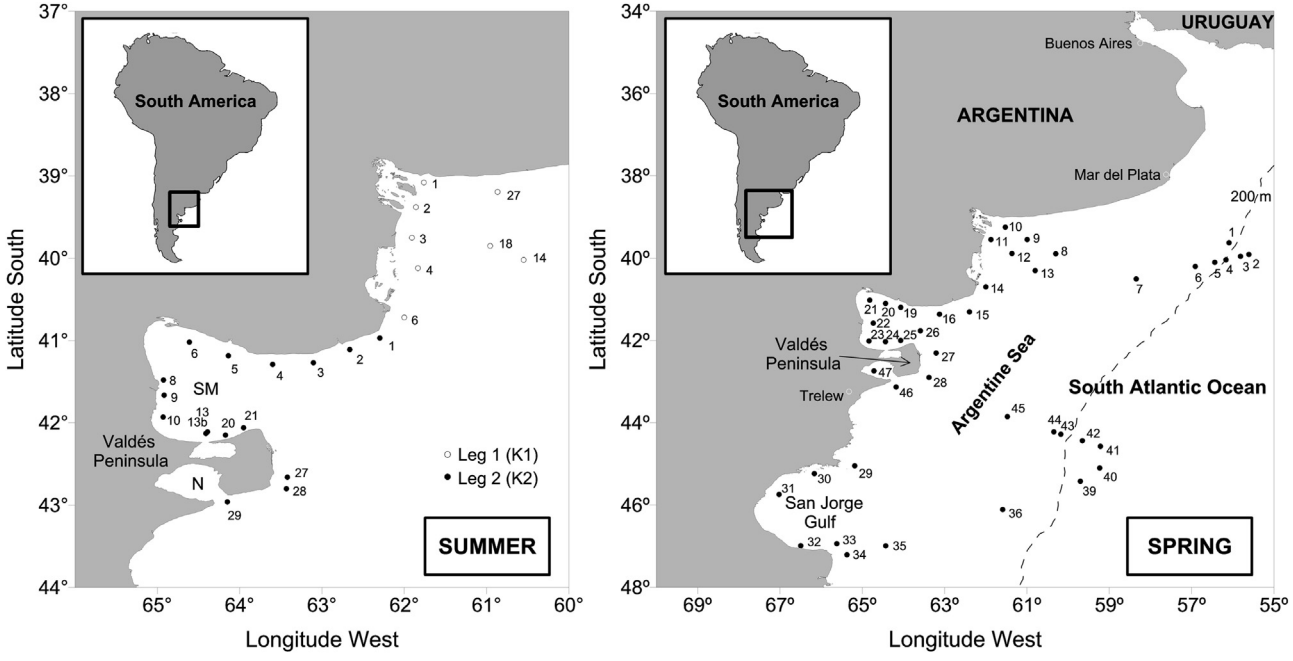


**Fig. 1.** Maps of the study area showing the location of sampling stations in the Argentine Sea during summer and spring cruises. SM = San Matías Gulf. N = Nuevo Gulf.