Occurrence of toxigenic microalgal species and phycotoxins accumulation in mesozooplankton in Northern Patagonian gulfs, Argentina

Running head: Phycotoxins accumulation in mesozooplankton from Argentina

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Data Accessibility

Data pertaining to this manuscript are available from the corresponding author (dagostino@cenpat-conicet.gob.ar).

Abstract

In the Northern Patagonian gulfs of Argentina, Golfo Nuevo (GN) and Golfo San José (GSJ), blooms of toxigenic microalgae and the detection of their associated phycotoxins are recurrent phenomena. The present study evaluated the transfer of phycotoxins from toxigenic microalgae to mesozooplankton in GN and GSJ throughout an annual cycle (December 2014-2015 and January 2015-2016, respectively). In addition, solid-phase adsorption toxin tracking (SPATT) samplers were deployed, for the first time in these gulfs, to estimate the occurrence of phycotoxins in the seawater between the phytoplankton samplings. Domoic acid (DA) was present throughout annual cycle in SPATT samplers, while no paralytic shellfish poisoning (PSP) toxins were detected. Ten toxigenic species were identified: *Alexandrium catenella, Dinophysis acuminata*, *D. acuta*, *D. tripos, D. caudata*, *Prorocentrum lima, Pseudo-nitzschia australis, P. calliantha*, *P. fraudulenta*, and *P. pungens*. Lipophilic and hydrophilic toxins were detected in phytoplankton and mesozooplankton from both gulfs. *Pseudo-nitzschia* spp. were the toxigenic species most frequent in these gulfs. Consequently, DA was the phycotoxin most abundantly detected and transferred to upper trophic levels. Spirolides were detected in phytoplankton and mesozooplankton for the first time

in the study area. Likewise, dinophysistoxins were found in mesozooplankton from both gulfs and this is the first report of the presence of these phycotoxins in zooplankton from the Argentine Sea. The dominance of calanoid copepods indicates that they were the primary vector of phycotoxins in the pelagic trophic web.

Keywords: Phycotoxins trophic transfer; Northern Patagonian gulfs; mesozooplankton accumulation; toxigenic phytoplankton; harmful algal blooms; phycotoxins

Introduction

Certain marine microalgae produce potent toxins that negatively affect human and ecosystem health, as well as economic activities that depend on marine resources when they are transported and accumulated through the marine food webs. In humans, phycotoxins can produce different types of gastrointestinal and neurological symptoms and even death due to the ingestion of contaminated seafood (Esteves et al. 1992; Shumway 1990; Lincoln et al. 2001). Impacts on marine organisms are generally observed as acute intoxications that include mass mortality of cultivated and wild organisms (such as fishes, birds and marine mammals) (Geraci et al. 1989; Anderson and White 1992; Gulland 1999; Núñez-Vázquez et al. 2004; Gayoso and Fulco 2006; Montoya and Carreto 2007; De la Riva et al. 2009; Fire et al. 2010). Although toxic microalgae are consumed by various marine organisms (Turner 2010), it has been reported that the main entry point for phycotoxins into the pelagic food web is copepods (Turner 2014).

Recently passive sampling techniques for monitoring the occurrence of phycotoxins and shellfish contamination events have been developed worldwide (MacKenzie et al. 2004; Lane et al. 2010; MacKenzie 2010). The techniques include the passive accumulation of phycotoxins from the water column onto porous synthetic resin filled sachets (solid-phase adsorption toxin tracking, SPATT) and their subsequent extraction and analysis (MacKenzie et al. 2004). The SPATT methodology has several advantages, such as that the adsorbed toxins are not subject to biotransformation and depuration unlike in shellfish, and time and space integrated sampling of toxins that simulates shellfish adsorption. Although the use of SPATT samplers to provide an early warning of lipophilic toxins has been questioned (Pizarro et al. 2013), several studies have demonstrated that SPATT samplers coupled to sensitive analytical technologies such as LC–MS/MS is a highly sensitive tool allowing for early information on the presence of this group of phycotoxins (Turrel et al. 2007; Reguera et al. 2012; McCarthy et al. 2014).

In the Argentine Sea, harmful algal blooms (HAB) occur frequently and repeat with varying intensities over the years (Gayoso and Fulco 2006; Almandoz et al.

2007; Montoya et al. 2010; D'Agostino et al. 2015; Carreto et al. 2016; D'Agostino et al. 2018). To date, five shellfish poisoning symptoms have been reported in Argentine Sea (reviewed by Krock et al. 2018). The North Patagonian Gulfs of Argentina, Golfo Nuevo (GN), Golfo San José (GSJ) and Golfo San Matías (GSM) (Figure 1), are some of the most productive areas of the Patagonian Shelf ecosystem (Carreto et al. 1986; Acha et al. 2004). These gulfs are characterized by an important biodiversity, with the presence of marine mammals (Harris and García 1990; Crespo and Pedraza 1991; Bastida and Rodríguez 2009), marine birds (Yorio et al. 1998), fishes (Elías 1998), natural shellfish banks (Orensanz et al. 2007; Amoroso et al. 2011) and seaweed beds (Boraso and Kreibohm 1984). In these gulfs, blooms of toxigenic microalgae as well as the detection of their respective toxins are recurring phenomena. In this sense, a HAB and Shellfish Toxicity Monitoring Program has been carried out in coastal waters of the North Patagonian gulfs since 2000. Paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP) toxins are monitored frequently and the shellfish fisheries in both GN and GSJ have been closed annually, because levels of paralytic shellfish toxins, produced by the dinoflagellate *A. catenella* (formerly named *A. tamarense*), were above the regulatory limit of 80 μg saxitoxin (STX) eq. 100 g^{-1} of mussel tissue (HAB and Shellfish Toxicity Monitoring Program; Wilson et al. 2015). On the other hand, only in a few years (2009, 2011, 2017, 2018 and 2019) the harvest of mollusks was closed either in GN and/or GSJ, because DSP toxin (produced by dinoflagellates of the genera *Dinophysis* and *Prorocentrum*) levels in shellfish field samples exceeded the regulatory limits (Harmful Algal Bloom and Shellfish Toxicity Monitoring Program; Gracia Villalobos et al. 2015). In contrast, they have not been closed due to domoic acid (DA), a neurotoxin produced by species of the diatom genus *Pseudo-nitzschia* (the regulatory limit for DA is 20 μg g^{-1} of mussel tissue). Although no cases of ASP events have been documented to date either in wildlife or in humans in the North Patagonian gulfs, previous research carried out in GN and GSJ showed the presence of DA in phytoplankton and mesozooplankton (mostly calanoid copepods) samples, as well as in fecal samples of whales (*Eubalaena australis*) collected at similar sites and on similar dates to those of the present study (D'Agostino et al. 2017). These findings indicate that the southern right whales are exposed to DA through the ingestion of contaminated zooplankton, mainly copepods, while they remain in their calving grounds in these gulfs. This could, in turn, become a risk for other species due the transfer of phycotoxins through the food web. In contrast, to date, spirolides (spiroimine shellfish poisoning, SSP) and azaspiracids (azaspiracid shellfish poisoning, AZP) have not been observed in these gulfs. In view of the above, the aim of the present study was to investigate the transfer of phycotoxins from toxigenic microalgae to mesozooplankton in GN and GSJ throughout an annual cycle. In this study levels of phycotoxins dissolved in the water column of North Patagonian gulfs were quantified and analyzed for the first time. The present study contributes baseline knowledge on the occurrence of phycotoxins and their accumulation in higher

trophic levels in GN and GSJ, and provides new information about the seasonal dynamics of phycotoxins in this area throughout an annual cycle.

