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Distribution of *Dinophysis* species and their association with lipophilic phycotoxins in plankton from the Argentine Sea

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ABSTRACT

Dinophysis is a cosmopolitan genus of marine dinoflagellates, considered as the major proximal source of diarrheic shellfish toxins and the only producer of pectenotoxins (PTX). From three oceanographic expeditions carried out during autumn, spring and late summer along the Argentine Sea (~38–56°S), lipophilic phycotoxins were determined by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) in size-fractionated plankton samples. Lipophilic toxin profiles were associated with species composition by microscopic analyses of toxigenic phytoplankton. Pectenotoxin-2 and PTX-11 were frequently found together with the presence of *Dinophysis acuminata* and *Dinophysis tripos*. By contrast, okadaic acid was rarely detected and only in trace concentrations, and dinophysistoxins were not found. The clear predominance of PTX over other lipophilic toxins in *Dinophysis* species from the Argentine Sea is in accordance with previous results obtained from north Patagonian Gulfs of the Argentine Sea, and from coastal waters of New Zealand, Chile, Denmark and United States. *Dinophysis caudata* was rarely found and it was confined to the north of the sampling area. Because of low cell densities, neither *D. caudata* nor *Dinophysis norvegica* could be biogeographically related to lipophilic toxins in this study. Nevertheless, the current identification of *D. norvegica* in the southern Argentine Sea is the first record for the southwestern Atlantic Ocean. Given the typical toxigenicity of this species on a global scale, this represents an important finding for future surveillance of plankton-toxin associations.

1. Introduction

Marine dinoflagellates of the genus *Dinophysis* include more than 120 phototrophic and heterotrophic species in marine waters over the entire world (Jensen and Daugbjerg, 2009). This cosmopolitan genus includes species with a wide geographical distribution, such as *Dinophysis acuminata*, which occurs within a wide range of temperature regimes (Kamiyama et al., 2010), whereas others, such as *Dinophysis norvegica* and *Dinophysis tripos*, apparently with more restricted environmental tolerances, mainly occur in boreal and tropical-temperate waters, respectively (Reguera et al., 2012).

Among all members of the genus *Dinophysis*, 10 species have been found to produce lipophilic polyether phycotoxins, known collectively as diarrheic shellfish toxins (DST), including okadaic acid (OA) and dinophysistoxin (DTX) derivatives. Seven of these analogs have been implicated as causative agents of diarrheic shellfish poisoning (DSP) (Reguera and Pizarro, 2008), an important human illness syndrome linked to consumption of contaminated shellfish. The first clinical report of diarrheic syndrome related to consumption of shellfish came from the Netherlands (Korringa and Roskam, 1961), but the causative organism was not determined until the 1980s, when this new toxic syndrome was described as DSP (Yasumoto et al., 1978) and *Dinophysis fortii* identified as the toxic agent (Yasumoto et al., 1980). Subsequent studies confirmed OA and DTX as the main compounds responsible for DSP (Murata et al., 1982).

In addition to toxigenic *Dinophysis* species, the heterotrophic dinophysoid *Phalacroma rotundatum* has been included in the list of DSP toxin-containing species (Reguera et al., 2014 and

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references therein), but the production of such toxins by this species has not been confirmed. González-Gil et al. (2011) suggested that *P. rotundatum* may act as a vector of toxins taken up from ciliate prey that have previously fed on co-occurring toxic *Dinophysis* spp. In contrast, the confirmed toxigenic *Dinophysis* species all possess plastids and hence they are capable of performing photosynthesis. Moreover, toxin production has been clearly linked to photosynthesis (Kim et al., 2008). Hence the capacity for *de novo* DST production in heterotrophic species remains highly questionable, and it is more likely that heterotrophic dinoflagellates only accumulate toxins rather than produce them.

Certain members of the genus *Dinophysis* also produce pectenotoxins (PTX), a large family of lipophilic polyether toxins originally associated with the DSP toxin complex. The PTX analogs produced by *Dinophysis* may be modified by metabolic activity within shellfish; for example PTX-2 can be modified to the corresponding seco-acid PTX-2sa (Lee et al., 1989; Suzuki et al., 1998; Ciminiello et al., 2010). Toxicological studies indicate that PTX are not diarrheagenic after oral administration to laboratory rodents, and hence are not true DST, but PTX-1 is hepatotoxic albeit at high acute concentrations (Terao et al., 1986). The risk to human health regarding this group of toxins remains under toxicological discussion and review by regulatory authorities.

Historical records on the occurrence of *Dinophysis* in the Argentine Sea include a large contingent of around 30 species, with *Dinophysis acuminata* cited as the most common and widely distributed species (Balech, 1988). Among these species, *D. acuminata*, *Dinophysis tripos*, *Dinophysis caudata* and *Dinophysis fortii* are included in the IOC-UNESCO reference list of toxic microalgae (Zingone and Larsen, 2014) as putative or confirmed producers of DST. Nevertheless, in spite of the common presence of *Dinophysis* spp. in Argentine coastal waters, confirmed reports of DSP are rather exceptional. The first documented case of human intoxication by DSP in Argentina occurred in 1999 in Chubut Province in Patagonia and was linked to consumption of bivalve shellfish that had been harvested in the Gulf of San José and Nuevo Gulf (≈42°S). This DSP event was related, however, to the presence of the benthic dinoflagellate *Prorocentrum lima* (Gayoso et al., 2002), which is also known to produce OA, DTX-1 and other variants (Quilliam and Ross, 1996).

More recently, a DSP outbreak was associated with the presence of *Dinophysis acuminata* and *Dinophysis caudata* on the northern coast of Buenos Aires Province (36.5–37°S) in summer 2010, and during which both OA and DTX-1 were detected in mussels by liquid chromatography with fluorescence detection (LC-FD) (Sar et al., 2010, 2012). Later, positive mouse bioassays for DSP were recorded in mussels collected in the same area during January and November, 2012, and related circumstantially to the presence of *D. acuminata* and *D. caudata*, respectively (Sunesen et al., 2014). Recent monitoring programs in the gulfs of north Patagonia obtained positive mouse bioassays for DST related to the presence of *D. tripos* (Gracia Villalobos et al., 2015). In addition, PTX-2 has been detected off the coast of Buenos Aires Province (Montoya et al., 2013), in the Gulf of San Jorge (Krock et al., 2015), in shelf waters from 40°S to 46°S (Fabro et al., 2015) and in the Gulf of San José and Nuevo Gulf (Gracia Villalobos et al., 2015). Finally, OA and DTX-1 have been recently detected by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) in shellfish collected in several coastal areas of Buenos Aires Province (Turner and Goya, 2015). The role of *Dinophysis* blooms in DSP events along the Argentine coast thus remains rather enigmatic and poorly defined, both with respect to associated species and known toxin composition.

Three oceanographic expeditions were conducted over an extended area (≈38–56 °S) of the coastal Argentine Sea and adjacent shelf and slope waters and in different seasons (autumn, late summer and spring) with the purpose of determining the potential biogeographical linkages between toxic microalgae and their associated toxins. In the analysis presented here the distribution and abundance of *Dinophysis* spp. in the Argentinean Sea is described, and relationships between their occurrence and their respective lipophilic toxin composition are established.

