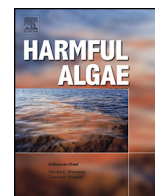


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## Co-occurrence of *Dinophysis tripos* and pectenotoxins in Argentinean shelf waters



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### ABSTRACT

The species *Dinophysis tripos* is a widely distributed marine dinoflagellate associated with diarrhetic shellfish poisoning (DSP) events, which has been recently identified as a pectenotoxin (PTX) producer. In two sampling expeditions carried out during austral autumns 2012 and 2013 along the Argentine Sea ( $\approx 38\text{--}56^\circ\text{S}$ ), lipophilic phycotoxins were measured by tandem mass spectrometry coupled to liquid chromatography (LC–MS/MS) in size-fractionated plankton samples together with microscopic analyses of potentially toxic phytoplankton. PTX-2, PTX-11 and PTX-2sa were recurrently detected in the 50–200  $\mu\text{m}$  fractions, in association to *D. tripos*. PTX-2 was also widely distributed among the 20–50  $\mu\text{m}$  fractions, mostly related to *Dinophysis acuminata*. Okadaic acid or its analogs were not detected in any sample. This is the first report of *D. tripos* related to PTX in the Argentine Sea and the first record of PTX-11 and PTX-2sa for this area. The morphological variability of *D. tripos*, including the presence of intermediate, small and dimorphic cells, is described. Also, the micro- and mesoplanktonic potential grazers of *Dinophysis* spp. were explored.

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### 1. Introduction

The genus *Dinophysis* Ehrenberg is represented by more than 120 species of marine dinoflagellates, including phototrophic and mixotrophic species (Hastrup-Jensen and Daugbjerg, 2009; Gómez et al., 2011). Of these, 10 toxic species are known as the main source of okadaic acid and its analogs, causative of diarrhetic shellfish poisoning (DSP) in humans (Reguera et al., 2014). A few hundred cells per liter are enough to generate intoxications in humans by consuming contaminated shellfish (Yasumoto et al., 1980). Up to date, dinoflagellate species of the genus *Dinophysis* have been recently recognized as the only known source of pectenotoxins (PTX), a large family of lipophilic toxins originally associated with the DSP toxin complex (Reguera et al., 2014). Toxicological studies indicate that PTX are not diarrheagenic after oral administration to laboratory rodents, but PTX-1 is highly

hepatotoxic and the other analogs are assumed to have similar effects (Terao et al., 1986). Reported lethal doses ( $\text{LD}_{50}$ ) by intraperitoneal injection (i.p.) in mice for PTXs are detailed in Domínguez et al. (2010). Cytotoxicity in different human cancer lines and induction of apoptosis in rat and salmon hepatocytes has been also attributed to some PTX (Domínguez et al., 2010). The existence of different published results reported by different groups has raised a controversy about the diarrhetic activity and oral toxicity of PTX.

Unlike DSP toxins as okadaic acid, which are known to accumulate through the food web and concentrate in somatic tissues of zooplanktonic organisms (Teegarden & Cembella, 1996; Suzuki et al., 1998; Maneiro et al., 2000; Tester et al., 2000), as far as we know there is no evidence of PTX bioaccumulation in zooplankton, although copepod grazing on *Dinophysis* spp. (Maneiro et al., 2002; Kozłowski-Suzuki et al., 2006) is well known. Pectenotoxin-2 was detected in copepods incubated with *Dinophysis* spp. (Setälä et al., 2009), but the absence of this toxin in individual copepods isolated from field zooplankton samples led the authors to conclude that a toxin transfer through the food web

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could not be proven (Setälä et al., 2011). However, PTX metabolized by enzymatic hydrolysis have been found in mussels (Miles et al., 2004b; Wilkins et al., 2006).

Pectenotoxins are cyclic polyether lactones that differ structurally from each other by different degrees of oxidation, different arrangements of the spiroketal ring system, and opening of the large lactone ring (Quilliam, 2003). These toxins are globally distributed and recurrently found at the southeast Pacific coast of Chile (Blanco et al., 2007; Krock et al., 2009; Trefault et al., 2011). In Argentinean waters, the presence of PTX-2 was recently detected in the Buenos Aires province coast (Montoya et al., 2013), and in the San Jorge Gulf (B. Krock, unpubl. data).

In the IOC-UNESCO reference list of toxic microalgae (Zingone & Larsen, 2014) *Dinophysis tripos* Gourret is one of the 10 *Dinophysis* species included. Detection of DTX-1 in picked cells from Kesenuma Bay (Japan) using liquid chromatography with fluorescence detection (LC-FLD) was the first record of toxin production by this species (Lee et al., 1989). However, these toxins were not detected later in *D. tripos* isolates from Japan analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Suzuki et al., 2009). More recently, the presence of PTX-2 in *D. tripos* was detected for the first time in field samples and cultures from Galician Rías also using LC–MS analyses (Rodríguez et al., 2012). In accordance, the production of PTX-2 and to a much lesser extent of DTX-1 was recently proven in Japanese cultures of *D. tripos* using LC–MS/MS (Nagai et al., 2013).

The species *Dinophysis tripos* is widely distributed in subtropical and tropical waters, but can also be observed in colder regions such as the northern Norwegian Sea and subantarctic waters of the South Atlantic (Johnsen & Lømsland, 2010). In the Argentine Sea, it is known to occur between  $\approx 36$  and  $55^\circ$  S (Balech, 2002), and it has been frequently observed along northern coastal waters of Buenos Aires Province ( $36$ – $37^\circ$  S), reaching densities up to  $3 \times 10^2$  cell  $L^{-1}$  (Sar et al., 2010). The distinctive cell shape and size, including the presence of two posterior hypothecal projections, allow the differentiation of *D. tripos* from other related species (Balech, 1988; Larsen & Moestrup, 1992). However, the presence of atypical intermediate and small cell forms of *D. tripos*, originally described as *Dinophysis diegensis* var. *curvata* Kofoid, as well as dimorphic forms result of “depauperating” cell division can also be observed (Reguera & González-Gil, 2001; Rodríguez et al., 2012).

