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Distribution of phycotoxins in Última Esperanza Province during the PROFAN expedition 2019

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A B S T R A C T

Harmful Algae Blooms pose an increasing threat to the public health and economic stability of Southern Chile, particularly to the aquaculture industries. This fieldwork performed during the PROFAN expedition from 12th to 22nd November 2019 extends the knowledge on the distribution of marine toxin-producing species in the difficult to access Última Esperanza Province in the Magallanes Region. Paralytic Shellfish Poisoning toxins with high relative abundances of saxitoxin and lipophilic toxins dominated by yessotoxins, pectenotoxins and domoic acid were detected at nearly each sampling station. The respective toxin-producing organisms are mainly from the genus *Alexandrium* and *Dinophysis*. Furthermore, the first detection of pinnatoxin-G (PnTx-G) in Chilean waters strongly indicates the presence of the dinoflagellate *Vulcanodinium rugosum*.

1. Introduction

Harmful algal blooms (HABs) present a worldwide threat to public health and coastal communities through intoxications and economic losses (Hallegraeff, 1993; Anderson, 2009; Hallegraeff, 2010). This proliferation of toxic species has been especially observed along the southern coasts of Chile over the last four decades (Díaz et al., 2014). This phenomenon appeared to be initially restricted to the southern Magallanes Region (53 °S) and from there has apparently spread northwards through the fjord and channel systems to the Regions of Aysén, Los Lagos and recently Los Ríos (Paredes-Mella et al., 2021; Mardones et al., 2010; Sunesen et al., 2021). This spread has probably occurred due to a combination of both natural (e.g. currents, winds) and anthropogenic (e.g. ship ballast water) causes (Hallegraeff, 1993; Hallegraeff and Bolch, 1991; Smayda, 2007). Along with this geographical expansion, there has been an apparent intensification of blooms, both in terms of the coastal areas affected and the abundance of toxic species detected (Hallegraeff, 1993; Anderson, 2009; Díaz et al., 2020). In February 2016, an intense bloom of the ichthyotoxic (fish toxic) microalgae *Pseudochattonella verruculosa* occurred in the Los Lagos Region in the northern end of the Chiloé Inland Sea and the Reloncaví Sound resulting in the largest mortality of farmed salmon (~27 million) ever recorded worldwide equivalent to an estimated export loss of 800 million US Dollar (Montes et al., 2018; Mardones et al., 2021; Trainer et al., 2020). Simultaneously, a bloom of the toxic dinoflagellate *Alexandrium catenella* was detected in the estuarine and marine ecosystems of southern Chile, which once more had a strong social and economic impact on the region (Montes et al., 2018). Apart from the afore mentioned species, additional toxic microalgae have recently proliferated and dispersed along the channel and fjord systems (Paredes-Mella et al., 2021). Among these are lipophilic toxin-producing dinoflagellates (mainly of the genus *Dinophysis*; *Azadinium*, *Karenia*, *Protoceratium*), amnesic toxin-producing diatom species (*Pseudo-nitzschia* spp.), raphidophytes, and dictyochophytes, which produce ichthyotoxic substances that pose an enormous threat to the extensive aquaculture industry in Chile (Sunesen et al., 2021; Adams et al., 2020; Díaz et al., 2019; Mardones et al., 2020). These blooms, apart from the impact on aquaculture, have caused massive mortalities of invertebrates and other higher organisms such as invertebrates, fish, and even whales, not only in the Los Lagos Region and Aysén, but also in the northern area of the Magallanes Region, in sectors such as Trinidad Channel and Abra Channel (Mardones et al., 2021; Díaz et al., 2019; Mardones et al., 2020; Häussermann et al., 2017; Álvarez et al., 2019). Finally, the occurrence of the genus *Azadinium* in the South eastern Pacific and its recent identification in the channel and fjord zone render this geographical location an important area to study marine toxin (phycotoxin) producing organisms (Adams

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et al., 2020; Tillmann et al., 2017; Álvarez et al., 2010). In addition, there is little information on HABs, especially toxin-producing ones, in remote or difficult to access areas, such as oceanic areas, ocean-exposed channels, turbulent central areas of channels and fjord-glacier interiors, as regular monitoring programs are mainly performed in aquaculture and harvest areas. A risk assessment of HAB species is additionally important with regards to the anticipated effects of global warming on plankton dynamics, particularly in the sub-Antarctic coastal regions, and increasing efforts to ensure the successful and safe exploitation of fishery and aquaculture resources. However, the knowledge of ecological and oceanographic factors controlling the distribution and population dynamics of marine toxin producing species is still limited for this area. Thus, the aim of this study was to gather more information on the occurrence and distribution of harmful microalgae and their toxins in the Magallanes Region as base for a risk assessment in the light of expanding aquaculture concessions over a wide area of the austral Chilean coast

2. Material & methods

2.1. PROFAN cruise

The cruise was conducted on the R/V AGS-61 "Cabo de Hornos" starting in Puerto Montt (41.5 °S) on the 15th of November 2019 and commenced south until 50.5 °S with the last station being sampled on the 21st of November 2019 (Fig. 1). A total of 16 stations were sampled for hydrophilic and lipophilic toxins between 48.5 °S and 50.5 °S, while SPATT sampling was performed during the whole cruise track (Fig. 2).

2.1.1. Plankton sampling

Phytoplankton was analyzed qualitatively by utilizing net-tow concentrates and quantitatively by utilizing samples taken by Niskin-bottles mounted on a conductivity-temperature-depth rosette (CTD). Samples were identified according to the morphological characteristics of each group and species, mainly identifying diatoms, dinoflagellates and silicoflagellates. A total of 8 stations (St) were sampled. For qualitative and toxin analysis, various vertical net-tows (2–14 net-tows, 20 m depth each) were taken from the water column using a 20 μm phytoplankton net (1.2 m length, 30 cm diameter). An aliquot of each net-tow concentrate was collected into a plastic bottle, filled up to a defined volume with filtered seawater (<5 μm) and aliquots were fixed with formaldehyde to a final concentration of 4%. Then, triplicate samples (0.1 mL) were analyzed on slides using a standard light microscope (BX-41, Olympus). Thecate dinoflagellates were analyzed by epifluorescence microscopy after staining with a calcofluor dye (CAS-18909, Merck, Santiago, Chile). For quantitative analysis samples were collected at four discrete depths (3, 5, 10 and 20 m) analogous to (Pizarro et al., 2005). Samples were pre-concentrated using a sedimentation chamber of 10 and 50 mL, fixed with 1% Bouins solution and then analyzed under an inverted light microscope (Axio Vert.A1, Zeiss) analogous to Pizarro et al. (Pizarro et al., 2005).

The remaining net-tow concentrate was size fractionated over a filter array to collect the > 200, >50 and > 20 μm fractions. Plankton of each size fraction were rinsed with 0.45 μm filtered seawater into 50 mL centrifugation tubes and adjusted to 45 mL. Of each size fraction two 15 mL aliquots for determination of hydrophilic and lipophilic toxins analysis were transferred into 15 mL centrifugation tubes and centrifuged for 15 min at 2680 × g at 4 °C (DLAB D1008, Ontario CA, USA). Cell pellets were frozen at −20 °C until further reprocessing. In addition, for azaspiracid (AZA) analysis, 3 L of four depths (surface, 5 m, 10 m, and 20 m) were screened through a 20 μm mesh and the filtrates pooled. At each station, 3 to 5 L of the pooled filtrates were filtered over 5 μm polycarbonate filters (Merck KGaA, Darmstadt, Germany). Each filter was placed into a 50 mL centrifuge tube and extracted by a series of