2. Material and methods

2.1. Field sampling protocols

The continental shelf waters of the Argentine Sea were sampled during three oceanographic expeditions. Expedition 1 was conducted in autumn onboard the *R/V Puerto Deseado* from March 30th to April 14th, 2012. A total of 47 stations were sampled between ≈38 and 56°S. The second expedition was carried out in late austral summer on the *R/V Bernardo Houssay* from March 11th to March 22nd, 2013, with 24 sampling stations located between ≈39 and 43°S. This cruise was divided in two legs K1 and K2, which comprise 8 and 16 sampling stations, respectively. The third expedition was conducted in austral spring aboard the *R/V Puerto Deseado*, from October 26 to November 09, 2013, with 47 sampling stations located between ≈40 and 47°S. The conductivity (salinity)/temperature/depth (CTD) data were available throughout all expeditions, except from leg K2 of Expedition 2, during which no CTD measurements were performed. During this leg, only surface water temperature was measured with a multiparameter probe TOA-DKK Model WQC.

Plankton samples were collected by vertical net tows through the upper 20 m of the water column with a 20 μm-mesh Nitex net of 60 cm diameter for both taxonomic and phycotoxin analysis. Each net haul concentrate was diluted up to 1 L with 0.2 μm-filtered seawater. An aliquot was fixed with acidic Lugol's iodine solution for species identification and enumeration. The rest was sequentially filtered through Nitex mesh of 200, 50 and 20 μm in PVC cylinders by gravity filtration and split into aliquots for toxin extraction. On Expedition 3, aliquots of each size-fraction were also taken for plankton analysis by microscopy. The total net sample was filtered through Nitex mesh of 200, 50 and 20 μm and re-suspended in 40 mL of filtered seawater. During all three expeditions, Niskin bottle samples were also taken from 3 and 10 m depth and mixed in equal volume for determination of total plankton community composition and quantitative analyses.

2.2. Phytoplankton analysis

Cell densities of *Dinophysis* species in net tow concentrates was determined by counting 1 mL of acidic Lugol's iodine fixed samples in Sedgewick-Rafter chambers (LeGresley and McDermott, 2010) with an inverted microscope (Leica DMIL LED). Data recovered from cell counting of the net samples concentrates was used as semi-quantitative information to compare cell densities and toxin concentrations. Cell densities are expressed per net tow (cells NT^{-1}), which corresponds to the total net harvest concentrate diluted up to 1 L. In plankton cell counting from Expeditions 1 and 2, 1 mL of total net material was used for semi-quantitative calculations, so the limit of detection of the counting method was 1000 cells NT^{-1}. During Expedition 3 the total net material (1 L) was filtered through three meshes (20, 50 and 200 μm) and re-suspended in 40 mL of filtered seawater, of which 1 mL from each fraction was counted for semi-quantitative estimations. In this case, the limit of detection was 40 cells NT^{-1}.

Cell densities of *Dinophysis* species in plankton samples collected by Niskin bottles was determined according to the Utermöhl (1958) inverted microscope method. Subsamples

(50 mL) from the Niskin bottles from fixed depths were left to settle for 24 h in a composite sedimentation chamber prior to counting. The limit of detection of this method was 20 cells L^{-1} for the three expeditions.

Further morphological examination of selected samples was conducted with a phase contrast/differential interference contrast optical microscope (Leica DM2500) equipped with a DFC420C camera, and under a scanning electron microscope (Jeol JSM-6360 LV SEM). For SEM analysis, samples were filtered onto 0.2 μm polyamide filters and sputter-coated with Au-Pd.

2.3. Toxin analysis

Cell pellets from the plankton net tow size-fractions were collected by centrifugation (3220 × *g*, 15 min at 4 °C), suspended in 500 μL methanol, and subsequently homogenized with 0.9 g of lysing matrix D by reciprocal shaking at maximum speed (6.5 m s^{-1}) for 45 s in a Bio101 FastPrep instrument (Thermo Savant, Illkirch, France). After homogenization, samples were centrifuged at 16,100 × *g* at 4 °C for 15 min. The supernatant was transferred to a spin-filter (0.45 μm pore-size, Millipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at 800 × *g*, followed by transfer to autosampler vials. Analysis of multiple lipophilic toxins was performed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), as described in Krock et al. (2008). Toxin concentrations are expressed as nanograms per net tow (ng NT^{-1}).

2.4. Toxin cell quotas

Cell quotas of lipophilic phycotoxins (calculated as toxin content divided by the number of cells) were estimated from samples containing only one putatively toxigenic *Dinophysis* species or where a single toxigenic species represented >90% of the cells of this genus. Following recommendations of the study of cell counting methods reported by ICES (2006), only those samples with cell abundances >10,000 cells NT^{-1} were considered for cell toxin quota estimates. PTX-2sa and isomers were excluded from the estimate of PTX-2 cell quotas. For samples collected during expeditions 1 (autumn) and 2 (late summer), *Dinophysis acuminata* and *Dinophysis tripos* cells were assigned to each size-fraction according to their cell dimensions.

3. Results

Microscopic examination of the field material from the three expeditions along the Argentine Sea revealed the presence of nine *Dinophysis* species, of which four are known to be toxigenic: *Dinophysis acuminata*, *Dinophysis caudata*, *Dinophysis norvegica*, and *Dinophysis tripos* (Fig. 1). As *D. norvegica* was found for the first time in South-Atlantic waters a description of the specimens from this area is provided.

Dinophysis norvegica cells were moderate in size, varying from 45 to 57 μm in length. The characteristic cell shape was presented by Argentine field specimens, exhibiting greatest cell width just above the transverse mid-line of the cell body and with a convex dorsal outline and concave ventral side toward the antapex (Fig. 2). The large hypothecal plates showed a coarse texture and were heavily areolated, and in some cells they possessed small irregular antapical knobs (Fig. 2C). The left sulcal list ended at the beginning of the ventral concavity and presented three ribs, R2 closest to R1 (Fig. 2A), and R3 directed antapically (Fig. 2B). The right sulcal list was rather small and triangular in shape.

Other *Dinophysis* species, not known to be toxigenic, including *Dinophysis truncata*, *Dinophysis operculata*, *Dinophysis cuneis*, *Dinophysis minuta* and *Dinophysis subcircularis* were present at some stations during Expedition 1 and/or 3, but were detected only in net samples and never dominant. Distribution of these species is shown in Fig. 3. The heterotrophic dinophysoid species *Phalacroma rotundatum* was also found in low cell densities.

3.1. Dinophysis acuminata *distribution and related toxins*

On Expedition 1 (autumn), *Dinophysis acuminata* was found in 80% of net samples, from stations between 38 and 56°S, representing an average of 88% of the toxigenic species of the genus. This species was present in 33% of Niskin bottle samples, exhibiting highest cell densities (100–160 cells L^{-1}) in the southern Argentine Sea and within San Jorge Gulf (Fig. 4). Pectenotoxin-2 was detected in 63% of the net samples in the 20–50 μm size-fraction, with the highest concentrations in the southern Argentine Sea; the analog PTX-2sa represented on average 14% of the total PTX-2 group (Supplementary Table S1). *Dinophysis acuminata* was observed at all stations where PTX-2 was also found within the 20–50 μm size-fraction (Fig. 5). The toxin quota estimated for *D. acuminata* varied from 0.2 to 15 pg $cell^{-1}$ (n = 12), with maximum values found in the southern range of the sampling area (52–54°S). Pectenotoxin was also detected in four samples of the 50–200 μm size-fraction, where *D. acuminata* was the only potentially toxic species detected.

