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From lipophilic to hydrophilic toxin producers: phytoplankton succession driven by an atmospheric river in western Patagonia

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Abstract:	Phytoplankton succession is related to hydroclimatic conditions. In this study we provide the first description of a toxic phytoplankton succession in the Patagonian Fjord System. The shift was modulated by atmospheric-oceanographic forcing and consisted of the replacement of the marine dinoflagellate Dinophysis acuta in a highly stratified water column during austral summer by the diatom Pseudo-nitzschia calliantha in a mixed water column during late summer and early autumn. This transition, accompanied by a change in the biotoxin profiles (from lipophilic dinophysis toxins to hydrophilic domoic acid), was induced by the arrival of an intense atmospheric river. The winds in Magdalena Sound may have been further amplified, due to its west-east orientation and its location within a tall, narrow mountain canyon. This work also documents the first known appearance of toxic P. calliantha in Northern Patagonian. The potential impacts of the biotoxins of this species on higher trophic levels are discussed.
Suggested Reviewers:	Antonella Luglié, PhD University of Sassari luglie@uniss.it Dr. Antonella Luglié (University of Sassari) is an internationally recognised expert in phytoplankton ecology with focus on harmful algal blooms. She has a long career and many publications on the topic, many of them focusing on the noxious and toxic phytoplankton species.
	Emilio Marañón, PhD University of Vigo em@uvigo.es Dr. Emilio Marañón (Universidade de Vigo) has a long career focused in ecology and biogeochemical role of phytoplankton. His studies focus on phytoplankton diversity and community structure.
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	marina.montresor@szn.it The research interests of Dr. Montresor focus on the diversity and ecology of marine phytoplankton, with particular attention on life histories of microalgae, including species of the genus Pseudo-nitszchia. These organisms have multiphasic life cycles that include dormant and sexual phases, which have implications for the dynamics and genetic structure of their populations.

Puerto Montt, April 18, 2023

Dr. Francois Galgani
Editor-in-Chief
Marine Pollution Bulletin

Dear Editor:

Attached, please, find a submission of our original manuscript entitled: "**From lipophilic to hydrophilic toxin producers: phytoplankton succession driven by an atmospheric river in western Patagonia**", that we would like you to consider for publication in *Marine Pollution Bulletin*.

Harmful Algal Blooms (HABs) constitute a worldwide problem, affecting aquatic ecosystems, public health and local economies. In the last decades, this problem appears to have increased in frequency, geographic extent and intensity due to the increase in nutrient discharges and climate variability, forcing affected sectors, as those related to the aquaculture industry, to think proactive strategies in order to avoid the increasing economic losses. The fjords system in the Chilean Patagonia, are model scenarios for the study of physical-biological interactions during HABs development. So far, most efforts in the country went to monitoring and management of the events and very little field work has been carried out with research purposes.

In the present paper, we describe for the first time a toxic phytoplankton succession in the Patagonian Fjord System (Puyuhuapi Fjord, Aysén Region) modulated by atmospheric-oceanographic forcing (Atmospheric River). The response of a phytoplankton community dominated by *Dinophysis acuta* (dinoflagellate) changing to one dominated by *Pseudo-nitzschia calliantha* (diatom) and the potential impact of its associated toxins on higher trophic levels is widely discussed. In addition, this work also documents the first known appearance of toxic *P. calliantha* in Northern Patagonian.

Our results contribute to a better understanding of HABs in the region and demonstrate the importance of hydroclimatic conditions in the toxic phytoplankton succession in a global "hotspot", which may alleviate their economic impact in the southern austral region and elsewhere.

We hope you find this contribution of interest for your journal.

Yours sincerely,

Dr. Patricio A. Díaz
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Highlights

- Toxic phytopkanton succession modulated by intense atmospheric river in northern Patagonia
- Transition from lipophilic dinophysis toxins to hydrophilic domoic acid.
- The first known appearance of toxic *Pseudo-nitzschia calliantha* in Northern Patagonian.

From lipophilic to hydrophilic toxin producers: phytoplankton succession driven by an atmospheric river in western Patagonia

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Abstract

Phytoplankton succession is related to hydroclimatic conditions. In this study we provide the first description of a toxic phytoplankton succession in the Patagonian Fjord System. The shift was modulated by atmospheric-oceanographic forcing and consisted of the replacement of the marine dinoflagellate *Dinophysis acuta* in a highly stratified water column during austral summer by the diatom *Pseudo-nitzschia calliantha* in a mixed water column during late summer and early autumn. This transition, accompanied by a change in the biotoxin profiles (from lipophilic dinophysis toxins to hydrophilic domoic acid), was induced by the arrival of an intense atmospheric river. The winds in Magdalena Sound may have been further amplified, due to its west-east orientation and its location within a tall, narrow mountain canyon. This work also documents the first known appearance of toxic *P. calliantha* in Northern Patagonian. The potential impacts of the biotoxins of this species on higher trophic levels are discussed.

Keywords: *Pseudo-nitzschia calliantha*; lipophilic toxins; amnesic shellfish poisoning; domoic acid; hydro-climatic modulation; Patagonian fjord system

1. Introduction

Chilean Patagonia is a vast geographical zone that extends nearly 1,500 km (42ºS–55ºS), covers an area of 240,000 km^2 and has a coastline of almost 80,000 km (Försterra, 2009). It constitutes a complex system of fjords, channels, gulfs, estuaries and bays and is affected by physical regimes that strongly modulate biological productivity (Iriarte et al., 2014). Harmful algal blooms (HABs) of the toxic dinoflagellate *Alexandrium catenella*, associated with the production of paralytic shellfish toxins (PST), are a frequent occurrence in this region (Díaz et al., 2014; Díaz et al., 2022b; Guzmán et al., 2002; Molinet et al., 2003; Molinet et al., 2010), but HABs of *Dinophysis acuta* and *D. acuminata*, associated with the production of the lipophilic compounds okadaic acid (OA), dinophysistoxins (DTX), and pectenotoxins (PTX) (Baldrich et al., 2021; Contreras and García, 2019; Díaz et al., 2022a; Díaz et al., 2021; García et al., 2012), and of *Protoceratium reticulatum*, associated with the production of yessotoxins (Alves de Souza et al., 2014), have also been reported. Local detections of these marine biotoxins have led to bans on the harvest of natural populations of different bivalve and gastropod species, in addition to interrupting aquaculture efforts, mainly for the mussel *Mytilus chilensis*, in the Aysén and Magallanes regions (Díaz et al., 2019). Ecosystem impacts in Chilean Patagonian waters associated with PST include the mass stranding of marine invertebrates in Cucao Bay, Chiloe Island (42º 38’S–74º 07´W) (Álvarez et al., 2019) and the mass mortality of Sei whales in Penas Gulf (46–48ºS) (Häussermann et al., 2017).

Biological processes occur under a wide variety of spatial and temporal scales and should thus be studied at the same spatial and temporal scales as the physical-chemical processes with which they interact (GEOHAB, 2010). The formation, maintenance and dissipation of HABs are subject to processes operating at multiple (macro-, meso-, micro-) scales (sensu Haury et al., 1978), all of which are exacerbated in highly heterogeneous systems such as the Patagonian fjord system (PFS),

where multiple niches promote the development or aggregation of different HAB species (Baldich et al., 2023; Baldrich et al., 2021).

The variability of atmospheric processes is one of the main drivers of the hydrodynamics in coastal and shelf areas, particularly in fjord systems. In the PFS, the wind, together with the tide, is one of the main sources of forcing, but it varies considerably, including at different time scales, due to atmospheric oscillations (Valle-Levinson, 2010; Valle-Levinson and Blanco, 2004). Thus, during the period of highest biological production (spring–summer) in the PFS, changes in the position of high-low pressure systems result in short-term variations (time scales of a few days) in the wind regime that promote cycles of stratification-mixing of the water column (Iriarte et al., 2007; Montero et al., 2011; Pérez-Santos et al., 2021; Pérez-Santos et al., 2019) and thereby modulate the seasonal succession of phytoplanktonic species, including those that cause HABs (Montero et al., 2017; Pérez-Santos et al., 2021).

The phytoplankton community is generally dominated by diatoms (Iriarte et al., 2007; Pizarro et al., 2005). In the PFS, the increase in the input of nutrients and in light availability that mark the end of winter and the beginning of spring activates phytoplankton activity, evidenced by blooms dominated by diatoms of the genus *Skeletonema.* In summer, when nutrients are depleted from the surface waters, phytoplankton sink as they follow the decaying nutrients until light becomes a co-limiting factor (1% of surface photosynthetically active radiance). The resulting subsurface chlorophyll *a* maximum coincides with the nutricline and the dominance of the diatom genus *Pseudo-nitzschia*. During mid- to late summer and early autumn, characterized by a high thermohaline stratification driven by extreme climatic anomalies (high pressure/temperature and low wind/rainfall), the phytoplankton community becomes dominated by dinoflagellates (Díaz et al., 2021) or phytoflagellates (Díaz et al., 2023; León-Muñoz et al., 2018).

