Comparison of phycotoxin composition and distribution in toxigenic plankton from the north and south Atlantic

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Abstract
Two oceanographic surveys for toxigenic phytoplankton in the South and North Atlantic Ocean, including the adjacent Irminger Sea and the Arctic coasts of Greenland and Iceland, were conducted for analysis of putative toxic microalgal species and their respective toxins. During both expeditions, plankton was sampled by phytoplankton net (20 µm mesh) vertical hauls with subsequent size-fractionation, and by filtration of Niskin bottle water samples from discrete depths. In addition, sediment samples at selected stations were taken for identification and analysis of organic-walled dinoflagellate cysts (dinocysts). Among the toxins detected in both areas were domoic acid (DA), pectenotoxins (PTXs), yessotoxin (YTX), and paralytic shellfish toxins (PSTs). In addition, in the northern hemisphere, dinophysistoxins (DTXs) and spirolides were present, but these toxins were not found in Argentinean waters. In the sediments of San Jorge Gulf of Argentina, cysts of the dinoflagellate species *Alexandrium tamarense* and *Protoceratium reticulatum* were found, and their respective toxins (PSTs and YTX) were associated with the planktonic samples from the same stations.

Keywords: algal toxins, phytoplankton, dinocysts, PSTs, DSTs

Introduction

The concept of latitudinal cosmopolitanism, e.g. that species occurring at high latitudes in both the Northern and Southern hemispheres may be similar if not identical, has rarely been applied to consideration of toxic marine microalgae and their toxins. The objective of this work was to compare the occurrence of toxic microalgae and associated toxins in the South and North Atlantic, Irminger Sea and the Arctic coasts of Greenland and Iceland. During field expeditions plankton samples were collected and size-fractionated for toxin analysis. Species composition information was supplemented with additionally sediment samples for dinocyst analysis from the southern Atlantic.

Methods

Two ship expeditions were carried out in the northern and southern hemisphere: along transects from Ushuaia (Tierra del Fuego) to Mar del Plata (Argentina) and from Uummannaq Fjord (Greenland) to Reykjavík (Iceland). The first expedition was carried out in the southern hemisphere in Argentinean shelf waters between latitudes 38°S and 56°S in March/April 2012, and the second expedition in the northern hemisphere in coastal waters of western Greenland and Iceland between 60°N and 71°N in July/August 2012.

Vertical net tows were conducted at each station through the upper 30 m (northern hemisphere) and the upper 20 m (southern hemisphere) of the water column with a 20 µm mesh Nitex plankton net. Net tow concentrates were filtered sequentially through Nitex mesh of 200, 55 and 20 µm by gravity filtration and split into size-fraction aliquots for extraction of lipophilic and hydrophilic toxins.

The cell pellets from the plankton net tows were harvested and extracted as described in Krock *et al.* (2008). In short, cells were harvested by

centrifugation and extracted by reciprocal shaking with methanol for lipophilic components and with 0.03 M acetic acid for hydrophilic toxins.

After filtration, hydrophilic extracts were analyzed for PSP toxins by separation of target analytes in reverse-phase mode by high-performance liquid chromatography with post-column derivatization and fluorescence detection (LC-FD), according to Krock *et al.* (2007). 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).