Dinophysis acuminata was found less frequently during Expedition 2 (late summer), than for the previous expedition, being present in ~38% of the net tow samples (Supplementary Table S2). In Niskin bottle samples, a single record of 20 cells L^{-1} was found in coastal waters from Valdés Peninsula (≈43°S) (Fig. 4). This species represented only an average of 8% of the cells of putatively toxigenic members of the genus; it was therefore not possible to estimate its relative contribution to the overall toxin content of the plankton.

On Expedition 3 (spring), *Dinophysis acuminata* appeared in 80% of net samples, representing an average of 98% of the cells of toxigenic *Dinophysis* species in the 20–50 μm size-fraction. In Niskin bottle samples, *D. acuminata* was present in 52% of the samples, with cell densities ranging from 20 to 1680 cells L^{-1}. The highest cell abundances were found at the continental shelf edge and in San Jorge Gulf (Fig. 4). From the 20–50 μm size-fraction, PTX-2 was detected at 41% of the stations (reaching the highest concentrations among the three sampling expeditions); PTX-11 was present at 23% of the stations, whereas OA was found only in a single instance (Supplementary Table S3). The highest concentrations of both PTX-2 and PTX-11 were found in the southern range (from 44°S to 46°S) and in waters at or near the continental slope. The analog PTX-2sa was also detected, representing on average 8.6% and 23% of the total PTX-2 analogs in the 20–50 and 50–200 μm size-fractions, respectively. *Dinophysis acuminata* co-occurred in almost all samples with PTX-2 and PTX-11, except for some stations where *D. acuminata* cells were found but no toxins were detected. In contrast, for St 14 no toxigenic *Dinophysis* species were found but PTX-2 and PTX-11 were detected (Fig. 6). The calculated PTX-2 cell quota for *D. acuminata* varied between 0 and 22 pg $cell^{-1}$ (n = 14) and the maximum value was detected at St 36, in waters near the slope adjacent to San Jorge Gulf (46°S). The second highest calculated value (5.7 pg $cell^{-1}$) occurred at 40°S, also within slope waters. Toxin cell quotas for PTX-11 ranged between 0.5 and 3.6 pg $cell^{-1}$ (n = 4), with the highest value found (as for PTX-2) at St 36. At the only station where OA was detected, *D. acuminata* cells were found and the toxin cell quota was estimated to be 0.5 pg $cell^{-1}$.

3.2. Dinophysis tripos *distribution and related toxins*

On Expedition 1 (austral autumn), *Dinophysis tripos* was found in net samples at only three stations (6% of total sampled) between

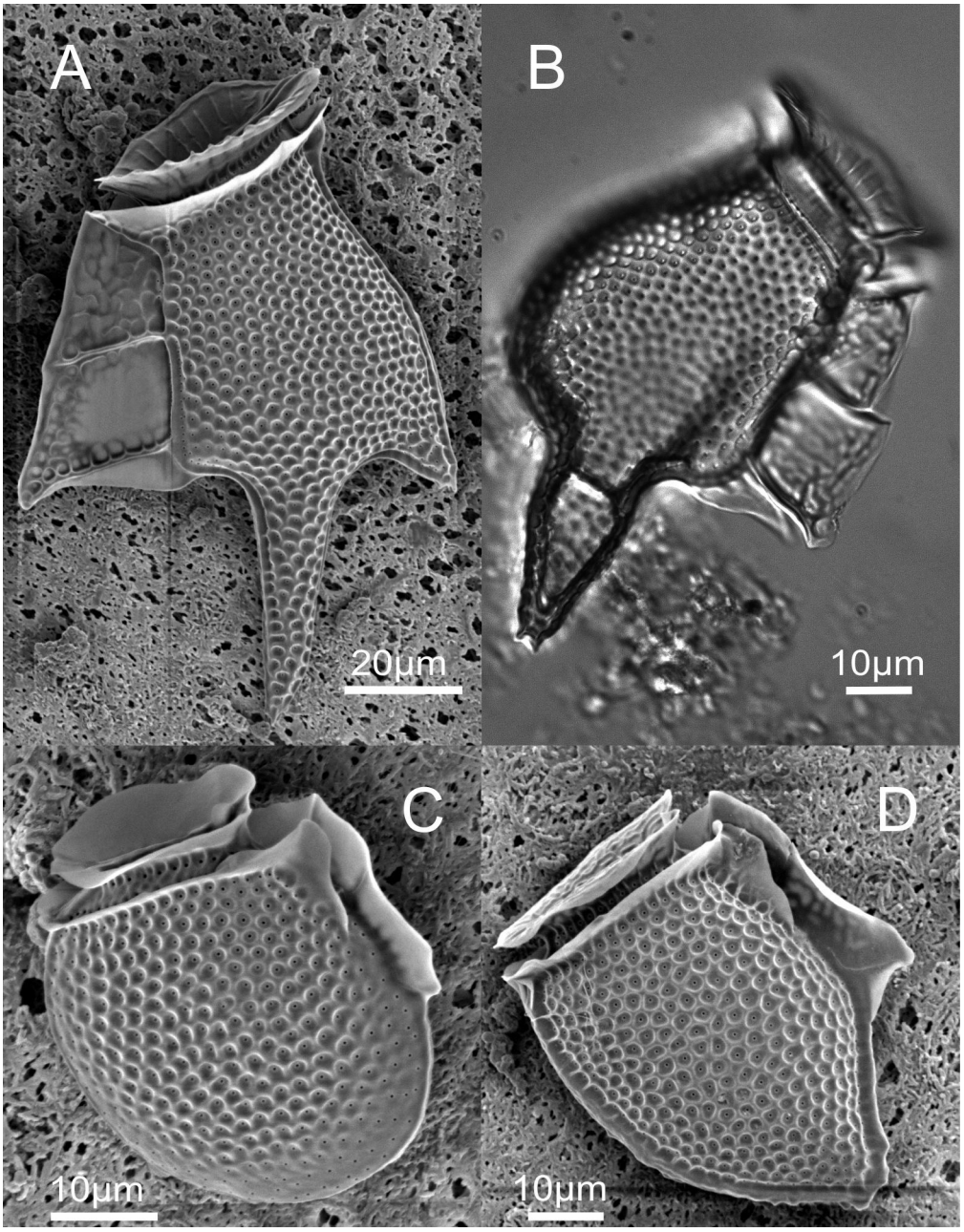

Fig. 1. Scanning electron microscopy (A, C and D) and light microscopy (B) images of putatively toxigenic *Dinophysis* species found on the three expeditions across the Argentine Sea. A: *D. tripos*; B: *D. caudata*; C: *D. acuminata*; D: *D. norvegica*.

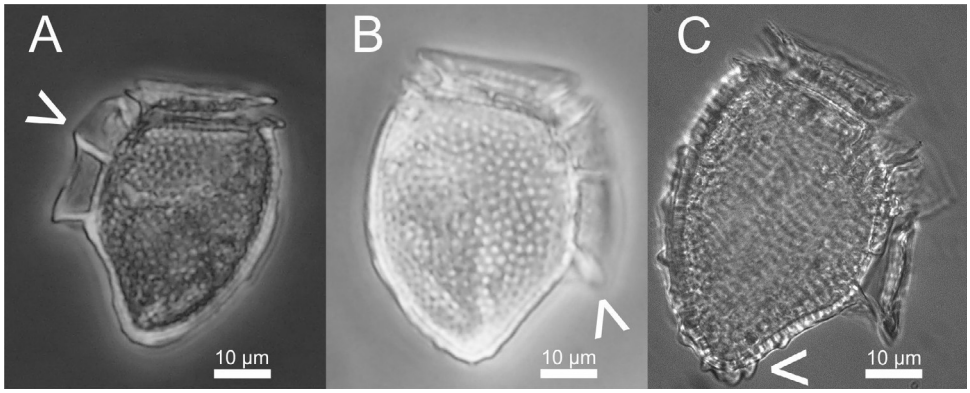

Fig. 2. Light microscopy images of different *Dinophysis norvegica* cells. (A) Left sulcal list supporting rib 2 (R2) closer to R1 than R3 (arrow). (B) R3 directed antapically (arrow). (C) Antapical knobs (arrow).