In this work, we provide the first description of a succession of toxic phytoplankton species in the PFS (Puyuhuapi Fjord, Aysén region) that was modulated by atmospheric-oceanographic forcing, with a community dominated by the dinoflagellate *D. acuta* replacing one dominated by the diatom *P. calliantha*. This change in community composition and the potential impact of the change in the associated toxins on higher trophic levels are discussed in detail.

2. Material and methods

2.1. Study area and climate background

Puyuhuapi Fjord, at 100 km one of the world's most extended fjord and channel systems (Fig. 1) (Pantoja et al., 2011), has an irregular bathymetry and a complex coastal morphology. The mean depth of its waters is 220 m, with a maximum of 350 m close to the mouth of Magdalena Sound (Fig. 1). The marked water column stratification is determined by freshwater input and by seasonal and latitudinal precipitation patterns, which include rainfall and ice melting (Pickard, 1971; Schneider et al., 2014). The primary freshwater input is from the Cisnes River (mean annual discharge ~218 $m^3 s^{-1}$, with peaks >500 $m^3 s^{-1}$), located in the middle of the fjord. The climate of northwestern Patagonia, including Puyuhuapi Fjord, is temperate and humid, as recurrent mid-latitude depressions embedded in the westerly wind belt (WWB) cause precipitation levels of 3500–4000 mm, occurring over 200 days per year (Sauter, 2020) along the coast but in some years reaching ~5000 mm inland due to the orographic uplift of the austral Andes (Viale and Garreaud, 2015). Many storms that reach northwestern Patagonia feature atmospheric rivers (ARs), defined as long filaments of high moisture transport (Viale et al., 2018) that connect the tropical oceans with mid-latitudes. Global and regional studies indicate that ARs frequently make landfall along the coast of southern Chile and their interaction with the Andes results in even more intense

precipitation events (>100 mm/day), abundant freshwater drainage (Valenzuela and Garreaud, 2019) and strong westerly winds.

2.2. Climate data

Rainfall, air temperature and river discharge data were obtained from Climate Explorer (http://explorador.cr2.cl/), which compiles quality-controlled records from the Chilean Weather Service (DMC) and Water Agency (DGA). Due to the low density of climate stations in Patagonia, additional data from the CR2Met dataset, comprising monthly gridded (0.05°× 0.05° lat-lon) precipitation and temperature fields over Chile from 1979 to date, were also used. CR2Met was built by the optimal interpolation of surface data using state-of-the-art European Centre reanalysis data (Álvarez-Garreton et al., 2018). The large-scale circulation during early 2018 was characterized using the ERA5 reanalysis of the European Centre for Medium-Range Weather Forecasts (Hersbach et al., 2020), with an hourly resolution on a 0.25° × 0.25° lat-lon grid and 137 vertical levels. The data were obtained through the Copernicus Climate Data Store archive (https://cds.climate.copernicus.eu/).

2.3. Field sampling

During summer and early autumn 2018, four one-day cruises of the *RV* Queen were carried out in the Puyuhuapi fjord area, Chilean Patagonia: on January 17, February 22, March 12 and April 24. On every cruise, samples were taken at a fixed sampling station located at the head of Magdalena Sound, to measure the physical-chemical properties of the water column and the fine-scale distribution of phytoplankton, with a focus on toxic species (Fig. 1).

Vertical profiles of temperature, salinity and *in vivo* chlorophyll *a* fluorescence were obtained using an AML Oceanographic CTD profiler (http://www.amloceanographic.com) model Metrec-XL equipped with a Turner Designs CYCLOPS-7 fluorometer (excitation 460 nm, emission, 620–715 nm). The absolute salinity (g kg^{-1}) and conservative temperature (°C) were calculated from the thermodynamic equation of seawater 2010 (TEOS-10) (IOC et al., 2010).

Water samples for quantitative analyses of microphytoplankton were collected in Niskin bottles deployed every 2 m, from the surface to 20 m depth. The samples were immediately fixed with acidic Lugol's solution (Lovegrove, 1960). During the February and March cruises, phytoplankton samples (0–20 m water depth) were obtained by vertical net hauls (20-µm mesh), which provided concentrated samples for marine toxins analyses. The samples were concentrated by filtration through 1.2-µm GF/F filters (13-mm diameter) and stored in aqueous methanol (50%) for the analysis of domoic acid (DA) and in 100% methanol for the analysis of lipophilic toxins.

2.4. Phytoplankton quantification

Quantitative phytoplankton analyses were conducted in 10-mL unconcentrated samples fixed with Lugol's acidic solution and left to sediment overnight. The phytoplankton were then analyzed under an inverted microscope (Olympus CKX41) according to the method described in Utermöhl (1958). The detection limit was 100 cells L^{-1} (i.e., one cell detected after examination of the entire surface of the sedimentation chamber base-plate). Microphytoplankton were identified to the species level when possible.

Pseudo-nitzschia species were separated into *P. delicatissima* and *P. seriata* complexes, based on their length and valve width (Hasle, 1965). The samples were identified using an inverted microscope (Zeiss, model Axio Vert. A1.).

2.5. Electron microscopy of Pseudo-nitzschia

Pseudo-nitzschia cells in Lugol were treated to remove the organic matter from their frustules, following the method described by Hasle (1978). Four mL of the cleaned sample was then filtered through a gravity system using polycarbonate track-etched membranes filters Nucleopore (13-mm diameter) (Whatman, UK). The filters were attached to stubs, sputter-coated with platinum/palladium, and the cells trapped on the filters were observed using a Zeiss Auriga 60 microscope operated at 3 kV.

2.6. Analysis of marine toxins

2.6.1. Toxin extraction

Phytoplankton net samples were sonicated in an ultrasonic cell disruptor (Branson Sonic Power 450; Danbury, CT, USA). The sonicate was then clarified by centrifugation (20,000 g; 15 min) and filtered through 0.20-µm Clarinert nylon syringe filters (13 mm diameter) (Bonna-Agela Technologies, Torrance, CA, USA). The filtrate was placed in amber vials and stored at −20°C for subsequent use in analyses of domoic acid (DA) and of free okadaic acid (OA) and other lipophilic toxins as described below. Samples for the analysis of esterified OA-group toxins were alkaline-hydrolyzed following the standard procedure of the EU Reference Laboratory for Marine Biotoxins (EURLMB, 2015), placed in amber vials and stored at −20°C.

2.6.2. Detection and quantification of toxins

Domoic acid was determined as previously described in Krock et al. (2008). In brief, the sample was first analyzed in a hybrid triple quadrupole-linear ion trap mass spectrometer (API 4000 QTrap; Sciex, Darmstadt, Germany) coupled to a liquid chromatograph (LC1100, Agilent; Waldbronn, Germany), after which the compounds of interest were separated by reversed-phase chromatography on a C8 phase column (Hypersil BDS), 3 µm, 120 Å (50×2 mm), (Phenomenex, Aschaffenburg, Germany) maintained at 20°C. The sample injection volume was 5 µL and the flow rate 0.2 mL min^{-1}. Gradient elution was performed using aqueous eluent A and organic eluent B (acetonitrile-water (95:5 v/v)), both containing 2.0 mM ammonium formate and 50 mM formic acid. DA was detected in positive selected reaction monitoring mode using the mass transitions *m/z* 312/266 and 312/161. For the detection of OA, mass transitions of *m/z* 822/223, for DTX-1 *m/z* 836/237, for PTX-2 *m/z* 876/213, and for PTX-2sa *m/z* 894/213 were used. All toxins were quantified by external calibration against standard solutions purchased from the certified reference material (CRM) program of the ICB-NRC, Halifax, NS, Canada.

3. Results

3.1. Regional climate conditions

The regional-scale climate conditions over southern Chile during summer–fall 2018 are summarized in the maps of bi-monthly rainfall anomalies shown in Figure 2. January and February, at the height of summer, were markedly dry in the Los Lagos and Aysén regions (40–47°S), with a rainfall deficit up to 70% (Fig. 2A). At Cisne Medio, the closest climatological station to Magdalena Island (about 40 km inland), the accumulation during those 2 months was 151 mm, well below the mean accumulation of 281 mm, with February 2018 as the driest month (50%

deficit). The low precipitation was accompanied by above average solar radiation reaching the surface during most of the summer (not shown). During early fall, precipitation across much of southern and austral Chile (38–55°S) was well above average, with rainfall anomalies increasing precipitation above the mean by 60–80% over central Patagonia (Fig. 1B). At Cisne Medio, nearly 500 mm of rainfall accumulated during March–April 2018, higher than the mean of 360 mm.

