



## Multiscale physical background to an exceptional harmful algal bloom of *Dinophysis acuta* in a fjord system



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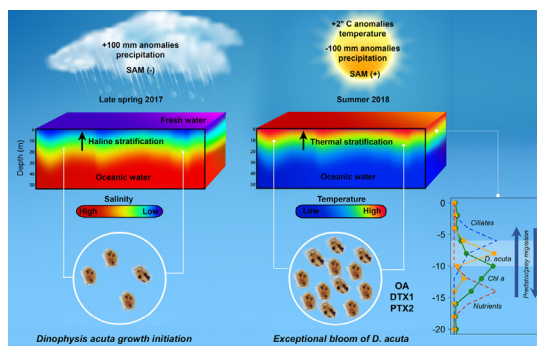
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### HIGHLIGHTS

- Exceptional bloom of *Dinophysis acuta* ( $>6 \times 10^5$  cell L<sup>-1</sup>) in thin layers in a fjord
- Late spring precipitation and summer SST (+) anomalies with high (+1.1 ha) SAM index
- Climate anomalies led to persistent haline (spring)-thermal (summer) stratification.
- Model simulations identified lowest flushing rate Puyuhuapi as epicentre of DSP events
- Multiple-scale drivers of *D. acuta* and diarrhetic shellfish (DSP) events identified

### GRAPHICAL ABSTRACT



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### ABSTRACT

*Dinophysis acuta* produces diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins (PTX). It blooms in thermally-stratified shelf waters in late summer in temperate to cold temperate latitudes. Despite its major contribution to shellfish harvesting bans, little effort has been devoted to study its population dynamics in Chilean Patagonia. In 2017–2018, mesoscale distribution of harmful algal species (75 monitoring stations) revealed the initiation (late spring) and seasonal growth of a dense *D. acuta* population in the Aysén region, with maximal values at Puyuhuapi Fjord (PF). Vertical phytoplankton distribution and fine-resolution measurements of physical parameters along a 25-km transect in February 16<sup>th</sup> identified a 15-km (horizontal extension) subsurface

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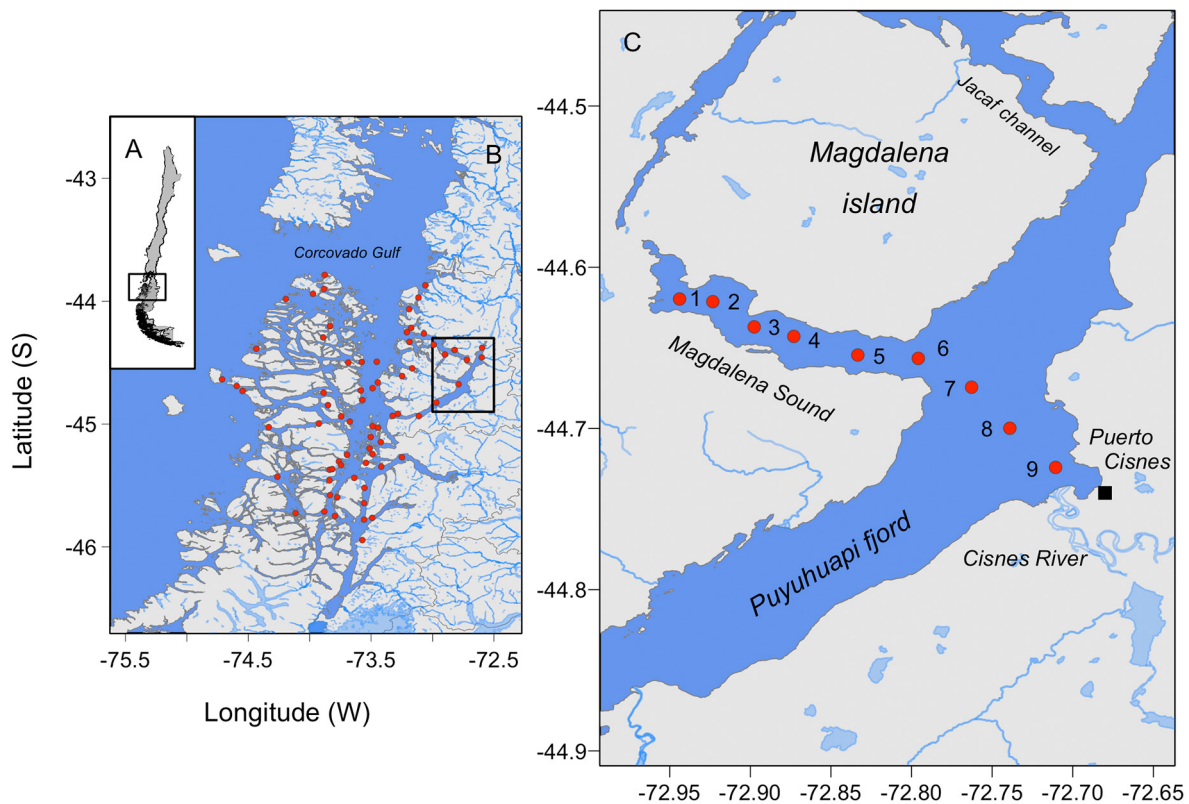
thin layer of *D. acuta* from 4 to 8 m depth. This layer, disrupted at the confluence of PF with the Magdalena Sound, peaked at the top of the pycnocline (6 m, 15.9 °C, 23.4 psu) where static stability was maximal. By February 22<sup>nd</sup>, it deepened (8 m, 15.5 °C; 23.62 psu) following the excursions of the pycnocline and reached the highest density ever recorded ( $664 \times 10^3$  cells L<sup>-1</sup>) for this species. *Dinophysis acuta* was the dominant *Dinophysis* species in all microplankton net-tows/bottle samples; they all contained DSP toxins (OA, DTX-1) and PTX-2. Modeled flushing rates showed that Puyuhuapi, the only fjord in the area with 2 connections with the open sea, had the highest water residence time. Long term climate variability in the Southern hemisphere showed the effects of a Southern Annular Mode (SAM) in positive mode (+1.1 hPa) overwhelming a moderate La Niña. These effects included positive spring precipitation anomalies with enhanced salinity gradients and summer drought with positive anomalies in air (+1 °C) and sea surface (+2 °C) temperature. Locally, persistent thermal stratification in PF seemed to provide an optimal physical habitat for initiation and bloom development of *D. acuta*. Thus, in summer 2018, a favourable combination of meteorological and hydrographic processes of multiple scales created conditions that promoted the development of a widespread bloom of *D. acuta* with its epicentre at the head of Puyuhuapi fjord.

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## 1. Introduction

In the last three decades there has been an apparent increase of Harmful Algal Blooms (HABs) events at a global scale, partly associated with cultural eutrophication, increased utilization of coastal waters for aquaculture (Masó and Garcés, 2006; Glibert, 2017; Zhou et al., 2008), and an exponential growth of observations by monitoring programmes (Hallegraeff, 1993; Reguera, 2002). HABs in Southern Chile (between 40 and 50°S) have followed this trend (Díaz et al., 2019a; Díaz et al., 2014a; Trainer et al., 2020). Until now, most research and monitoring efforts in Chile have been focused on *Alexandrium catenella*, which produces Paralytic Shellfish Poisoning (PSP) toxins. Blooms of this species, first reported in Magallanes in 1972 (Guzmán et al., 1975), and in 1992 in Aysén (Molinet et al., 2003) have led to world records of shellfish

toxicity and human fatalities (Díaz et al., 2019a; Guzmán et al., 2002). Blooms of endemic species of *Dinophysis*, mainly *Dinophysis acuminata* and *D. acuta*, which produce diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins (PTX) are also frequent in Southern Chile. A gastrointestinal outbreak in 1970 affecting over 100 people after eating mussels, *Aulacomya ater*, from the Reloncavi Estuary in Los Lagos (Fig. 1), was the first time a *Dinophysis* species (*D. acuta*) was associated with a toxic syndrome (Guzmán and Campodónico, 1975). *Dinophysis acuta*, and to a lesser extent *D. acuminata*, are now recognized as threats to public health, artisanal fisheries and the mussel industry in Southern Chile, in particular in the region of Los Lagos, site of 95% of national shellfish production over  $3 \times 10^5$  t y<sup>-1</sup> (Sernapesca, 2017). Nevertheless, with the exception of a few research cruises (Alves de Souza et al., 2014; Díaz et al., 2011; Lembeye et al., 1993) and the official monitoring carried



**Fig. 1.** Map showing: A) Chile (box delimits Aysén region); B) Northwest Patagonia (box delimits Puyuhuapi fjord system) and IFOP monitoring sampling stations (red circles); C) Magdalena Sound and PF and sampling stations (red circles) on a 25-km transect (Magdalena Sound to mouth of Cisnes river) visited during the cruises in February and March 2018. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

out by the Fisheries Institute (Instituto de Fomento Pesquero, IFOP), little attention has been paid to the taxonomy, toxinology and bloom development of *Dinophysis* species in Chilean fjords.