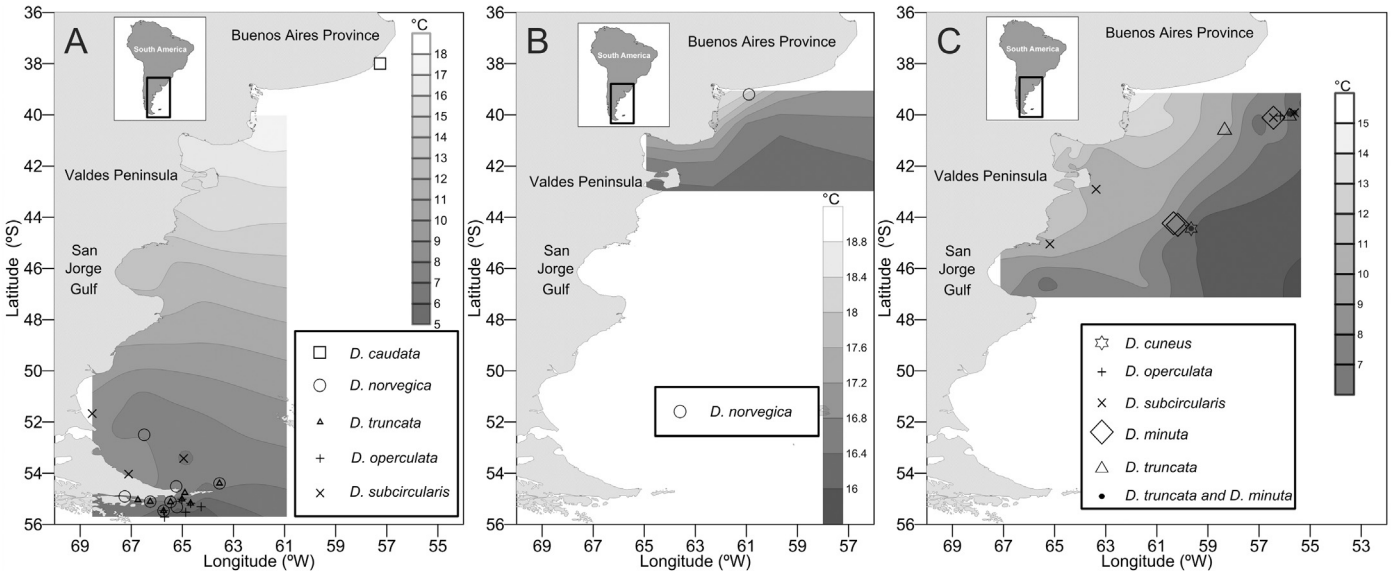

Fig. 3. Distribution of *Dinophysis* species found only in semi-quantitative net samples from autumn Expedition 1 (A), summer Expedition 2 (B) and spring Expedition 3 (C).

38 and 41°S. In Niskin bottle samples, this species was detected only at St I50 and I51 (40 and 39°S, respectively) and at low cell densities (Fig. 7). At St I50, *D. tripos* was the only toxigenic *Dinophysis* species found, occurring together with PTX-2 in the 50–200 μm size-fraction. The estimated cell quota was 0.5 pg cell^{-1}.

During Expedition 2 (late austral summer), *Dinophysis tripos* was the dominant species of the genus, averaging 70% of total *Dinophysis* cells in net tow samples where the genus was present. The species was found in 50% of the net samples, but occurred only within a geographically restricted area from ≈41 to 43°S.

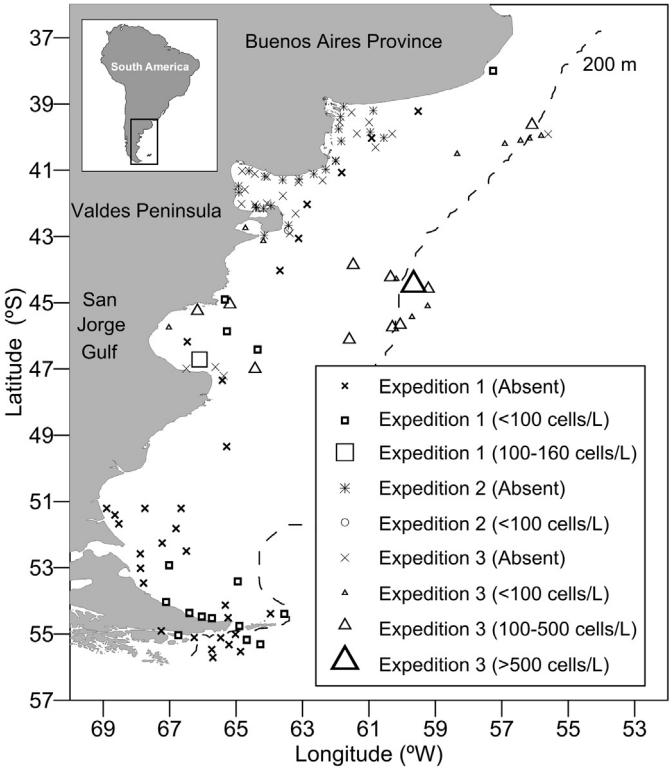

Fig. 4. Distribution and density of *Dinophysis acuminata* in Niskin bottle samples from the Argentine Sea during the three expeditions.

Dinophysis tripos was also present in 42% of the Niskin bottle samples at cell densities ranging from 20 to 1560 cells L^{-1} (Fig. 7), with highest values at 41°S. Pectenotoxin-2 was present at 46% of the stations (from ≈41 to 43 °S) within the 20–50 μm size-fraction (Supplementary Table S2). Pectenotoxin-11 was detected in the smallest size-fraction at only one station. Small and/or intermediate *D. tripos* cells (*sensu* Rodríguez et al., 2012) with dorso-ventral cell dimensions ranging between 25 and 40 μm were present in PTX-containing samples in the 20–50 μm size-fraction (Fig. 8). The PTX-2 cell quotas estimated for *D. tripos* small/intermediate cells ranged between 0.1 and 0.9 pg cell^{-1} (n = 3). On this expedition, the analog PTX-2sa accounted for 38% of the total PTX-2 analogs in the smallest size-fraction.

During Expedition 3 (austral spring), *Dinophysis tripos* was found in 14% of net samples, occurring in coastal waters from 41°S to 42°S. In Niskin bottle samples, this species was present at 7% of the stations in low cell densities, ranging from 20 to 40 cells L^{-1} (Fig. 7). *Dinophysis tripos* was the only toxigenic species present together with PTX-2 and PTX-11 in sample 19 from the 50 to 200 μm size-fraction.