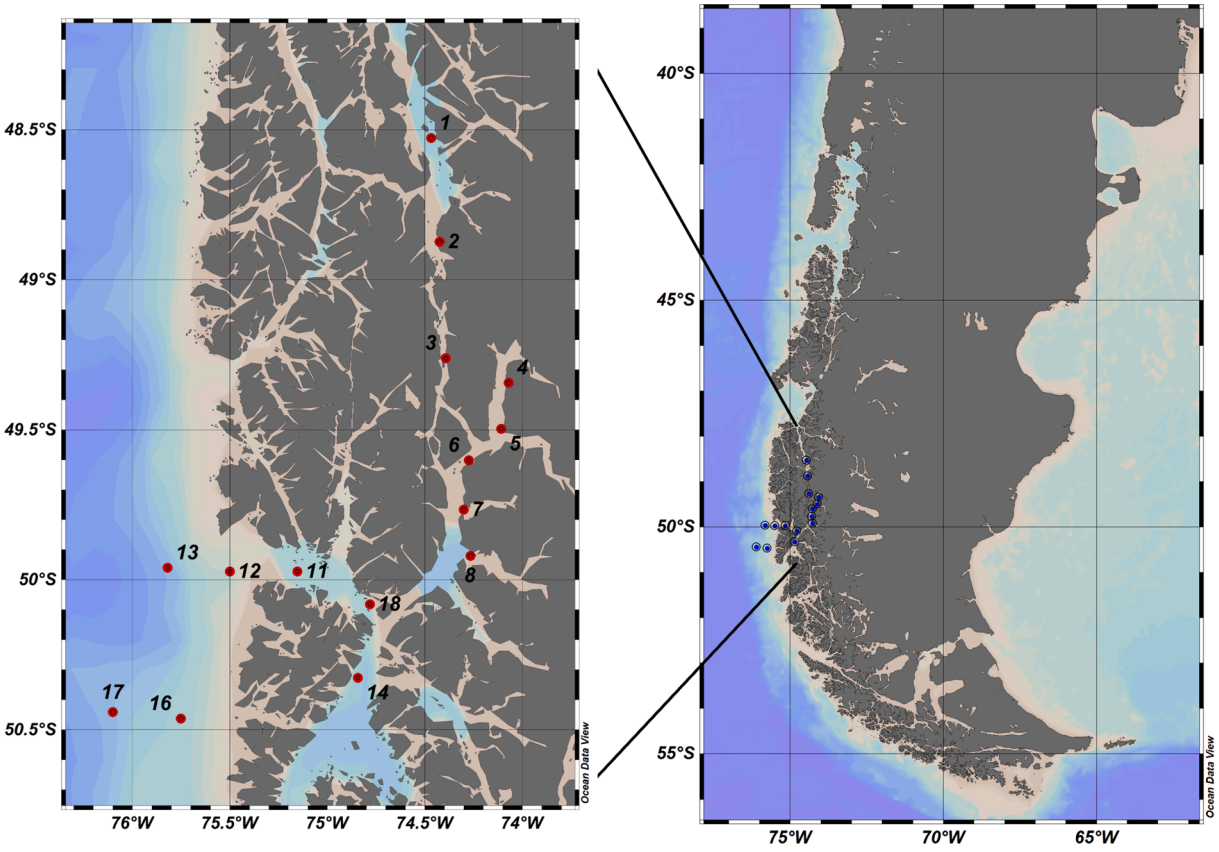

Fig. 1. Sampling stations on the PROFAN expedition in the Última Esperanza Province in southern Chile starting from Puerto Montt in November 2019.

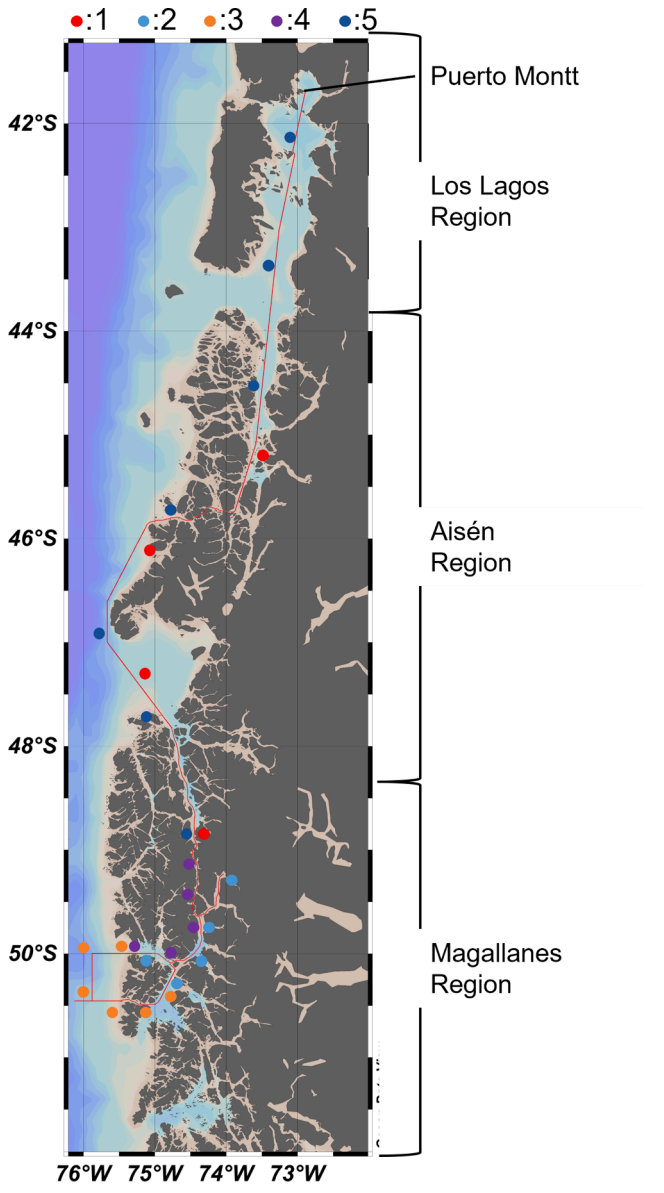

Fig. 2. SPATT Bag sampling areas including relevant geographic regions.; corresponding sampling conditions are shown in table A.1 and toxin contents in table 6.

0.5–1 mL methanol washes until complete filter discoloration.

After the expedition, cell pellets of the 20–50 μm size fractions were re-suspended in 1 mL 0.03 M acetic acid or methanol, respectively, and transferred to FastPrep tubes containing 0.9 g lysing matrix D (Thermo-Savant, Illkirch, France) for analysis of hydrophilic and lipophilic phycotoxins. Samples were homogenized by reciprocal shaking for 45 s at maximal speed (6.5 m s^{-1}) in a FastPrep instrument (Thermo-Savant) and subsequently centrifuged for 15 min (10 °C, 16,100 × g). Supernatants were transferred into spin filters (Ultra-free, Millipore, Eschborn, Germany) and filtered by centrifugation for 30 s at 10 °C and 5000 × g. Filtrates were transferred to HPLC vials and stored at −20 °C until analysis.

2.1.2. Isolation of *A. catenella*

Two cultures of the dinoflagellate *A. catenella* were established after single-cell isolation from station 3 (AC_PROFAN_01 strain) and station 7 (AC_PROFAN_02) (Fig. 1). Both strains were kept in culture at the CREAN-IFOP algae collection in Puerto Montt, Chile. Non-axenic cultures were grown in L1 medium at 12 °C in sterile (0.22 μm filtered) seawater (30 PSU) at 120 μmol photon m^{-2} s^{-1} (cool white fluorescence lamps) and under a 16:8h light:dark cycle (Guillard and Ryther, 1962).

2.1.3. Reagents and standards of toxin measurements of field samples

Water was deionized and purified (Milli-Q, Millipore, Eschborn, Germany) to 18 MV cm^{-1} or better quality. Formic acid (90%, p.a.), acetic acid (p.a.) and ammonium formate (p.a.) were purchased from Merck (Darmstadt, Germany). The solvents, methanol and acetonitrile, were high performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). Domoic acid (DA), gymnodimine A (GYM), 13-desmethyl spirolide C (SPX-1), okadaic acid (OA), dinophysistoxin-1 (DTX-1), dinophysistoxin-2 (DTX-2), pectenotoxin-2 (PTX-2), yessotoxin (YTX), and paralytic shellfish toxins (PST) including C-1- and C-2-toxin (C-1/C-2), B1-toxin (B1), gonyautoxins-1/4 (GTX-1/4), gonyautoxins-2/3 (GTX-2/3), decarbamoylgonyautoxins-2/3 (dcGTX-2/3), saxitoxin (STX), decarbamoylsaxitoxin (dcSTX), and neosaxitoxin (NEO) were purchased from the Certified Reference Materials (CRM) Program of the Institute of Marine Biology, National Research Council, 1411 Oxford Street, Halifax, NS, B3H 3Z1, Canada. Azaspiracid-1, −2, and −3 (AZA-1, −2, −3) were provided by Jane Kilcoyne of the Marine Institute, Oranmore, Co. Galway, H91 R673, Ireland. Goniodomin A (GDA) was provided by Thomas Harris, Vanderbuilt University, Nashville; TN, USA. A standard Mix of pinnatoxin G (PnTx-G), pinnatoxin E (PnTx-E), and pinnatoxin F (PnTx-F) was provided by Andrew Selwood, Cawthron Institute, 98 Halifax Street East, Nelson, New Zealand. All standards and corresponding mass spectrometric parameters are listed in table A.4.