Phytoplankton growth including harmful blooms in fjord systems is driven by complex physical-biological interactions on multiple scales (Roy et al., 2018; Haury et al., 1978; Lucas and Largier, 2013). Chilean fjords are characterized by strong salinity gradients and high rainfall exceeding 3000 mm/year. Multiannual variability of these features and the resulting water column stability are affected by macroscale processes — El Niño Southern Oscillation (ENSO) and the Southern Annular Mode (SAM) — either directly through changes in local wind patterns and rainfall, or indirectly through changes in glacier ice melt, one of the main freshwater sources in this region (Calvete and Sobarzo, 2011; Dávila et al., 2002). Stratification and mixing of the water column in coastal areas are mainly controlled by local atmospheric processes (winds, rainfall, solar incidence). Mixing and nutrient concentrations were identified by Margalef (1978) in his classic Mandala as the two key factors controlling succession of phytoplankton assemblages because they affect light and resource availability and create the physical habitat. Changes in local wind patterns and precipitation and their effects on annual phytoplankton succession in the Patagonian fjords have been described (González et al., 2010; León-Muñoz et al., 2018; Montero et al., 2017).

Further development of Margalef's Mandala led to the Reynold's In-taglio which emphasizes that microturbulence and stratification can favour dinoflagellates able to exploit light and resources by vertical migration (Smayda and Reynolds, 2001). It is the current view that abiotic factors shape dinoflagellate communities (revised in Rengefors, 2020). A challenging problem is to understand how different morphological and physiological traits of individual species and their interactions with the abiotic factors select those species that grow fast enough to form blooms (Carstensen et al., 2015). Some dinoflagellate blooms have been linked to climatic anomalies in temperate coastal waters of the north (Cloern et al., 2005; Du et al., 2011; White et al., 2014) and south Pacific (Álvarez et al., 2019; León-Muñoz et al., 2018). Likewise, *Dinophysis* blooms have been associated with anomalies in coastal waters of the northeast — Spain and Portugal (Díaz et al., 2013; Díaz et al., 2016; Moita et al., 2016); Ireland (Farrell et al., 2012; Raine, 2013; Whyte et al., 2014) — and southwest — Brazil, (Mafra et al., 2019; Mendez et al., 2016) — Atlantic, and of southern Chile (Díaz et al., 2020; Lembeye et al., 1993). Díaz et al. (2016) showed that summer blooms of *Dinophysis acuta* in north-western Spain in 1989 and 1990 were linked to large-scale hydroclimatic anomalies — high positive sea surface temperature (SST) anomalies and the North Atlantic Ocean (NAO) — which affects most of the Atlantic coasts of western Europe. At the mesoscale, a northward shift of the Azores High pressure centre led to positive SST anomalies (>2 °C), moderate upwelling rates, persistent thermal stratification and a drought in June–August in the Galician Rías. These conditions were associated with a northwards displacement of the epicentre of *D. acuta* populations, from northern Portugal (40.64°N) to the Galician shelf off the Rías Baixas (42.43°N) (Díaz et al., 2019b).

The importance of sub-mesoscale (>10 km) and high frequency processes (e.g. microturbulence, tidal cycles and circadian rhythms) in fjords and coastal embayments needs to be emphasized (Díaz et al., 2011; Roy et al., 2018; Lucas and Largier, 2013). Higher resolution observations of physical and biological processes and their interactions are needed to understand these processes (Raine et al., 2018; Roy et al., 2018). In the case of *Dinophysis*, classified as a “low biomass HAB” species, microscale (<5 m) variability in their vertical distribution hinders their detection and/or underestimates their density (Escalera et al., 2012). This variability can be amplified in highly heterogeneous systems, such as the Patagonian fjords, where multiple micro-environments promote development and aggregation of phytoplankton (Alves de Souza et al., 2019; Alves de Souza et al., 2014; Díaz et al., 2011). Vertical gradients promote the formation of thin layers of phytoplankton in calm weather (Raine et al., 2018); in these layers, cell numbers can be several

orders of magnitude higher than in the water layers immediately above or below (McManus et al., 2003). Thin layers of *Pseudo-nitzschia* —in the Galician Rías, NW Spain (Díaz et al., 2014b; Rines et al., 2002; Velo-Suárez et al., 2008) and Washington, NW USA (Rines et al., 2002) — and *Dinophysis* — *D. acuta* in Portugal, NE Atlantic (Moita et al., 2006; Sjöqvist and Lindholm, 2011) — have been observed in fjords and embayments.

We describe the development of an exceptionally dense bloom of *Dinophysis acuta*, possibly triggered by extreme climatic anomalies in Chilean Patagonia during the austral spring-summer 2017–2018. Our objectives were to elucidate: i) the physical-biological interactions leading to subsurface aggregations of *Dinophysis acuta*; ii) the site-specific features of the regional epicentre of *D. acuta* blooms and iii) the macroscale patterns of variability associated with blooms of this species in the Chilean Fjords. Understanding these processes is needed to improve early warning systems and to forecast specific HAB events.

## 2. Material and methods

### 2.1. Study area

Puyuhuapi Fjord (PF) is located in Aysén, a region of Chilean Patagonia with a very humid climate (Fig. 1) (Pantoja et al., 2011). This 100-km long fjord system has a mean depth of 220 m and a maximum of 350 m close to the mouth of Magdalena Sound. It is characterized by complex coastal morphology and variable water column stratification determined by runoff from ice-melt in late spring (November–December), and by persistent rainfall (>3000 mm/year; Sauter, 2020). Weather systems there, embedded in the Southern Hemisphere (SH) westerly wind belt, reach the coast year round. Precipitation is further enhanced over the western slopes of the Austral Andes (Viale and Garreaud, 2015). On interannual time scales, climate in NW Patagonia is modulated by the El Niño Southern Oscillation (ENSO) and the Southern Annular mode (SAM). El Niño/La Niña conditions are associated with precipitation deficit/excess (Montecinos and Aceituno, 2003), and positive SAM with drier conditions in NW Patagonia (Gillett et al., 2006).

The main freshwater input to PF is the Cisnes River (annual mean discharge  $\sim 220 \text{ m}^3 \text{ s}^{-1}$ ), that flows into the middle part of the Fjord ([www.dga.cl](http://www.dga.cl)). Additional freshwater inputs from minor rivers (Ventisquero, Marta and Uspallante) contribute to the haline stratification of the surface layer. During the ice-melt, river discharge rates can exceed  $700 \text{ m}^3 \text{ s}^{-1}$ , as in November 2017 (data available in <http://chonos.ifop.cl/aguadulce/visor>). This discharge lowers surface salinity in the entire channel dividing it at around 44.6°S into northern low salinity (5–10) and southern medium salinity (15–25) sections. The presence of Modified Subantarctic Water (MSAAW) resulting from the mixing of the estuarine water and the Subantarctic water mass (SAAW), is frequently detected at a subsurface layer inside the fjord (Schneider et al., 2014; Sievers and Silva, 2008).