With the purpose of studying potentially toxic microalgae and their toxins in Argentinean shelf waters, two expeditions took place covering an extended area ( $\approx 38$ – $56^\circ$  S). In addition, the occurrence of possible predators of *Dinophysis tripos* was also analyzed in order to study possible toxin transfer through the food web. In this work, we describe the recurrent finding of pectenotoxins associated with the dinoflagellate *D. tripos*, providing the first report linking this species with toxins in the Argentine Sea. A description of different morphological forms of *D. tripos* observed in the area is also presented.

## 2. Materials and methods

### 2.1. Sampling

The continental shelf waters of the Argentine Sea were sampled during two expeditions. The first one was carried out onboard the R/V “Puerto Deseado” from March 30th to April 14th, 2012. A total of 46 stations were sampled between  $\approx 38^\circ$  and  $55^\circ$  S (Fig. 1). The second was performed on the R/V “Bernardo Houssay” from March 11th to March 22nd, 2013 with 24 sampling points located between  $\approx 39^\circ$  and  $43^\circ$  S. It consisted of two legs, K1 (eight stations) and K2 (16 stations).

Plankton samples were collected by vertical net tows through the upper 20 m of the water column with a  $20 \mu\text{m}$ -mesh Nitex net

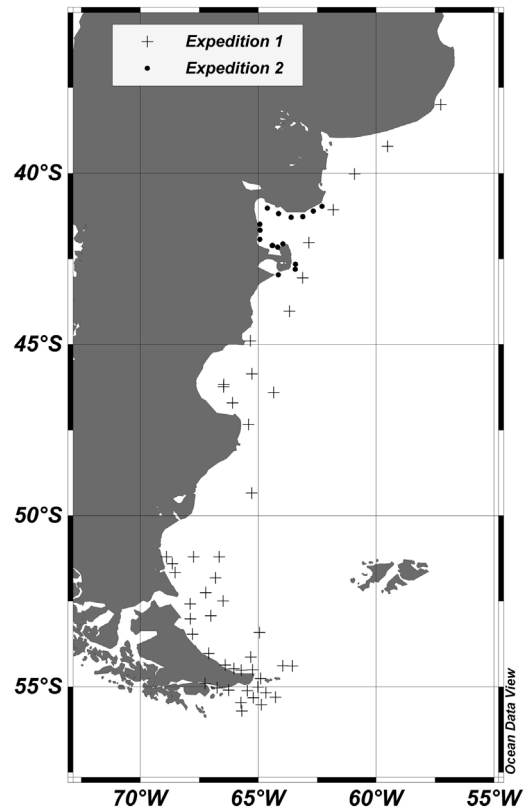


Fig. 1. Map of the study area showing the location of sampling points.

for both plankton and phycotoxins analysis. Each net haul was taken up to 1 L with  $0.2 \mu\text{m}$  filtered sea water, of which 20 mL and 100 mL were fixed with Lugol's solution during expedition 1 and 2 respectively, for species determination and enumeration. The rest was sequentially filtered through Nitex mesh of 200, 50 and  $20 \mu\text{m}$  by gravity filtration and split into aliquots for extraction of toxins.

### 2.2. Phytoplankton analysis

Cell abundance of *Dinophysis* spp. in net tow concentrates was determined by counting 1 mL of Lugol fixed samples using Sedgewick–Rafter chambers (LeGresley & McDermott, 2010) with an inverted microscope (Leica DMIL LED). Further morphological examination was made with a phase contrast/differential interference contrast Leica DM2500 microscope equipped with a DFC420C camera, and a scanning electron microscope (FEI Quanta FEG 200, Eindhoven, Netherlands).

### 2.3. Zooplankton analysis

Samples ( $20 \mu\text{m}$ -mesh net) from the leg K2 of expedition 2 ( $n = 16$ ) were also analyzed for microzooplankton ( $20$ – $200 \mu\text{m}$  and  $>200 \mu\text{m}$  in size) analysis. The latter were identified to the low taxonomic possible level and enumerated following the Utermöhl method (Utermöhl, 1931) using appropriate literature (Boltovskoy, 1981; Balech, 1988; Montagnes et al., 1988; Montagnes & Lynn, 1991; Montagnes & Taylor, 1994; Agatha & Riedel-Lorje, 1997; Alder, 1999; Petz, 1999; Kogan, 2005). Each sample was well homogenized and 10 mL allowed settling for 24 h. After that, all organisms in the base chamber were identified and counted under a Nikon Eclipse TS100 inverted microscope. Some possible predators of *Dinophysis* spp. present in samples (including those of  $>200 \mu\text{m}$  of size) were examined in more detail under the

mentioned inverted microscope, a Nikon SMZ 1500 stereomicroscope and a Nikon Eclipse 80i microscope sometimes using methylene blue stain to get better contrast. Images from them were obtained using a Nikon DXM 1200F digital camera which were later processed with Image Pro plus software (Media Cybernetics).

#### 2.4. Toxin analysis

Cell pellets from the plankton net tow size fractions were collected by centrifugation ( $3220 \times g$ , 15 min at  $4^\circ\text{C}$ ), suspended in 500  $\mu\text{L}$  methanol, and subsequently homogenized with 0.9 g of lysing matrix D by reciprocal shaking at maximum speed ( $6.5 \text{ m s}^{-1}$ ) for 45 s in a Bio101 FastPrep instrument (Thermo Savant, Illkirch, France). After homogenization, samples were centrifuged at  $16,100 \times g$  at  $4^\circ\text{C}$  for 15 min. The supernatant was transferred to a spin-filter (0.45  $\mu\text{m}$  pore-size, Millipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at  $800 \times g$ , 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).