2.1.4. Analysis of hydrophilic toxins

Paralytic shellfish poisoning toxin (PST) analysis was performed by ion pair chromatography coupled to post-column derivatization and fluorescence detection (LC-FLD) according to van de Riet et al. with slight modifications (Van De Riet et al., 2009). LC-FLD analysis was performed on a LC-1100 series liquid chromatography system equipped with a Phenomenex Luna C-18 reversed-phase column (250 mm × 4.6 mm internal diameter (id), 5 μm pore size) (Phenomenex, Aschaffenburg, Germany) with a precolumn (SecuriGuard, C-18, 4 mm × 3.0 mm id, Phenomenex). The column was coupled to a PCX 2500 post-column derivatization system (Pickering Laboratories, Mountain View, CA, USA). Eluent A contained 6 mM octane-sulfonic acid, 6 mM heptane-sulfonic acid, 40 mM ammonium phosphate, adjusted to pH 6.95 with dilute phosphoric acid, and 0.75 % tetrahydrofuran. Eluent B contained 13 mM octane-sulfonic acid, 50 mM phosphoric acid, adjusted to pH 6.9 with ammonium hydroxide, 15 % acetonitrile and1.5 % tetrahydrofuran. The flow rate was 1 mL min^{-1} with two isocratic elution steps: 0–15 min 100% A, 15–16 min switch to 100 % B, 16–35 min 100% B, 35–36 min switch back to 100 % A, 36–45 min 100% A. The injection volume was 20 μL and the autosampler was cooled to 4 °C. The eluate from the column was oxidized with 10 mM periodic acid in 555 mM ammonium hydroxide before entering the 50 °C reaction coil, after which it was acidified with 0.75 M nitric acid. Both the oxidizing and acidifying reagents entered the system at a rate of 0.4 mL min^{-1}. The toxins were detected by dual-monochromator fluorescence (l_{ex} 333 nm; l_{em} 395 nm) and the data were processed with Chemstation software (Agilent Technologies). External calibration curves were recorded with seven dilutions of a PST standard mix containing PST specified in section 2.1.3 in the 1–500 nM concentration range, which were used to quantitate individual PST concentrations. The obtained toxin concentrations of the plankton extracts were normalized to the total volume of the net-tow concentrate by division of the volume used for toxin analysis by the total volume to account for removed fractions used for microscopy. In addition, toxin abundances were normalized to toxin per net-tow by dividing with the number of net-tows taken at each respective station.

2.1.5. Analysis of lipophilic toxins

LC-MS/MS analysis for lipophilic toxins was performed on a reversed

phase C18 column (Purospher STAR RP-18 end-capped (2 μm) Hibar HR 50–2.1, Merck, Darmstadt, Germany) equipped with a guard column (EXP Pre-column Filter Cartridge, Merck) and thermostated at 40 °C according to Brãna-Magdalena et al with slight modifications (Brãna-Magdalena et al., 2014). Mobile phase A consisted of 500 mL water with 955 μL formic acid and 75 μL 25 % ammonia. Mobile phase B consisted of 475 mL acetonitrile, 25 mL deionized water, 955 μL formic acid and 75 μL 25 % ammonia. The flow rate was 0.6 mL min^{-1}, and the injection volume was 0.5 μL. Elution was carried out with the following gradient: 0–5 min with 95:5 % A:B, followed by a linear gradient of 2.0 min to 100 % B and 3.0 min 100 % B elution prior to return to initial conditions. Mass spectrometric experiments were performed in the selected reaction monitoring (SRM) mode in positive polarity on a Xevo TQ-XS triple quadrupole mass spectrometer equipped with a Z-Spray Ion source (Waters). Standard solutions of YTX (500 pg μL^{-1}), OA (500 pg μL^{-1}), DTX-1 (500 pg μL^{-1}), DTX-2 (500 pg μL^{-1}), DA (50 pg μL^{-1}), GDA (500 pg μL^{-1}), Pntx-E (20 pg μL^{-1}), PnTx-F (20 pg μL^{-1}), PnTx-G (20 pg μL^{-1}), PTX-2 (100 pg μL^{-1}), SPX-G (100 pg μL^{-1}) and AZA-1 (100 pg μL^{-1}) were used for external calibrations and the determination of detection limits. For quantification, 20-methyl SPX-G and PnTx-G were expressed as SPX-1 equivalents, PTX-2-sa and its isomer as PTX-2 equivalents and homo-YTX and all hydroxy-homo-YTXs as YTX equivalents. Toxin abundances were normalized as described in 2.1.4.

2.1.6. Solid phase adsorption toxin tracking sampling

SPATT bags (5 × 5 cm) were hand sewed of 50 μm mesh gauze and filled with 1 g Diaion HP20 (Sigma, Deisenhofen, Germany) (MacKenzie et al., 2004). Filled SPATT bags were conditioned by immersion in methanol and stirring overnight. Conditioned SPATTs were washed with deionized water, individually placed moist into a zip-lock bag, and kept at 4 °C until usage. SPATTs were suspended in a continuous water-flow from the ships' seawater pump located at 4 m depth below sea surface for two days (Table A.1) and stored moist at 4 °C until extraction of phycotoxins. For toxin extraction SPATT bags were first rinsed with deionized water and dried in an oven for one hour at 50 °C. Dry SPATT bags were opened, and the resin was transferred into 50 mL centrifugation tubes. 25 mL methanol was added and left overnight. The next day each resin-methanol mixture was poured into a glass chromatography column (270 mm length, 13 mm id, filled with 20 mm glass wadding and 10 mm quartz sand). Additional 15 mL methanol were used to rinse the centrifugation tube and added to the column. Methanol was eluted dropwise from the column until the supernatant reached the top of the filling and then another 25 mL of methanol were added to the column and subsequently eluted. The eluate was concentrated to approximately 0.5 mL in a rotary evaporator, transferred to an Eppendorf tube, fully concentrated under nitrogen gas flow, and then re-suspended in 250 μL methanol. The suspension was vortexed twice and the supernatant was spin-filtered and transferred to HPLC vials for analysis according to Fux et al. with slight modifications (Fux et al., 2009).

2.2. PST analysis of A. catenella strains

2.2.1. Toxin extraction

The established cultures of the two isolated *A. catenella* strains (AC_PROFAN_01 and AC_PROFAN_02) were harvested in the exponential growth phase (1.29×10^6 and 1.31×10^6 cells) by filtration. Cells were then lyophilized for 36 h (Thermo Sabant Modulyo-D) and maintained in dry and dark conditions until toxin extraction. Filters were then immersed in acetic acid (500 μL, 0.05 M), vortexed (1 min) and centrifuged (20,000 × g; 10 min). The supernatant was transferred and the residue was re-suspended in acetic acid (500 μL, 0.05 M) and centrifuged (20,000 × g; 10 min) again. Both supernatants were combined, filtered (0.22 μm) and stored at −20 °C until analysis.

2.2.2. Toxin analysis

LC-FLD analysis for PSP toxins was performed by the post-column derivatization method with liquid chromatography (LC-10ADvp, Shimadzu) coupled to a fluorescence detector (RF-551, Shimadzu) according to Franco & Fernández-Vila (Franco and Fernández-Vila, 1993). All PST specified in section 2.1.3 were analysed.

3. Results

3.1. Plankton taxonomy and cell counts

The qualitative analyses of net-tow concentrates evidenced a high heterogeneity of species (Table 1), which may have shifted along changes in the vertical and horizontal water column due to potential varying abiotic conditions such as rivers, snowdrifts, coastal runoffs, rainfall and/or distance to fresh water sources. A total species richness of 60 and 49 species was observed at stations 7 and 8, respectively, corresponding to the maximum species richness observed in this study. In terms of cell density, the diatom communities were generally dominated by the genera *Asterionella* (max = 2.4×10^5 cells L^{-1}), *Guinardia* (max = 1.8×10^6 cells L^{-1}), *Leptocylindrus* (max = 6.4×10^5 cells L^{-1}), *Rhizosolenia* (max = 4.3×10^5 cells L^{-1}), *Chaetoceros* (max = 9.2×10^5 cells L^{-1}) and *Thalassiosira* (max = 1.1×10^6 cells L^{-1}). The only toxic diatoms were from the *Pseudo-nitzschia seriata* complex (max = 1.3×10^5 cells L^{-1}) and *delicatissima* complex (max = 1.0×10^5 cells L^{-1}) (Table 2/3). Changes in temperature and salinity in close proximity to glacier-marine systems (St. 4, 5, 6) were found to be correlated (Pearson Correlation, $p < 0.01$ and $p < 0.1$, respectively) with a more diverse spectrum of centric diatoms mainly of the *Thalassiosira* and *Chaetoceros* group (Table 1).