Materials and methods

Sample collection

Plankton and seawater samples were collected on an approximately monthly basis at three sites (S) in GN (S1 = 42.61°S, 64.29°W; S2 = 42.63°S, 64.27°W; S3 = 42.56°S, 64.34°W) and GSJ (S1 = 42.40°S, 64.12°W; S2 = 42.37°S, 64.08°W; S3 = 42.33°S, 64.08°W) (Figure 1). Sampling was carried out in GN from December 2014 to December 2015 (n_{phyto} S1 = 11, S2 = 10, S3 = 9; $n_{mesozoo}$ S1 = 11, S2 = 10, S3 = 9), and in GSJ from January 2015 to January 2016 (n_{phyto} S1 = 10, S2 = 10, S3 = 10; $n_{mesozoo}$ S1 = 10, S2 = 10, S3 = 10). The PSP toxin analyses of plankton (phyto- and mesozooplankton) were performed from samples collected in GN from July 2015 to December 2015 (n_{phyto} S1 = 5, S2 = 4, S3 = 3; $n_{mesozoo}$ S1 = 5, S2 = 4, S3 = 3) and in GSJ from August 2015 to January 2016 (n_{phyto} S1 = 5, S2 = 5, S3 = 5; $n_{mesozoo}$ S1 = 5, S2 = 5, S3 = 5). At each sampling site, surface seawater temperature was measured *in situ* with a portable thermometer (for details see D'Agostino et al. 2018). Seawater was subsequently sampled at 3 m and 10 m depths using a 2.5 L Van Dorn bottle from a boat. One liter from each depth was mixed and 500 mL were taken for the analysis of chlorophyll *a* (Chl-*a*) and phaeopigments (Phae), and a 250 mL aliquot was fixed with Lugol's solution at a final concentration of 0.4 mL 100 mL^{-1} (Ferrario et al. 1995) for quantitative analysis of toxigenic species. Phytoplankton samples for both relative abundance and phycotoxin analysis were collected using oblique net tows from 20 m depth to the surface using a 20 µm mesh net while traveling over a 7 min period at a speed of 2 knots. Samples were collected in 500 mL plastic bottles. Net tow samples for relative abundance analysis were fixed with 4% formaldehyde, whereas samples for phycotoxin analysis were placed in portable coolers and immediately processed after returning to the laboratory (see below). Mesozooplankton samples for quantitative taxonomic and phycotoxin analysis were collected using a 335 µm mesh net equipped with a flow meter (General Oceanics Model 2030R) on mouth of the net. Net tows were performed obliquely from a depth of 30 m to the surface for 7 min period at a speed of 2 knots and the samples were put in 250 mL plastic flasks. Mesozooplankton samples for taxonomic analysis were fixed with 4% formaldehyde, whereas samples for phycotoxin extractions were placed in portable coolers and immediately processed after returning to the laboratory (see below). The mesozooplankton samples collected in October in GN could not be used in the analyses of phycotoxins, because of an intense *P. australis* bloom and long chains of this species were found in the respective mesozooplankton samples which made it impossible to attribute any phycotoxins to mesozooplankton.

Phycopigment analysis

For Chl-*a* determination, the seawater samples were filtered through GF/F filters (25 mm and 0.7 μm in pore size) which were stored frozen at -20 °C. Chl-*a* was extracted during 24 h at 4 °C with 5 ml 90% acetone in darkness. Extracts were centrifuged at 1,680 x *g* for 5 minutes. Chl-*a* and Phae were subsequently quantified using a spectrofluorophotometer (Shimatzu RF-5301PC) at λEx/λEm: 430/671 nm calibrated with a standard of *Anacystis nidulans* and concentrations were estimated according to Holm-Hansen et al. (1965) equations. Chl-*a* is a photosynthetic pigment common to all autotrophic phytoplankton organisms. Concentration data from high performance liquid chromatography (HPLC) analysis were therefore used to estimate phytoplankton biomass (Almandoz et al. 2011; Gonçalves-Araujo et al. 2016). Chl-*a* values were corrected for Phae by acidification with HCl (0.1 N). Phae (mainly phaeofitin) was used as a principal indicator of Chl-*a* degradation as a result of herbivorous zooplankton grazing (Lorenzen 1967; Helling and Baars 1985; Head and Harris 1992).

Species identification and abundance of planktonic organisms

Microalgae species in bottle samples were enumerated with an inverted microscope (Leica DMIL) at × 200 magnification following Utermöhl (1958). For toxigenic diatoms identification, net samples were cleaned (Hasle and Fryxell, 1970). Naphrax mounted slides (Ferrario et al. 1995) were observed with an optical microscope equipped with phase contrast at × 400 and × 1000 magnification. Scanning electron microscopy observations of the samples were made with a Jeol JSM-6360 LV at the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, and with Zeiss Supra 40 at the Advanced Microscopy Center of the Universidad de Buenos Aires (UBA) in order to identify *Pseudo-nitzschia* species. Taxonomic phytoplankton identifications were based on the specific literature (Balech 1995, 2002; Boltovskoy 1995; Tomas 1997; Ferrario et al. 1999, 2002; Lundholm et al. 2003; Fryxell and Hasle 2004; Sar et al. 2006; Almandoz et al. 2007; Reguera et al. 2012; Guiry and Guiry 2017). Phytoplankton abundances were expressed as cells per liter (cells L^{-1}). For qualitative estimation, net samples were standardized into an abundance scale. Abundance estimates were obtained by counting the number of cells for toxic species in three 0.1 mL aliquots. The abundance classification was performed using a relative abundance scale between 0 and 6 (0 = absent and 6 = bloom [> 1,000,000]; for details see Gracia Villalobos et al. 2015) according to the abundance of this species in natural populations. For data presentation the abundance of toxigenic species identified in bottle samples were standardized to the same relative abundance scale. When the same species was identified in both the bottle and net samples at the same site, the highest abundance was used in subsequent analyses.

A Pearson correlation was used to investigate the relationships between phytoplankton biomass and phytoplankton abundances. In all tests, the threshold

for significance was set at 0.05. Statistical analyses were conducted in InfoStat (free version) software packages.

Mesozooplankton samples for species identification and enumeration were examined under a stereo microscope Nikon SMZ645 at × 30, × 40 and × 50 magnification. Potential consumers of toxic microalgae were identified to the lowest possible taxonomic level using appropriate literature (Boltovskoy 1981, 1999; Kirkwood 1982; Cervellini 1988; Harris et al. 2000; Young 2002). According to the abundance of organisms observed *a priori* in the samples, total or aliquot counts were applied. In the latter case, samples were subsampled (1/10) (Boltovskoy 1981) and all individuals were then identified and counted. Mesozooplankton abundances were expressed as number of individuals per cubic meter (ind m^{-3}).