### 3. Results

#### 3.1. Plankton and phycotoxin analysis

##### 3.1.1. Expedition 1

The most frequent *Dinophysis* spp. observed along expedition 1 was *Dinophysis acuminata* ( $\approx 37$ – $45 \mu\text{m}$ ), occurring in 82% of the stations in abundances from  $1 \times 10^3$  to  $168 \times 10^3 \text{ cells NT}^{-1}$ . However, the maximum *Dinophysis* abundance ( $20.7 \times 10^4 \text{ cells NT}^{-1}$ ) was reached by *Dinophysis tripos* ( $\approx 70$ – $90 \mu\text{m}$ ) at station I50 ( $\approx 40^\circ\text{S}$ ), while no other *Dinophysis* species was observed at this sampling point. In fact, the presence of *D. tripos* during the expedition 1 was almost confined to station I50, since it appeared in only two more stations in very low abundances ( $1$ – $2 \times 10^3 \text{ cells NT}^{-1}$ ) (Fig. 2A).

Analysis of phycotoxins revealed a very high abundance of PTX-2sa ( $2396 \text{ ng NT}^{-1}$ ) in the 50– $200 \mu\text{m}$  fraction size at station I50

(Fig. 2B). Pectenotoxin-2 seco acid appeared in this size fraction in only five more samples but in lower concentrations (from 104 to  $266 \text{ ng NT}^{-1}$ ). Also low concentrations of PTX-2 (from 106 to  $578 \text{ ng NT}^{-1}$ ) were detected in four samples in this fraction size (Fig. 2B). In contrast, in the 20– $50 \mu\text{m}$  size fractions, the most distributed toxin was PTX-2, occurring in 61% of the stations (data not shown). In this fraction, also PTX-2sa was detected in 15 samples in trace concentrations ( $< 35 \text{ ng NT}^{-1}$ ) and in two samples in low values (56 and  $78 \text{ ng NT}^{-1}$ ). Pectenotoxin-2 seco acid was the only toxin present in the  $> 200 \mu\text{m}$  size fraction, with a maximum value at station I50 ( $107 \text{ ng NT}^{-1}$ ). No okadaic acid or its analogs were detected in any size fraction.

##### 3.1.2. Expedition 2

The most common *Dinophysis* species observed along expedition 2 was *Dinophysis tripos* ( $\approx 79$ – $100 \mu\text{m}$ ), being present in 12 stations located between  $\approx 41^\circ$  and  $43^\circ\text{S}$  (leg K2). It represented an average of 83% of the total abundance of the genus, with densities ranging between  $11 \times 10^3 \text{ cells NT}^{-1}$  and  $3800 \times 10^3 \text{ cells NT}^{-1}$  (Fig. 3A). The species *Dinophysis acuminata* ( $\approx 25$ – $45 \mu\text{m}$ ) was also present in nine of total samples, but in lower abundances, from 1 to  $22 \times 10^3 \text{ cells NT}^{-1}$ .

Toxin analyses showed the presence of three PTX in the 50– $200 \mu\text{m}$  size fractions: PTX-2, PTX-11 and PTX-2sa. The latter was the most abundant toxin, being present in 50% of the samples and confined to the same 12 stations where *Dinophysis tripos* was found, whereas PTX-2 and PTX-11 were detected in 46% and 21% of stations, respectively (Fig. 3B). The maximum levels of PTX-2sa, PTX-2 and PTX-11 were  $3002$ ,  $1317$  and  $512.5 \text{ ng NT}^{-1}$  respectively (Table 1). In the 20– $50 \mu\text{m}$  size fractions only trace concentrations of the three toxins were found; PTX-2 appeared at 12 stations, PTX-2sa at 10 stations, and PTX-11 at only 1 station (data not shown). In the  $> 200 \mu\text{m}$  size fractions, PTX-2 and PTX-2sa were detected also in trace concentrations in two and nine samples respectively, but PTX-2sa also appeared in one sample showing a higher value ( $630 \text{ ng NT}^{-1}$ ).

High and significant ( $p < 0.05$ ) Spearman correlations coefficients were found between *Dinophysis tripos* abundance and PTX-2 ( $r = 0.91$ ), and PTX-2sa ( $r = 0.96$ ) concentrations. No okadaic acid or its analogs were detected in any size fraction.

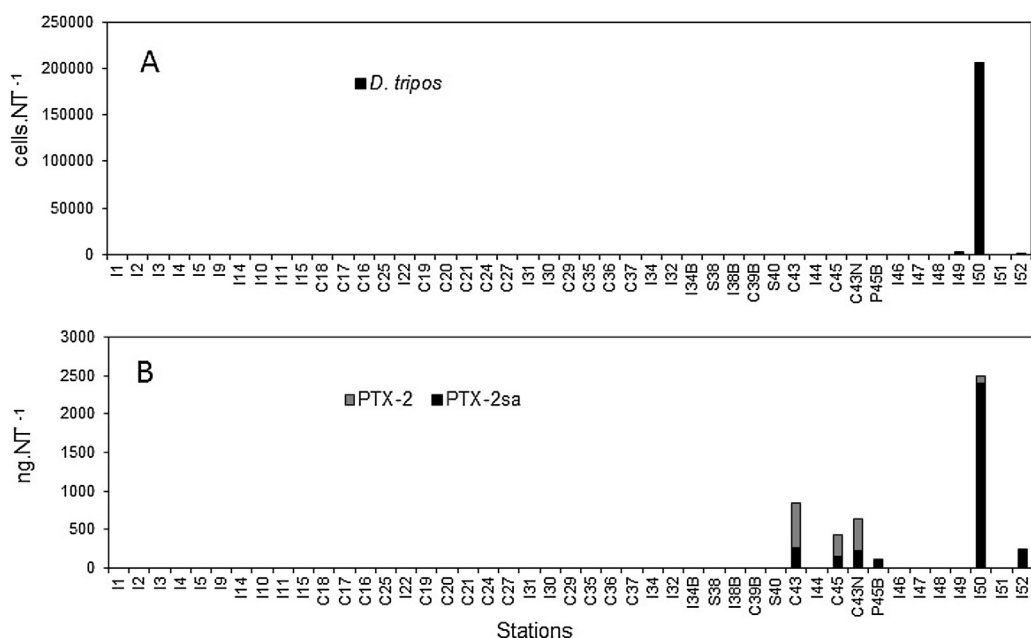


Fig. 2. Distribution of PTXs concentration in the 50– $200 \mu\text{m}$  fraction size and *D. tripos* abundances, during the first expedition in the Argentine Sea.