### 2.2. Climate data

Rainfall, air temperature and river discharge data were obtained from the Climate Explorer (<http://explorador.cr2.cl/>) which compiles quality-controlled records from the Chilean Weather Service (DMC) and the Water Agency (DGA). Due to the low-density of climate stations in Patagonia, additional data from the CR2Met dataset, comprising monthly gridded ( $0.05^\circ \times 0.05^\circ$  lat-long) precipitation and temperature fields over Chile, from 1979 to date, were also used. CR2Met was built by optimal interpolation of surface data upon state-of-the-art European Centre reanalysis data (Álvarez-Garretón et al., 2018). Large-scale atmospheric conditions were described using monthly means of sea level pressure (SLP) and geopotential height at selected pressure levels from the National Centres for Environmental Prediction (NCEP)-National Centre for Atmospheric Research (NCAR) Reanalysis, available from 1948 onwards on a  $2.5^\circ \times 2.5^\circ$  latitude-longitude grid (Kalnay et al., 1996).

### 2.3. Field sampling

#### 2.3.1. IFOP phytoplankton monitoring

Between October 2017 and March 2018 monthly sampling was carried out at 75 stations in Aysén (Fig. 1B) as part of the IFOP Monitoring Programme. Integrated water-column samples for quantitative analyses of phytoplankton are collected with a dividable hose sampler from 0 to 10 and 10 to 20 m (Lindahl, 1986) and immediately fixed with acidic Lugol's solution (Lovegrove, 1960). For quantitative analyses of phytoplankton, 10 mL of the hose-samples are left to sediment overnight and analysed under an inverted microscope (Olympus CKX41) using the method described in Utermöhl (1958). To enumerate large but less abundant species, such as *Dinophysis* spp., the whole surface of the chamber is scanned at a magnification of  $\times 100$ , so that the detection limit is 100 cells  $L^{-1}$ .

#### 2.3.2. Cruise sampling overview

During late summer (February–March) 2018, three one-day cruises were carried out in PF on board R.V. *Queen*. On the first cruise, on February 16<sup>th</sup>, nine stations aligned in a 25-km transect were sampled; on the second (February 22<sup>nd</sup>) and third (March 13<sup>th</sup>), only station 1, at the head of Magdalena Sound, was sampled (Fig. 1C).

Vertical profiles of temperature, salinity and in vivo chl *a* fluorescence were obtained with 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). This fluorescence sensor outputs an analog voltage proportional to the chl *a* concentration. CTD fluorescence data from a northern Patagonian fjord (41.6° S/72.85° W) and chl *a* in bottle samples collected at standard depths (0, 5, 10, 15, 25, 50, 75, 100, 150, and 200 m) were highly correlated ( $R^2 = 0.987$ ), and used to convert the CYCLOPS-7 output voltage into chl *a* concentration. The CTD was also equipped with an optical sensor for dissolved oxygen (DO), and other sensors for turbidity and pH. All these parameters were measured from 0 to 500 m, at a sampling rate of 24 Hz. Water samples for inorganic nutrients and chl *a* analyses were taken at four stations, from four discrete depths (0, 4, 10 and 20 m) with 5-L Niskin bottles. Water samples for ammonia analyses were omitted for logistic reasons, i.e., the impossibility to ensure analyses of this labile molecule very soon after collection in remote areas in southern Chile. Samples for spectrophotometric nutrient analyses were filtered through Whatman GF/F fiberglass filters (47 mm  $\varnothing$ , 0.7- $\mu$ m nominal pore size) (Whatman, Maidstone, England) and the filtered material frozen at  $-20^\circ C$  until analysis. Samples for quantitative analyses of microphytoplankton were collected at each station with Niskin bottles every 2 m, from surface to 20 m depth. Fixation and storage was as described in Section 2.3.1.

Two kinds of samples were collected for lipophilic toxin analysis. Plankton nets (20- $\mu$ m mesh), were towed vertically from 0 to 20 m to collect integrated water column samples and obtain semiquantitative information of lipophilic toxin (content per net tow) (Fabro et al., 2016). The collected net-tow sample was adjusted to a final volume of 50 mL and the concentrated microplankton suspension placed in a Falcon tube, stored in the vessel's deep-freeze and later transported in a portable fridge before final storage in the laboratory at  $-20^\circ C$  until analysis. For vertical distribution of particulate lipophilic toxins, 1-L aliquots from the bottle samples were collected at 5 fixed depths (0, 4, 8, 10 and 20 m) and filtered through Whatman GF/F filters, each filter placed in a cryotube with 1 mL analysis grade methanol, and kept at  $-20^\circ C$  until analysis.

#### 2.4. Analysis of nutrients, chl *a* and microphytoplankton

Concentrations of nitrate, orthophosphate and silicic acid were determined following Strickland and Parsons (1968). For chl *a*, 200 mL seawater were filtered through the same kind of filters described

above and immediately frozen at  $-20^\circ C$  until analysis with a calibrated Turner Design (TD-700) fluorometer, following pigment extraction with acetone (90%, v/v), according to standard procedures (Parsons et al., 1984).

For quantitative analyses of microphytoplankton, 10 mL of fixed bottle samples were left to sediment overnight and analysis carried out as those described in Section 2.3.1.

#### 2.5. Extraction and analysis of lipophilic toxins

To extract toxins from the net-tow samples, Falcon tubes were centrifuged (4000g; 10 min), the pellet obtained mixed with 1 mL of MeOH (100%) and cells disrupted with a Branson Ultrasonic 250 (Danbury, CT, USA). The extract was clarified by centrifugation (20,000g; 20 min) and filtered through 0.22  $\mu$ m Clarinert nylon syringe filters (13 mm diameter) (Bonna-Agela technologies, Torrance, CA, USA).

For the analysis of particulate lipophilic toxins at fixed depths, filtered samples kept in MeOH 100% at  $-20^\circ C$  were defrosted, extracted by sonication, clarified by centrifugation (20,000g, 20 min), and filtered through 0.22  $\mu$ m nylon syringe filters. To analyse free okadaic acid (OA) and other lipophilic toxins, a 0.5-mL aliquot from each sample was placed in an amber vial and stored at  $-20^\circ C$  until further processing. Finally, the 0.5 mL aliquots were subjected to alkaline hydrolysis following the standard procedure of the EU Reference Laboratory for Marine Biotoxins (EURLMB, 2015), placed in amber vials and stored at  $-20^\circ C$  until analysis.

##### 2.5.1. Toxin analysis

Phytoplankton toxins analyses were carried out according to Regueiro et al. (2011) with minor modifications (a shorter column and allowing enough time for the elution of all the toxins) with a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Sunnyvale, CA, USA). Toxin detection was carried out with a high-resolution mass spectrometer Q Exactive Focus equipped with an electrospray interphase HESI II (Thermo Fisher Scientific, Sunnyvale, CA, USA). The presence of lipophilic toxins was confirmed by comparing the retention time, exact mass and fragmentation spectra with those of certified reference solutions from the NRC, Canada. Toxin was quantified by comparing the area of the peaks obtained in the chromatograms with those of certified reference materials. The detection (LOD) and quantification (LOQ) limits of the LC-MS/MS method were 3.82 ng  $mL^{-1}$  and 1.73 ng  $mL^{-1}$  respectively, for OA; 2.93 ng  $mL^{-1}$  and 1.67 ng  $mL^{-1}$  for DTX1 and 1.90 ng  $mL^{-1}$  and 0.82 ng  $mL^{-1}$  for PTX2.

#### 2.6. Model simulations

A high-resolution 3D hydrodynamic model, MIKE 3 FM, was used to determine flushing times in the fjords of northwestern Patagonia. This model uses the finite volume method to solve the Navier-Stokes equations (DHI, 2016; it was initialed with bathymetric information from the Chilean Navy Hydrographic Service (SHOA), river discharges from the General Water Directorate (DGA) and atmospheric forcing provided by the Weather Research and Forecasting (WRF) meteorological model (Skamarock et al., 2008), a component of the IFOP Modeling Systems. The hydrodynamic simulation was for one year, from April 2018 to March 2019. Currents, temperature and salinity field data are available for viewing or downloading on the Chonos oceanographic information platform (<http://chonos.ifop.cl/atlas>). A stabilization or spin-up period of 3 years was used to avoid spurious results from the model due to the characteristics of the fjords. This period depends mainly on the initial water temperature and salinity conditions (density) resulting from the propagation of the boundary conditions and river discharge for the spin-up period. After verification of the model stability, the final simulation of temperature and salinity was extracted and incorporated as a new initial condition. More details on the configuration and validation of this model can be found in Pinilla et al. (2019) and in the Supplementary

material. The MIKE 3 model, with a different configuration, has been used in PF to determine different circulation and transport features (Pinilla et al., 2020).