The marked contrast between the dry summer and wet fall was consistent with the disparate number of synoptic-scale disturbances that reached southern South America during that period and well captured by the meteorological time series over Magdalena Island from ERA5. The precipitation series (Fig. 3A) revealed few and minor rainfall events during January–February, with the dry-wet transition occurring very rapidly, around the first week of March, and frequent (1–2 per week) and major events during March–April (Fig. 3A). Most of these rainfall events during March–April 2018 were associated with ARs, in sharp contrast to the lack of ARs in the preceding two months.

During the study period, the first AR reached the region on 7–8 March 2018 (Figure 3D). The moist air filament, largely concentrated in the first 2–3 km above sea level (asl), was driven by intense WNW winds that also signaled a low-level dome of cold air progressing from south to north. These two features and the resulting copious precipitation are commonly associated with landfalling ARs and were also evident in the time series of the zonal wind and air temperature at 850 hPa (~1500 m asl) over Magdalena Island (Fig. 3B-C). Early March thus marked a shift in the regional meteorological conditions, from an exceptionally dry, sunny, warm and calm (weak westerlies) summer to an anomalous wet, cloudy, cold and windy (strong westerlies) fall. The mechanism and impact of these dramatic changes are discussed in the next sections.

3.2. Hydrographic conditions

The temporal evolution of the meteorological conditions that occurred in summer and early autumn 2018 were also apparent in the water column structure (Fig. 4). Early summer (January–February) was characterized by strongly thermo-haline-stratified conditions with a typical two-layered structure (Fig. 4A, B). A maximum temperature gradient of 5.3ºC 10 m^{-1} and a salinity gradient of 17.2 10 m^{-1} were detected in January. Towards the end of summer–beginning of autumn, the water column became more homogeneous, with temperature and salinity gradients of 0.4ºC 10 m^{-1} and 7.0 10 m^{-1} in March. In general, surface (0–2 m) temperatures and salinities ranged from 18.2 to 10.6ºC and from 11.9 to 22.9 g kg^{-1}, respectively. The minimum temperature, reached in April, was probably associated with the start of the thermal inversion process (Fig. 4A). Strong stratification in the upper 10 m was accompanied by a Brunt-Väisälä frequency that in January reached a maximum (60–100 cycles h^{-1}) in the inter-phase between estuarine fresh water (salinities 11–21 g kg^{-1}) and estuarine salty water (21–31 g kg^{-1}) (Fig. 4C). High levels of dissolved oxygen (>6 ml L^{-1}; >90% saturation) and a high pH (>8) were likewise detected in this upper layer (Fig. 4D, E). Chlorophyll *a* (fluorescence) levels were highest in a layer extending from the surface to 10 m depth, with a maximum of 15.2 µg L^{-1} recorded on April 23, 2018 at 4 m depth and forming an intense thin layer between 2 and 6 m depth (Fig. 4F).

3.3. Phytoplankton community succession and marine biotoxins

The phytoplankton analysis revealed a phytoplankton community dominated by diatoms (>96%) at the beginning of the summer (Fig. 5). On January 17, 2018, the phytoplankton community reached a maximum cell density of 450 × 10^3 cells L^{-1} and was dominated by the diatom *Pseudo-nitzschia delicatissima* complex (96.9%), associated with a strong thermo-haline stratification (Figs. 5A–C). One month later, on February 16, 2018, the structure of phytoplankton community

underwent a significant change, with a maximum cell density of 109 × 10^3 cells L^{-1} and a clear dominance of the dinoflagellate *D. acuta* (96.3%). Fine-scale vertical resolution sampling showed an intense thin layer made up of cells of this species, with a maximum density of 105.4 × 10^3 cells L^{-1}, located at 6 m depth. The detection coincided with maximum temperature and salinity gradients (0.49ºC m^{-1} and 1.49 g kg^{-1} m^{-1}) (Fig. 5D–F). The analysis of net tow extracts by liquid chromatography coupled to mass spectrometry (LC-MS) revealed the presence of diarrhetic shellfish toxins (OA and DTX-1) and pectenotoxins (PTX-2, PTX-2sa, and PTX-2sa isomer) in different proportions (Fig. 6). The highest concentrations were those of DTX-1 and OA (231 and 72 ng NT^{-1}, respectively) followed by PTX-2 (20 ng NT^{-1}). On March 12, the phytoplankton composition changed again, with the resumption of a clear dominance of diatoms (>99%) that coincided with the arrival of an AR (Fig. 3). Thus, the intense February bloom of *D. acuta* was significantly reduced (max. 2.5 × 10^3 cells L^{-1}), as it gave way to an intense bloom of *P. delicatissima* complex, with a maximum cell density of 1.560 × 10^3 cells L^{-1} at 2 m. A second maximum of 1.300 × 10^3 cells L^{-1} was detected at 16 m depth (Fig. 5G–I). LC-MS/MS analysis of net tow extracts prepared from the *Pseudo-nitzschia* bloom showed the presence of DA, at a concentration of 45 ng NT^{-1}, as well as DTX-1, OA, and PTX-2, albeit at lower concentrations of 28 and 10 ng NT^{-1}, respectively (Fig. 6). On April 23, the density of the *P. delicatissima* complex bloom increased. The cells formed a well-defined thin layer between 2 and 6 m (Fig. 5J–L), with a maximum of 3,382 × 10^3 cells L^{-1} at 4 m, that followed the vertical structure of the chlorophyll *a* concentration, which reached a maximum of 15.4 µg L^{-1} at the same depth (Fig. 5J, K). In March, the phytoplankton community was still dominated by *P. delicatissima* complex (>99% at the thin layer depths; Fig. 5L).

3.4. Taxonomic identification of Pseudo-nitzschia

Light microscopy (LM) of the samples confirmed the dominance of cells from *P. delicatissima* complex. The length and width of the cells ranged from 66 to 86 μm (77.7±3.9 μm) and from 1.1 to 2 μm (1.5±0.3 μm), respectively. Scanning electron microscopy analysis revealed the presence of *P. calliantha* (Fig. 7). The cells were linear in valve view (Fig. 7A), with an apical axis of 66–80 μm, a transapical axis of 1.3–1.8 mm (Fig. 7B, C) and a large central nodule (Fig. 7D, E). The fibulae and striae (19–22 and 37–39 per 10 μm, respectively) were regularly spaced. The striae were formed by one row of round poroids with a density 4–6 per 1 μm. The hymen of the poroids was perforated to form 6–12 sectors (Table 1).

4. Discussion

The spatial and temporal distributions of phytoplankton assemblages in estuarine and shallow coastal water are closely linked to atmospheric and hydrographic conditions. In the latter, nutrient availability and turbulence play fundamental roles in phytoplanktonic community structure (Margalef, 1978; Margalef et al., 1979; Smayda and Reynolds, 2001). Although some aspects of phytoplankton succession in the study area are relatively well known, mainly those related to turbulence-induced transitions between toxic dinoflagellates and diatoms, our results substantially expand current knowledge about the relationships between toxic phytoplankton successions and atmospheric-hydrographic parameters, as they showed that mid-latitude storms and ARs are important factors triggering population shifts in phytoplankton. We also present the first report of a toxic species of *Pseudo-nitzschia* in NW Patagonia. The information provided by our study will

help in evaluating the threats to shellfish farming posed by HABs and will improve predictions of toxic events, by incorporating environmental parameters.

4.1. Broad-scale climate forcing

The warmest and driest conditions in northern Patagonia occur at the height of the austral summer, and the coldest wettest conditions in winter. This climate transition was particularly marked in 2018 because of an anomalous dry and warm summer that was followed by an equally anomalous wet and cold fall. The shift in the regional climate took place in early March, fostered by the arrival of several ARs, each accompanied by large amounts of precipitation, cold air and strong westerly winds (Fig. 3A–C).