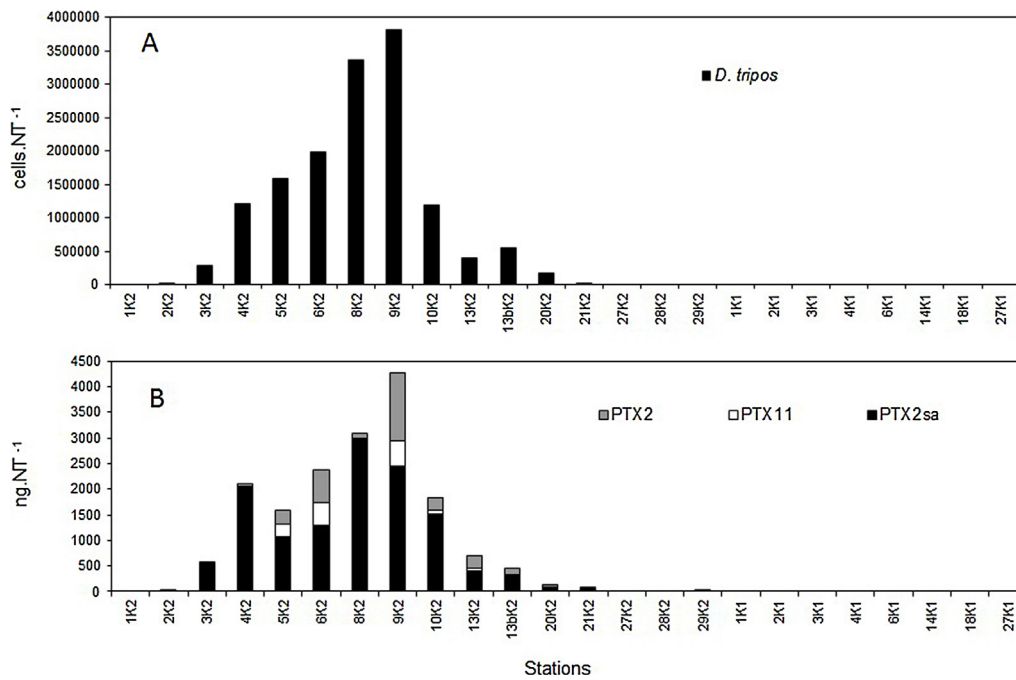


Fig. 3. Distribution of PTXs concentration in the 50–200  $\mu\text{m}$  fraction size and *D. tripos* abundances, during the second expedition in the Argentine Sea.

**Table 1**  
Co-occurrence of pectenotoxins (PTXs) and *D. tripos*, and estimated toxin cell quotas in the 50–200  $\mu\text{m}$  size fractions of net samples collected during the second expedition in the Argentine Sea.

Sample	PTX2sa (ng NT <sup>-1</sup> )	PTX2 (ng NT <sup>-1</sup> )	PTX11 (ng NT <sup>-1</sup> )	Total (ng NT <sup>-1</sup> )	<i>Dinophysis tripos</i> (cells 10 <sup>3</sup> NT <sup>-1</sup> )	PTX2sa (pg cell <sup>-1</sup> )	PTX2 (pg cell <sup>-1</sup> )	PTX11 (pg cell <sup>-1</sup> )	Total (pg cell <sup>-1</sup> )
2K2	27.0	0.0	0.0	27.0	13	2.07	0.00	0.00	2.07
3K2	560.7	6.9	0.0	567.6	273	2.05	0.03	0.00	2.08
4K2	2057.4	55.9	0.0	2113.3	1180	1.74	0.05	0.00	1.79
5K2	1069.7	280.6	245.1	1595.4	1570	0.68	0.18	0.16	1.02
6K2	1282.4	639.3	459.2	2380.9	1970	0.65	0.32	0.23	1.21
8K2	3001.9	100.4	0.0	3102.3	3350	0.90	0.03	0.00	0.93
9K2	2441.2	1316.9	512.5	4270.7	3800	0.64	0.35	0.13	1.12
10K2	1505.3	229.5	90.5	1825.3	1133	1.33	0.19	0.08	1.61
13K2	414.5	238.8	48.1	701.3	405	1.02	0.06	0.12	1.73
13bK2	323.1	123.7	0.0	446.8	295	1.10	0.04	0.00	1.51
20K2	81.5	42.5	0.0	124.0	170	0.48	0.03	0.00	0.73
21K2	68.1	3.4	0.0	71.6	11	6.20	0.03	0.00	6.51

**Table 2**  
Abundances of potential predators of *D. tripos* present in the 20–200  $\mu\text{m}$  size fractions during the leg K2 of expedition 2 in the Argentine Sea.