3.3. *Distribution of other toxigenic* Dinophysis *species and* Phalacroma rotundatum

Among toxin-associated *Dinophysis* species other than *Dinophysis acuminata* and *Dinophysis tripos*, *Dinophysis norvegica* was perhaps the most significant. Nevertheless, *D. norvegica* was never present at high cell densities, as it was not detected in Niskin bottle samples in any season, and in net samples 2 × 10^3 cells NT^{-1} was the highest density detected. This species appeared in net samples from Expedition 1 (autumn) at 17% of stations, all of them located southward of 52°S and from Expedition 2 (late summer) in only one sample at 39°S. As shown in the distributional map (Fig. 3), it was not found during Expedition 3 (spring). In samples with detectable levels of toxins the species always co-occurred with *D. acuminata*.

Dinophysis caudata was only found during Expedition 1 (autumn) in one net sample at northern extent of the sampling area (38°S) but where no toxins were detected. It was absent from quantitative samples (Fig. 3).

Phalacroma rotundatum was found at 11% of the stations from Expedition 1 (autumn). This species was mostly confined

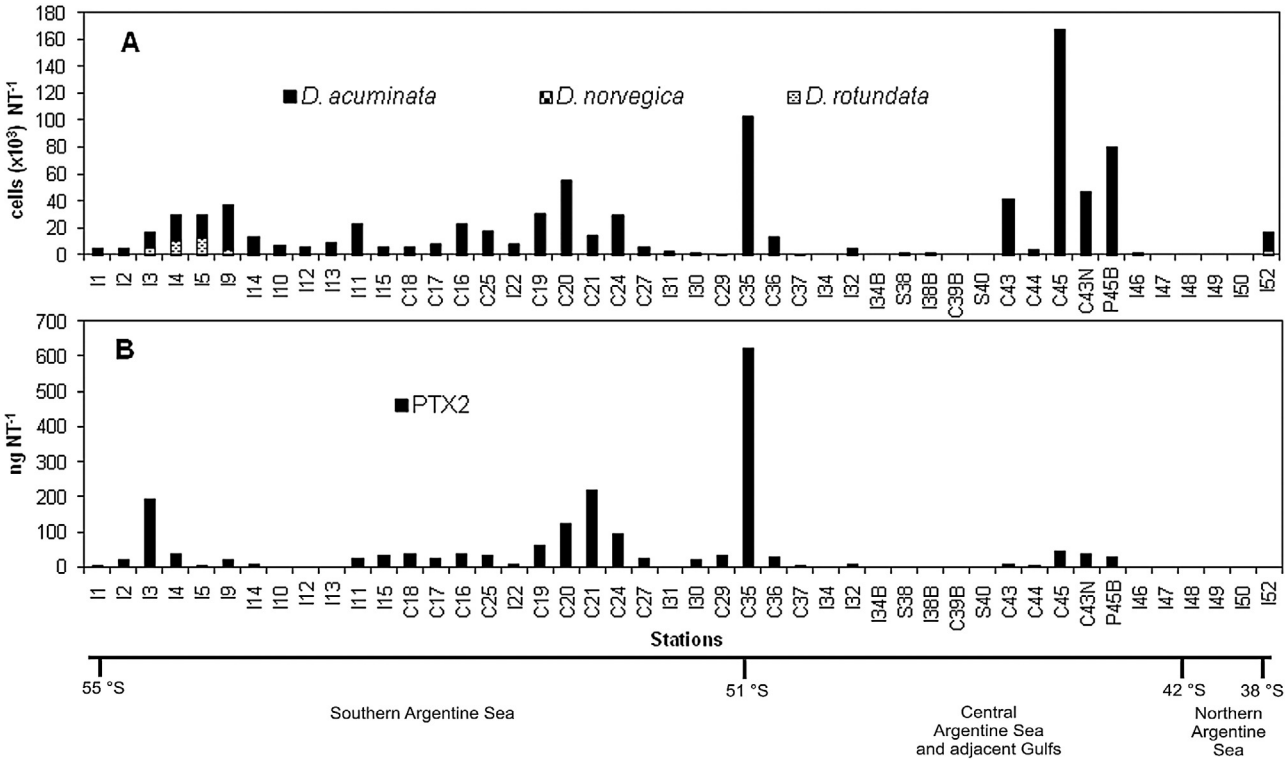

Fig. 5. Densities of *Dinophysis* spp. (<50 μm cell size) in total net samples (A) and distribution of PTX-2 concentration in the 20–50 μm size-fraction (B), during Expedition 1 (autumn). No toxin data were available for stations I12 and I13.

to those samples collected around 55°S, but it was also observed at one station at 38°S. In spring (Expedition 3), *P. rotundatum* was present at 26% of the net sampled stations. This species did not appear in the quantitative samples of any expedition.

3.4. Environmental parameters related to distribution of Dinophysis spp.

Among all *Dinophysis* species found during the three expeditions, *Dinophysis acuminata* was represented over the widest

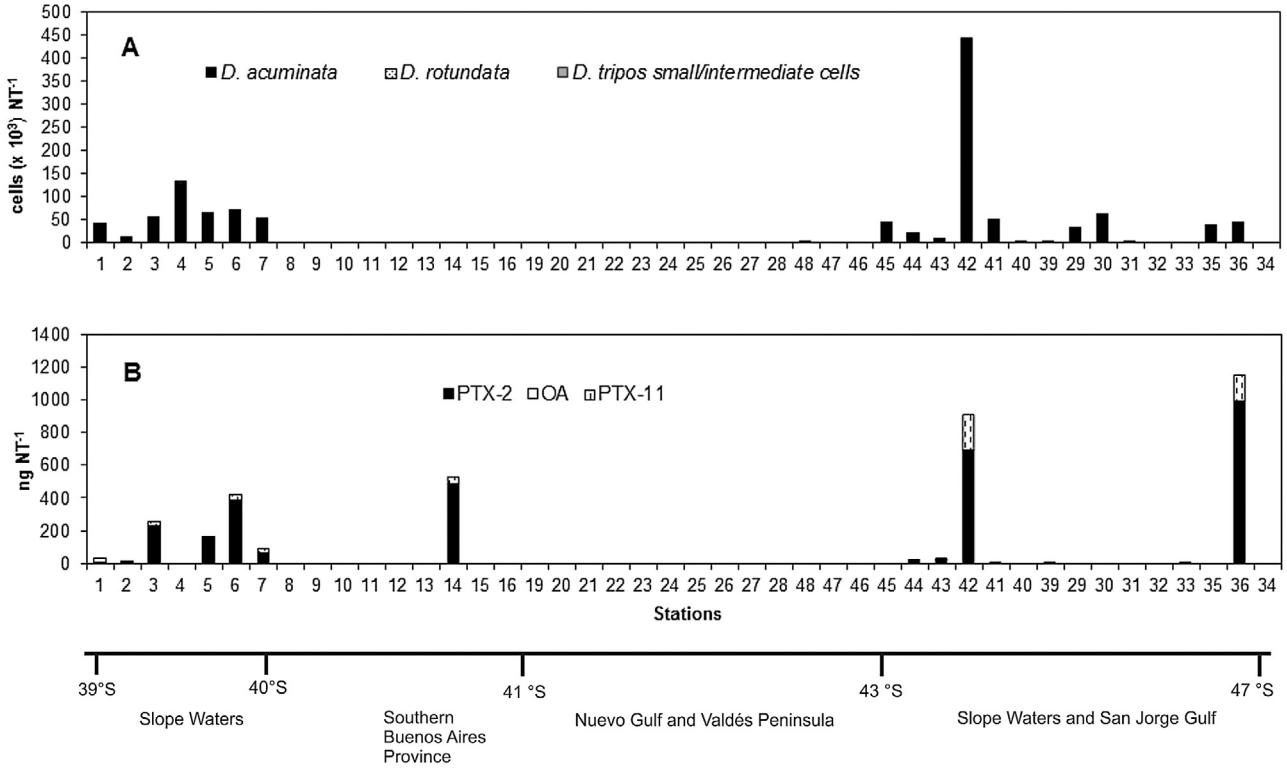

Fig. 6. *Dinophysis* spp. cell densities (A) and toxin concentrations (B) in the 20–50 μm size-fraction during Expedition 3 (spring).