The contrasting conditions between summer and fall are regional manifestations of large-scale circulation changes, illustrated by the sea-level pressure anomalies in the inset maps of Figure 2A, B. During January–February, a persistent band of high pressure extended from the Pacific to the Atlantic at about 45°S, hindering the passage of mid-latitude weather systems and weakening the westerlies against the southern Andes, resulting in a rainfall deficit. On a hemispheric scale, negative anomalies prevailed at high latitudes and positive anomalies at mid-latitudes, i.e., the fingerprint of the positive phase of the Southern Annular Mode (SAM, Thompson and Wallace, 2000), whose index (Marshall, 2003) reached +1.1 hPa in the summer of 2018 (Fig. 2C). This highly positive value was in part due to the tendency of the SAM to maintain the positive polarity that has prevailed during the last three to four decades (Fogt and Marshall, 2020; Thompson and Wallace, 2000). The positive SAM trend in summer has been linked to the anthropogenically forced increase in greenhouse gas concentrations in the troposphere and to the decrease in the ozone layer in the polar stratosphere (Arblaster and Meehl, 2006; Gillett et al., 2013); it also explains much of

the contemporaneous drying in northern Patagonia during summer (Garreaud et al., 2013; Gillett et al., 2006). In early fall, the large-scale pattern reversed, with positive pressure anomalies prevailing over Antarctica and negative pressure anomalies across the mid-latitudes of the Southern Hemisphere, causing a steep drop of the SAM index to –0.6 hPa (Fig. 2C). A change in SAM polarity between January–February and March–April is not uncommon, given the high variability and low autocorrelation of the SAM at monthly time scales as well as the weaker trend in the SAM in fall (Fogt and Marshall, 2020). Within the negative SAM pattern during March–April 2018, a cyclonic anomaly developed near the southern tip of the continent (Fig. 2B), increasing the strength of the westerlies at mid-latitudes that drive ARs toward southern South America, resulting in excess precipitation across Patagonia.

4.2. Toxic phytoplankton succession and hydroclimatic modulation

According to Margalef's mandala model (Margalef, 1978), turbulent environments are associated with diatoms and stable conditions with dinoflagellates. Diatoms tend to dominate during periods of mixing and high nutrient concentrations, because of the numerous advantages conferred by their higher growth rates, higher photosynthetic rates and improved ability to compete for nitrogen at low cell densities (Estrada et al., 1987; Estrada and Berdalet, 1997). Conversely, dinoflagellates prevail in more stable, oligotrophic systems, because turbulence has harmful effects on their morphology (Berdalet and Estrada, 1995), cleavage rate (Thomas and Gibson, 1990a, b; Thomas et al., 1995) and growth (Gibson and Thomas, 1995). Thermo-haline stratification of the water column facilitates the accumulation of dinoflagellates at more stable depths, such as the pycnocline (Díaz et al., 2021).

Based on the models proposed by Margalef (1978) and Reynolds (2002), Alves de Souza *et al.* (2008) suggested that the distribution and abundance of phytoplankton assemblages in southern Chile are mostly determined by the stability of the water column and the nutrient concentration. The present work documented the succession of toxic phytoplankton communities in Puyuhuapi Fjord during early 2018. *D. acuta* (producer of lipophilic toxins) was dominant in summer (January–February), as it thrives in a highly stratified water column, while *Pseudo-nitzschia calliantha* (producer of hydrophilic DA) bloomed and was dominant in March–April, when the water column became mixed. However, our study also identified forces acting outside of Margalef´s mandala, as the change in water column stability could be directly linked to regional meteorological conditions as the driver. Indeed, the prominent stratification of the water column in summer 2018 was consistent with the extremely dry, sunny, warm, calm (weak winds) conditions that prevailed during those months, which in turn were caused by anticyclonic anomalies over the southern tip of South America and the positive phase of SAM. Likewise, the mixed water column during March–April was coincident with the rainy, cold, windy conditions of early fall, in connection with a reversed large-scale atmospheric pattern (negative phase of SAM). The hydrographic cross-sections (Fig. 4) showed a smooth transition, although the monthly-scale sampling schedule should be noted. By contrast, the sub-daily meteorological data revealed a sharper transition between summer and fall conditions during the first half of March (Fig. 3A–C). Of particular relevance was the arrival of several ARs in northern Patagonia, with the first strong AR reaching the study area during 6–8 March 2020 (Fig. 3D). In addition to bringing copious rainfall and cold air, the ARs were accompanied by strong winds blowing from the northwest. As Magdalena Sound is oriented in the west-east direction and located within a tall, narrow mountain canyon (Fig. 1C), the large-scale northwest winds during the ARs were likely amplified over the

sound. The resulting strong surface wind stress may have been the main driver of the water column mixing that ultimately caused the toxic phytoplankton succession reported herein. However, high-resolution coupled ocean-atmosphere simulations need to be carried out to confirm the role of this abiotic component in the succession.

4.3. Pseudo-nitzschia calliantha and the potential impact of DA

The few taxonomic identifications of *Pseudo-nitzschia* species in the study area include those in comprehensive studies by Hasle (1972) and Rivera (1985). However, *Pseudo-nitzschia* includes cryptic species that cannot be readily identified by light microscopy, such that in recent oceanographic studies the specimens were categorized within two main complexes: *Pseudo-nitzschia seriata* and *Pseudo-nitzschia delicatissima* (Alves de Souza et al., 2008; Cassis et al., 2002; Montero et al., 2017; Pizarro et al., 2018). Among the toxins produced by *Pseudo-nitzschia* are DA, with its first detection in southern Chile occurring in clams from Chiloé Island during the austral summer of 1997, in association with the presence of *P. australis* (Suárez-Isla et al., 2002). In 2000, a major outbreak of amnesic shellfish poisoning, which is caused by DA, in the same area resulted in the closure of a local natural mussel bank for more than a month, because the mussel DA content exceeded the regulatory limit (20 mg kg^{-1}) (Suárez-Isla et al., 2002). Again, the responsible species was likely *P. australis*, although a morphologically similar cell belonging to the *Pseudo-nitzschia delicatissima* complex was also present in some areas (Díaz et al., 2019).

Given that *Pseudo-nitzschia* is responsible for nearly all toxic algal bloom events in upwelling regions around the world and the serious threat it poses to mariculture and wild fauna, the need for knowledge of the conditions that promote these events is clear (Torres-Palenzuela et al., 2019). However, as noted above, a precise classification of the species comprising *Pseudo-nitzschia* by

light microscopy is difficult, such that most reports on *Pseudo-nitzschia* blooms do not include a precise identification. Specifically, *P. calliantha* is found worldwide but was previously described as toxic and non-toxic (see Bates et al., 2018 and references therein; Lelong et al., 2012; Trainer et al., 2012), although the presence of this species in Chilean coastal waters and its production of DA were previously described by (Álvarez et al., 2009). In addition to being the first report of toxic *P. calliantha* in NW Patagonia, our study establishes a relationship between the presence of this species and the occurrence of ARs. Thus, in addition to important knowledge on the composition of the toxic phytoplankton community in the PFS, it provides insights into the general factors governing toxic phytoplankton successions and potential sources of DA in upwelling systems around the world.

4.4. Lipophilic toxins, biotransformation and its potential negative impacts on the early stages of shellfish development

In Chilean Patagonia, *D. acuta* is the most harmful of the lipophilic toxin producers and its blooms are always associated with the occurrence of OA, DTX1 and PTXs, detected in shellfish and plankton samples (reviewed by Díaz et al., 2022a). Profiles of plankton samples from Puyuhuapi Fjord are similar to those determined in previous studies of the fjord (Baldich et al., 2023; Baldrich et al., 2021; Díaz et al., 2021). However, as PTX2 is the only pectenotoxin found in *D. acuta*, the detection of PTX-2sa, and PTX-2sa isomers suggests that they are produced via the biotransformation of PTX2 by species of higher trophic levels, such as zooplankton (Blanco et al., 2007).

While the conditions leading to toxic bloom events by *Dinophysis* and *Pseudo-nitzschia* in the PFS are not uncommon, the consequences for fisheries, aquaculture and human health have been

scarcely evaluated. The main reason is that most of the toxic outbreaks thus far have been caused by *Alexandrium catenella*, responsible for the accumulation of paralytic shellfish toxins (PST) in natural banks of shellfish (Díaz et al., 2022b; Molinet et al., 2010) and the considerable socio-economic impacts of these toxic events. In Chile, the potential negative impacts of *Dinophysis* species and/or their associated toxins on marine invertebrates, including shellfish species, have received little attention. However, the exposure of mussels to OA reduces protein phosphatase activity and larval viability in *Mytilus edulis* (De Rijcke et al., 2015) whereas neither the activity nor the viability of the eastern oyster *Crassostrea virginica* is affected (Pease et al., 2022). In Pacific oysters (*Magallana gigas*) exposed to PTX2, fertilization success is reduced and larval mortality rates are increased (Gaillard et al., 2020). Similarly, in *C. virginica* larvae, PTX2 exposure induces immobilization and rapid mortality (nearly 50%) (Pease et al., 2022).

Finally, further research into the interplay of atmospheric and hydrographic conditions in the PFS is needed in order to understand their combined impact on plankton communities in this semi-enclosed environment. A better understanding of plankton dynamics and the negative effects of lipophilic toxins on bivalve species, especially juvenile stages, will lay the basis for more accurate assessments of HAB-related risks and the more effective management of seafood aquaculture in this region.