Transport time scales, coupled to the hydrodynamic simulation, were estimated through the flushing time metrics with a passive tracer. Although the simulation period did not coincide with the study period, it covered a broad spatial dimension and included places, such as the Magdalena Sound, with longer water retention times. Water renewal time, defined as the time for the total mass within the area of interest (in this case the Aysén fjords) to be reduced by a factor of  $1/e$  ( $\sim 37\%$ ) (Prandle, 1984), was estimated according to Takeoka (1984) and Monsen et al. (2002). The initial concentration values assigned were 1 for the inner and 0 for the outer limit of the area. During the simulation, the water mass from the original basin was gradually replaced by inputs through the open boundaries, as well as from the rivers. This variable shows the amount of original water contained on each element within the domain of interest at a given time. Therefore, it can be expected to identify the areas with lower flushing rates within the modeled basins (Andrejev et al., 2004).

### 3. Results

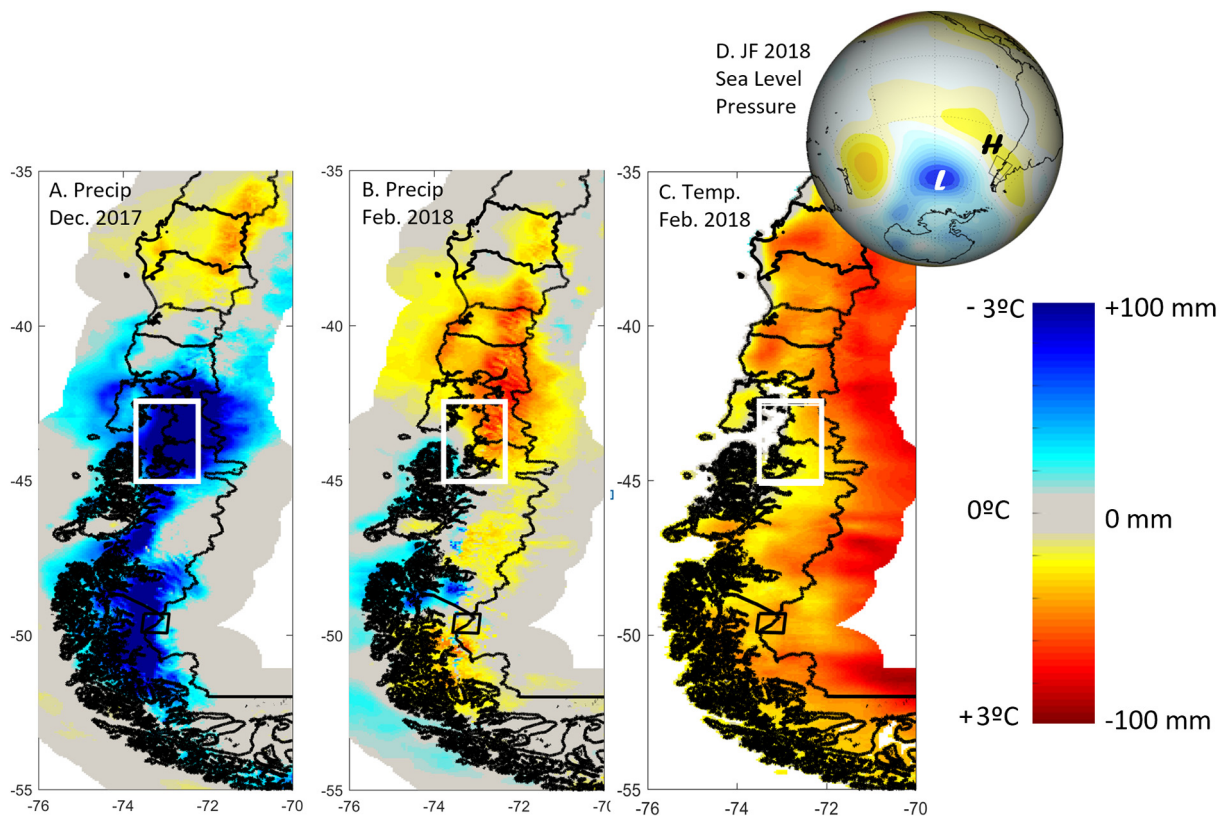
#### 3.1. Meteorological conditions during summer 2018

Precipitation anomalies of opposite sign, i.e., positive in late spring (Dec 2017) and negative in summer (Jan–Feb 2018), were recorded in Aysén during the study period. Despite higher pressure zones prevailing at mid-latitudes over the southeast Pacific, above average cumulative values of precipitation were observed in December 2017 (Fig. 2A) in connection with two strong atmospheric rivers that impacted northwestern

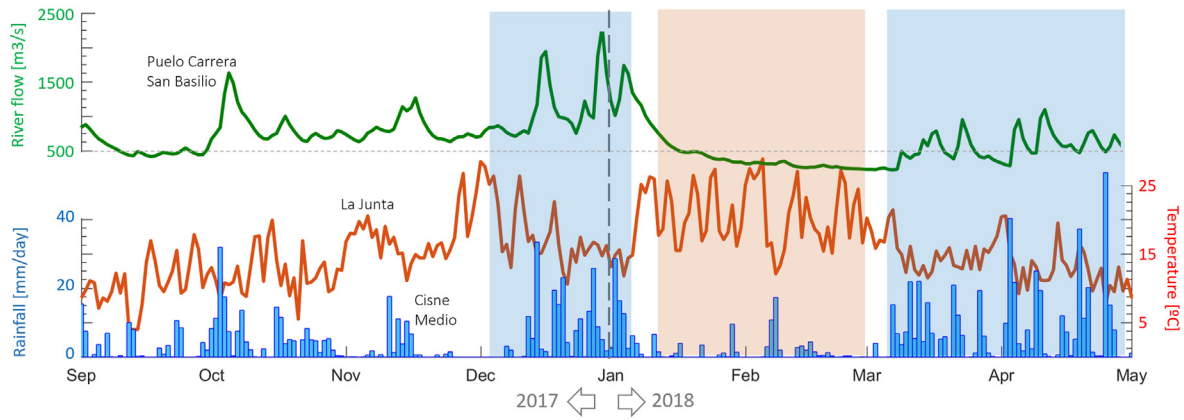
Patagonia on December 15<sup>th</sup> and 23<sup>rd</sup>. Monthly rainfall plus increased ice melt due to warmer air temperatures led to substantial runoff that reached a maximum in late December (Fig. 3). These conditions intensified the characteristic haline stratification in the fjords. Anticyclonic conditions in January and February (Fig. 2D) led to drier conditions in northwestern Patagonia, especially during February, when rainfall deficits ranged between 30 and 90 mm, i.e.,  $\sim 30\%$  below average (Fig. 2B). Consequently, runoff dropped to a minimum by late February (Fig. 3). During the anticyclone of summer 2018, air temperature anomalies exceeded  $+1^\circ\text{C}$  during February (Fig. 2C). This scenario enhanced thermal water-column stratification. The seasonal drought was ended abruptly in March with cyclonic conditions in southern South America and heavy precipitation over western Patagonia (Fig. S1).