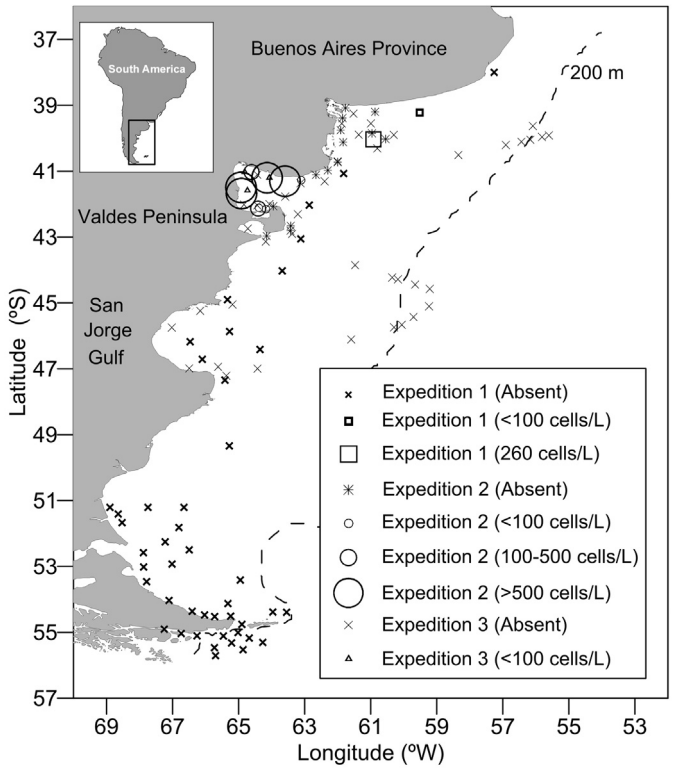

Fig. 7. Distribution and density of *Dinophysis tripos* in Niskin bottle samples from the Argentine Sea during the three expeditions.

temperature range (5.7–18.4 °C), although maximum cell densities in quantitative samples were found between ~8 and ~13 °C. *Dinophysis tripos* was found only within temperate waters, at temperatures above 12 °C, but yielded highest cell densities at ~17 °C. The only record of *Dinophysis caudata* among the three expeditions was from waters at 18.8 °C. On the other hand, *Dinophysis norvegica* and *Dinophysis operculata* were both found in cooler waters, at temperatures <9 °C (there is one record of *D. norvegica* at 18 °C, but this corresponded only to one empty theca of the species). *Dinophysis truncata*, *Dinophysis subcircularis* and *Phalacroma rotundatum* were all detected in this study within similar temperature ranges from ~6 to 12 °C (Table 1).

Dinophysis acuminata and *Dinophysis norvegica* were the two species found within the widest salinity range (31.2–34.2) among the three expeditions. All other *Dinophysis* species and *Phalacroma rotundatum* were present within a more limited salinity range (~33 to 34) (Table 1).

4. Discussion

Our results show that the genus *Dinophysis* is commonly found in the Argentine Sea throughout various seasons and over a wide geographical range. The most widely distributed species was *Dinophysis acuminata*, which is in accordance with previous reports (Balech, 1988; Sar et al., 2010; Negri et al., 2013; Sunesen et al., 2014). This species was the dominant taxon of the genus during Expeditions 1 and 3 (austral autumn and spring, respectively) and exhibited the highest cell densities at the southern extent of the sampling area, in slope waters and in the San Jorge Gulf, where surface temperatures ranged between ~8 and ~13 °C. This distributional pattern coincides with the historical predominance of this species in sub-antarctic waters at temperatures lower than 14 °C (Balech, 1988). The higher cell densities found in the San Jorge Gulf may result from the specific hydrographic conditions present in this area, such as a strongly stratified water column known to favor dinoflagellate cell accumulations (Cloern et al., 2005; Jephson and Carlsson, 2009; Krock et al., 2015). The salinity range (33.2–33.9) in which the highest cell densities were detected corresponds to Argentine shelf waters, which consist mainly of subantarctic waters diluted by continental discharge (Piola and Falabella, 2009).

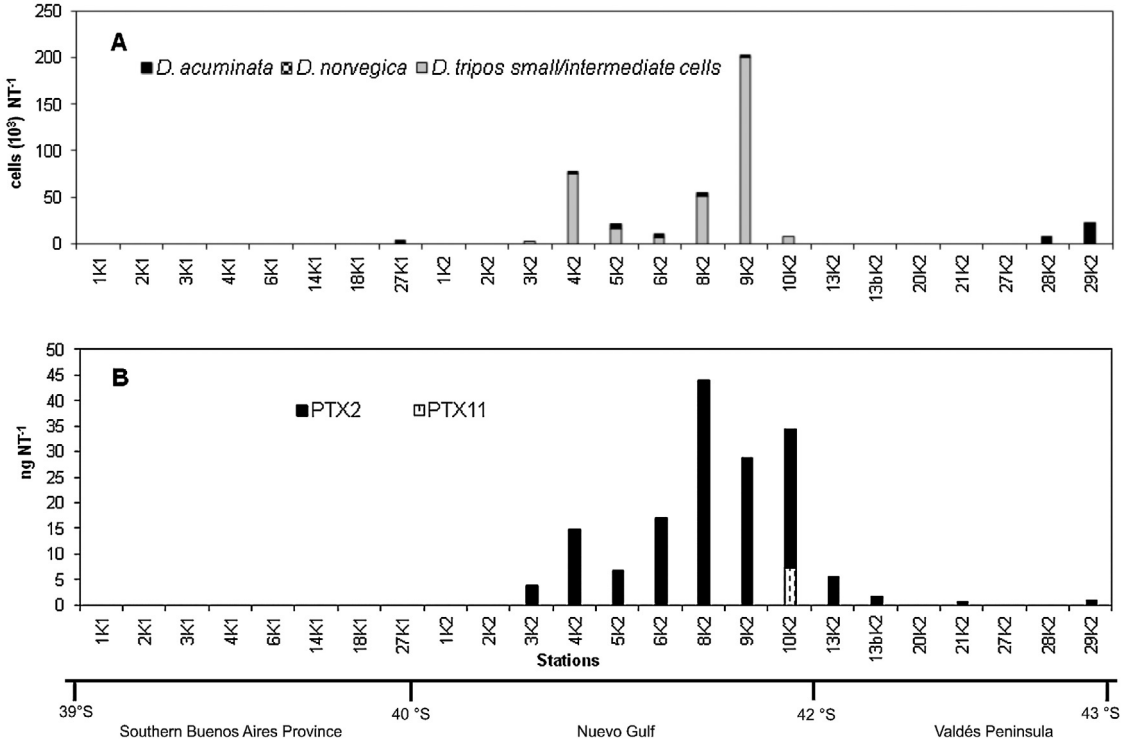

Fig. 8. Abundances of *Dinophysis* spp. <50 μm in total net samples (A) and toxin concentrations in the 20–50 μm size-fraction (B) during Expedition 2 (late summer).