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Table 1. Morphological characteristics of *Pseudo-nitzschia calliantha* found in Chile and Denmark.

Author	Year	Locality	Fibula/10 µm	Inter-striae/10 µm	Central interspace	Rows of poroids (n)	Poroids/ 1 µm	Apical axis (µm)	Transapical axis (µm)	Evaluated cells (n)
Lundholm et al.	2003	Denmark	15–21	34–39	+	1	4 -6	41–98	1.3–1.8	
Álvarez et al.	2009	Northern Chile	15–22	34–39	+	1	4–8	41–98	1.3–1.8	
Pinto-Torres et al.	2023	Southern Chile	18–22	36–39	+	1	4–6	65–80	1.9–2.3	6
This study	2023	Southern Chile	18–22	36–39	+	1	4–6	65–80	1.3–1.8	
			(20.4 ± 1.8)	(38 ± 1.2)			(4.9 ± 0.9)	(74.8 ± 5.9)	(1.5 ± 0.2)	10

Figure legends

Figure 1. Map showing: A) Chile (the box delimits Northwest Patagonia); B) Northwest Patagonia (the box delimits Puyuhuapi Fjord); C) Puyuhuapi Fjord and Magdalena Sound. The red circle indicates the sampling station.

Figure 2. Regional and hemispheric conditions in early 2018. Maps of precipitation anomalies over southern Chile for (A) January–February and (B) March–April 2018. The anomalies are the bi-monthly average minus the long-term mean, divided by the long-term mean, expressed as a % (data source: CR2Met). The globe inset shows the surface pressure anomalies (departure from the long-term mean, in hPa) for the corresponding months. (C) Average Southern Annular Mode (SAM) index (hPa) for January–February and March–April. The values for 2018 are highlighted by the red line.

Figure 3. Left panels: Time series (every 6 hr) from ERA5 data interpolated for Magdalena Island (45°S, 73°W) from January 1 to April 30, 2018. From top to bottom: (A) Precipitation rate (mm/6 hr), (B) 850 hPa (~1500 m ASL) zonal wind (west to east, m/s) and (C) 850 hPa air temperature (°C). In (b) and (c), the horizontal line is the seasonal average. Periods of stronger westerlies/colder temperatures are shown in blue, and periods of weaker westerlies/warmer temperatures in orange. Right panel (D): Image (MODIS Terra True Color) obtained around midday of March 8, 2018, showing an atmospheric river (AR) making landfall in northern Patagonia. The semi-transparent shading is the 24-hr accumulated precipitation determined by the IMERG satellite product (light rain in green, heavy rain in yellow). The blue arrow indicates the predominant WSW flow in

the lower and middle troposphere, signaling the marked contrast between cold air to the south and warm air to the north of the AR.

Figure 4. Vertical distribution of A) conservative temperature (ºC); B) absolute salinity (g kg^{-1}); Brunt-Väisälä frequency (cycles h^{-1}); D) dissolved oxygen (mL L^{-1} and % saturation) and dissolved oxygen (mL L^{-1}); E) pH and F) fluorescence (Volts, from 0 to 50) at a fixed sampling station at the head of Magdalena Sound from January to April 2018.

Figure 5. Vertical distribution (0–20 m) of: **upper panel**: temperature (blue line), salinity (red line) and fluorescence (green line); **middle panel**: cell density (cells L^{-1}) of *Dinophysis acuta* (orange circles) and *Pseudo-nitzschia calliantha* (green circles); **lower panel**: Percentage of the total phytoplankton contribution (%) at a fixed sampling station on A) January 17, B) February 16, C) March 12 and April 23, 2018.

Figure 6. Toxin contents determined in vertical net hauls from February 16 and March 12, 2018. The green bar indicates domoic acid and blue shades indicate lipophilic toxins. ND = not detected.

Figure 7. Scanning electron microscopy image of *Pseudo-nitzschia calliantha* isolated from Magdalena Sound during a bloom in February 2018.

Figure S1. Selected reaction monitoring chromatograms shown the transitions *m/z* 312/266 and 312/161 of: A) a standard solution of domoic acid (DA) and B) a phytoplankton net sample from Magdalena Sound on March 12, 2018.

Figure_1

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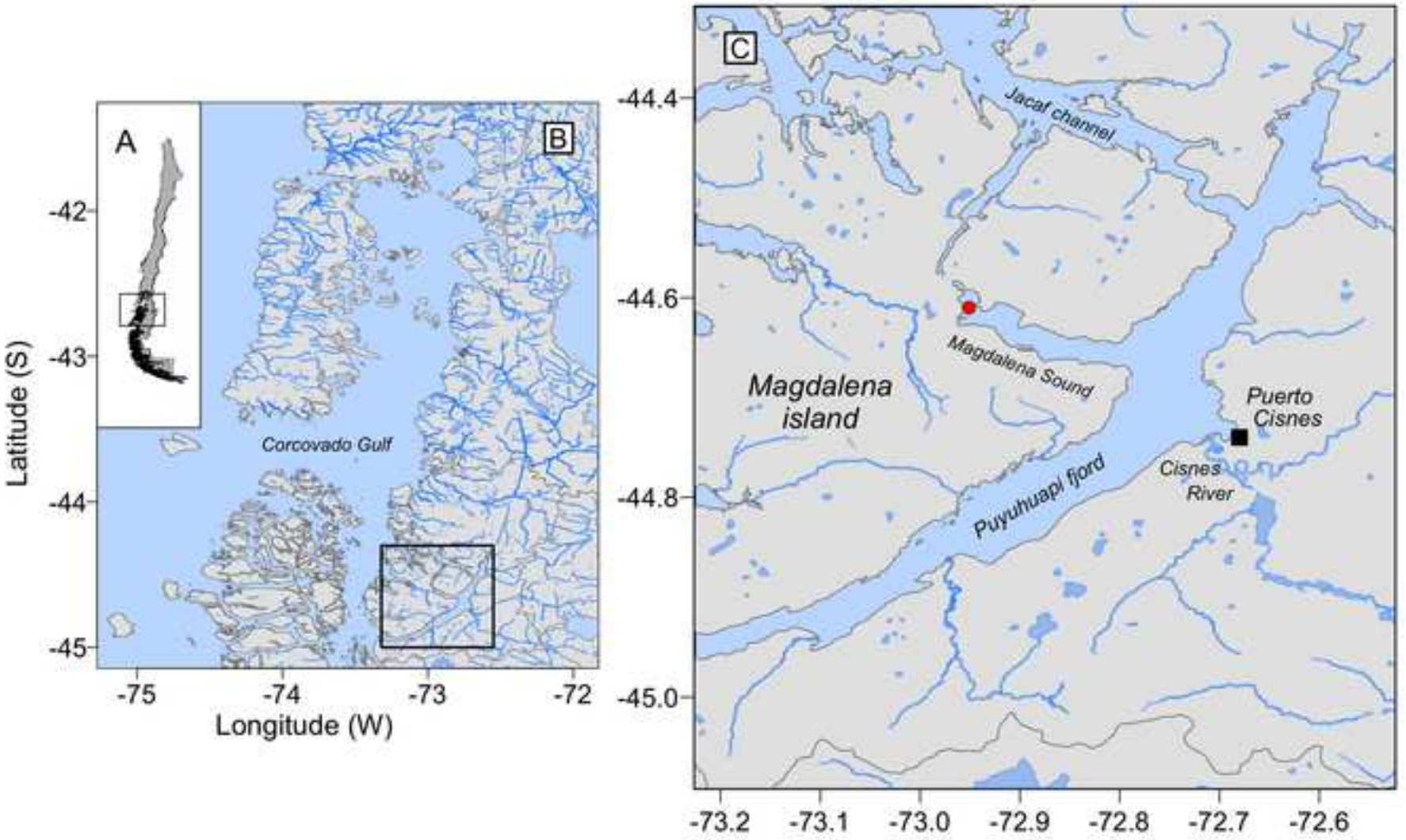