With increasing distance of stations from the coastline and the glaciers (St. 7, 8, 14, 16), an increase in dinoflagellates of the genera *Tripos* (max = 2.8×10^4 cells L^{-1}), *Protoperidinium* (max = 1.5×10^5 cells L^{-1}) and *Heterocapsa* (max = 3.7×10^5 cells L^{-1}) was observed (Table 3). The dinoflagellate communities were generally dominated by the genera *Heterocapsa*, *Protoperidinium*, *Tripos*, *Dinophysis*, and *Alexandrium*, whereby only the latter two are known to produce phycotoxins. *Heterocapsa* and *Protoperidinium* species reached cell densities comparable to diatoms. However, the two toxic genera *Alexandrium* and *Dinophysis* were only observed at lower cell densities with *Alexandrium* only being observed at the stations 5–8. *Alexandrium* species ranged between non-detectable and 4.0×10^2 cells L^{-1}, while *Dinophysis* species reached maximum cell densities of 2.4×10^3 cells L^{-1} (Table 3).

The sole additional toxic dinoflagellate genus that was identified was *Azadinium* (Table 3). However, this genus was only detected at three stations (8, 16, and 18) with low cell densities, reaching up to 1.8×10^3 cells L^{-1}.

3.2. Hydrophilic toxins

Paralytic Shellfish Poisoning (PSP) toxins were detected at each sampling station, except for station 4 (stations 5, 10, and 15 were not

Table 1
Distribution and diversity of the different observed phytoplankton groups as observed by qualitative analysis of net-tow concentrates (20 m vertical depth) on the PROFAN expedition; corresponding sampling stations in Fig. 1.

Groups	Station							
	4	5	6	7	8	14	16	18
Radial centric diatoms	6	5	6	12	6	8	4	4
Polar centric diatoms	8	9	16	22	14	16	5	4
Arraphid pennate diatoms	1	0	1	1	3	1	1	2
Raphid pennate diatoms	2	2	3	5	5	2	2	2
Dinoflagellates	2	9	11	19	16	6	7	5
Silicoflagellates	1	1	1	1	1	1	1	1
Total Taxa	20	26	38	60	49	34	20	18

Table 2
Qualitative phytoplankton counting data of a subset of sampling stations of net-tow concentrates (20 m vertical depth) on the PROFAN expedition; only potentially toxin-producing species are shown; 1 = detected, 0 = not detected; corresponding sampling stations in Fig. 1.

Species	Station							
	4	5	6	7	8	14	16	18
Pseudo-nitzschia seriata complex	1	1	1	1	1	1	0	0
Pseudo-nitzschia delicatissima complex	0	0	0	1	1	1	0	0
Alexandrium catenella	0	1	1	1	1	0	0	0
Alexandrium ostenfeldii	0	0	1	1	0	0	0	0
Azadinium (group)	0	0	0	0	1	0	1	0
Dinophysis acuminata	0	0	1	1	1	0	1	0
Dinophysis infundibulum	0	0	0	1	1	0	0	0
Dinophysis truncata	0	0	0	0	0	0	1	0
Dinophysis spp.	0	0	0	0	1	0	0	0
Gymnodinium spp.	0	0	0	0	1	0	0	0

Table 3
Quantitative phytoplankton counting data [cells L^{-1}] integrated over four discrete sampling depths (3, 5, 10 and 20 m) of a subset of sampling stations of seawater collected with a CTD-rosette on the PROFAN expedition; only potentially toxin-producing species are shown; corresponding sampling stations in Fig. 1; nd = not detected.

Species	Station							
	4	5	6	7	8	14	16	18
Pseudo-nitzschia seriata	2.0 × 10^2	9.3 × 10^3	1.8 × 10^3	1.2 × 10^5	1.0 × 10^5	3.1 × 10^4	nd	nd
Pseudo-nitzschia delicatissima	nd	nd	nd	9.0 × 10^4	1.0 × 10^5	nd	nd	nd
Alexandrium catenella	nd	1.6 × 10^2	4.0 × 10^2	1.6 × 10^2	2.0 × 10^2	nd	nd	nd
Alexandrium ostenfeldii	nd	nd	nd	1.6 × 10^2	nd	nd	nd	nd
Azadinium (group)	nd	nd	nd	nd	6.4 × 10^2	nd	9.2 × 10^2	1.8 × 10^3
Dinophysis acuminata	nd	nd	1.3 × 04	1.9 × 10^4	6.5 × 10^3	nd	1.9 × 10^4	nd
Dinophysis infundibulum	nd	nd	nd	1.2 × 10^2	2.4 × 10^2	nd	nd	nd
Dinophysis truncata	nd	nd	nd	nd	nd	nd	2.3 × 10^3	2.4 × 10^3
Dinophysis spp.	nd	nd	nd	nd	1.2 × 10^2	nd	nd	nd

sampled), however with strongly differing relative toxin compositions and abundances (Fig. 3, Table 4). No decarbamoyl-derivatives were found at any stations. According to their geographical distribution and toxin composition, toxin profiles can be divided into four categories, which are open ocean stations (13, 16, 17), stations of Trinidad Channel (11, 12, 18), of Wilde Channel (5, 8, 14) and of Messier Channel (1, 2, 3, 6, 7) (Fig. 3). At some stations, toxin abundances of C-1/C-2 and gonyautoxin-1/4 (GTX-1/4) fall slightly under the limit of detection (LoD) (Table 4) but remain illustrated in Fig. 3 to not distort the relative toxin contributions. However, even though these toxins were present in the field samples, the accuracy of the measured abundances is reduced. The open ocean stations had very low total PSP-toxin abundances (<5 ng) and only the carbamoyl derivatives GTX-2/3 could be detected (Table 4). In Trinidad Channel, connecting open ocean and inner channels, the toxin levels were slightly higher, but still low (<10 ng). However, the corresponding *N*-sulfocarbamoyl derivatives of GTX-2/3, namely C-1/C-2, were also detected in addition to GTX-2/3. More diverse toxin profiles were found in inner channels Wilde Channel and Messier Channel (Fig. 3). The toxin profiles of Wilde Channel in addition to GTX 2/3 and C-1/C-2 contained high relative proportions of the severely toxic PSP-variant saxitoxin (STX) being the major contributor (40–70%) to these PSP toxin profiles even though it was completely absent in the samples of the open ocean and Trinidad Channel. Finally, the most diverse PSP toxin profiles were detected at the stations of Messier Channel (Fig. 3). While total PSP toxin abundances were still low in Wilde Channel (<30 ng), total toxin contents were 10–100 times higher in Messier Channel in comparison to other stations (Table 4). Here, the highest relative toxin contributors were GTX 1–4, with only very small contributions of C-1/C-2 and STX. Furthermore, these were the only stations where neosaxitoxin (NEO) could be detected. From stations 3 and 7 in the Messier Channel a single *Alexandrium catenella* cell was isolated (AC_PROFAN_1 and AC_PROFAN_2, respectively) and cultures were established. PST analysis of the laboratory cultures showed differences to the PST profiles of the respective stations, where *A. catenella* was isolated. AC_PROFAN_1 contained high relative contributions of C-1/C-2 and GTX-1/4 with minor contributions of GTX 2/3 and B1 (GTX 5) (Fig. 3), while AC_PROFAN_2 only contained GTX-2/3 and GTX-1/4. B1 was only measured in strain AC_PROFAN_1 and not in the other strain nor any field sample of this expedition. All screened toxins and their quantified transitions are shown in table A.2 and A.3, including retention times (RT), LoDs and the detected abundance ranges.