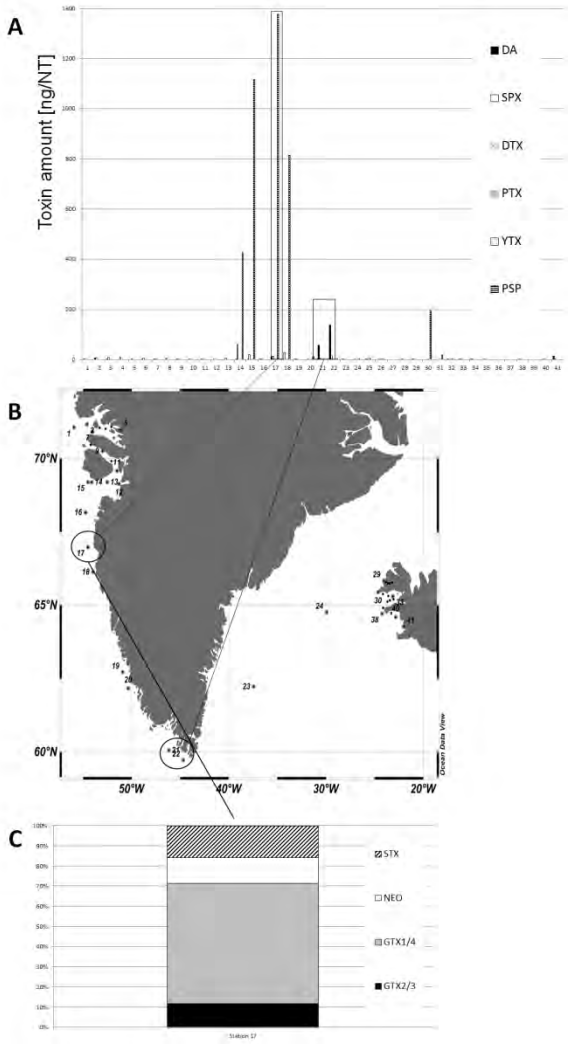

Fig. 1 A) toxin groups detected in the 20-50 µm size-fractions of plankton net tows collected on the Greenland coast; B) geographical location of sampling stations in the Arctic and Irminger Sea along the Greenland and Iceland coast; C) PSP toxin profile (% composition) at Station 17.

Surface sediments were collected with either a Van Veen or a Shipek grab sampler. The top 0-1 cm sediment and the flocculent layer samples were kept in dark cool (4 °C) conditions until analysis.

A 5 cm^3 wet sample from each station was sieved sequentially through 150 µm and 10 µm Nitex screens and the fraction retained on 10 µm was analyzed for the presence of dinocysts. One calibrated tablet of the spore-bearing plant *Lycopodium clavatum* spores was added as exotic markers before treatment to allow calculations of concentrations of cysts/g of dry sediment. Samples were treated by cold 10% hydrochloric acid and heavy-liquid separation with $ZnCl_2$. The residues were finally sieved and collected on a 10 µm mesh and mounted in glycerin jelly. Dinocysts were counted by light microscopy (Nikon Eclipse 600) under 600 x and 1000 x magnification. Slides containing the illustrated specimens are stored at the Laboratorio de Palinología (INGEOSUR-UNS), Bahía Blanca, Argentina.

Results and Discussion

The Arctic transect

Toxins found at stations along the northern transect were dominated by PSP toxins, with values up to 1400 ng per net tow (ng/NT), followed by domoic acid (DA) at the southern tip of Greenland (Stations 21, 22; Fig. 1B) and spirolides (SPX) in the Disko Bay area. These toxin results for the coastal Arctic imply a prevalence of *Alexandrium* and *Pseudo-nitzschia* spp. among toxigenic microalgae, especially in the Disko Bay region of western Greenland. In fact, the presence of toxic *Pseudo-nitzschia* from this coastal region has been described recently (Hansen *et al.* 2011).

In contrast, dinophysistoxins (DTXs) and pectenotoxins (PTXs) produced typically by *Dinophysis* spp., and yessotoxin (YTX), usually associated with the dinoflagellates *Protoceratium reticulatum*, *Lingulodinium polyedrum* or *Gonyaulax spinifera*, were only detected sporadically and at low concentrations <50 ng/NT (Fig. 1A).

The PSP toxin profile in the 20 – 50 µm size-fraction of Station 17 (Fig. 1B) was characterized mainly by gonyautoxins (GTXs) 1-4, low percentages of neosaxitoxin (NEO) and saxitoxin (STX) and the absence of N-sulfocarbamoyl toxins (Fig. 1C). This toxin profile closely matches the composition of several *Alexandrium tamarense*, isolates from Attu and Maniitsoq on the Greenland west coast (Baggesen *et al.* 2012).