#### 3.2. Mesoscale variability of the spatial-temporal distribution of *D. acuta*

Data from the Chilean Monitoring Programme (IFOP) (integrated hose-sampler) in Aysén Region (75 stations) showed that *D. acuta* populations, with cell densities ranging from 100 to 400 cells  $\text{L}^{-1}$ , were first detected in the spring (October and November 2017) off Aysén Fjord and in all the stations in Puyuhuapi (Fig. 4). A relevant increase in *D. acuta* cell densities was observed in the same areas in December, in particular in Jacaf Channel and the northern half of PF, with a cell maximum of 6600 cells  $\text{L}^{-1}$  (Fig. 4C). Maximal bloom development and a southward spreading of the population was observed in January, with a peak of 12,500 cells  $\text{L}^{-1}$  close to the head of PF (Fig. 4D). February showed the bloom decline inside the fjords and high densities ( $>10^3$  cells  $\text{L}^{-1}$ ) persisted in the more neritic Jacaf (3600 cells  $\text{L}^{-1}$ ) and Moraleda channel stations (Fig. 4E) before the population dispersion off the fjords in March (Fig. 4F).



**Fig. 2.** Anomalies (related to 30 years, 1980–2010, mean) for selected months over southern Chile: A) positive precipitation anomalies in December 2017 showing the above average values over western Patagonia; B) negative precipitation anomalies in February 2018 (similar dry conditions were observed in January 2018); C) Surface air temperature anomalies in February 2018 showing warm conditions over Patagonia; D) Sea level pressure (SLP) anomalies in January–February 2018. Letters H and L indicate positive and negative SLP anomalies, respectively. Precipitation and temperature from CR2Met dataset. SLP from NCEP–NCAR Reanalysis.

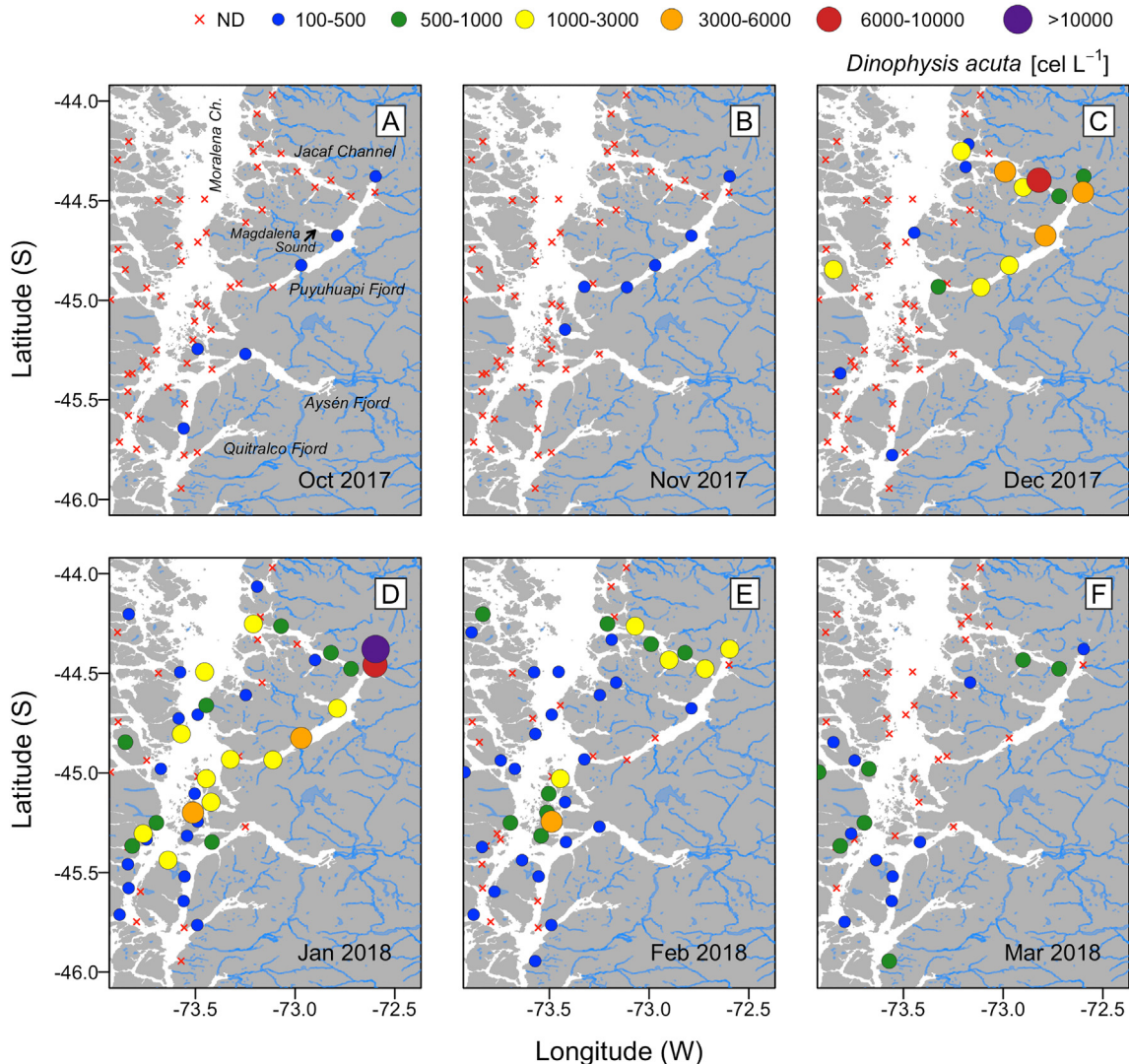


**Fig. 3.** Local conditions near study area from late (spring) 2017 to early (summer) 2018. Top panel: Discharge of Puelo River at Carrera San Basilio (41.6°S, 72.2°W, 5 m ASL) (green line) and maximum air temperature at La Junta (43.9°S, 72.4°W, 45 m ASL) (red line). Bottom panel: Rainfall at Cisne Medio (44.6°S, 72.4°W, 180 m ASL) (blue bars). Light-blue (orange) rectangle shows particularly wet/cold (dry/warm) periods. All daily data from DMC/DGA Chile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Oceanographic conditions during the *D. acuta* bloom

CTD data from a transect along Magdalena Sound and across PF on February 16<sup>th</sup> showed a characteristic two-layered structure. The top

(0–10 m) layer of warmer (12.6 °C–17.9 °C) brackish (11.4–29.7) water had density gradients of 0.2 °C m<sup>-1</sup> and 1.4 psu m<sup>-1</sup> (max. 0.49 °C m<sup>-1</sup> and 1.64 psu m<sup>-1</sup> between 6 and 7 m). The bottom (10–50 m) layer of saltier (28.0–32.7) water had temperatures ranging



**Fig. 4.** Mesoscale distribution of *Dinophysis acuta* cells (cells L<sup>-1</sup>) recorded in the monthly monitoring programme carried out in Aysén region (southern Chile) from October 2017 to March 2018.

from 14.2 °C at 11 m to 10.5 °C at 50 m (Fig. 5A,B). Salinity in the top 2 m ranged from 11.4 to 18.5 with a minimum (11.4 at 1 m) at station 9 off the mouth of Cisnes River (Fig. 5B). Modified Subantarctic Water (MSAAW), according to the description of Schneider et al. (2014), was found between 20 and 50 m. A fluorescence maximum, with values much higher than those reported by Schneider et al. (2014) for this area, extended from 3 m to the bottom of the pycnocline (>20 m). Maximal values of chl *a* fluorescence were observed in a layer extending from the outer reaches of Magdalena Sound to the middle part of PF, between stations 5 and 7. This maximum was located between ~5 and 15 m depth and extended for 14 km. A smaller isolated maximum was found at st. 1 (Fig. 5C). Inorganic nutrient concentrations at 4 stations

were extremely low at the surface, with maximal values – nitrate ( $6.33 \mu\text{mol L}^{-1}$ ), nitrite ( $0.07 \mu\text{mol L}^{-1}$ ) and phosphate ( $0.9 \mu\text{mol L}^{-1}$ ) – at 20 m depth in Modified Subantarctic Water (Fig. 6A–D). In contrast, silicate concentrations were maximal at the surface, in particular at the head of Magdalena Sound, where a maximum of  $9.1 \mu\text{mol L}^{-1}$  was detected (Fig. 6A).