Station/cells NT <sup>-1</sup>	1K2	2K2	3K2	4K2	5K2	6K2	8K2	9K2	10K2	13K2	13bK2	20K2	21K2	27K2	28K2	29K2
<i>Cyrodinium</i> spp.	0	0	0	0	0	0	0	0	6195	0	3640	0	0	0	0	0
<i>Tiarina fusus</i>	0	0	7735	13,350	5005	30,030	3540	0	1035	27,880	28,220	620	0	0	670	1098
<i>Strombidinopsis acuminata</i>	0	0	0	1335	0	0	0	0	0	410	415	0	0	0	0	122
<i>Strombidinopsis elongata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61
<i>Cyclotrichium gigas</i>	0	276	910	1335	0	1365	0	0	0	1230	1245	0	940	0	0	61
<i>Codonellopsis obesa</i>	0	4920	840	0	0	0	0	0	0	0	0	0	0	0	1530	20,235
<i>Favella taraikaensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	570
<i>Favella ehrenbergi</i>	0	168,100	8400	2765	21,060	810	0	0	9292	5040	12,775	4095	840	1890	0	11,970

Zooplankton analyses revealed the presence of heterotrophic dinoflagellates, tintinnids, aloricate ciliates, different marine invertebrate larvae, cladocerans as well as nauplius larvae and copepodites of cyclopid, calanoid and harpacticoid copepods. Taking into account their size, small and medium dinoflagellates, ciliates, some eggs and first nauplii of copepods belonged to microzooplankton (<200  $\mu\text{m}$  size fraction), while the large heterotrophic dinoflagellate *Noctiluca scintillans*, copepod nauplii,

copepodites and some adults, cladocerans and invertebrate larvae belonged to mesozooplankton (>200 fraction). According to body measures, the potential predators of *Dinophysis* spp. (e.g., mainly *Dinophysis tripos* which was the dominant species of this genus in samples) in the 20–200  $\mu\text{m}$  size fraction and their abundances, are presented in Table 2. They belong to dinoflagellates, aloricate ciliates, and tintinnids, all microzooplanktonic groups. None of these taxa showed a distribution pattern related to that of PTX in

**Table 3**

Abundances (cells NT<sup>-1</sup>) of potential predators of *D. tripos* present in the >200 µm fraction size during the leg K2 of expedition 2 in the Argentine Sea, and PTX-2sa concentration (ng NT<sup>-1</sup>) in the same fraction.

Sample	1K2	2K2	3K2	4K2	5K2	6K2	8K2	9K2	10K2	13K2	13bK2	20K2	21K2	27K2	28K2	29K2
PTX2sa (ng NT <sup>-1</sup> )	0	0	18	7	4	8	630	50	17	23	4	47	0	0	0	0
<i>Noctiluca scintillans</i> (cells NT <sup>-1</sup> )	0	0	0	0	60,750	22,680	51,450	2,975	15,488	0	1,820	1,365	0	68,040	8,670	0
<i>Copepodites Cyclopoida</i> (cells NT <sup>-1</sup> )	5,000	11,480	3,360	3,950	15,390	17,010	6,370	5,100	8,850	0	2,555	780	0	1,800	2,550	7,980
<i>Copepodites Calanoida</i> (cells NT <sup>-1</sup> )	13,000	27,880	15,120	15,010	33,210	38,070	14,210	17,000	24,485	15,120	25,550	7,540	2,520	18,720	7,650	19,380
<i>Copepodites Harpacticoida</i> (cells NT <sup>-1</sup> )	0	4,100	10,080	0	0	0	0	0	0	0	0	0	2,520	0	510	0

**Table 4**

Length (*L*) and dorso-ventral depth (*E*) of the hypothecal plates of normal, intermediate and small cell forms of *D. tripos* observed in the Argentine Sea.

	Normal cells	Intermediate cells	Small cells
<i>L</i> range (µm)	79–100	72–87	59–70
<i>E</i> range (µm)	42–50	28–40	25–32
<i>L</i> average (µm)	94	79	65
<i>E</i> average (µm)	45	34	28

the 50–200 µm size fraction. Copepod fecal pellets were also frequently observed in this size fraction of samples but were not quantified. In the >200 size fraction *Dinophysis* spp. grazers as *N. scintillans* and other potential ones as different development stages of copepods appeared at all stations (Table 3).

### 3.2. *Dinophysis tripos* morphology

*Dinophysis tripos* normal cells are easily distinguished from other species by their typical cell shape, which includes two big posterior prolongations, one dorsal and the other ventral. Also the cell size is characteristic, ranging between 94 and 125 µm. Cingular lists are well developed and the left sulcal list is bigger than the right one (Balech, 1988).

Besides the normal shape of *Dinophysis tripos* another two different forms, intermediate and small cells according to Rodríguez et al. (2012), were commonly observed along the second expedition. These two morphotypes differ from the typical form in terms of length and dorso-ventral depth of the larger hypothecal plates (Table 4); development of dorsal horn, absent in small cells and reduced in intermediate cells (Fig. 4); and areolation, marked in normal cells but absent in small cell forms (Fig. 5).

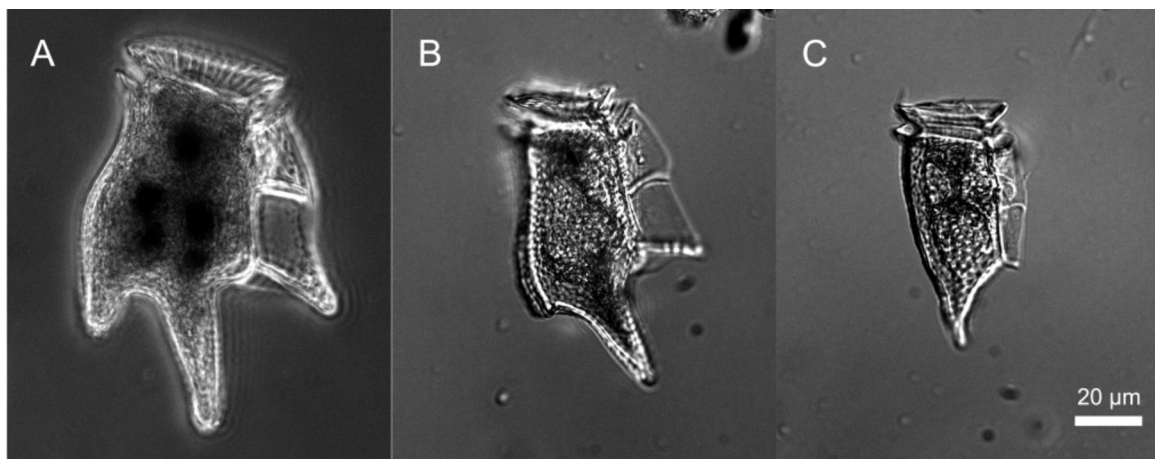
Intermediate and small forms were present in 9 of 12 samples in which *Dinophysis tripos* was recorded. However, they represented only 0.18–2.54% (average of 0.86%) of the total abundances of *D. tripos*. On the other hand, in the three samples in which only normal cells were found, the abundance of *D. tripos* was considerably low (maximum of  $170 \times 10^3$  cells NT<sup>-1</sup>).