3.3. Lipophilic toxins

Lipophilic toxins were also detected at each sampling station and showed high variabilities in composition and total toxin content (Fig. 4, Table 5). In general, domoic acid (DA), yessotoxins (YTXs) and pectenotoxins (PTXs) were the most abundant lipophilic toxins detected in the water column (Fig. 4). DA was only detected at a total of four stations (3, 6, 7, 14) that are all located inside of the channel system. However, only low quantities of DA as the only detected toxin were found at station 14. In contrast, at the other three stations of Messier Channel the DA content greatly exceeded all other toxins with values of >100 ng per net-tow representing the highest toxin abundance measured in this study. The second most abundant toxin group featuring a diverse structural variety of variants are YTXs. YTX and its congener homo-YTX, featuring a one-carbon elongated aliphatic side chain, were found at approximately a third of the surveyed stations with combined values of a few nanograms per net-tow. Furthermore, three hydroxylated variants of homo-YTX (provisionally named 45-OH homo-YTX a/b/c) were detected with the variant 45-OH homo-YTX a being the most abundant (Table 5). The third most abundant toxin was the pectenotoxin (PTX) PTX-2 that is related to the group of dinophysistoxins (DTXs) as both toxin classes can be produced by the same species. Interestingly, at some stations only the corresponding seco acid PTX-2sa, resulting of hydrolysis of the intramolecular lactone bridge of PTX2, and a further corresponding isomer could be detected (Table 5). The macrocyclic imine gymnodimine A (GYM-A) and related analogues were not detected (LoD = 25 pg NT^{-1}). However, 20-methyl spirolide G (20-Me SPX-G), belonging to the related group of macrocyclic imine toxins, could be detected at some stations in very low quantities (Fig. 4). Low levels of AZA-2 were detected in net-tow samples at stations 11, 13, 16, and 17, but not in water samples. Any other lipophilic toxins, including DTXs (LoD < 649.1 pg NT^{-1}) and PnTxs (LoD < 170.4 pg NT^{-1}) were not detected at any station in net-tow samples. All screened toxins are shown in table A.4, including RTs, LoDs and the detected abundance ranges.

3.4. Solid phase adsorption toxin tracking (SPATT) sampling

In addition to plankton sampling for the analysis of particulate phycotoxins, five passive samplers (SPATT) for the detection of

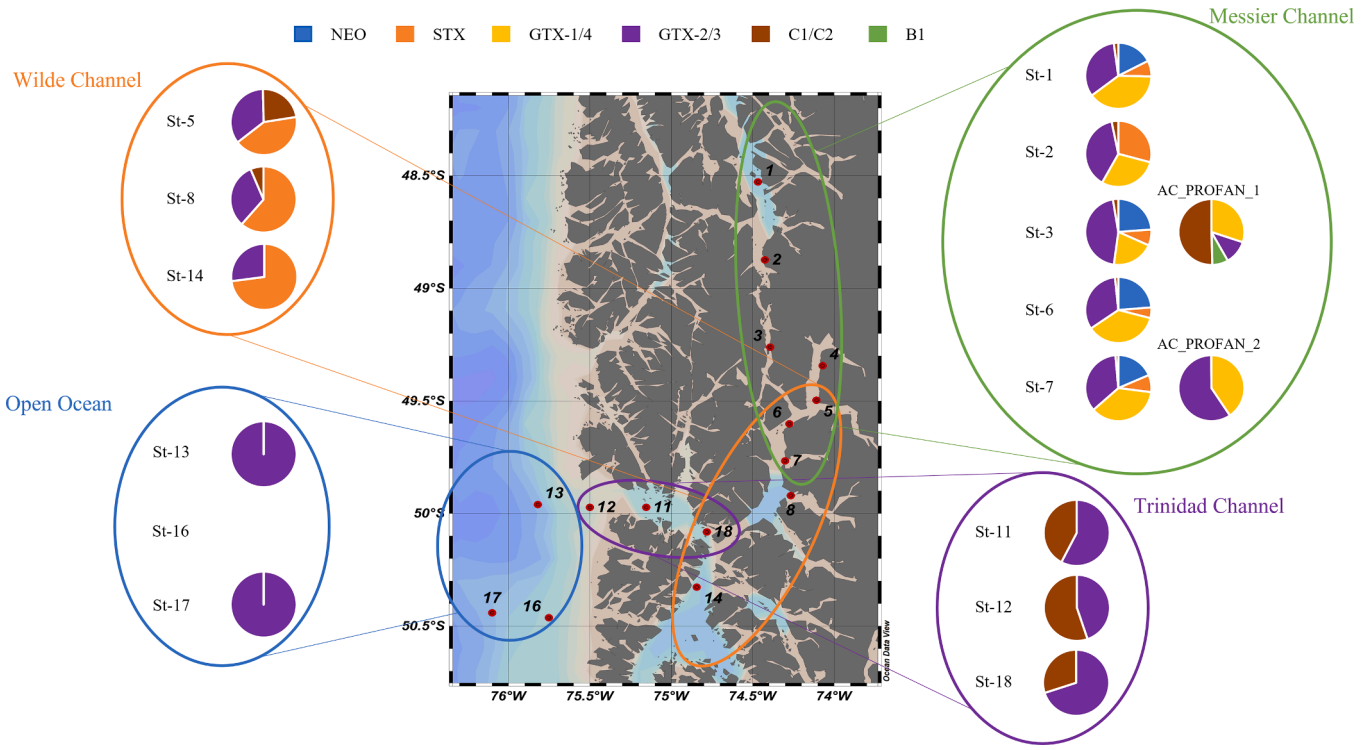

Fig. 3. PSP-toxin profiles [%] as determined by LC-FLD analysis of net-tow concentrates (20 m vertical depth) and two *A. catenella* isolates of sampling stations on the PROFAN expedition excluding stations 13, 16, and 17; profiles were grouped (denoted by colored circle) according to toxin composition and geographical appearance in the open Ocean (blue), Wilde Channel (orange), Messier Channel (green) and Trinidad Channel (purple); NEO = neosaxitoxin, STX = saxitoxin, GTX = gonyautoxin.

Table 4
Relative PST content [%] of sampling stations on the PROFAN cruise as determined by LC-FLD analysis of net-tow concentrates (20 m vertical depth) including total toxin content [ng NT^{-1}]; only detected toxins are shown;*= values slightly below the LoD that were included to not distort the relative PST profiles; corresponding sampling stations in Fig. 1; PST = paralytic shellfish toxin, GTX = gonyautoxin, NEO = neosaxitoxin, STX = saxitoxin, NT = net townet-tow, nd = not detected, ns = not sampled.

Station	PST profile [%]						Total Toxin content [ng NT^{-1}]
	C-1/C-2	GTX-1/4	GTX-2/3	NEO	STX	B1	
1	2.2	39.3	32.8	17.5	7.7	nd	233.8
2	3.2*	28.5*	37.7	nd	28.5	nd	65.4
3	2.6*	20.4*	44.8	23.6	7.7	nd	138.8
4	nd	nd	nd	nd	nd	nd	nd
5	22.9*	nd	35.1	nd	42.0	nd	5.9
6	1.6*	36.6	32.8	23.7	5.2	nd	165.4
7	1.4	36.6	35.0	18.6	8.4	nd	505.9
8	6.4*	nd	32.1	nd	61.5	nd	26.0
9	ns	ns	ns	ns	ns	ns	ns
10	ns	ns	ns	ns	ns	ns	ns
11	42.3*	nd	57.7	nd	nd	nd	4.4
12	55.2*	nd	44.8	nd	nd	nd	4.4
13	nd	nd	100.0	nd	nd	nd	1.9
14	nd	nd	27.1*	nd	72.9	nd	3.7
15	ns	ns	ns	ns	ns	ns	ns
16	nd	nd	nd	nd	nd	nd	nd
17	nd	nd	100.0	nd	nd	nd	4.7
18	30.0	nd	70.0	nd	nd	nd	9.9
AC_PROFAN_1 (St 3)	50.4	30.0	11.7	nd	nd	7.9	/
AC_PROFAN_2 (St 7)	0.1	40.6	59.3	nd	nd	nd	/

dissolved phycotoxins were deployed sequentially during the cruise (Fig. 2). In accordance with toxins derived from plankton sampling, PTX-2 including its corresponding seco acid PTX-2sa and YTXs were the toxins with the highest abundances on each SPATT sampler (Fig. 5, Table 6). Furthermore, low quantities of 20-Me SPX G could also be identified on four of the five SPATT samplers complementing its presence in net-tow samples (Table 6). In addition, spirolide SPX-1 could be detected north of 49 °S, which was outside the working area and accordingly not sampled by net-tows. More noteworthy, some striking differences were found between toxin data of the SPATT samplers and of net-tows. First, even though no OA or DTX-1 were detected in any plankton samples, these toxins were present in water samples during the entire cruise transect. Second, AZA-2 was detected at low concentrations in water samples during the entire cruise transect and thus seems to be the prevalent AZA variant in southern Chile with no other AZA detection in plankton samples. However, traces of AZA-2 could be detected in net-tows of a few stations that fell under the limit of detection (LoD = 48.8 pg NT^{-1}) and can thus not be confidently identified (Fig. 4). Nevertheless, it is an indication for the presence of AZA-2 in southern Chile and thus complements the SPATT sampler data. Last, the neurotoxin PnTx-G belonging to the cyclic imine group was detected for the first time in Chilean waters (Table 6).