SPATT samplers

SPATT samplers were deployed to complement sporadic phytoplankton and toxin sampling with integrated toxin sampling over an extended period. The purpose of the deployment of SPATT samplers was also to evaluate the presence/absence of phycotoxins in the study area and to confirm that no phycotoxin classes were missed by discrete phytoplankton sampling. The SPATT samplers were prepared from 95 µm polyester mesh that contained ~ 10 g (dry weight) of DIAION HP20 (Sigma, Deisenhofen, Germany) resin. For activation, SPATT samplers were conditioned in 100% MeOH for approximately 24 h. Then, they were rinsed several times with distilled water and kept wet and refrigerated between 4-6 °C until they were placed on the study site in GN and GSJ (Figure 1). In each gulf, two SPATT samplers were deployed at a fixed sampling site (GN = 42.61°S, 64.27°W; GSJ = 42.41°S, 64.11°W; Figure 1), shallow (≈ 15 m) and anchored approximately 1 m from the seabed. One of the SPATT samplers was used for the analysis of hydrophilic PSP toxins, while the other was used for analysis of DA and lipophilic toxins. The SPATT samplers were replaced monthly (at the same time of plankton and seawater samples were collect) and kept at 4 °C until extraction.

Phycotoxin analysis

Phytoplankton samples for phycotoxins extraction were filtered through GF/F filters (25 mm and 0.7 µm in pore size) and frozen (-20 °C) until analysis. Filters were transferred into FastPrep tubes containing 0.9 g of lysing matrix D (Thermo Savant, Illkirch, France) and 0.5 mL of methanol was added to extract multiple lipophilic toxins (such as DA, gymnodimine (GYM), spirolides (SPXs), dinophysistoxins (DTXs), okadaic acid (OA), pectenotoxins (PTXs), yessotoxins (YTXs), and azaspiracids (AZAs)) and 1 mL 0.03 M acetic acid was added to extract hydrophilic PSP toxins. The samples were homogenized 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

(Eppendorf 5415 R, Hamburg, Germany) at 16,100 × *g* at 4 °C for 15 min. Supernatants were transferred to centrifugation filters (0.45 μm pore-size, Millipore Ultrafree, Eschborn, Germany) and centrifuged at 800 × *g* at 4 °C for 30 s, and then transferred to autosampler vials (Krock et al. 2008).

Mesozooplankton samples for phycotoxin extraction were filtered through GF/F filters (47 mm and 0.7 μm in pore size) and frozen (-20 °C) until analysis. Filters for lipophilic toxins extraction were cut in half and transferred into FastPrep tubes containing 0.9 g lysing matrix D (Thermo Savant, Illkirch, France) and 1 mL methanol was added. Samples were homogenized as described above. Filtrates of the same samples were combined and dried in a gentle nitrogen stream and reconstituted with methanol to a final volume of 0.5 mL. Subsequently, the extracts were filtered through centrifugation filters (0.45 μm pore-size, Millipore Ultrafree, Eschborn, Germany) at 16,100 × *g* at 4 °C for 5 min. Samples were transferred into an autosampler vial for LC-MS/MS analyses (Tammilehto et al. 2012). For extraction of hydrophilic toxins, filters were cut in small fractions and transferred into centrifuge tubes and phycotoxins were extracted in acetic acid 0.03 M (4 mL) with the use of ultrasonication (Sonopuls HD 2070, Bandelin, Berlin, Germany; 2 min/7 cycles/ 96% power). Samples were vortexed (Heidolph Reax top) for 2 min, centrifuged (Eppendorf 5810R) at 3220 × *g* at 4 °C for 10 min, and the supernatant was transferred into centrifuge tubes and stored at 4 °C until used (Leandro et al. 2010). The residues were reextracted once as described above. Then, extracts were combined and evaporated using a rotary evaporator (Heidolph Rotary Evaporator, Laborota 4002), and the volumes were adjusted to 1 mL with 0.03 M acetic acid. Finally, the concentrates were filtered through centrifugation filters (0.45 μm pore-size, Millipore Ultrafree, Eschborn, Germany), and centrifuged at 3,000 × *g* at 4 °C for 30 s, and then transferred to autosampler vials.

Each SPATT sampler for lipophilic toxin extractions was rinsed three times with 500 mL of Milli-Q water. SPATT samplers were subsequently dried on filter paper for 3 h at 50 °C in a drying oven. Then, dry SPATT samplers were opened, and the resins were transferred to 50 mL centrifuge tubes and 30 mL of 100% MeOH was added. The mixture was vortexed for 1 min, and the tubes were left overnight in order to extract the lipophilic toxins adsorbed by the resins. Subsequently, the mixture of resins with MeOH were transferred into chromatographic glass columns (27 cm length, 13 mm ID, packed with a 2 cm layer of quartz wool and 1 cm of quartz sand), and the centrifugation tubes were rinsed with an additional 15 mL methanol. Methanol was eluted dropwise from the column until the liquid reached the top column layer and subsequently another 25 mL were added to each column for complete extraction of toxins. Finally, fractions were combined and methanol evaporated using a rotary evaporator (Heidolph Rotary Evaporator, Laborota 4002) to a final volume of approximately 0.5 mL. The concentrates were transferred to HPLC vials and adjusted with methanol to 1 mL. The extracts were filtered through centrifugation filters (0.45 mm pore-size, Millipore Ultrafree, Eschborn, Germany)

at 3000 × g at 4 °C for 0.5 min. Samples were transferred into an autosampler vial for lipophilic toxin analysis (Fux et al. 2008).

PSP toxins accumulated in SPATT samplers were extracted following the methodology described in Rodríguez et al. (2011) with little modification. Each SPATT sampler was opened and the resin was transferred to a chromatographic column. The resin was then rinsed with 7 mL of Milli-Q water and the PSP toxins were eluted twice with a solution of 10% MeOH (3.5 mL) containing 2% of 100% acetic acid. Finally, the rinsed water fraction and the two methanolic fractions were combined and evaporated using a rotary evaporator (Heidolph Rotary Evaporator, Laborota 4002) until approximately 0.5 mL, then the final volume was adjusted to 1 mL with acetic acid (0.03 N). Subsequently, the extracts were filtered through centrifugation filters (0.45 mm pore-size, Millipore Ultrafree, Eschborn, Germany) at 3000 × g at 4 °C for 0.5 min at 4 °C. Samples were transferred into an autosampler vial for PSP toxin analysis.

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). In brief, samples were analyzed in the selected reaction monitoring mode by single injection and quantified against external standard solutions of DA, DTX-1, PTX-2, PTX-11 and SPX-1 purchased from the Certified Reference Material Program of the IMB-NRC (Halifax, NS, Canada). As no analytical standard of 20-methyl spirolide G is commercially available, 20-methyl spirolide G values were expressed as SPX-1 equivalents assuming a similar molecular response factor. The filtrates of the acetic acid extraction were analyzed for PSP toxins after separation of target analytes in reverse-phase mode by high-performance liquid chromatography with post-column derivatization and fluorescence detection (LC-FD), according to the method described by Krock et al. (2007). PSP toxins were analyzed by single injection and quantified against an external four point calibration curve of PSP mix solutions containing (C1/2, GTX1/4, GTX2/3, dcGTX2/3, B1, dcSTX, NEO and STX). All toxins were purchased from the Certified Reference Material Program of the IMB-NRC (Halifax, NS, Canada). Phycotoxin levels in SPATT samplers were expressed as nanograms per SPATT and in plankton samples as nanograms per net tow (ng NT^{-1}). A list of monitored phycotoxins is given in Table 1.