During the second cruise (February 22<sup>nd</sup>), the pycnocline had deepened about 2 m, the maximal gradient was between 8 and –9 m (2.2 psu; 0.33 °C) and nutrient concentrations were much lower (Fig. 6E), particularly in subsurface layers, where a chl *a* fluorescence peak ( $6.4 \mu\text{g L}^{-1}$ ) occupied the layer with the maximal density gradient (Figs. 6F,G). On March 12<sup>th</sup>, after return of cyclonic conditions and heavy

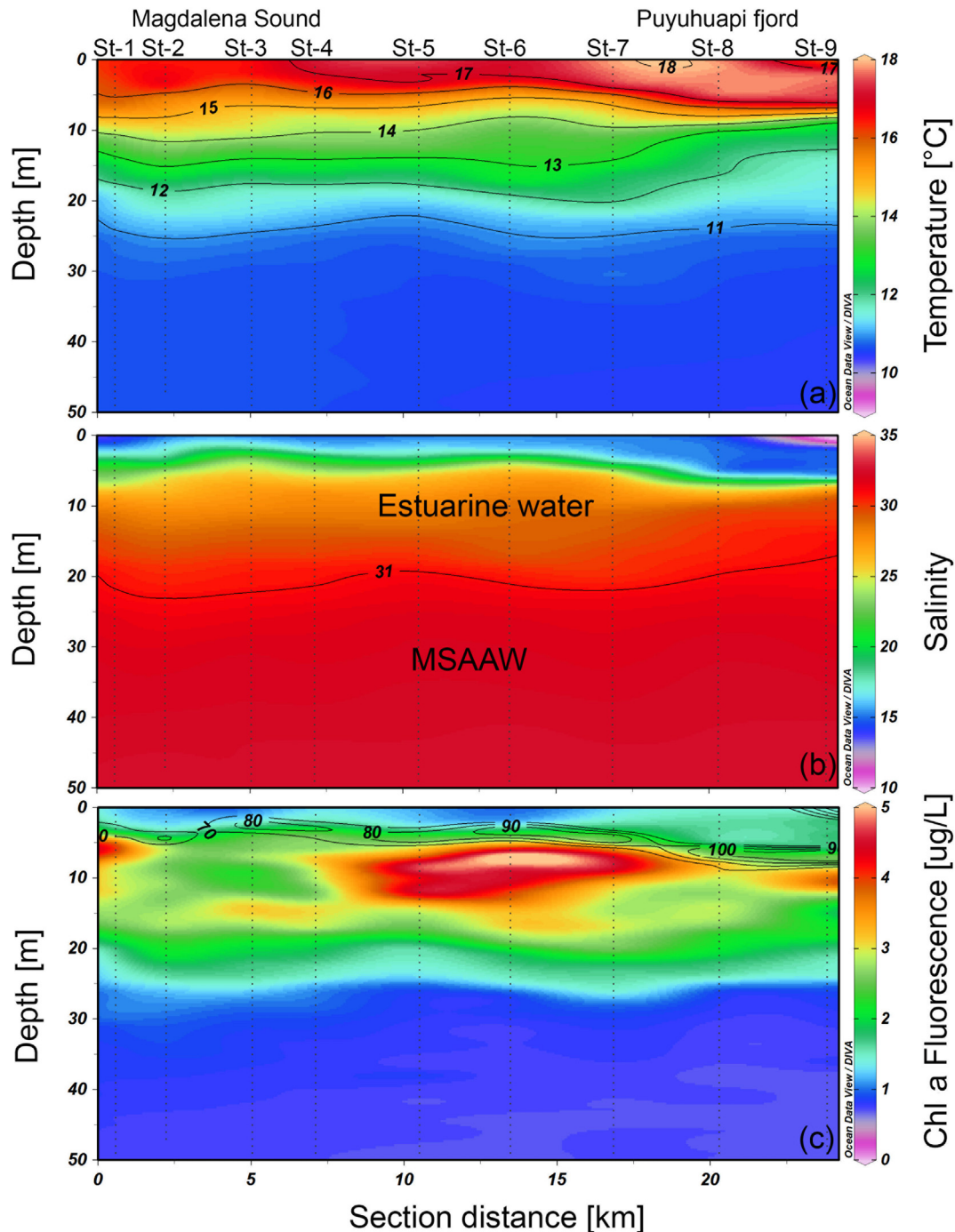
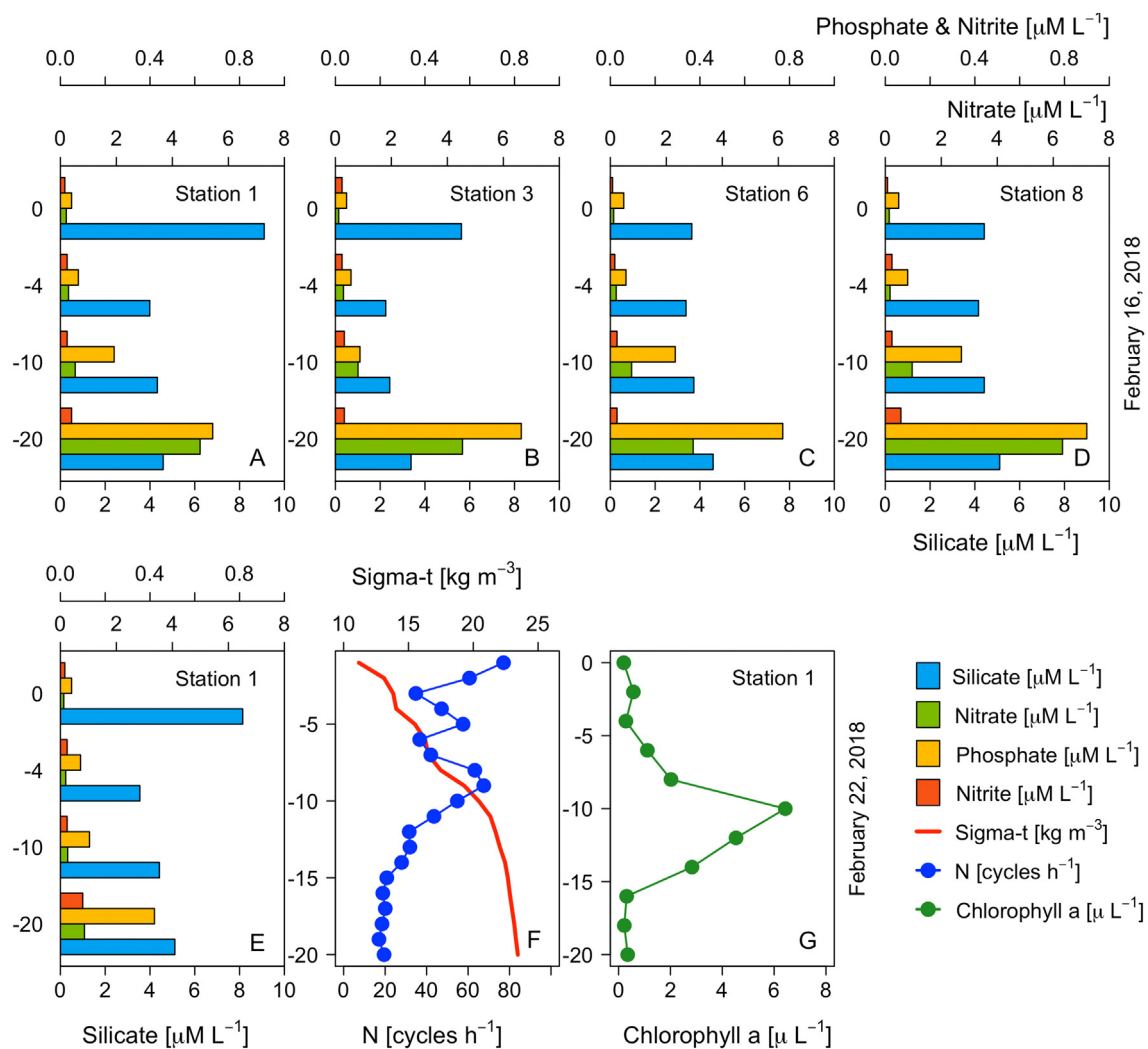


Fig. 5. Vertical distribution of temperature (top), salinity (middle) and fluorescence (bottom) with the Brunt-Vaisala frequency (black lines) at nine sampling stations in a 25-km transect during cruise on February 16<sup>th</sup>, 2018.