Recently divided vegetative cells, lacking the anterior or the posterior left sulcal list, were observed in all samples that contain *Dinophysis tripos* normal cells (Fig. 6A). In addition, dimorphic cells, result of depaupering division (Reguera & González-Gil, 2001), were found. They presented different hypothecal plates in the same individual, one of them with normal appearance and the other one with intermediate or small cell characteristics (Fig. 6B and C).

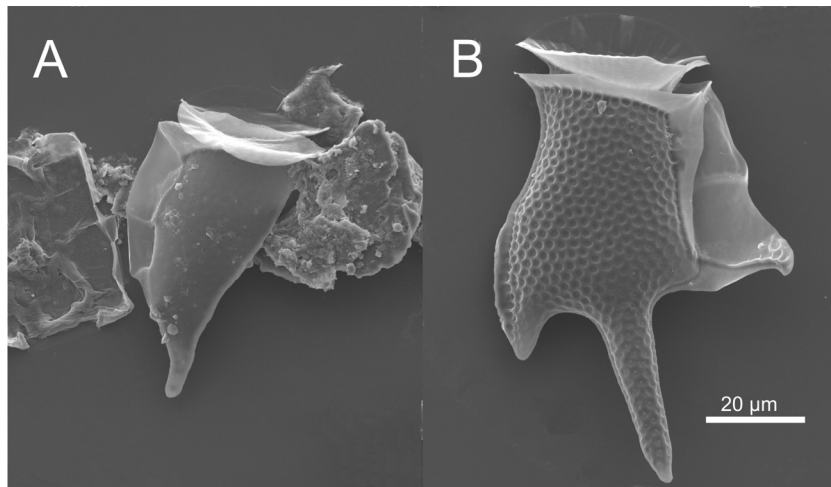
## 4. Discussion

The dinoflagellate genus *Dinophysis* is considered as the only source of PTX in marine waters (Reguera et al., 2014). Several PTX analogs have been found in culture conditions, field water samples and shellfish (Domínguez et al., 2010). The species *Dinophysis fortii* was the first one identified as a PTX producer (Lee et al., 1989; Draisci et al., 1996), but PTX have been later found in *Dinophysis acuta* (Suzuki et al., 2003), *Dinophysis norvegica* (Miles et al., 2004a; Suzuki et al., 2009), *Dinophysis acuminata* (MacKenzie et al., 2005), *Dinophysis caudata* (Fernández et al., 2006) and *Dinophysis tripos* (Rodríguez et al., 2012; Nagai et al., 2013).

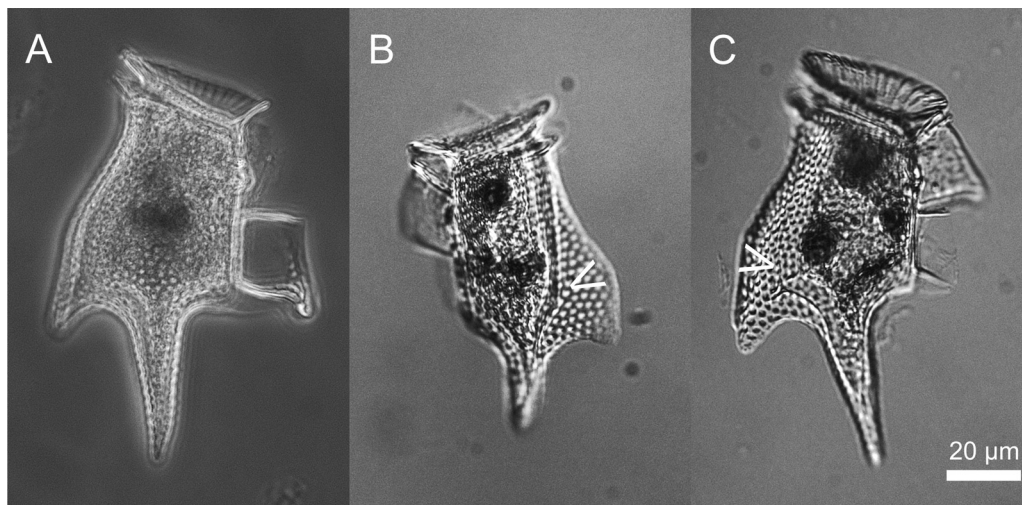
In the present study, the occurrence of PTX in the 50–200 µm size fractions was observed together with the presence of two potentially toxic *Dinophysis* species, *Dinophysis tripos* and *Dinophysis acuminata*. However, cells of *D. acuminata* ranging in size from 39 to 53 µm (Balech, 2002) and from ≈25 to 45 µm (this



**Fig. 4.** Light micrographs (phase contrast) of the different morphological forms of *D. tripos* found in field samples, normal (A), intermediate (B) and small sized cells (C).



**Fig. 5.** Electronic micrographs of small (A) and normal (B) cells of *Dinophysis tripos*, showing the different hypothecal areolation.