The Argentinean transect

In general, toxin abundances (ng/NT) were higher in the southern than in the northern Atlantic and Arctic. The PTXs were the predominant toxin group found in Argentinean shelf waters, at concentrations up to 3500 ng/NT and dominated by PTX-2. These toxins were found in the entire Tierra del Fuego and Southern Patagonia coastal and shelf regions up to the San Jorge Gulf (Stations I1 to P45B; Fig 2A). The San Jorge Gulf, characterized by nutrient rich, cold waters from the south overlaid by warm, nutrient-depleted coastal waters is a region of high primary productivity (data not shown). In this region (Stations C43 to P45B), a massive bloom of the non-toxic dinoflagellate *Ceratium* spp. was observed, but this plankton assemblage also coincided with the highest toxins concentrations of the entire transect, with PSP toxins > 5000 ng/NT. From Station C43 two isolates of *A. tamarense* displayed the same toxin profiles as already described for Argentinean coastal strains (Montoya *et al.* 2010). In addition two isolates of *Protoceratium reticulatum* from the same Station C43 proved to be YTX-producing.

Dinocysts of potentially toxic species were also found in sediment samples of San Jorge Gulf, with the *Gonyaulax spinifera* species complex dominating in the samples analyzed from three stations. However, there was a significant difference between the cyst assemblage at Station C45, in the central area of San Jorge Gulf, and at Stations C43 and C43N in the mouth region of the Gulf. Cysts of *Alexandrium* spp. were constrained to the sediments of the inner gulf, and their abundance followed the abundance of the *G. spinifera* complex in this sample. *P. reticulatum* cysts reached the highest proportion (only ~13% of the total cyst population) at this inner station.

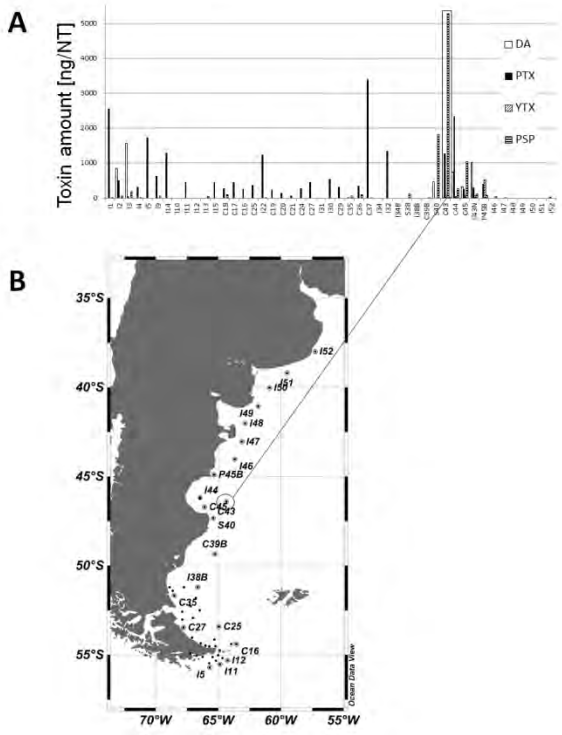

Fig. 2 A) Phycotoxin abundances and B) location of sampling stations along the Argentine coast and shelf region.

Significantly, vegetative cells of *A. tamarense* were isolated at Station C43 where the highest PSP toxin concentrations were also measured, but no *Alexandrium* cysts could be detected in the sediments. In contrast, *Alexandrium* cysts were found at station C45, where only moderate PSP toxin levels were measured. This uncoupling of the distribution of toxins from vegetative cells and of corresponding benthic cysts may reflect either small-scale temporal-spatial patchiness or the dominance of advective processes.

References

Baggesen C., Moestrup Ø., Daugbjerg N., Krock B. et al. (2012). Harmful Algae 19:108-116.

Hansen L.R., Soylu S.í., Kotaki Y. et al. (2011). Harmful Algae 10: 689-696.

Krock B., Seguel C.G. & Cembella A.D. (2007). Harmful Algae 6:734-744.

Krock, B., Tillmann, U., John, U. et al. (2008). Analytical and Bioanalytical Chemistry 392:797-803.

Montoya N.G., Fulco V.K., Carignan M.O. et al. (2010). Toxicon 56:1408-1418.