4. Discussion

4.1. Hydrophilic toxins

In general, PSP toxin levels were higher in the inner fjords and channels than at the open ocean stations (Fig. 3, Table 4), which is in accordance with the fact that dinoflagellates generally prefer more stratified water columns, where they can possibly outcompete faster growing, but passively floating diatoms by diel vertical migration (Fauchot et al., 2005; Ji and Franks, 2007). This observation was further

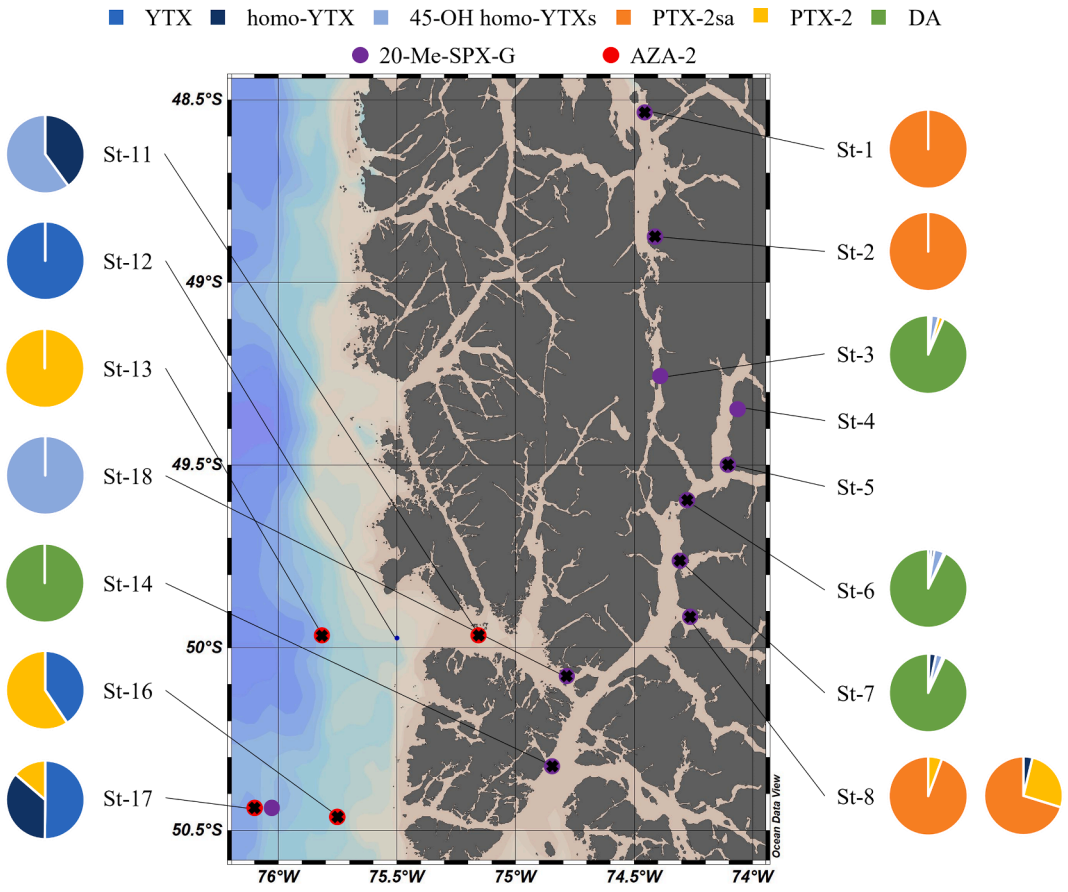

Fig. 4. Lipophilic toxin profiles [%] as determined by LC-MS/MS of net-tow concentrates (20 m vertical depth) of sampling stations on the PROFAN expedition; colored circles indicate traces of 20-Me SPX-G (purple) and AZA-2 (red) that fell under the LoD if they contain a cross; SPX = spirolide, AZA = azaspiracid, YTX = yessotoxin, PTX = pectenotoxin, DA = domoic acid.

Table 5
Lipophilic toxin abundance [pg NT^{-1}] of sampling stations on the PROFAN expedition as determined by LC-MS/MS analysis of net-tow concentrates (20 m vertical depth); only detected toxins are shown; nd = not detected; corresponding sampling stations in Fig. 1; NT = net-tow, ns = not sampled, nd = not detected; DA = domoic acid, SPX = spirolide, PTX = pectenotoxin, YTX = yessotoxin, sa = seco acid.

Station	Lipophilic toxin abundance [pg NT^{-1}]									
	DA	20-Me-SPX-G	PTX-2	YTX	homo- YTX	45-OH homo- YTX a	45-OH homo- YTX b	45-OH homo- YTX c	PTX-2sa isomer	PTX-2sa
1	nd	nd	nd	nd	nd	nd	nd	nd	343.2	266.2
2	nd	nd	nd	nd	nd	nd	nd	nd	368	329
3	411,366	151	8,734	2,718	3,695	13,014	nd	nd	198	345
4	nd	125	nd	nd	nd	nd	nd	nd	nd	nd
5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	92,774	nd	766	1,258	1,326	3,991	nd	nd	nd	nd
7	112,149	nd	1,116	548	3,344	3,405	nd	nd	nd	200
8a	nd	nd	507	nd	nd	nd	520	nd	5,544	2,865
8b	nd	nd	3,497	nd	506	542	nd	nd	4,417	5,024
9	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
11	nd	nd	nd	nd	678	nd	nd	520	nd	nd
12	nd	nd	nd	708	nd	nd	nd	nd	nd	nd
13	nd	nd	389	nd	nd	nd	nd	nd	nd	nd
14	4,553	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
16	nd	nd	1,523	1,042	nd	nd	nd	nd	nd	nd
17	nd	nd	771	2,820	2,029	nd	nd	nd	nd	nd
18	nd	nd	nd	nd	nd	nd	nd	440	nd	nd

supported by the generally good agreement of the spatial distribution of PSP toxins and their potential producing organisms *Alexandrium catenella* and *A. ostenfeldii* in this area (Table 2/3).

In contrast to these anticipated findings, a surprisingly high variability of different PSP toxin profiles was found in the relatively small sampling area (Table 4), even though toxin profiles of stations with low total PST content may not be complete due to relatively high detection limits of GXT1/4 and NEO. Profiles consisting of GTX-2/3 and

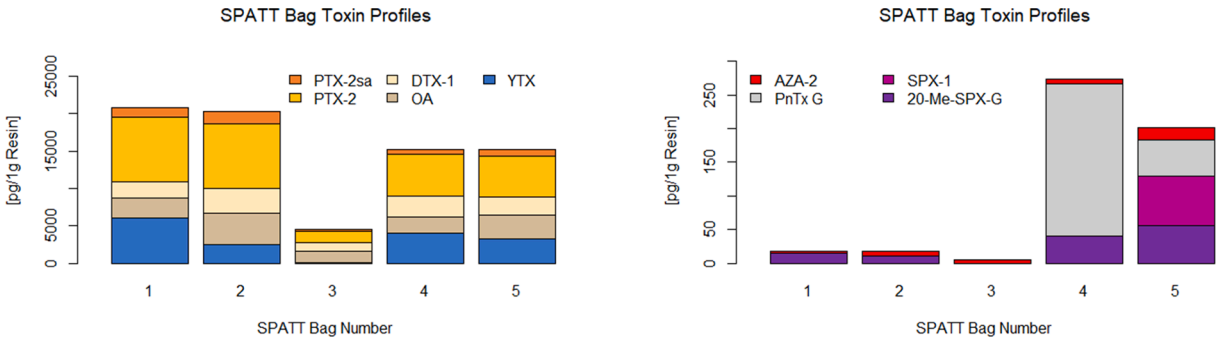

Fig. 5. SPATT bag toxin content [pg g^{-1} Resin] as determined by LC-MS/MS on the PROFAN expedition; high and low abundant toxins are depicted right and left, respectively; corresponding sampling area and conditions shown in Fig. 4 and table A.1; SPATTs were suspended in a continuous water-flow from the ships' seawater pump located at 4 m depth below sea surface for two days; PTX = pectenotoxin, YTX = yessotoxin, DTX = dinophysistoxin, OA = okadaic acid, PnTx = pinnatoxin, SPX = spirolide.

