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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