Results

Phytoplankton biomass and occurrence of toxigenic *Pseudo-nitzschia* species

In GN, phytoplankton biomass (Chl-*a*) ranged between 0.23-4.45 µg L^{-1} throughout the sampling period, with minimum values found during late spring (0.23 µg L^{-1}; Dec-14) and early summer (0.39 µg L^{-1}; Jan-15) (Figure 2a). A clear correlation between phytoplankton biomass and diatom abundances (Pearson's

correlation: $r = 0.97$, $p < 0.05$, $n = 11$) was found. The maximum values were observed during late winter (1.04 µg L^{-1}; Sep-15) and spring (4.45 µg L^{-1}; Oct-15) (Figure 2a). This peak was associated with an intense bloom of *P. australis* that occurred in October 2015 in this gulf (Figure 2c). Total diatom abundances (cells L^{-1}) peaked in early summer (9.5×10^4 cells L^{-1}, Jan-15) and spring (6.72×10^5 cells L^{-1}, Oct-15) and attained their minimum in late spring and early winter (Dec-14 and Jul 15 = 4.4×10^2 cells L^{-1}) (Figure 2a). *Pseudo-nitzschia* spp. were found throughout the annual cycle except for July 2015, from low densities ranging from 1.1×10^2 cells L^{-1} in bottle samples and 2 in concentrated net samples to bloom densities (6.06×10^5 cells L^{-1} and 6 in relative scale abundance) (Figure 2c). During October 2015 *Pseudo-nitzschia* spp. were dominant, representing > 90% of total diatoms (Figure 2a). The *Pseudo-nitzschia* species that were detected with the highest frequency and abundance during the study period in GN were *P. calliantha* and *P. australis*, which reached densities of 1.49×10^5 and 4.56×10^5 cell L^{-1}, respectively in October 2015 (Figure 2c).

In GSJ, phytoplankton biomass ranged from 0.74-2.69 µg L^{-1}, throughout the annual cycle (Figure 2b). Lowest values were found in late summer (0.84 µg L^{-1}, Mar-15) and during winter (0.74 and 0.85 µg L^{-1}, Jul-15 and Aug-15, respectively), and they were found to be highest in autumn (2.7 µg L^{-1}, Apr-15) and spring (2.56 µg L^{-1}, Oct-15) (Figure 2b). No significant correlation between phytoplankton biomass and diatom abundances (Pearson's correlation: $r = -0.173$, $p > 0.05$, $n = 10$) was found. The highest diatom abundances were detected in late summer (1.93×10^5 cells L^{-1}, Mar-15) and spring (7.33×10^5 cells L^{-1}, Nov-15) which did not coincide with the highest biomass values found in this gulf, and were lowest during the winter (1.73×10^3 cells L^{-1}, Jul-15) (Figure 2b). *Pseudo-nitzschia* spp. were present throughout the period studied (Figure 2d), ranging from 4.4×10^2 to 1.32×10^4 cells L^{-1} in bottle samples and from 2 to 4 in concentrated net samples (Figure 2d). The most abundant species were *P. pungens* and *P. calliantha* with maximum cell abundances of 1.32×10^4 cells L^{-1} observed in March 2015 and 1.23×10^4 cells L^{-1} in April 2015, respectively (Figure 2d).

In general, values of Phae in GN and GSJ showed a pattern similar to that of Chl-*a* levels (Figure 2a and b). In GN, the highest values of Phae (1.22 µg L^{-1}) were observed in October 2015 coincident with the bloom of *P. australis* and the highest Chl-*a* levels registered in this gulf (Figure 2a). Likewise, in GSJ, the highest mean values of Phae were detected in April and October 2015 (1.99 and 1.29 µg L^{-1}, respectively) coincident with the peaks of Chl-*a* (Figure 2b).

Toxigenic phytoplankton species and phycotoxin abundance

Taxonomic analyses showed the presence of 10 potentially toxic phytoplankton species, six of them dinoflagellates: *Alexandrium catenella*, *Dinophysis acuminata*, *D. acuta* (only in GSJ), *D. tripos*, *D. caudata* (only in GN), *Prorocentrum lima* (only in GN) (Figure 3a and b), and the other four diatoms: *Pseudo-nitzschia*

australis, *P. calliantha*, *P. fraudulenta*, and *P. pungens* (Figure 2c and d). In GN *A. catenella*, the recognized source of PSP in the study area, was observed only during summer (Feb-15) and spring (Oct-15) in the net samples, with abundances of 1 and 2 respectively in the abundance scale (Figure 2a) and was not identified in the bottle samples from this gulf. Three out of five phytoplankton samples were positive for PSP toxins at low levels. In October 2015 a correlation between *A. catenella* and PSP toxins in phytoplankton samples was found (Figure 3a). The genus *Dinophysis*, the source of PTX toxins in marine waters (Reguera et al. 2014), was observed to reach bloom densities of 10^3 cells L^{-1} (Maneiro et al. 2000). This genus was represented by *D. acuminata*, *D. caudata*, and *D. tripos*, which all have been associated with DSP events in the Argentine Sea (Fabro et al. 2015; Gracia Villalobos et al. 2015; Turner and Goya 2015) (Figure 3a). The most abundant *Dinophysis* species in bottle samples was *D. caudata*, which reached a bloom density (1.32 x 10^3 cells L^{-1}) in January 2015 and *D. acuminata* in net samples in February 2015, with an abundance of 2 on the abundance scale (Figure 3a). PTX2 was detected in 27.27% samples from this gulf and only in February 2015 was there a correlation between *Dinphysis* spp. and PTX2. (Figure 3a). The genus *Pseudo-nitzschia* was found in 90.9% of the phytoplankton samples (Figure 2c). Domoic acid was the most abundant toxin, being present in 54.54% of samples, with levels between 1.47 and 6,140 ng NT^{-1} (Figure 2c). The highest DA abundances (6,140 ng NT^{-1}) coincided with the bloom of *P. australis* observed in bottle and net samples in October 2015 (Figure 2c). On the other hand only traces of 20-methyl spirolide G (20-Me-SPX-G), 13-desmethyl spirolide C (SPX1) and pectenotoxin-11 (PTX11) were detected (< LOD (limit of detection), data not shown).