Table 6
SPATT Bag Toxin content [pg g^{-1} Resin] on the PROFAN cruise including LoD-values [pg $sample^{-1}$]; only detected toxins are shown; corresponding sampling area and conditions shown in Fig. 2 and table A.1; SPATTs were suspended in a continuous water-flow from the ships' seawater pump located at 4 m depth below sea surface for two days; PTX = pectenotoxin, YTX = yessotoxin, DTX = dinophysistoxin, OA = okadaic acid, PnTx = pinnatoxin, SPX = spirolide, nd = not detected.

SPATT #	SPATT Bag Toxin Content [pg g^{-1} Resin]								
	20-Me SPX-G	AZA-2	DTX-1	OA	PnTx- G	PTX-2	PTX-2sa	SPX-1	YTX
1	14.7	3.7	2,114	2,652	nd	8,666	1,293	nd	6,062
2	10.8	6.8	3,313	4,115	nd	8,691	1,719	nd	2,511
3	nd	5.7	1,035	1,515	nd	1,583	206	nd	134
4	40.5	7.6	2,858	2,131	2251	5,521	717	nd	3,997
5	55.5	18.6	2,421	3,1291	54.0	5,466	823	74.0	3,308
LoD [pg $sample^{-1}$]	8.6	4.5	60.1	47.6	4.1	14.6	29.1	8.6	35.0

C-1/C-2 were found in the Trinidad Channel, profiles consisting of GTX-2/3, C-1/C-2, and STX were found in Wilde Channel and toxin profiles consisting of GTX-1/4, GTX-2/3, C-1/C-2, STX, and NEO in Messier Channel. Plankton taxonomy and cell counting support the presence of PSP toxins at most stations but did not enable to assign the local STX producer. *A. catenella* and *A. ostenfeldii*, both known PSP-producers, were found in the Wilde Channel and Messier Channel (Table 2). Even though *A. ostenfeldii* is generally known as producer of spirolides, some strains have been shown to produce PSP toxins, typically in coastal areas with low salinities (Ciminiello et al., 2006; Gribble et al., 2005; Hakanen et al., 2012; John et al., 2003; Moestrup and Hansen, 1988; Van de Waal et al., 2015). However, there has been one publication of a PSP producing *A. ostenfeldii* strain in open ocean waters as well (Guinder et al., 2018). Nevertheless, the high relative abundance of STX in Wilde Channel does not fit the previously published PSP toxin profiles of a cultured strain of *A. ostenfeldii* from the Isla Vergara and of *A. catenella* isolated near Puerto Edén (Salgado et al., 2015; Varela et al., 2012). Be that as it may, the published data only represents a single strain from the respective geographical region and thus the significance is limited (Salgado et al., 2015; Varela et al., 2012). Nonetheless, three different studies analysing the toxin profile of 24 different strains in total, isolated between 43 °S and 49 °S of the Chilean waters, have not reported a single profile dominated by STX (Salgado et al., 2015; Varela et al., 2012; Aguilera-Belmonte et al., 2011). Therefore, the toxin profiles found in northern Magallanes cannot result from an overlap of already known and published Chilean profiles. Several explanations for this phenomenon are possible. First, the published toxin profiles from *A. catenella* and *A. ostenfeldii* might originate of a different strain than the toxin profile presented in this study. However, two strains of *A. catenella* isolated from stations 3 and 7 do not contain any saxitoxin either and thus it is unlikely that this strain of *A. catenella* contributed the high relative abundance of saxitoxin. Second, the PSP toxin profile of the producing organism might have changed over time. However, this is rather unlikely, as significant changes in PSP toxin profile composition have not been reported in strains cultured over few years except of a total loss of STX production reported after more than four decades (Martins et al., 2004). Nevertheless, toxin composition in the genus *Alexandrium* is generally a highly stable characteristic and can thus be utilized as a chemotaxonomic marker (Anderson et al., 2012). With respect to the cell counts of *A. catenella* and *A. ostenfeldii* found in this study and the fact that the latter was only detected at two stations (Table 2), it is most likely that unknown strains of *A. catenella* were responsible for the detected PSP toxins. The only other potential PSP producer, which might be responsible for the high abundancy of STX, is *Gymnodinium catenatum*, as there are no other PSP producing *Gymnodinium* spp. known (Hallegraeff et al., 2012). However, no species-specific counting of the *Gymnodinium* complex was conducted and there was only a single appearance of the whole complex. In addition, *G. catenatum* is mostly known from tropic to temperate regions and has not been reported for Chile so far (Hallegraeff et al., 2012). Thus it is unlikely that this organism is responsible for the high relative abundancy of STX in Patagonia. The two isolated and analysed *A. catenella* strains in this study (AC_PROFAN_1 and AC_PROFAN_2) can partly explain the observed toxin profiles of the stations that they were isolated at (3 and 7, respectively). However, discrepancies between toxin profiles of field samples and monoclonal strains of the same area are common as local populations of certain species usually show a high genetic diversity, which is reflected in varying toxin profiles. While plankton field samples represent the whole genetic diversity of a given location, monoclonal strains only represent one individual genotype. Nonetheless, the appearance of STX and NEO cannot be explained by the cultured strains and thus hint to another PSP-producer. With respect to STX being the most toxic PSP congener, further work is needed to identify the producing organism and to perform a reasonable risk assessment of the PSP toxin threat in this geographical region.

4.2. Lipophilic toxins

The most abundant lipophilic toxins including DA found in net-tow concentrates were DA, PTXs and YTXs (Table 5, Fig. 4). Domoic acid

is a rare neurotoxic amino acid, structurally related to glutamic acid and produced by several species of the diatom genus *Pseudo-nitzschia* (Bates et al., 2018; Lelong et al., 2012). This toxin has been associated with amnesic shellfish poisoning (ASP) in humans (Bates et al., 1989). DA could only be identified at four different stations (3, 6, 7, 14) with three of these located in the Messier Channel (3, 6, 7). *Pseudo-nitzschia* species belonging to the *seriata* and the *delicatissima* complex could be identified at each of these stations, however, in strongly differing abundances (Table 2/3). DA abundances of station 6 and 7 were very similar, while at station 6 less than one percent of *Pseudo-nitzschia* cells in comparison to station 7 were observed. Furthermore, similar amounts of *Pseudo-nitzschia* cells were observed at station 8 in comparison to station 6, however no DA could be detected. Several possibilities for these observations are feasible. First, there have been DA containing and not containing strains published for both *Pseudo-nitzschia* complexes (Bates et al., 2018). Thus, high cell counts do not necessarily correlate to high particular levels of DA. Furthermore, it has been shown that herbivorous grazers can induce DA production in *Pseudo-nitzschia* spp. (Lundholm et al., 2018; Harðardóttir et al., 2015). Hence, differences in DA levels might reflect local differences of the zooplankton communities in addition to *Pseudo-nitzschia* populations in a given area.