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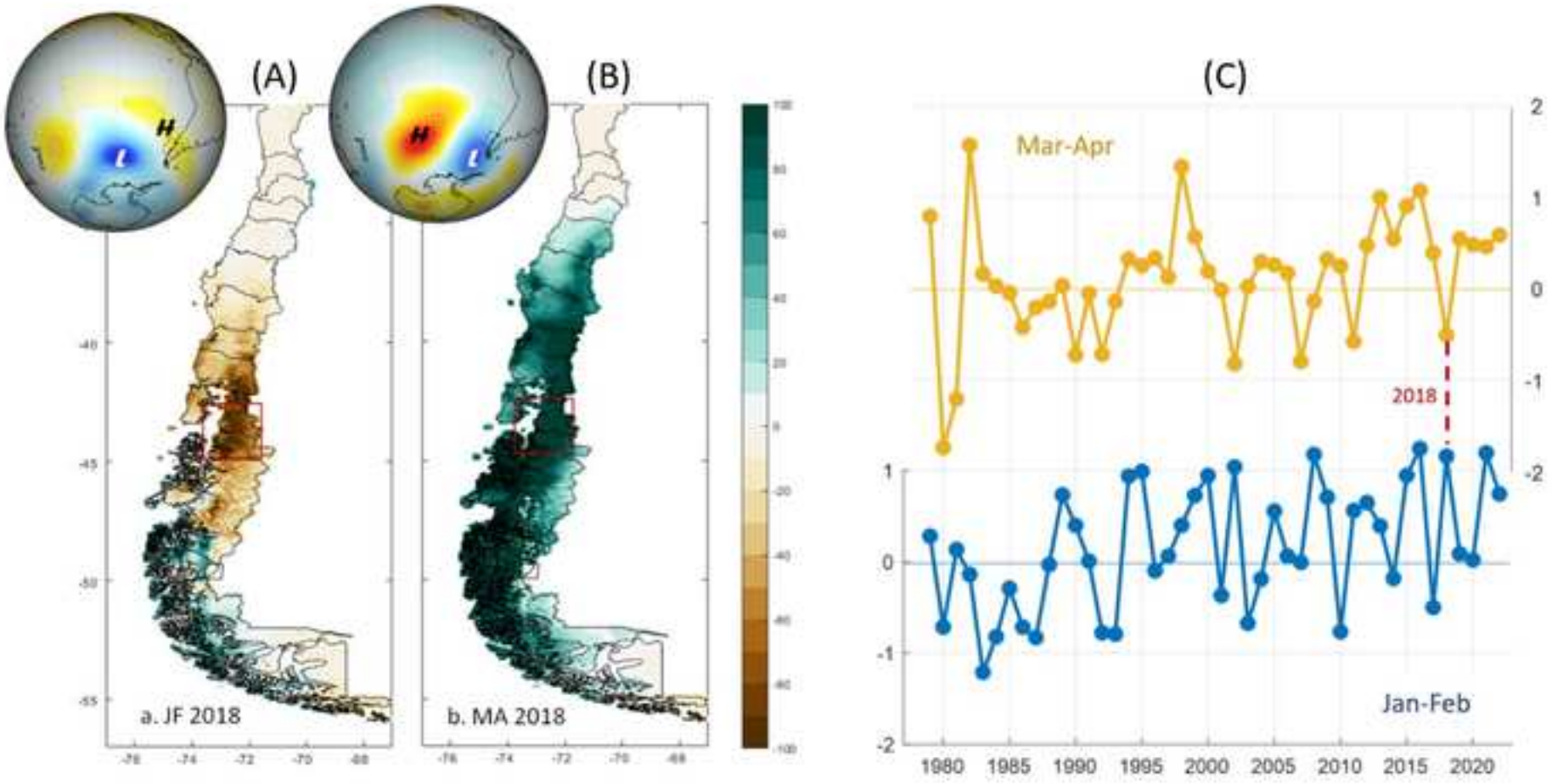

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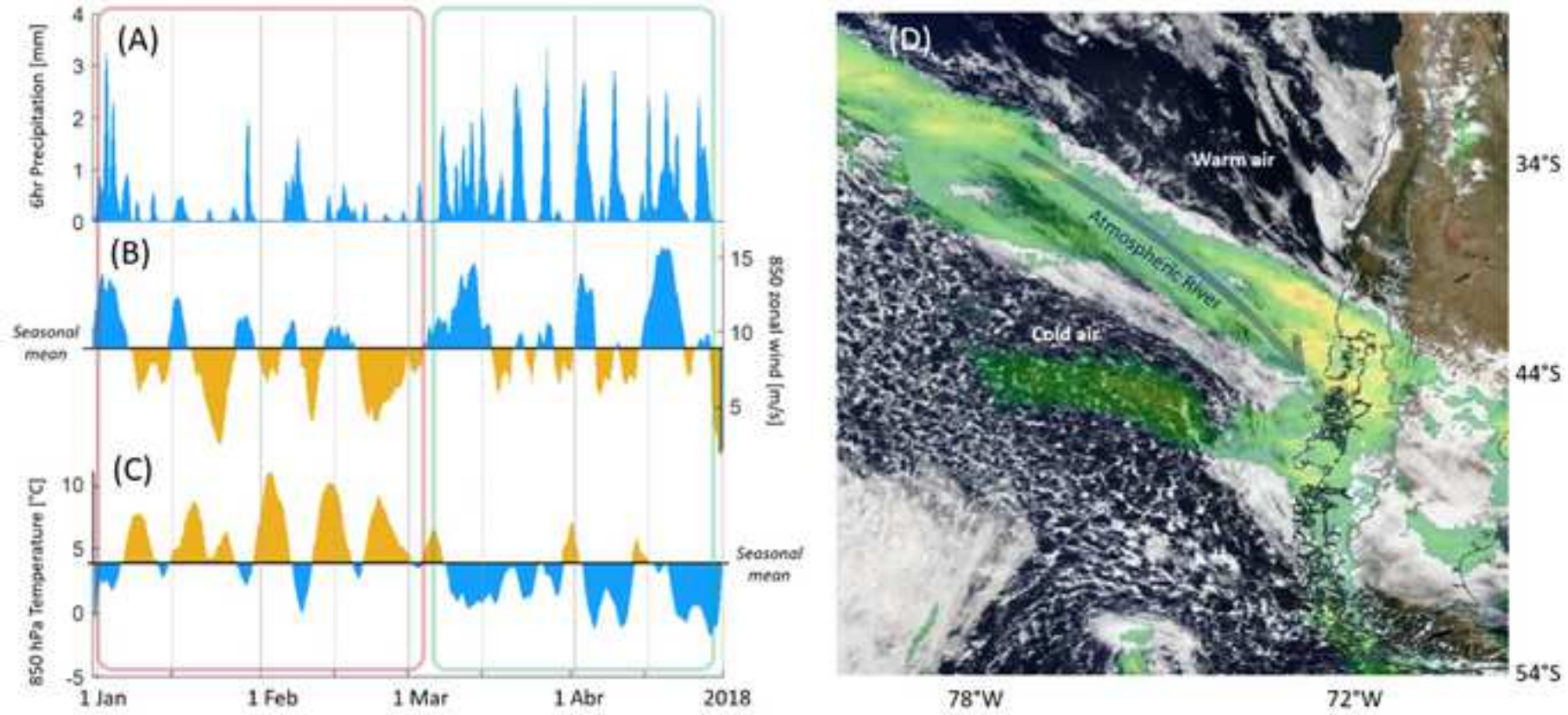