Toxigenic dinoflagellates were the most frequently found in GSJ (Figure 3b). The most frequent species found both in bottle and net samples were *A. catenella* and *D. tripos* that reached maximum cell densities of 4.4 x 10^2 and 1.76 x 10^3 cells L^{-1} in bottle samples and abundance levels of 2 and 3 in concentrated net samples, respectively (Figure 3b). *A. catenella* was identified in 60% of phytoplankton samples, from late autumn to spring 2015 with densities in bottle samples of 2.2 x 10^2 and 4.4 x 10^2 cells L^{-1} and of 1 and 3 on the abundance scale (Figure 3b). The PSP toxin pairs C1/2 and GTX2/3 were detected in all analyzed phytoplankton samples, except for the sample collected in January 2016 (Fig. 3b). C1/C2 abundances ranged between 36.8 and 266 ng NT^{-1} and GTX2/3 between 23.8 and 283 ng NT^{-1} (Figure 3b). Maximum levels of both toxins were detected in mid-winter (Aug-15, Figure 3b). In this gulf, the presence of *A. catenella* coincided with the detection of PSP toxins in the phytoplankton samples analyzed for PSP toxins (Figure 3b). The genus *Dinophysis* was observed in 50% of samples, with densities in bottle samples ranging between 4.4 x 10^2 and 1.76 x 10^3 cells L^{-1} and with levels between 1 and 3 in concentrated net samples (Figure 3b). The most frequently found *Dinophysis* species in this gulf was *D. tripos* (Figure 3b). Two pectenotoxins (PTX) were detected in this gulf, PTX2 and PTX11 (Figure 3b). PTX2 was

detected in 60% of samples and PTX11 in 20% of them (Figure 3b). A general concurrence between the presence of *D. tripos* and PTX2 was observed (Figure 3b). In fact, the maximum level PTX2 (634 ng NT^{-1}) was detected with the presence of a bloom of *D. tripos* (1.76 x 10^3 cells L^{-1}) in April 2015 (Figure 3b). The genus *Pseudo-nitzschia* was observed in 100% of phytoplankton samples, with densities ranging between 4.4 x 10^2 and 1.32 x 10^4 cells L^{-1} and with abundances between 2 and 4 in concentrated net samples (Figure 2d). Phycotoxin analyses showed the presence of DA in all phytoplankton samples, with abundances ranging between 1.3 and 1,053 ng DA NT^{-1} (Figure 2d). 20-Me-SPX-G was detected in mid-spring (Nov-15) and early summer (Jan-16), whereas SPX1 was found during winter (Jul and Aug-15); both spirolides were found in very low abundances (Figure 3b). On the other hand, trace levels of DTX1 were detected (<LOD, data not shown).

Potential zooplankton vectors and phycotoxin content

Analysis of mesozooplankton samples revealed the presence of primarily herbivorous organisms (appendicularians, ascidian larvae, cirripedian larvae, echinoderm larvae and mollusc larvae), predominantly omnivorous (copepods (copepodites and adults) and euphausiids (larvae, juveniles and adults)) and predominantly carnivorous (amphipods (only in GN), cnidarians, chaetognaths, decapod larvae, polychaete larvae and fish larvae) species as possible vectors of phycotoxins (Figure 4a and b).

In GN, calanoid copepods dominated the mesozooplankton community throughout the annual cycle, except during summer 2015 when appendicularians (*Oikopleura* sp. and *Fritillaria* sp.) were the dominant group (Figure 4a). In general, the most abundant copepod species were the small copepods *Ctenocalanus vanus* and *Paracalanus parvus* and the large copepods *Calanus australis* and *Calanoides carinatus* (data not show). Phycotoxin analysis of mesozooplankton samples showed the presence of low levels of DA, DTX1, 20-Me-SPX-G and GTX2/3 (Figure 4a). Domoic acid was the most frequent toxin, being present in almost 50% of the mesozooplankton samples (Figure 4a) ranging from 0.71 to 138 ng NT^{-1}. The maximum level of DA was detected in April (138 ng NT^{-1}) (Figure 4a) when the copepod *C. australis* was the most abundant species (35.12 ind m^{-3}). In addition, PTX2 and dcGTX3 were detected at trace levels (< LOD, data not shown).

In GSJ, among the zooplankton that could act as potential phycotoxin vectors, calanoid copepods were the most abundant group throughout the annual cycle (Figure 4b). The most abundant species were *C. vanus* and *P. parvus* among the small copepods and the large abundant calanoids were *C. carinatus* and *C. australis* (data not shown). Analysis of phycotoxins revealed the presence of DA, DTX1, PTX2, GTX2/3 (Figure 4b) and trace levels of 20-Me-SPX-G and dcGTX3 (< LOD, data not shown). DA was the most frequent toxin, being present in 100% of the mesozooplankton samples ranging from 0.69 to 64 ng NT^{-1} (Figure 4b). This

latter value was detected in May 2015 when *C. carinatus* was the dominant specie in mesozooplankton community (271.97 ind m^{-3}). DTX1 was only detected in May 2015 and was the phycotoxins most abundant in the mesozooplankton (Figure 4b). The other phycotoxins only were detected sporadically and at low levels on a few sampling dates (Figure 4b).

Adsorption of phycotoxins by SPATT (Solid Phase Adsorption Toxin Tracking) samplers

Domoic acid was present in all SPATT samplers from GN and all but one (Jan-15) from GSJ at low levels (Table 2). The maximum levels both in GN and in GSJ were found during spring (Dec-15 and Sep-15, respectively) (Table 2). In contrast, no PSP toxins or its analogs were detected in SPATT samplers from GN and GSJ during the study period.

Discussion and conclusions

This study represents the most complete analysis carried out to date on the dynamics of phycotoxins produced by phytoplankton organisms and their accumulation in higher trophic levels both in GN and GSJ. It is also important to highlight that this is the first study in which SPATT (Solid Phase Adsorption Toxin Tracking) samplers were used to determine the phycotoxins dissolved in the water column in both GN and GSJ in addition to sporadic phytoplankton sampling and toxin determination. SPATT samplers are complementary to toxin determinations in plankton, because SPATT, despite not being quantitative in a strict analytical sense, provides integral data on toxin occurrence over an extended time period and therefore capture important toxin occurrences between individual plankton samplings. It is noteworthy that only DA was detected on SPATT samplers. This clearly indicated that DA-producing *Pseudo-nitzschia* species constitute a regular component of the phytoplankton communities in GSJ and GN. In contrast, the absence of other phycotoxins on the exposed SPATT samplers indicate that the toxic species found in plankton samples, did not dominate the plankton community over the study period. Nevertheless, this does not imply that these species might not form blooms under favorable conditions. In order to achieve a higher temporal resolution of toxic species and their associated toxins in the area, future investigations should consider the replacement of these samplers weekly. This would improve our knowledge of the phycotoxins present in North Patagonian gulfs, their persistence in the column water and frequency of the toxic episodes.

Both in GN and GSJ, only the PSP toxins C1/2 and GTX2/3 were found. Contrary to previous studies carried out in these gulfs (Reyero et al. 1998; Andrinolo et al. 1999a), our findings showed higher levels of C1/2 than of GTX2/3 several times in both GN and GSJ. Both studies (Reyero et al. 1998; Andrinolo et al. 1999a) found trace levels of C1/2 in field samples from GN and GSJ or these toxins were not detected in phytoplankton samples. Interestingly, those studies have documented

that the profiles of PSP toxins were dominated almost exclusively by GTX1/4. This dominance of GTX1/4 in field samples from GN and GSJ was also found in a study carried out during the winter and spring (Cadaillón 2012). Therefore, those results also differ from the findings found in the present study in GN and GSJ for the same seasons.