Table 1
Surface (2 m depth) temperature and salinity ranges in which *Dinophysis* spp. and *Phalacroma rotundatum* were found during the three expeditions.

	D. acuminata	*D. tripos*	*D. caudata*	*D. norvegica*	*D. truncata*	*D. subcircularis*	*D. operculata*	*D. minuta*	*D. cuneis*	*P. rotundatum*
Expedition 3 (spring)										
T (°C)	7.4–14.8	12.1–13.6	Not found	Not found	7.8–11.4	7.8–11.8	8–8.3	7.8–9.7	8	7.4–11.4
Salinity	32.6–34.2	33.9–34.1	Not found	Not found	33.6–34	33.1–34	33.8	33.7–34	33.8	33.6–34.2
Expedition 2 (summer)										
T (°C)	16.2–18.4	14.4–17.9	Not found	18.4	Not found	Not found	Not found	Not found	Not found	Not found
Salinity	32.2	no data	Not found	32.2	Not found	Not found	Not found	Not found	Not found	Not found
Expedition 1 (autumn)										
T (°C)	5.7–15.7	17.3–17.7	18.8	6–8.9	6.8–8.4	5.7–9.6	5.8–7.8	Not found	Not found	5.9–8.4
Salinity	31.2–34.2	33.8	34.1	31.2–34.2	33.2–34.2	32.6–34.2	33.6–34.2	Not found	Not found	33.3–34.3
All expeditions										
T (°C)	5.7–18.4	12.1–17.9	18.8	6–8.9 (18.4)	6.8–11.4	5.7–11.8	5.8–8.3	7.8–9.7	8	5.9–11.4
Salinity	31.2–34.2	33.8–34.1	34.1	31.2–34.2	33.2–34.2	32.6–34.2	33.6–34.2	33.7–34	33.8	33.3–34.3

In size-fractionated net samples, most *Dinophysis acuminata* cells were found in the 20–50 μm size-fraction, although a small percentage were also detected in the 50–200 μm fraction, likely as a result of partial net obstruction caused by high cell concentrations in the plankton concentrate. This may also explain the presence of PTX-2 in this larger size-fraction during the autumn expedition in samples where *D. acuminata* was the only *Dinophysis* species found. Nevertheless, transmission through the food web to heterotrophic species cannot be excluded as the proximal source of this toxin in the larger cell size-fraction. The presence of PTX has been detected in isolated cells of the heterotrophic dinoflagellates *Protoperidinium divergens*, *Protoperidinium depressum*, and *Protoperidinium crassipes* that were previously observed to feed on *Dinophysis* spp., suggesting that transfer of toxin to these heterotrophs had occurred (Miles et al., 2004).

Other food web toxin transfer vectors may also account for DST compartments in net tow or Niskin bottle size-fractions. Some copepod species (*Oithona nana* and *Temora longicornis*) can actively feed on toxic *Dinophysis* species, although the lack of correlation between abundance of this copepods and concentration of toxins led Maneiro et al. (2000) to suggest that transmission of DST by those species is not important. On the other hand, in the same work, these authors found that the tintinnid *Favella serrata* is able to accumulate DSP toxins when high cell density populations feed selectively on *Dinophysis*. Moreover, PTX might be released to seawater and remain attached to particulate matter and debris (Fux et al., 2011; Pizarro et al., 2008) that could be collected in the net tows. This non-living particulate component and/or the presence of vector microzooplankton and heterotrophic dinoflagellates can also lead to detection of toxins in samples where *Dinophysis* cells are no longer present, as in the case of St 14 (20–50 μm size-fraction) from Expedition 3, and in samples from the larger size-fraction from Expedition 1 in autumn.

Lipophilic toxin analysis revealed a wide geographical distribution of PTX-2 associated with the presence of *Dinophysis acuminata* during both autumn (Expedition 1) and spring (Expedition 3). In addition, PTX-11 was also detected in association with *D. acuminata* during spring. The high variability (estimated from 0 to 22 pg cell^{-1}) calculated for PTX-2 cell quotas in the present field study is in accordance with results from natural populations in Galician Rías, where toxin content of the same dinoflagellate species can vary considerably (Fernández et al., 2006). In this context, it is noteworthy that at three stations from Expedition 3, moderate *D. acuminata* cell concentrations were found in net samples (41, 47 and 131 × 10^3 cells NT^{-1}), although no toxins were detected. This might imply the presence of non-toxigenic strains in natural populations of the species. Alternatively, cell disruption or even more minor physical stress may lead to toxin leakage from the plankton slurries collected on meshes during the filtration processes (Johansen and Rundberget, 2006). Plankton collection and filtration for toxin analyses during this study were carried out as rapidly as possible, and in the same manner for each sampling point, but some losses for the reasons mentioned cannot be ruled out (Pizarro et al., 2008) and could lead to underestimates of cell toxin quota.

Dinophysis tripos represented the second most important species in terms of cell densities among the three expeditions in the Argentine Sea. This species predominated during Expedition 2 (late summer) and its distribution was circumscribed to northward of about ≈42°S. *D. tripos* was frequently found in San Matías Gulf, a semi-enclosed basin influenced by the Negro river (Guerrero and Piola, 1997), that is characterized by a high tidal energy (Tonini et al., 2007) and a vertical mixing (Palma et al., 2004). Moreover, multi-year records (2009–2011) from the Argentine Sea have reported this species throughout all seasons in the gulfs of northern Patagonia (≈41–43°S), with the species appearing mainly during autumn and winter (Gracia Villalobos et al., 2015). Furthermore, maximum cell densities in this study were detected at ∼17 °C, which agrees with known primary occurrence in tropical to temperate seas (Reguera et al., 2012). Co-occurrence of PTX-2 and PTX-11 in the smaller size-fraction (20–50 μm) and small and intermediate *D. tripos* cells (<50 μm) from Expedition 2 (late summer) agrees with results from our previous work, which showed a strong association of *D. tripos* large cells and occurrence of the same PTX in the 50–200 μm size-fraction (Fabro et al., 2015). This is further supported by comparison of the toxin profile of *D. tripos* from natural populations in the Gulf of San José and Nuevo Gulf, which was characterized by PTX-2 and PTX-11 (Gracia Villalobos et al., 2015).

The current finding of *Dinophysis norvegica* in the Argentine Sea is, as far as we know, the first record for the southwestern Atlantic Ocean. Considering the extensive work of Enrique Balech on dinoflagellates of the Argentine Sea and the southwest Atlantic (Balech, 1988), the fact that *D. norvegica* was never reported before from this area could indicate a recent introduction. It is also possible that this species may have been previously overlooked because even in our study, where it was found occasionally in net tow concentrates, cell densities were so low that it was never detected from Niskin bottle samples. Previous misidentification is unlikely, as *D. norvegica* can be easily distinguished from *Dinophysis acuminata*, primarily by the ventral rounded antapex of the latter species and also because in *D. acuminata* the most antapical rib of the sulcal list is not directed antapically, as is characteristic for *D. norvegica*. The latter species might be confused with *Dinophysis acuta*, which is known to occur in Chilean waters (Balech, 2002), but *D. acuta* is typically larger in size (54–100 μm) and the maximum width is below the mid-line of the cell, which was never the case for the cells found in this study.