**Fig. 6.** Light micrographs (phase contrast) of recently divided normal cell lacking the anterior portion of the left sulcal list (A) and recently divided dimorphic cells (B and C). Arrows show the small (B) and intermediate (C) part of the thecae.

study) are expected to be collected in the 20–50 µm size fraction, but most PTX were detected in the 50–200 µm fraction, which argues against a substantial contribution of *D. acuminata* to the PTX detected. In addition, in six samples from the second expedition in which PTX-2sa was detected, *D. tripos* was the only toxic species found in any of these size fractions. In five samples containing PTX-2 and in two with presence of PTX-11 *D. tripos* also was the only toxic species found. In contrast, *D. acuminata* was detected always co-occurring with *D. tripos* in the samples with PTX from expedition 2.

Pectenotoxin-11 has been reported from New Zealand (Suzuki et al., 2003), the Galician Rías, Spain (Pizarro et al., 2008), North Sea (Krock et al., 2008), along the Chilean coast (Blanco et al., 2007; Krock et al., 2009; Trefault et al., 2011) and from the Benguela Current, South Africa (Pitcher et al., 2011), but this is the first report for the Argentinean Sea. Although this toxin was already associated with *Dinophysis acuta* (MacKenzie et al., 2005; Suzuki et al., 2006) and *Dinophysis acuminata* (MacKenzie et al., 2005), our data do not strongly support *Dinophysis tripos* being a producer of PTX-11, because there was little correlation between the toxin and the species ( $r = 0.67$ ). A possible explanation for this low correlation is that *D. tripos* might feed on the original producer of the toxin which could be *D. acuminata*, as it was present in some stations. Although dinoflagellates are known to feed on diverse taxa including other

dinoflagellates (Tillman, 2004), this feeding behavior has not been reported for *D. tripos*, but it cannot be entirely excluded, as the genus *Dinophysis* is known to be mixotrophic and that growth of *Dinophysis* spp. are based on prey–predator interactions (Park et al., 2006; Nishitani et al., 2008). However, as *D. tripos* was the only toxic species in two samples from expedition 2 that contained PTX-11, cell quotas in those samples were calculated (0.08 and 0.12 pg cell<sup>-1</sup>).

The finding of PTX-2 production associated to *Dinophysis tripos* in the Argentine Sea is in accordance with recent experimental and field observations in other regions (Rodríguez et al., 2012; Nagai et al., 2013). The PTX-2 cell quota estimated in our field study (0.03–0.35 pg cell<sup>-1</sup>) is considerably lower than those obtained in Japanese cultures (0.8 ± 29.1 pg cell<sup>-1</sup> at the beginning of incubation and 1235.6 ± 96.1 pg cell<sup>-1</sup> at the end of incubation) by Nagai et al. (2013). However, the high PTX cell quota found in Japanese cultures could be a result of measurement of intra and extracellular toxin content. Pectenotoxin-2 cell quotas obtained in field (45–90 pg cell<sup>-1</sup>) and culture (179–232 pg cell<sup>-1</sup>) samples from the Galician Rías (Rodríguez et al., 2012) are also higher than cell quotas in our study.

Variability in cell toxin content and PTX composition can be high among different *Dinophysis* spp. populations (Lee et al., 1989), even within the same species and geographical area, as was found

between the coasts of the western and the eastern North Sea (Krock et al., 2008), and differential production of toxins in the same species can vary considerably even within specimens collected in the same locality (Fernández et al., 2006). Although variation in toxin production is very common, the cell quotas of PTX-2 found in this study are strikingly low. The average PTX-2 cell quota of *Dinophysis tripos* found during the second expedition was calculated as  $0.1 \text{ pg cell}^{-1}$ . This is less than 10% of PTX-2sa, cell quota  $1.6 \text{ pg cell}^{-1}$ , which is dominating the profile. This is quite unusual, because to date PTX-2 always has been found to be the most abundant variant in PTX profiles of the genus *Dinophysis* (Reguera et al., 2014 and citations therein). However, it seems that, other than in most *Dinophysis* spp., *D. tripos* either metabolizes PTX-2 further to PTX-2sa or the biosynthesis of PTX-2 in this species is only a minor biosynthetic pathway and PTX-2sa is biosynthesized independently as the main metabolic product.

Pectenotoxin-2sa is commonly associated to enzymatic hydrolysis of PTX-2 in mussels (Miles et al., 2004b; Wilkins et al., 2006) or to enzymatic conversion after phytoplankton cell rupture (Fernández et al., 2006). In this sense, association between PTX-2sa and *Dinophysis caudata* has been previously mentioned, but it was interpreted as a result of cell rupture due to sampling methodology or transportation of the samples (Takahashi et al., 2007). However, this can be excluded in our case, because at stations from expedition 1 with dominance of *Dinophysis acuminata* PTX-2 was the most abundant PTX in the 20–50  $\mu\text{m}$  fraction size and only traces of PTX-2sa were detected. Given that samples from both expeditions (and different size fractions) were treated the same way, it is extremely unlikely that hydrolysis of PTX-2 has occurred only in those samples containing *Dinophysis tripos*, but not in those containing *D. acuminata*. Another possibility to explain PTX-2sa content is that *D. tripos* has been feeding on other *Dinophysis* species (e.g. *D. acuminata*) that produce PTX-2 and it has hydrolyzed the toxin through enzymatic digestive reactions (MacKenzie et al., 2012), but this has not been proven at the present. A third possible explanation would be copepod fecal pellets (i.e., assuming copepod grazing on *Dinophysis* species) (Maneiro et al., 2002) that were frequently observed occurring in the 20–200  $\mu\text{m}$  size fractions. However, the lack of quantitative data prevent this can be a proof. Finally, a fourth explanation would be the micrograzer predation on *D. tripos*.