In addition, previous studies have reported the absence of C1/C2 or lower levels than those of the GTX2/3 in natural populations of *A. catenella* (formerly described as *A. tamarense*) from the Argentine Sea (Carreto et al. 2001; Montoya et al. 2010; Krock et al. 2015). According to these authors, the profiles of the PSP toxins obtained from the analysis of field samples of *A. catenella* were dominated by the GTX2/3 epimers, and even Krock et al. (2015) found only GTX2/3 toxins and trace amounts of STX. Notwithstanding Montoya et al. (2010) and Krock et al. (2015) reported the presence of elevated levels of C1/2 toxins followed by GTX1/4, in culture strains of *A. catenella* (described as *A. tamarense*) that were isolated from the same area where the natural populations of this dinoflagellate were sampled during their studies. These differences in C1/2 levels between natural and cultured strains of *A. catenella* cells found by these and others authors (Oshima et al. 1992; Anderson et al. 1996) led to the hypothesis that the production of toxin by this dinoflagellate changes according to whether it is studied in natural or laboratory conditions (Andrinolo et al. 1999b; Montoya et al. 2010). However, the results obtained in our study show that this dinoflagellate is able to synthesize C1/2 in natural conditions. Another possible explanation for the absence of C1/2 in field samples, could be related with the time that elapses between the collection of the samples at the study sites and the phycotoxin analyses in the laboratory, which would be sufficient for the chemical conversion of *N*-sulfocarbamoyl toxins (C toxins) to gonyautoxins (GTXs) (Santinelli et al. 2002; Krock et al. 2015). In line with this, Krock et al. (2015) indicated that the detection of only GTX2/3 toxins in field samples could be associated with the transformation of C1/2 toxins, which could have occurred within the time (three months) between the collection of plankton samples in the study area and their analysis in the laboratory. However, in the present study this period of time sometimes was greater (between 1 and 7 months). Therefore, it should not be ruled out that the differences between the profiles of PSP toxins observed here and in previous studies carried out in Argentine Sea, could be due to an unexplored diversity of PSP-producing organisms in the South West Atlantic.

With respect to toxicity it has been demonstrated that GTXs have an intermediate toxicity compared to STX, while the toxins of the sulfocarbamoyl group (B and C toxins) are the least toxic ones (Kwong et al. 2006). But it must be taken into account that the *N*-sulfocarbamoyl group is chemically labile and the sulfonyl group is easily cleaved off at low pH and/or metabolic activity of the ingesting organisms, which converts the almost non-toxic *N*-sulfocarbamoyl B- and C-toxins into the more toxic carbamoyl toxins such as gonyautoxins (GTX) (Krock et al.

2007). Via this mechanism a priori low toxic *Alexandrium* blooms can become more toxic through ingestion and biotransformation by vectors.

In both GN and GSJ, only one sample of mesozooplankton was positive for PSP toxins in each gulf. In both cases, low levels of the epimers GTX2/3 were detected. The absence of C toxins in these samples is in line with the biotransformation of *N*-sulfocarbamoyl toxins as discussed above. In addition, decarbamoyl toxin dcGTX3 was detected in five samples (3 from GN and 2 from GSJ) at trace levels (<LOD = 15 ng dcGTX3 NT^{-1}). As *A. catenella* has never been reported to produce decarbamoyl toxins (Montoya et al. 2010; Krock et al. 2015), the detection of dcGTX3 in mesozooplankton can been seen as an indication that copepods (most abundant group among the potential toxin vectors in GN and GSJ) also decarbamoylate carbamoyl toxins as it has been described for filter feeding mollusks (Artigas et al. 2010; Turner et al. 2013). At this point the observation of decarbamoyl toxins in copepods is only a first indication and the hypothesis of decarbamoylation of PSP toxins in zooplankton needs to be confirmed by feeding experiments.

Grazing experiments have documented that copepods select their prey based on levels of PSP toxins, feeding on dinoflagellates when the levels of toxins present in them were low, and when the concentrations of toxins in the dinoflagellates increased the copepods fed on non-toxic dinoflagellates (Turriff et al. 1995; Shaw et al. 1997; Teegarden 1999; Guisande et al. 2002). This selective behavior of copepods towards non-toxic dinoflagellates, could be an explanation for the absence of PSP toxins recorded in the present study in the mesozooplankton samples dominated by copepods species. Evidence of this could be the presence of PSP toxins in the phytoplankton samples analyzed, and the closure of the shellfish fisheries from the detection of PSP toxins in the study area during the period analyzed in the present study (Harmful Algal Bloom and Shellfish Toxicity Monitoring Program). By contrast, several investigations have reported that mesozooplankton, and especially copepods, are capable of feeding on toxic dinoflagellate species (Turrif et al. 1995; Turner et al. 2000; Teegarden et al. 2001; Durbin et al. 2002; Doucette et al. 2006) and accumulate PSP toxins produced by them (White 1981; Turriff et al. 1995; Lincoln et al. 2001; Bargu et al. 2002; Durbin et al. 2002; Hamasaki et al. 2003; Teegarden et al. 2003; Doucette et al. 2006). Therefore, future studies should analyze, through grazing experiments, whether the dominant copepod species both in GN and GSJ are the key vector for the transfer of PSP to higher trophic level organisms.

In addition to the transfer of hydrophilic PSP toxins, this study also addressed the transfer of lipophilic toxins in the marine food web. Only one study showed the association between *Dinophysis* spp. and DSP toxins in shellfish from GN and GSJ by the analysis of samples collected by the Harmful Algal Bloom and Shellfish Toxicity Monitoring Program as well as in phytoplankton samples collected in

February 2005 in these gulfs (Gracia Villalobos et al. 2015). In agreement with our results, Gracia Villalobos et al. (2015) found that the DSP toxin profiles from phytoplankton samples consisted mostly of PTX2 and PTX11. These authors observed a clear association of *D. tripos* with PTX2 and PTX11, which is in agreement with our results from GSJ and -to a lesser extent- GN. Our findings support the hypothesis that *D. tripos* could be the major PTX toxin producer species in North Patagonian gulfs (Gracia Villalobos et al. 2015). During the study period, PTX2 was detected more frequently and in higher levels in GSJ than in GN. The highest level of this toxin of phytoplankton in GSJ were recorded in April (634 ng PTX2 NT^{-1}), coincident with a bloom of *D. tripos* (1.76 x 10^3 cells L^{-1}), and the only detection of PTX2 in the mesozooplankton samples (26 ng PTX2 NT^{-1}). Although the PTX2 level found in the mesozooplankton sample in April was low, this represents the first detection of PTX2 in the mesozooplankton, mostly copepods, in the study area reported to date. Likewise, trace levels of this toxin were detected in mesozooplankton from GN. Therefore, these findings demonstrated that PTX toxins are transferred and accumulated by copepods in the study area. In agreement with this, several researchers have demonstrated that the copepods ingest toxic species of *Dinophysis* during natural blooms of these dinoflagellates (Jansen et al. 2006; Kozlowsky-Suzuki et al. 2006) and accumulate their toxins (Setälä et al. 2009).