Records of *Dinophysis norvegica* cells in this study were almost restricted to the southern and cooler waters, which agrees with its wide distribution in cold-temperate waters of the northern hemisphere, including the Baltic, Norwegian, North and Arctic Seas (Okolodkov and Dodge, 1996; Meyer-Harms and Pollenhe, 1998; Edvardsen et al., 2003; Jansen et al., 2006). Although *D. norvegica* is known to be frequently toxigenic around the world, our results do not allow a clear association between *D. norvegica* and particular lipophilic toxins, as this species was always found at lower cell densities than other putatively toxigenic species from the genus in samples that contained toxins.

The species *Phalacroma rotundatum* was found widely distributed in the Argentine Sea (from 39.6°S to 55°S), although it was never detected at high cell densities. Even though *P. rotundatum* has never been unambiguously proven to produce toxins, it is important to have in mind that this species might act as a vector of toxins produced by mixotrophic co-occurring *Dinophysis* spp. (González-Gil et al., 2011).

Finally, *Dinophysis caudata*, a species typically distributed in tropical to warm temperate waters (Taylor, 1976), was confined to the north of the sampling area in the Argentine Sea. *D. caudata* is reported to be toxigenic, but in our study this species was rare and present in too low densities for any linkage to *Dinophysis* toxins.

Among *Dinophysis* species that have never been associated with toxic outbreaks (reviewed by Reguera et al., 2014 and references therein), five species (*D. truncata*, *D. operculata*, *D. cuneis*, *D. minuta* and *D. subcircularis*) were found in our samples from Argentine waters. Nevertheless, as far as we know, these species have not been analyzed for toxin composition. Although *D. truncata* was the dominant species of the genus in Parsons Bay, Tasmania and co-occurred with detection of OA and DTX-1 in the blue mussel *Mytilus edulis*, Wallace (2011) concluded that it was unlikely that *D. truncata* was the cause of the low toxin levels found in the mussels. The DST concentrations were decreasing over time, despite periods of increasingly high *D. truncata* cell concentrations. This suggests that at least the Tasmanian strains of *D. truncata* are weakly toxic (if toxic at all).

The repeated observation of several different toxigenic *Dinophysis* species in the Argentine Sea and adjacent waters, but the almost total absence of OA and DTX, is particularly significant. In fact, among the three expeditions, comprising a total of 112 stations sampled during three different seasons (austral spring, late summer and autumn) and covering a wide geographical area (from ≈38–56°S), toxigenic *Dinophysis* species were found at 91 stations, but OA was detected at just one station, and there only in trace concentrations. By contrast, pectenotoxins (PTX) were recorded in 67 samples, and showed a widespread distribution. This agrees with results from monitoring programs in the gulfs of north Patagonia, where PTX-2, PTX-11 and PTX-2sa were detected in plankton samples, but OA was absent (Gracia Villalobos et al., 2015).

A similar situation was observed from several populations from the south Pacific coast of Chile. Results of a study conducted along a transect of the Chilean coast showed a wide distribution of PTX in plankton over almost the entire transect, whereas OA was absent and DTX-1 was detected only within a narrow area at three sampling stations (Trefault et al., 2011). Likewise, DTX and OA were not detected in waters of northern Chile where *Dinophysis acuminata* was present, but PTX-2 and analogs were found (Krock et al., 2009). In Bahía Inglesa, Chile, no DTX or OA but high concentrations of PTX-2 (180 pg cell^{-1}) were detected in *D. acuminata* cell isolates (Blanco et al., 2007). Moreover, neither OA nor DTX-1 was found in isolates of *Dinophysis* spp. from Reloncaví Estuary, Chile (Fux et al., 2011).

The predominance in the production of PTX over OA and its derivatives seems to be a recurrent pattern in some *Dinophysis acuminata* strains from other locations as well. Cell concentrates of this species from New Zealand yielded much more PTX than DTX (PTX/DTX ratio >22) (MacKenzie et al., 2005). A similar situation was observed among seven strains of *D. acuminata* from Denmark, which produced PTX-2, whereas none produced OA or DTX (Nielsen et al., 2012). Similarly, a *D. acuminata* culture isolated from Eel Pond, Woods Hole, Massachusetts, USA exposed to two different irradiance treatments (dark and light) showed a cellular PTX-2 content an order of magnitude greater than that of OA and DTX-1 in both treatments (Smith et al., 2012). On the other hand, strains that showed DST profiles dominated by OA and/or DTX have been found in western Europe (Moroño et al., 2003; Marcaillou et al., 2005), as is the case for *D. acuminata* cells from concentrated water samples from the Limfjord, Denmark (Jørgensen and Andersen, 2007).

The absence or only trace abundance of OA and DTX derivatives in samples from the three Argentine Sea expeditions cannot be attributed to low cell densities of *Dinophysis* species, because moderate to high densities of *Dinophysis acuminata* and *Dinophysis tripos* (1680 and 1560 cells L^{-1}, respectively) were found in bottle samples and in fact were much higher in net tow samples.

In a recent study on the occurrence of lipophilic toxins in shellfish harvested along the Argentine Sea, toxins of the OA/DTX group were detected in 43 samples, whereas PTXs were detected in only 8 of a total of 69 samples examined (Turner and Goya, 2015). The authors found that PTX concentration was low and remarked upon the fact that 5 of the 8 samples with PTX did not contain OA. In addition, OA was commonly detected in shellfish collected in northern waters of the Argentine Sea (≈36–38°S). In our study, OA was also confined to the northern waters of the Argentine Sea but it was only rarely found in plankton samples. In this sense, the toxin composition in bivalve shellfish, such as mussels, tends to be different than in phytoplankton (Pavela-Vrančič et al., 2001; Moroño et al., 2003; Blanco et al., 2007; Alves-de-Souza et al., 2014). The proportion of toxins retained inside the producer cells versus excreted or leaked into seawater will affect not only relative concentration in seawater but also retention in shellfish. In the case of the PTX versus OA and DTX, the former group has a higher tendency to remain inside the dinoflagellate cell or remain strongly associated with cellular debris as a culture or natural bloom collapses (Nagai et al., 2011; Smith et al., 2012), which might lead to a higher proportion of PTXs toxins versus OA and DTX in plankton samples than in shellfish.

5. Conclusions

The genus *Dinophysis* showed a widespread distribution in the Argentine Sea and was mostly represented by *Dinophysis acuminata*; nevertheless, *Dinophysis tripos* was also commonly found and sometimes occurred in great cell densities. These two species appeared as the main species related to lipophilic toxins, primarily associated to PTX-2 and PTX-11. By contrast, *Dinophysis caudata*, *Phalacroma rotundatum* and *Dinophysis norvegica* were less commonly detected and at lower cell densities and thus no clear association to toxin distribution could be revealed.

Dinophysis species from Argentine coastal and shelf waters co-occurred primarily with PTX, as OA was rarely detected and at very low levels, whereas DTX was absent throughout the three expeditions. The general scarceness of OA and derivatives in our study might reflect a biogeographical tendency of *Dinophysis* populations from Argentine waters of the southwest Atlantic to produce primarily PTX as a result of a regionally specific combination of genetic and environmental factors. Hence more field and culture studies are required to elucidate the toxin profile of *Dinophysis* species and thus increase knowledge on the causative mechanisms of DSP outbreaks in this region. In any case, the

distribution and abundance of *Dinophysis* and *Phalacroma* species detailed in this work, in addition to the lipophilic toxins detected together with the toxigenic species, provides a useful dataset to future monitoring programs and to predict association of specific dinoflagellates with the presence of lipophilic toxins in the Argentine Sea.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hal.2016.09.001.

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