Dinophysistoxins (DTXs) including okadaic acid (OA) and pectenotoxins (PTXs) are not only chemically related, but additionally often produced by the same phytoplankton species. DTXs are produced worldwide by a total of ten species of *Dinophysis*. Therein, the most prevalent representatives are *D. acuminata* and *D. acuta*. Generally, DTXs are produced by *D. acuta*, while *D. acuminata* is known to mainly produce PTXs (Díaz et al., 2020). However, this only represents the general trend of toxin production in the *Dinophysis* genus with exceptions being published for both *D. acuta* and also *D. acuminata* (Kamiyama and Suzuki, 2009; Lee et al., 1989; Uchida et al., 2018). In addition, the dinoflagellate *Prorocentrum lima* has been shown to produce DTXs as well, however with no publication available about the production of PTXs (Bravo et al., 2001; Gayoso et al., 2002; Vale et al., 2009). In contrast to *Dinophysis, P. lima* has a benthic life cycle and is therefore rarely found in the water column. Thus, it is generally unlikely that this organism is responsible for any toxins analyzed in net-tow concentrates and in accordance with that it was not found during microscopy analysis either (Table 2). No DTXs were found in the net-tow concentrates, but PTXs were detected at various stations (Fig. 4). *D. acuminata* was found at all sampling stations featuring high abundancy of PTXs and *D. infundibulum*, also a known PTX producer, at a subgroup of these stations (Table 2) (Uchida et al., 2018; Nishitani et al., 2008). Thus, the local producers of PTXs and their corresponding seco-acid including an isomer are probably *D. acuminata* and *D. infundibulum* with *D. acuminata* most likely being the main contributor, since the abundance of *D. acuminata* in the water column was significantly higher and it was found at all stations corresponding to PTX-2 detection (Table 3). Furthermore, PTX-2 has already been associated with *D. acuminata* in central Chile strengthening this hypothesis (Díaz et al., 2020).

The last main toxin group that was detected at approximately a third of the sampling stations were yessotoxins (YTXs). The currently known organisms responsible for YTX production are the dinoflagellates *Protoceratium reticulatum*, *Lingulodinium polyedra, Gonyaulax spinifera* and *Gonyaulax taylorii* (Paz et al., 2008; Paz et al., 2004; Álvarez et al., 2016; Rhodes et al., 2006; Riccardi et al., 2009). However, *G. taylorii* has only been published once to be a YTX producer and the taxonomy of this organism is doubtful (Álvarez et al., 2016). Álvarez et al. definitely found the organism responsible for the production of YTX as it made up >95% of the biomass in the net haul, however, the identification is solely based on morphological traits and remains arguable until a gene sequence can be derived for verification (Álvarez et al., 2016). A similarly complicated taxonomy exists with *G. spinifera*, the second dinoflagellate species of the *Gonyaulax* species complex that also produces YTXs. In this case, however, there have been various phylogenetic and toxigenic analyses of single-cell isolates confirming its identification and the production of YTXs, after the first connection was made by single-cell analyses utilizing the ELISA method (Rhodes et al., 2006; Riccardi et al., 2009; Rajotte et al., 2019). Structurally speaking approximately one hundred YTX analogues have been reported worldwide, however YTX and homo-YTX are generally dominating the toxin profiles (Paz et al., 2008). *P. reticulatum* usually produces YTX as main congener and only a few strains have been reported to produce homo-YTX, featuring a one carbon elongated aliphatic side chain, as the most prominent toxin (Paz et al., 2008; Suzuki et al., 2007; Paz et al., 2007). However, no vegetative cells of any known YTX- producing species (*Gonyaulax, P. reticulatum,* and *L. polyedra*) were found in both net-tow and water samples. Nevertheless, *P. reticulatum* has already been described in this geographical region and thus it is quite likely at least partly responsible for the presence of YTXs (Satake et al., 1998). *L. polyedra*, however, has been shown to produce similar amounts of YTXs including homo-YTX variants and therefore this dinoflagellate is probably responsible for the presence of the homo-YTX variants, which are rarely found in *P. reticulatum*. In addition, resting cysts of this dinoflagellate have already been reported in the Los Lagos Region and thus make its appearance further south plausible (Salgado et al., 2011). Solely at station 18, only OH-YTX c could be detected, which does not fit any published data of the two previously described organisms and hints to either different strains or additional YTX producing organisms (Table 5). However, the measured level was just above the detection limit and thus other potential variants might have been undetectable. With no detection of any YTX-producing organisms in the plankton samples and no isolates available, confirmation of the YTX-producer in this geographic region is unfeasible and thus further work is needed to identify the regional producer.

The macrocyclic imine GYM and related analogues that primarily occur in combination with the dinoflagellates *Karenia selliformis* or *Alexandrium ostenfeldii* were not detected. However, non-gymnodimine producing *Karenia selliformis* strains have also been connected with ichthyotoxic characteristics, probably through the concomitant action of polyunsaturated fatty acids (PUFAs) and reactive oxygen species (ROS) (Mardones et al., 2020). Nonetheless, no species of the *Karenia* complex were detected during phytoplanktonic analysis. Despite no detection of GYM, 20-Me SPX-G belonging to the related group of macrocyclic imine toxins known as SPX originating from *A. ostenfeldii* could be detected at some stations (3 and 4) in very low abundances (Fig. 4). At these stations, however, no *A. ostenfeldii* was detected (Table 2). This is most likely a result of very low cell densities in accordance with low 20-Me SPX-G levels of approximately 100 pg NT^{-1}. The presence of a different spirolide producer is unlikely as no other SPX producers than *A. ostenfeldii* are doubtlessness known to date. The only other species that has been associated with SPX production is the closely related species *Alexandrium peruvianum* (Borkman et al., 2012; Van Wagoner et al., 2011). However, there has been doubt expressed over the concomitant existence of both *Alexandrium* species as a result of only subtle morphological differences in plate structure that do not always conform to phylogenetic relationships (Suikkanen et al., 2013). Later, *A. peruvianum* has been shown to be conspecific with *A. ostenfeldii* (Kremp et al., 2014).

4.3. Solid phase adsorption toxin tracking (SPATT) sampling

SPATT samplers allow the integration of data over temporal and spatial scales, while the analysis of phytoplankton net-tows represents only a snapshot of phycotoxins present at one certain location and time point. Thus, SPATT sampling of dissolved phycotoxins in the water column is complemental to phycotoxin analysis of plankton samples. The comparison of phycotoxins detected in plankton and SPATT samples on one hand confirmed the presence of phycotoxins in plankton samples but on the other hand extended the available data on the occurrence of phycotoxins in southern Chile. Thus, the combined use of both sampling techniques allowed for a broader analysis of phycotoxins and their

producing organisms.

Even though no OA/DTX-1 were detected in plankton samples, these toxins were present in water samples during the entire cruise transect (Fig. 5) and thus indicate the presence of either *Dinophysis acuta* or *Prorocentrum lima* in the northern Magallanes, Aysén and southern Los Lagos Regions, where both organisms have been previously, but never as far south as in this study (Cassis et al., 2002; Díaz et al., 2011; Hoppenrath et al., 2013; Salgado et al., 2012). The study reporting *P. lima* in southern Chile described its appearance both in the form of cysts and also of vegetative cells, however, the formation of resting or temporary cysts by *P. lima* remains unclear (Hoppenrath et al., 2013; Salgado et al., 2012). During phytoplankton analysis no differentiation was made between *D. acuta* and the general *Dinophysis* genus and thus the SPATT bag data can only be used as an indication. Yet, if *D. acuta* was responsible for the detection of OA/DTX-1 during the entire cruise transect, then it seems rather unlikely that it was never found in net-tow concentrates. Also, *Dinophysis* spp. were only found at a single station (8). Furthermore, it is rather unlikely that *D. acuminata* or *D. infundibulum* are responsible for the detection of OA/DTX-1 since then these toxins should have also been detectable in net-tow concentrates at stations featuring these dinoflagellates. Thus, from the chemo taxonomical analysis it seems very plausible that the widely distributed species *P. lima* was responsible for the detection of DTXs in the study area of Southern Chile. This hypothesis is in agreement with the benthic life cycle of *P. lima* that could explain the presence of OA/DTX-1 on SPATT samplers and their absence in net-tows (Hoppenrath et al., 2013). Furthermore, the neurotoxin pinnatoxin G (PnTx-G) was detected for the first time in Chilean waters, which strongly indicates the presence of the dinoflagellate *Vulcanodinium rugosum*, which currently is the only known producer of PnTx (Fig. 5, Table 6) (Rhodes et al., 2011). *V. rugosum* has two important life cycle stages, including a short motile and thecate stage and a longer more stationary epibenthic stage, in which the species is non-motile for most of the time (Hernández-Becerril et al., 2013; Zeng et al., 2012). Thus, similar to the previously mentioned dinoflagellate *P. lima*, it is improbable to sample this species in net-tow concentrates and thus not surprising that this toxin was only found on the SPATT samplers. Once more, the temporal and spatial variable data obtained from SPATT samplers proved useful to detect low abundant toxins or toxins produced by benthic organisms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pocean.2022.102851.

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