Diatoms of the genus *Pseudo-nitzschia* were the potentially toxic species most frequently found in these gulfs during the studied annual cycle, with concentrations up to 4.56 x 10^5 cells L^{-1}. Consequently, DA was the phycotoxin that was most abundant and transferred to upper trophic levels in both gulfs. The highest DA level recorded in phytoplankton samples was detected concurrently with the bloom of *P. australis* in GN in October 2015. The attribution of toxicity to a species usually involves the proof of the presence of toxins in cultures or isolated cells (Álvarez et al. 2009). However, in the present study the identification of a bloom of *P. australis* in net tow samples and the high cell densities of this species observed in bottle samples in GN in October, suggest that this species was the main producer of the highest levels of DA. In the Argentine Sea *P. australis* has been suggested to be the major producer of DA (Almandoz et al. 2017). Furthermore, *P. australis* is considered to be a strong DA producer (12-37 pg $cell^{-1}$) (Bates 2000; Kotaki et al. 2000) and for this reason it has been reported to be the most toxic species of the genus *Pseudo-nitzschia* (Trainer et al. 2000) and primarily responsible for ASP problems worldwide (Bates 2000; Fire et al. 2010). Therefore, the simultaneous detection of the highest levels of DA and major densities of *P. australis* in the Argentine Sea (Negri and Inza 1998; Sastre et al. 2001; Negri et al. 2004; Almandoz et al. 2007, 2017) are worth being highlighted, because proliferation of this species may pose a risk for fishery activities in the region that are focused on Tehuelche scallop (*Aequipecten tehuelchus*) in GSJ (Orensanz et al. 2007) and on several species of marine animals that feed and reproduce in both gulfs.

The present findings indicate that calanoid copepods were the main potential vectors for the trophic transfer of DA both in GN and GSJ during the studied period. Evidence of this is the dominance of this group among the potential consumers of *Pseudo-nitzschia* spp. throughout the year in both gulfs, including those months in which the highest levels of DA were recorded in phytoplankton and mesozooplankton samples. In the present study, *C. vanus* was the calanoid copepod that coincided with highest DA concentrations during April, June and October 2015 in the plankton (phyto- and mesozooplankton) samples from GN. In GSJ the highest levels of DA were recorded in the plankton samples during January 2015, May 2015 and January 2016 when *C. australis*, *C. carinatus* and *Acartia tonsa* were the most abundant species, respectively. These copepod species have been defined as herbivores or omnivores (Boltovskoy 1981, 1999; Lombard et al. 2010; D'Agostino 2013; Antacli et al. 2014 and reference herein), therefore they could act as effective phycotoxins vectors, either by the direct consumption of DA-producing species or by ingestion of organisms of lower trophic levels contaminated with toxins. Consequently, vectorial intoxication of pelagic food webs could occur either by the grazing of copepods on toxic microalgae and also by predation of organisms contaminated with DA. In addition, taking into account that Phae are related to degradation of Chl-*a* due to grazing by zooplankton, the detection of the highest levels of Phae together with the highest abundances of *Pseudo-nitzschia* spp. in both gulfs (including the bloom of *P. australis* and the highest levels of DA observed in phytoplankton samples in GN), show other evidence that copepods were the main vector of DA through the food web.

Although spirolides have been previously detected in the Argentine Sea (Almandoz et al. 2014; Turner and Goya 2015; Fabro et al. 2017; Krock et al. 2018), in this study we report for the first time the presence of SPX1 and 20-Me-SPX-G in plankton (phyto- and mesozooplankton) samples from GN and GSJ. However, *Alexandrium ostenfeldii* the only known source of spirolides (Cembella et al. 2001; Franco et al. 2006) was not detected in phytoplankton samples from this study. Until now, *A. ostenfeldii* was only found in the Beagle Channel, southern Argentina (Almandoz et al. 2014) and in Argentinean slope waters (Fabro et al. 2017; Guinder et al. 2018) and it has not been previously observed in the Northern Patagonian gulfs. However, the identification of these spirolides in phytoplankton samples suggests the presence of *A. ostenfeldii* in the study area. On the other hand, the detection of 20-Me-SPX-G in mesozooplankton is evidence that zooplankton, mainly copepods, accumulate these neurotoxins. It has been demonstrated that spirolides produce strong neurotoxic symptoms when they are administrated to laboratory rodents (Guéret and Brimble 2010). Therefore, even when low levels of spirolides were detected in the present study, the presence of SPX1 and 20-Me-SPX-G in plankton indicates that it is important for monitoring programs to assess the transfer of these neurotoxins through food webs. This will generate knowledge about the impacts of spirolides exposure in marine fauna as well as on human health by consumption of seafood contaminated with these phycotoxins.

In the present study, dinophysistoxins, DTX1, was recorded for the first time in mesozooplankton samples from the Argentine Sea. To date, the presence of these phycotoxins had been demonstrated only in phytoplankton and shellfish from the Argentine Sea (Gracia Villalobos et al. 2015; Fabro et al. 2018; Krock et al. 2018). Although dinophysistoxins were absent in phytoplankton samples from GN and GSJ, the presence of DTX1 in mesozooplankton samples indicate that toxigenic strains of *P. lima* were present in the phytoplankton and were consumed by the mesozooplanktonic organisms.

Our findings indicate that there are transfers of phycotoxins to higher trophic levels in North Patagonian gulfs. In line with this, the present study highlights the need for understanding the mechanisms of transfer of phycotoxins from phytoplankton producer-species to higher trophic levels in the study area. The important role that copepods play in pelagic food webs of Northern Patagonian gulfs (Hoffmeyer et al. 2010; D'Agostino et al. 2016, 2018) justifies the need to conduct studies under controlled conditions that evaluate the grazing of main copepod species on toxigenic microalgae species present in the area. This research will allow one to know if copepods select among toxic and nontoxic phytoplankton species, as well the rate and the time of phycotoxin retention in their body. Likewise, future studies should focus on phycotoxin content of copepod body tissues, as well as to know if there are biotransformations of the toxins by copepods following ingestion of toxic microalgae.

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Table 1: Toxins screened by LC-MS/MS

Chemical classification, and polarity	Syndrome	Toxin group	Toxins	Mass transition (m/z)
hydrophilic, amino acid	ASP	DA	domoic acid	312>266 312>161
lipophilic, cyclic imine	SSP	GYM	gymnodimine A gymnodimine B gymnodimine C gymnodimine D 12-methyl gymnodimine A 16-desmethyl gymnodimine D	508>490 526>508 526>508 524>506 522>504 510>492
	SSP	SPX	spirolide A spirolide B spirolide C spirolide D 13-desmethyl spirolide C 13-desmethyl spirolide D spirolide G 20-methyl spirolide G	692>150 694>150 706>164 708>164 692>164 694>164 692>164 706>164
	SSP	PnTx	pinnatoxin E pinnatoxin F pinnatoxin G	784>164 766>164 694>164

lipophilic		GD	goniodomin A	786>607
	DSP	OA	okadic acid	822>223
		DTX	dinophysistoxin-1	836>237
			dinophysistoxin-1b	836>237
			di-hydrodinophysistoxin-1	638>237
			dinophysistoxin-2	822>223
	AZP	AZA	azaspiracid-1	842>824
			azaspiracid-2	856>838
			azaspiracid-3	828>810
		PTX	pectenotoxin-2	876>213
			pectenotoxin-2 seco acid	894>213
			pectenotoxin-11	892>213
			pectenotoxin-12	874>213
		YTX	yessotoxin	1160>965
			45-hydroxy yessotoxin	1176>981
			homo yessotoxin	1174>979
			45-hydroxy homo yessotoxin	1190>977

Table 2: Domoic acid (DA) adsorbed by SPATTs (Solid Phase Adsorption Toxin Tracking) in Golfo Nuevo (GN) and Golfo San José (GSJ). ND = no detected

GN		GSJ	
Date	DA ng/SPATT	Date	DA ng/SPATT
Jan-15	3.20	Jan-15	ND
Feb-15	5.45	Apr-15	6.47
Apr-15	10.77	May-15	3.61
Jun-15	9.90	Jul-15	5.34
Jul-15	6.24	Aug-15	12.16
Sep-15	5.82	Sep-15	17.89
Oct-15	6.59	Oct-15	9.29
Nov-15	5.02	Nov-15	17.46
Dec-15	71.91	Jan-16	16.33

Figures

Figure 1: Site locations of sampling in Golfo Nuevo and Golfo San José (S1, S2, S3). 〈〉 SPATTs (solidphase adsorption toxin tracking).