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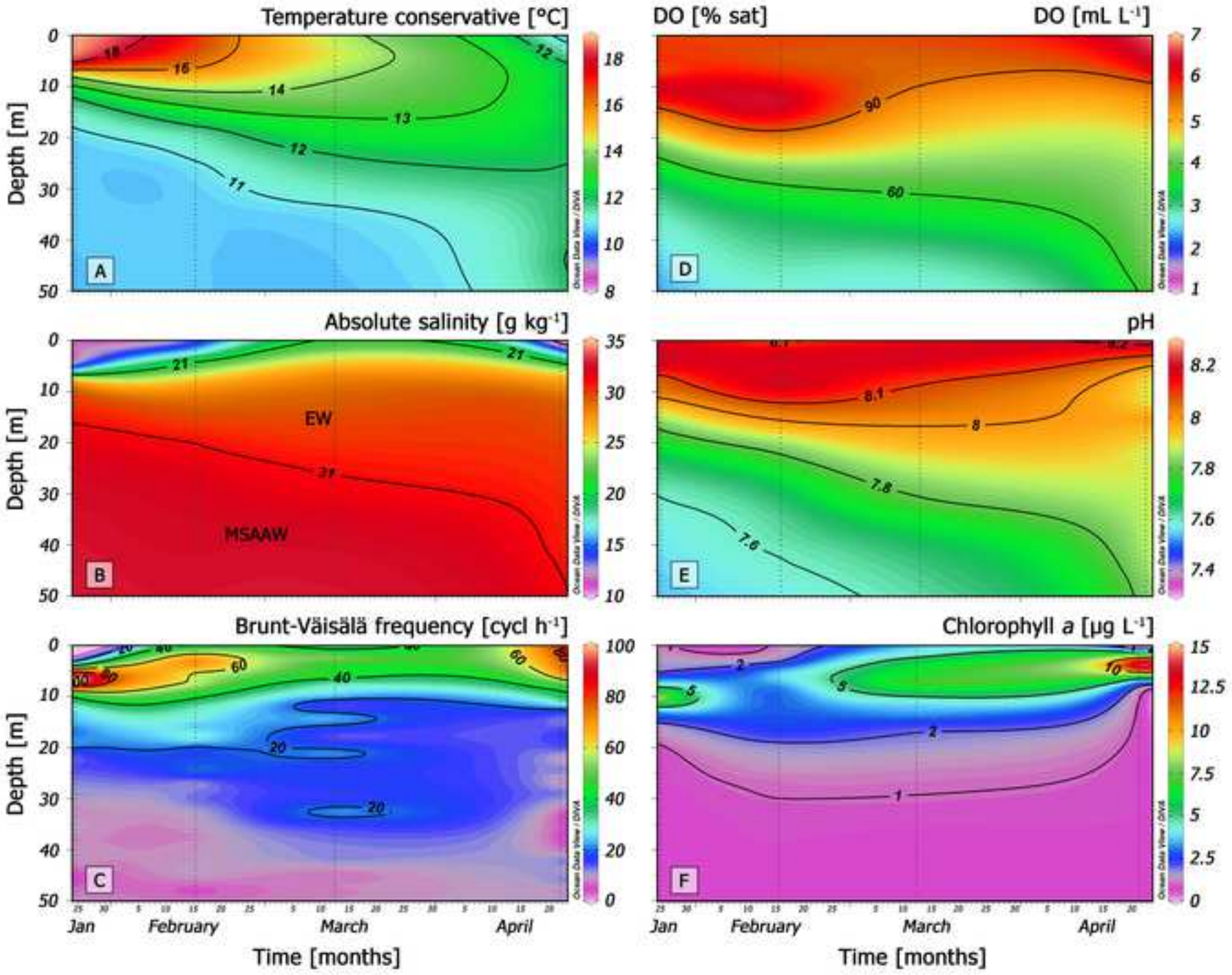

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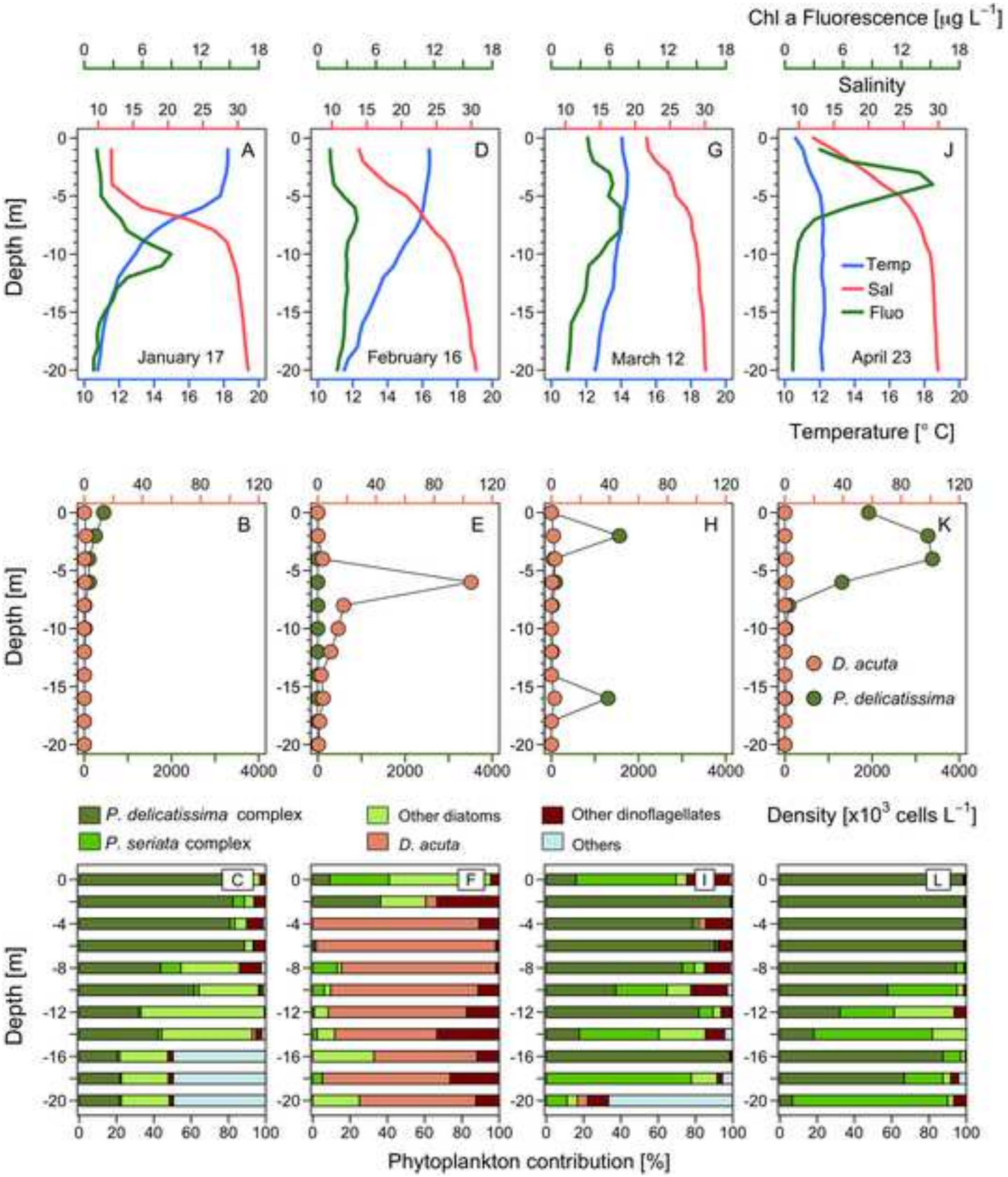

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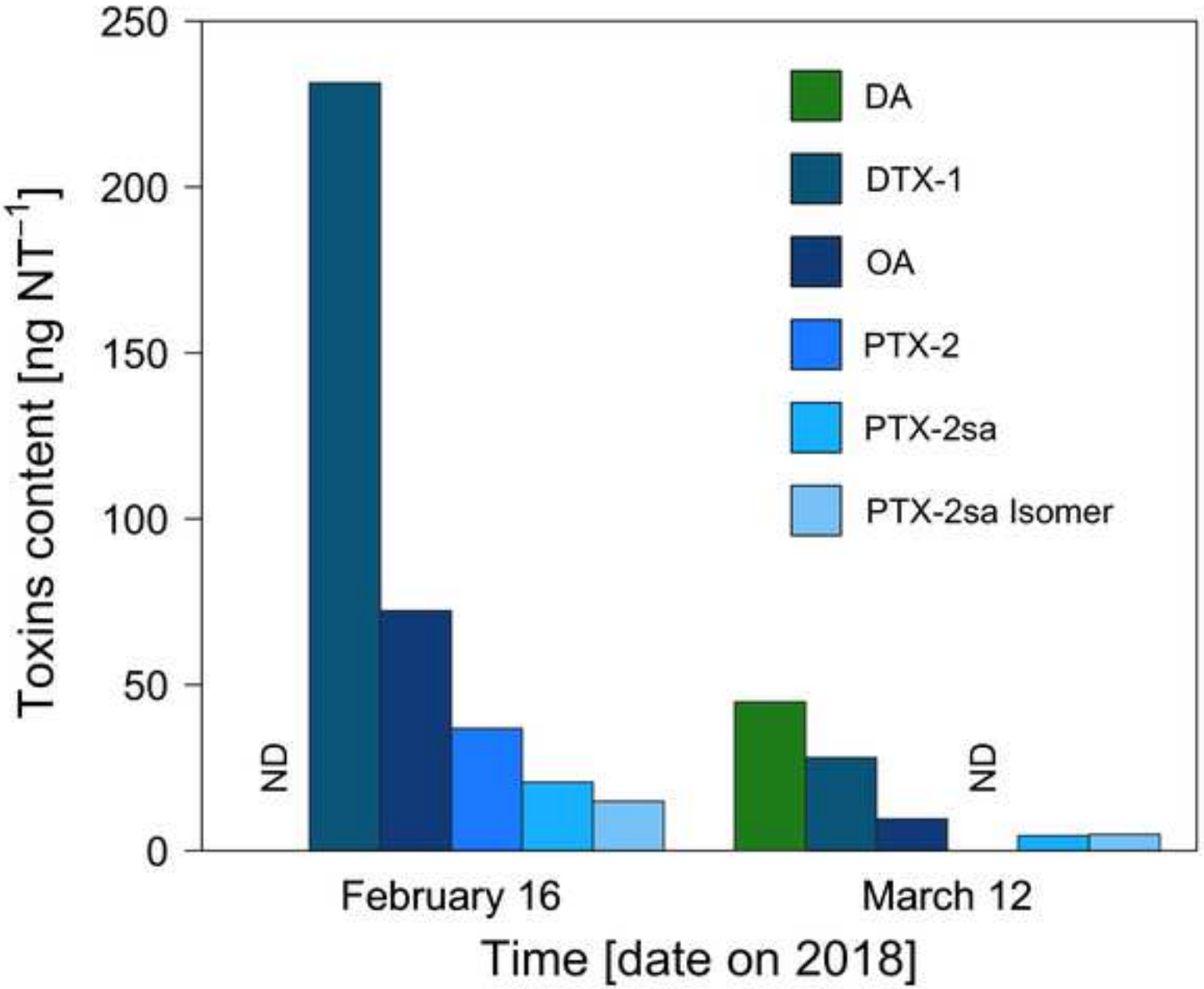