Several potential predators of *Dinophysis* were observed in the 20–200  $\mu\text{m}$  size fractions of some samples (Table 2). Among the possible predators, we postulate the ciliate *Tiarina fusus* which has been mentioned as consuming autotrophic dinoflagellates by Hansen (1991). Also, *Favella ehrenbergii* and *Favella serrata*, within tintinnids, which have also been signaled as predators on the

nanoflagellate *Azadinium* spp. (Krock et al., 2009) and *Dinophysis acuminata* (Maneiro et al., 2000) respectively. However, the association between these potential predators of *Dinophysis tripos* and PTX-2sa was not significant ( $r_{\text{max}} = 0.45$ ). Also in samples 8K2 and 9K2, with the highest values of PTX-2sa, potential predators were not detected or present at reduced abundances (Table 2).

In summary, considering that the four previous hypotheses are not conclusive to explain the high amount of PTX-2sa in the 50–200  $\mu\text{m}$  size fraction, and the high correlation observed between PTX-2sa concentration and *Dinophysis tripos* abundances, our data suggest this species as a producer of PTX-2sa, showing cell quotas ranging between 0.48 and  $6.2 \text{ pg cell}^{-1}$  in the second expedition, and a higher value ( $11.6 \text{ pg cell}^{-1}$ ) at station I50 from expedition 1. This relation between PTX-2sa and *Dinophysis* spp. ( $8.16 \text{ pg cell}^{-1}$ ) was previously reported for natural plankton food suspensions rich in *Dinophysis norvegica* cells (Kozłowski-Suzuki et al., 2006). However, additional field studies as well as culture establishment are required to unambiguously elucidate the toxin profile of this species and the possible production of PTX-2sa by this genus, as well as to test its bioaccumulation in the food web.

With the purpose to explore a possible transfer of PTX through the food web, the  $>200 \mu\text{m}$  size fractions were analyzed for putative *Dinophysis tripos* predators. Numerous individuals of the large heterotrophic dinoflagellate *Noctiluca scintillans*, with body diameter range of 200–750  $\mu\text{m}$  according to Sato et al. (2004), were observed containing partial or completely empty *D. tripos* thecae (Fig. 7), so predation of *N. scintillans* on this dinoflagellate seems clear. This finding agrees with the report of Sato et al. (2004) which mentions *D. tripos* being part of the diet of *N. scintillans* at a coastal fixed station (EPEA,  $38^{\circ}28'S$ ,  $57^{\circ}41'W$ ) from the Argentine Sea shelf. These authors reported that *N. scintillans* is an opportunistic predator grazing on the most abundant microplankton items present in the water column such as copepod eggs, diatoms, tintinnids and *D. tripos*. As such *N. scintillans* has also been recorded to feed on *Dinophysis acuta* and *Dinophysis caudata* (Escalera et al., 2007) in the Galician Rías. In addition, this is a cosmopolitan heterotrophic dinoflagellate producer of red tides in numerous coastal areas in the world during spring-summer (Montani et al., 1998; Fonda-Umani et al., 2004). It has been classified as phagotrophic and its feeding mechanism is by engulfing preys (Hansen & Calado, 1999). The occurrence of *N. scintillans* as well as different copepodites would explain the observed levels of PTX-2sa toxin in the  $>200 \mu\text{m}$  fraction size. However, it is not possible to exclude the possibility that some *D. tripos* cells may have been retained on the 200  $\mu\text{m}$  mesh and contributed to the PTX-2sa presence in this fraction.

Morphological analyses of *Dinophysis tripos* revealed different morphotypes in almost all populations of the Argentinean Sea. This was already pointed for other populations, as formation of intermediate and small cell forms and dimorphic cells have been recorded for a number of species of the genus *Dinophysis* (Reguera & González-Gil, 2001). Presence of recently divided cells lacking part of the left sulcal list was also detected in our field samples, which is a sign of recent cellular division. In our material all these forms were commonly observed as part of *D. tripos* populations, and their characteristics were in accordance with those previously described from culture and field samples by Rodríguez et al. (2012). However, intermediate and small cells represented only a small percentage of total abundance (0.18–2.54) when compared with other values observed in *Dinophysis* spp. (10–45%) by Reguera & González-Gil (2001).

## 5. Conclusion

This is the first record of association between *Dinophysis tripos* and toxins in Argentine Sea, where DSP events and detection of

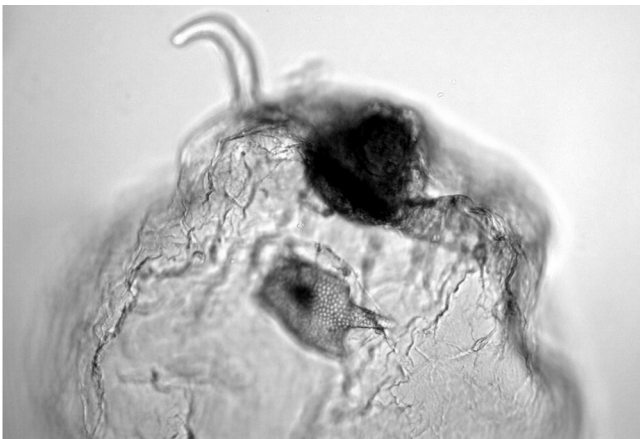


Fig. 7. Image of *Noctiluca scintillans* (length: 300  $\mu\text{m}$ ) (20 $\times$ ) containing a *D. tripos* partially empty thecae.

DTX-1 and OA have been previously linked to *Dinophysis acuminata* and *Dinophysis caudata* (Sar et al., 2012). Pectenotoxin-11 and PTX-2sa are found for the first time in this area. The recurrent finding of different PTX but lack of OA or its analogs agrees with results from the Baltic Sea (Setälä et al., 2011) and from the Chilean coast (Blanco et al., 2007; Krock et al., 2008).

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