**Fig. 6.** Vertical distribution (0–20 m) of dissolved nutrients (nitrates, nitrites, phosphates and silicates) on A–D) 16<sup>th</sup> February at four stations (St. 1, St. 3, St. 6 and St. 8) in 25-km transect; E) 22<sup>nd</sup> February at station 1 (inner fjord); vertical distribution of F) Sigma-t (red line; kg m<sup>-3</sup>) and Brunt-Vaisala frequency (blue line; cycles h<sup>-1</sup>); and G) fluorescence chlorophyll a (μL<sup>-1</sup>) at station 1 on 22<sup>nd</sup> February 2018. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rain, the pycnocline shoaled, and gradients weakened (0.03 °C m<sup>-1</sup> and 0.7 psu m<sup>-1</sup> in top 10 m) with a maximum between 3 and 4 m (0.15 °C m<sup>-1</sup> and 1.82 psu m<sup>-1</sup>).

### 3.4. Development of a thin layer (TL) of *Dinophysis acuta*

On February 16<sup>th</sup>, maximal cell densities of *D. acuta* occurred in Magdalena Sound, where an intense thin layer (between 4 and 8 m) was formed. This thin layer, with a maximum of 118 × 10<sup>3</sup> cells L<sup>-1</sup> at station 1 (inner fjord), at 6 m (15.88 °C, 23.36 psu) was above the pycnocline, where thermal gradient and static stability maxima were found (Fig. 7A, B); it had a horizontal extension of 15 km and ended (6.9 × 10<sup>3</sup> cells L<sup>-1</sup>) at the confluence of the Sound with PF (stations 6 and 7). Another *D. acuta* patch was found at station 8 (6 m depth) with a maximum of 45.5 × 10<sup>3</sup> cells L<sup>-1</sup> (Fig. 7A).

On February 22<sup>th</sup>, the thin layer was again at the top of the deepened thermocline; it was more intense, reaching a maximum of 664 × 10<sup>-3</sup> cells L<sup>-1</sup> of *D. acuta* at 8 m, at station 1 (Fig. 7C). Thermohaline properties at the depth of the cell maximum were very similar (15.43 °C; 23.62 psu) to those registered on February 16<sup>th</sup> at the 6 m maximum.

On March 12<sup>th</sup>, density gradients were considerably weaker (0.03 °C m<sup>-1</sup> and 0.7 psu m<sup>-1</sup> in the top 10 m) and a significant decline in cell densities was observed at the head of Magdalena Sound (station 1). Unlike the vertical distribution patterns observed on the two

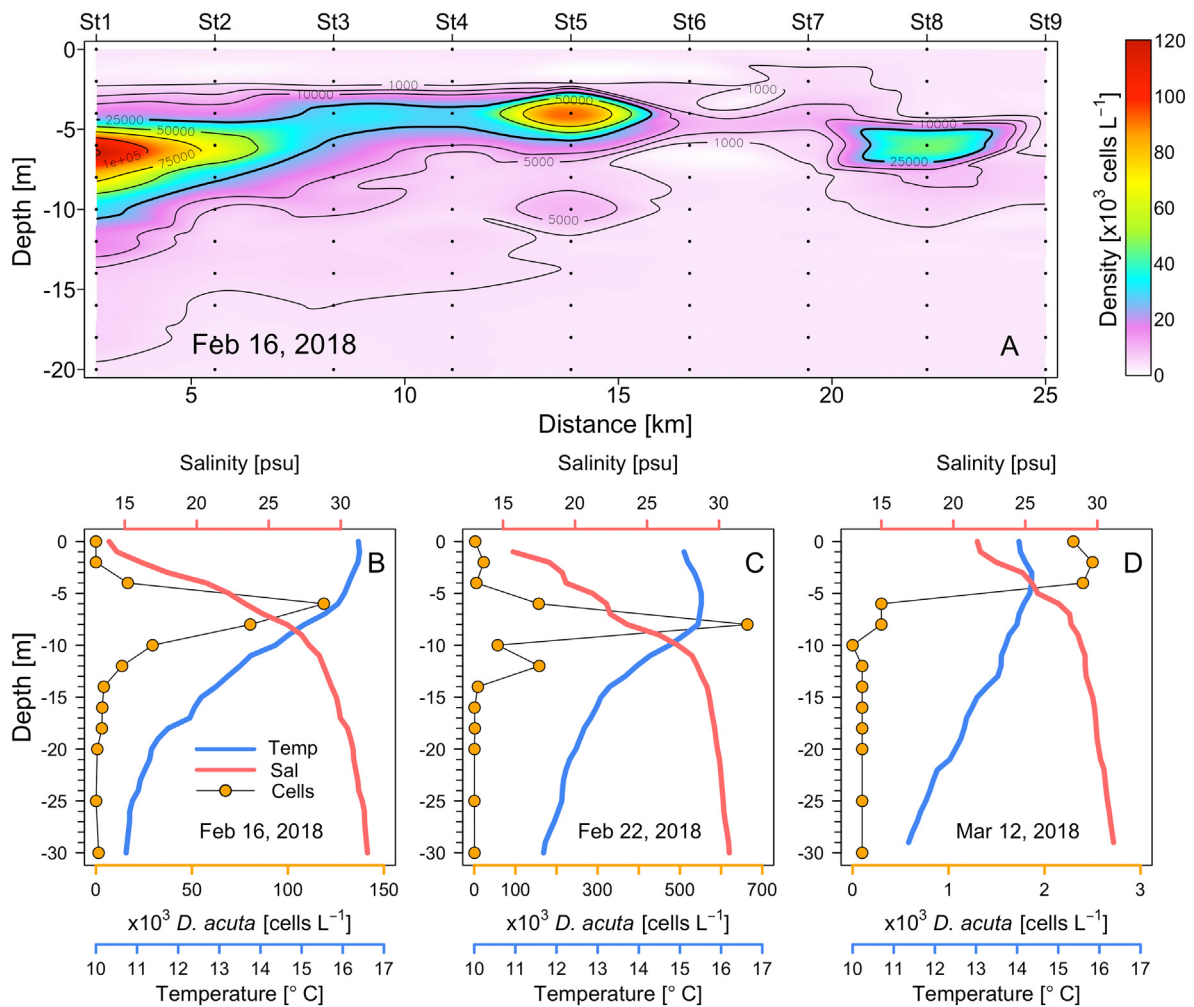
previous cruises (February 16<sup>th</sup> and 22<sup>th</sup>), *D. acuta* cells during this cruise were mainly in the surface layer (0–4 m) with a maximum of 2.5 × 10<sup>3</sup> cells L<sup>-1</sup> at 2 m (14.2 °C; 22.97 psu).

### 3.5. Lipophilic toxin analysis and distribution in plankton samples

Toxin analysis by LC-MS showed a first chromatographic peak with a retention time of 5.77 min corresponding to the ion [M-H]<sup>-</sup> 803.4591 m/z. The fragmentation mass spectrum of the 803.4505 ion confirmed the identification of OA since the characteristic fragment 255.1211 m/z was obtained (Fig. S2A, B). A second chromatographic peak with a retention time of 6.56 min with a [M-H]<sup>-</sup> at m/z 817.4744, was identified as dinophysistoxin-1 (DTX1); its presence was confirmed by its characteristic MS/MS fragment at m/z 255.1211 (Fig. S2C,D). Finally, the last chromatographic peak detected at 8.21 min with a parent mass [M-H]<sup>+</sup> 876.5086 m/z corresponded to PTX2; its presence was confirmed by its characteristic MS/MS fragment at m/z 841.4723, 823.4618, 805.4514 and 787.4406 m/z (Fig. S2E, F).