Figure_7

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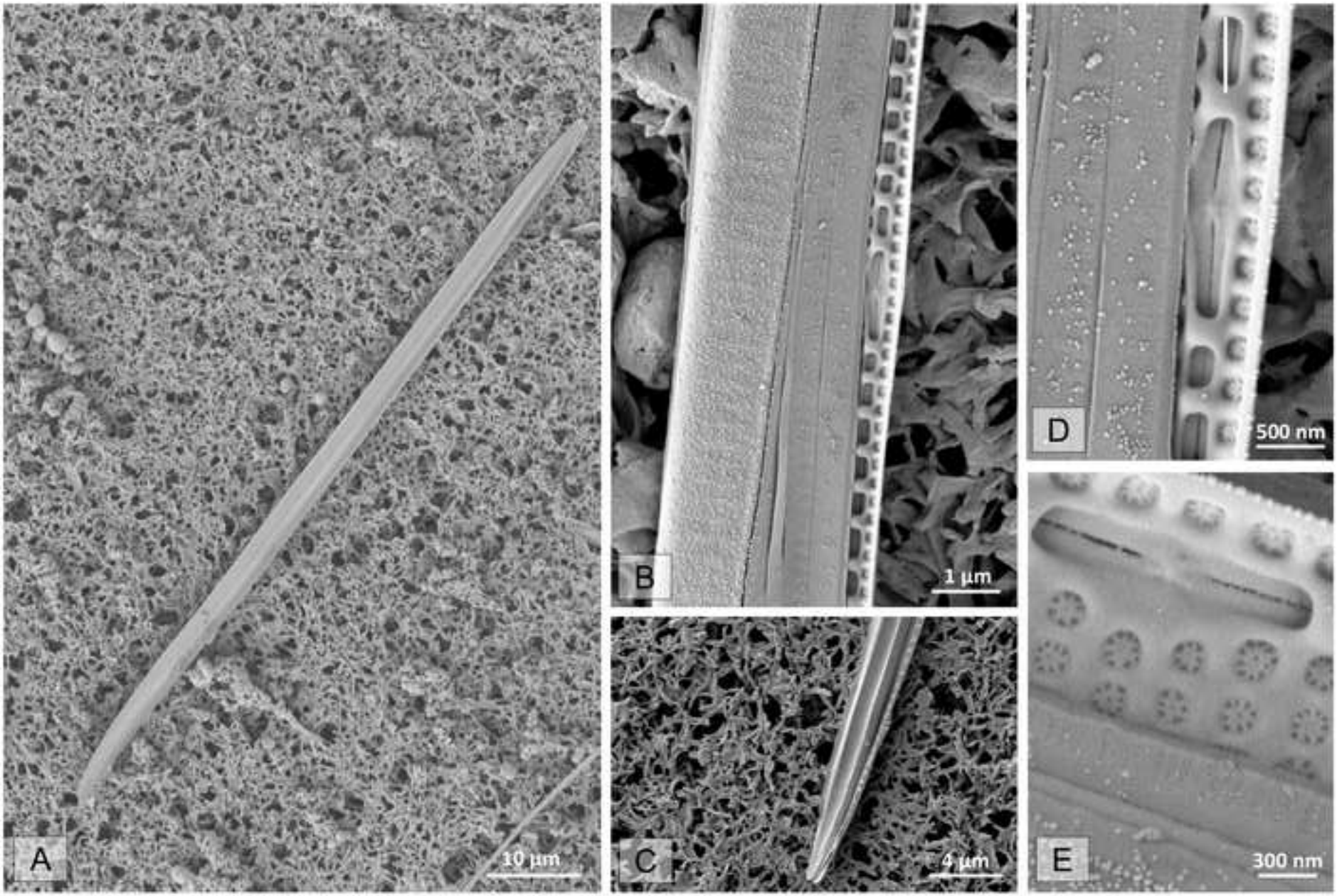
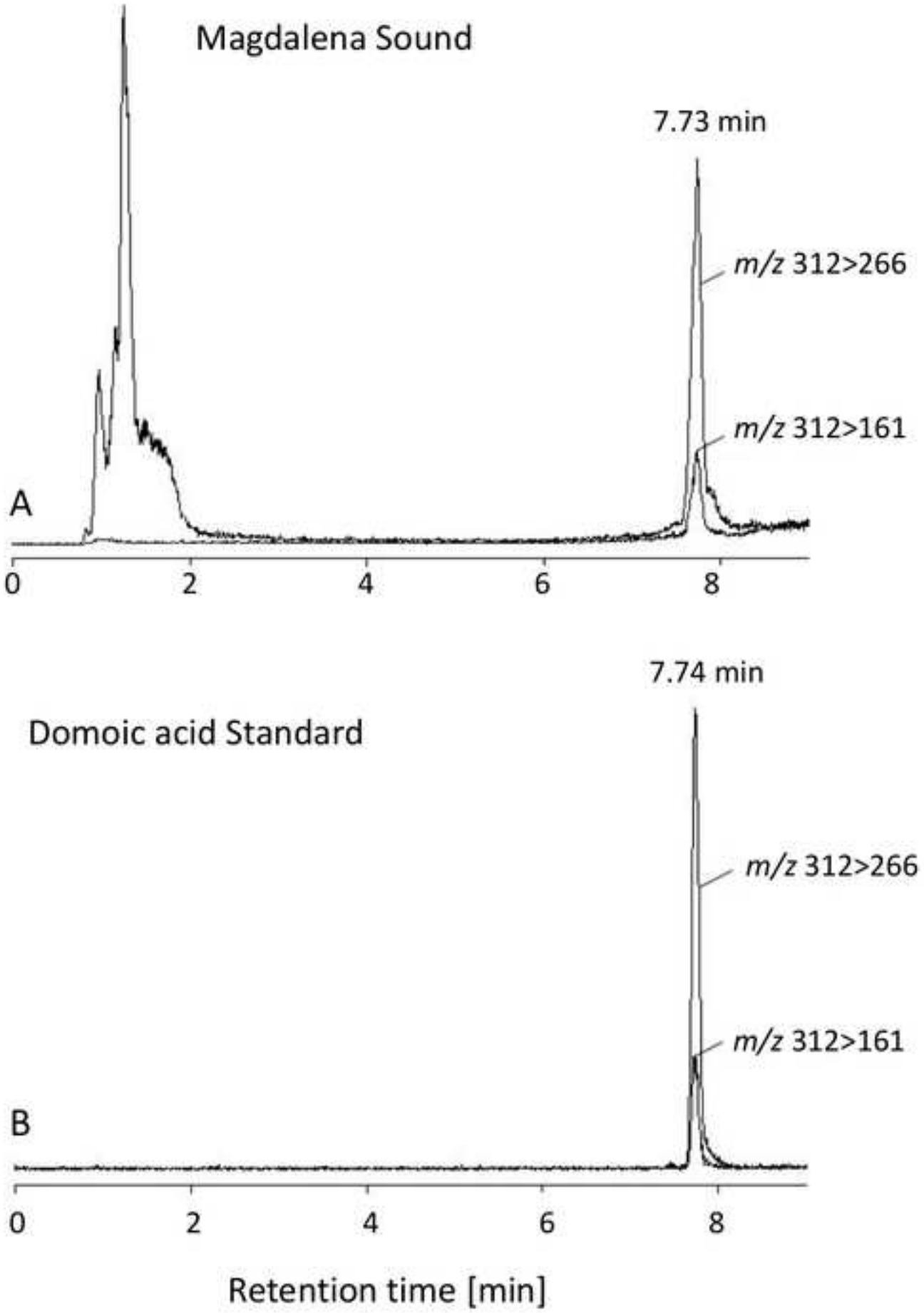


Supplementary_figure_S1



Declaration of interests

☒ 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.

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