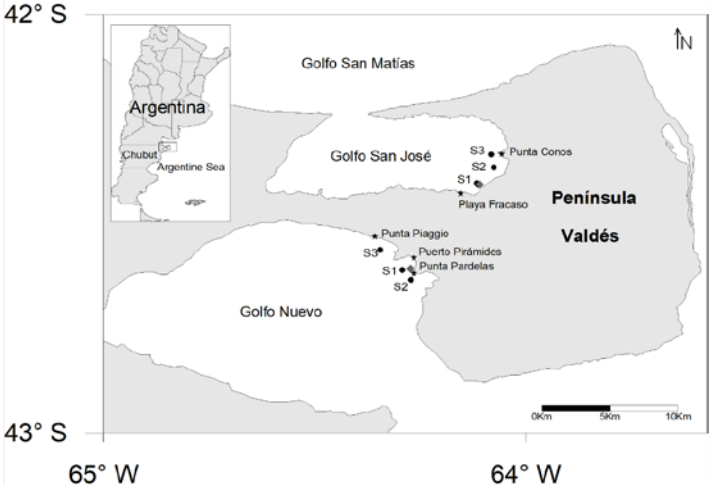

Figure 2: Seasonal variability in Golfo Nuevo and Golfo San José of (a and b) total diatoms; total cell densities of *Pseudo-nitzschia* species; chlorophyll a (Chla-a) and phaeopigments (Phae), and (c-d) relative cell abundances of Pseudo-nitzschia species, and levels of domoic acid (DA). For data presentation the abundance of *Pseudo-nitzschia* spp. identified in bottle samples were standardized in the relative abundance scale (see Gracia Villalobos et al., 2015).

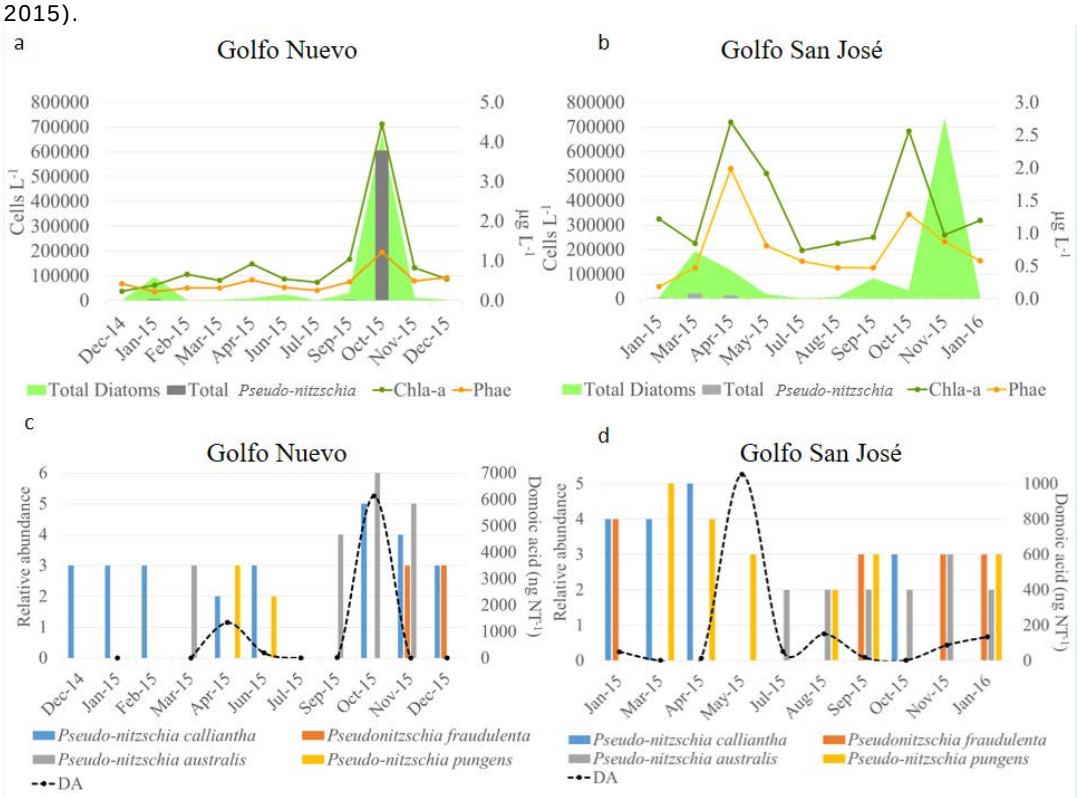

Figure 3: Relative cell abundances of toxigenic dinoflagellates and phycotoxins levels detected in Golfo Nuevo (a) and Golfo San José (b). For data presentation the abundance of dinoflagellates identified in bottle samples were standardized in the relative abundance scale (see Gracia Villalobos et al., 2015). C = Nsulfocarbamoyl toxins. GTX = Gonyautoxins. PTX = Pectenotoxins. SPX = Spirolides.

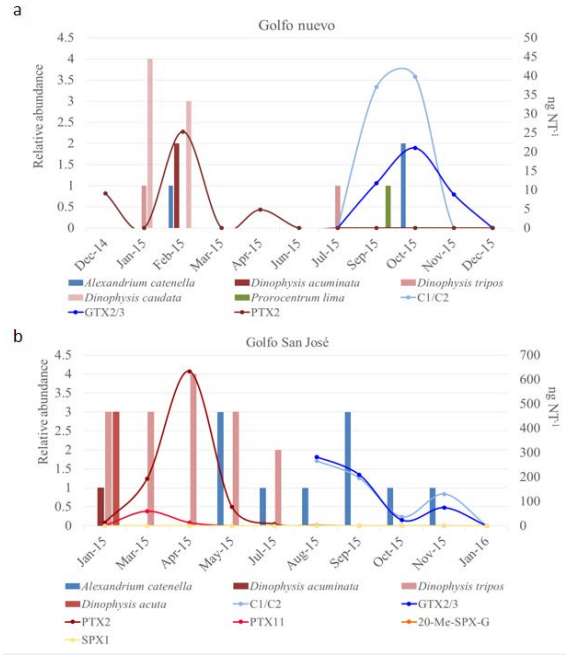

Figure 4: Abundances (ind m-3) of potential vectors of phycotoxins and phycotoxins levels present in mesozooplankton samples from Golfo Nuevo (a) and Golfo San José (b). Domoic acid (DA) data from June-December 2015 in GN and July-November 2015 in GSJ were taken from D'Agostino et al. (2017). C = Nsulfocarbamoyl toxins. GTX = Gonyautoxins. PTX = Pectenotoxins. SPX = Spirolides. DTX = Dinophysistoxins. Note that abundances (ind m-3) is displayed on a logarithmic scale.