Diarrhetic shellfish toxins (OA and DTX-1) and pectenotoxins (PTX-2) in different proportions were present in all net and bottle samples (Fig. 8). DSP toxins (OA + DTX-1) were dominant in the net samples on February 16<sup>th</sup> when only traces of PTX2 were detected. On February 22<sup>nd</sup>, total toxin per net sample was 50 times higher and PTX-2 made up a 20.3% of the total. Finally on March 12<sup>th</sup>, the net sample content





**Fig. 7.** A) Vertical distribution (0–20 m) of *Dinophysis acuta* (cells  $L^{-1}$ ) at nine stations in 25-km transect on February 16<sup>th</sup>, 2018 (A) and vertical profiles of salinity, temperature and *D. acuta* cell densities at station 1 during the three one day cruises: A) February 16<sup>th</sup>, 2018; B) February 22<sup>th</sup>, 2018; D) March 12<sup>th</sup>, 2018.

dropped over an order of magnitude, but the proportions were similar (Fig. 8A).

On February 22<sup>nd</sup> *D. acuta* represented >97% of total *Dinophysis* cells at all depths and the toxin profile at 8 m (32.1% OA, 42.7% DTX1 and 21.2% PTX2), in the thin layer (*D. acuta* > 99%), was very similar to the profile from the net sample (Fig. 8B,C). *Dinophysis acuta* was the overwhelmingly dominant (>96%) *Dinophysis* species in the filtered bottle samples collected from 5 depths on the first two cruises, but the situation changed in March, when *D. acuminata* represented between 30% and 57% of the total between 0 and 8 m depth.

### 3.6. Water renewal time

Passive tracer simulations were used to estimate water renewal times of each fjord in northwestern Patagonia (Fig. 9) and showed the remaining percentage of water from the beginning of the simulation. For day 100, Aysén fjord, with a concentration of original water close to 35%, had the shortest renewal time. The equivalent figure of 80% at the head of Puyuhuapi, revealed its low flushing rate (Fig. 9A). By day 150, Quintralco and Cupquelán fjords reached values of 30 and 35% respectively, whereas Aysén Fjord gave values between 15 and 20%, and PF values remained high (~50%) at its northernmost area (Fig. 9B). By day 250, estimates of original water concentration had dropped to 13% for Aysén Fjord, to 15% and 20% for Quintralco and Cupquelán fjords respectively, and to 35–40% for PF. Maximal values within PF (50%) were estimated at Magdalena Sound (Fig. 9C). By day 300, estimates

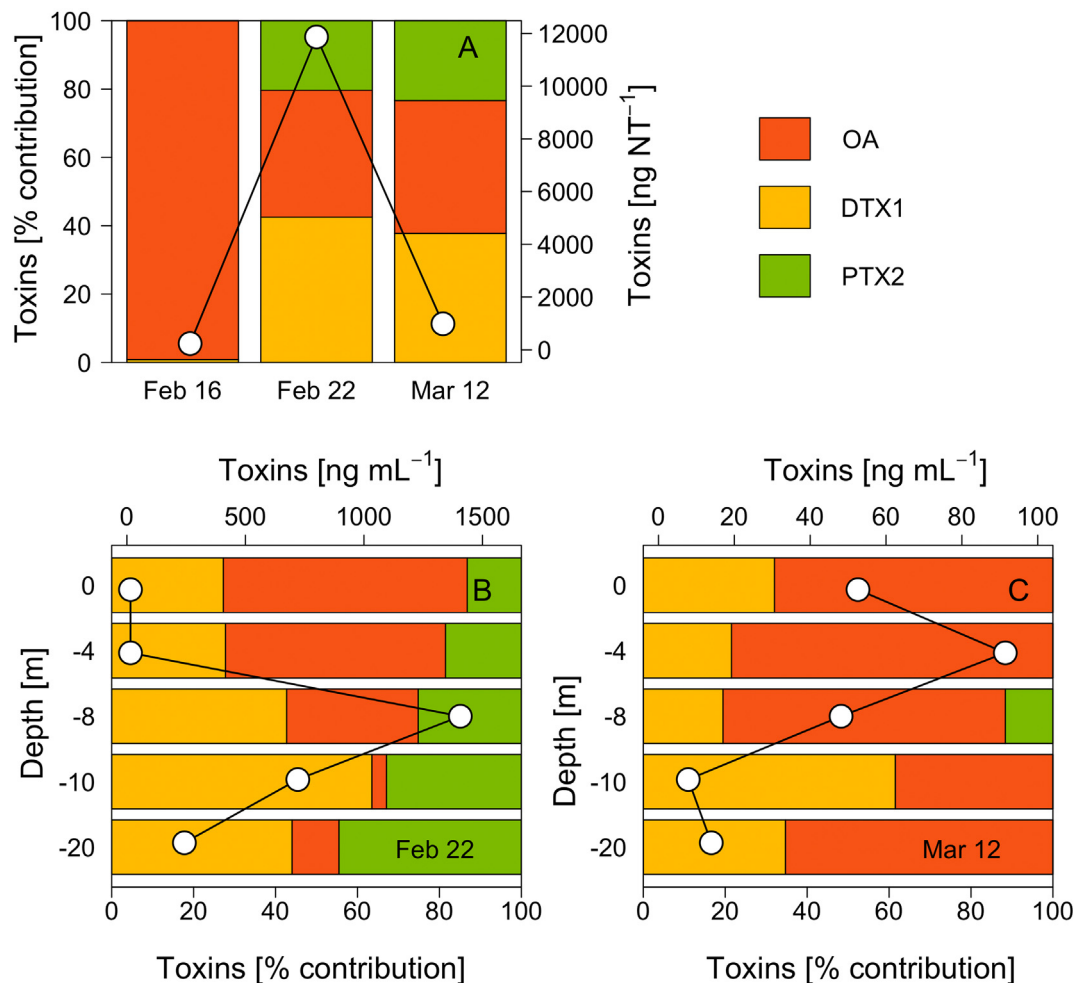
of original water concentrations for all basins were below the established threshold (35%). Puyuhuapi Fjord, in particular Magdalena Sound, showed the lowest flushing rates and Aysén Fjord the highest (Fig. 9D).

## 4. Discussion

Exceptional dinoflagellate blooms have often been related to large scale climatic anomalies, and increased stratification (Cloern et al., 2005; Hernández et al., 2016; León-Muñoz et al., 2018; Trainer et al., 2020), but the challenge is to identify the regional and site specific mechanisms underlying bloom development of a single species (Wells et al., 2015). An extremely dense bloom of *Dinophysis acuta* developed in January–February 2018 in Putuhuapi Fjord, northwest Patagonia. We discuss the large scale climatic factors, regional meteorology and hydrodynamics that accompanied the bloom.

### 4.1. Climatic anomalies and *D. acuta* bloom

During the last 3–4 decades, northwest Patagonia has experienced a decline in summer and autumn precipitation of 5–7% per decade (Aguayo et al., 2019; Boisier et al., 2018; Garreaud et al., 2013). This is well illustrated by the DJF rainfall accumulation at the reference station in Puerto Montt (42°S) during the study period (Fig. 10A). This drying trend has been attributed to long-term changes of the Southern Annular Mode (SAM), characterized by a circumpolar ring of increased pressure



**Fig. 8.** A) Profile and total amount of lipophilic toxins per phytoplankton net-tow. (B–C) Profile and total toxin per ml in concentrated bottle samples obtained on 22<sup>th</sup> February and 12<sup>th</sup> March, 2018.

at midlatitudes (Boisier et al., 2018; Gillett et al., 2006). During austral summer (Fig. 10B) this trend has been attributed to stratospheric ozone ( $O_3$ ) depletion and increased Greenhouse Gas (GHG) effect (Arblaster and Meehl, 2006; Gillett and Thompson, 2003; Gonzalez et al., 2014). This trend and the subsequent droughts in southern Chile are expected to continue during the rest of the 21<sup>st</sup> century (Aguayo et al., 2019; Boisier et al., 2018).

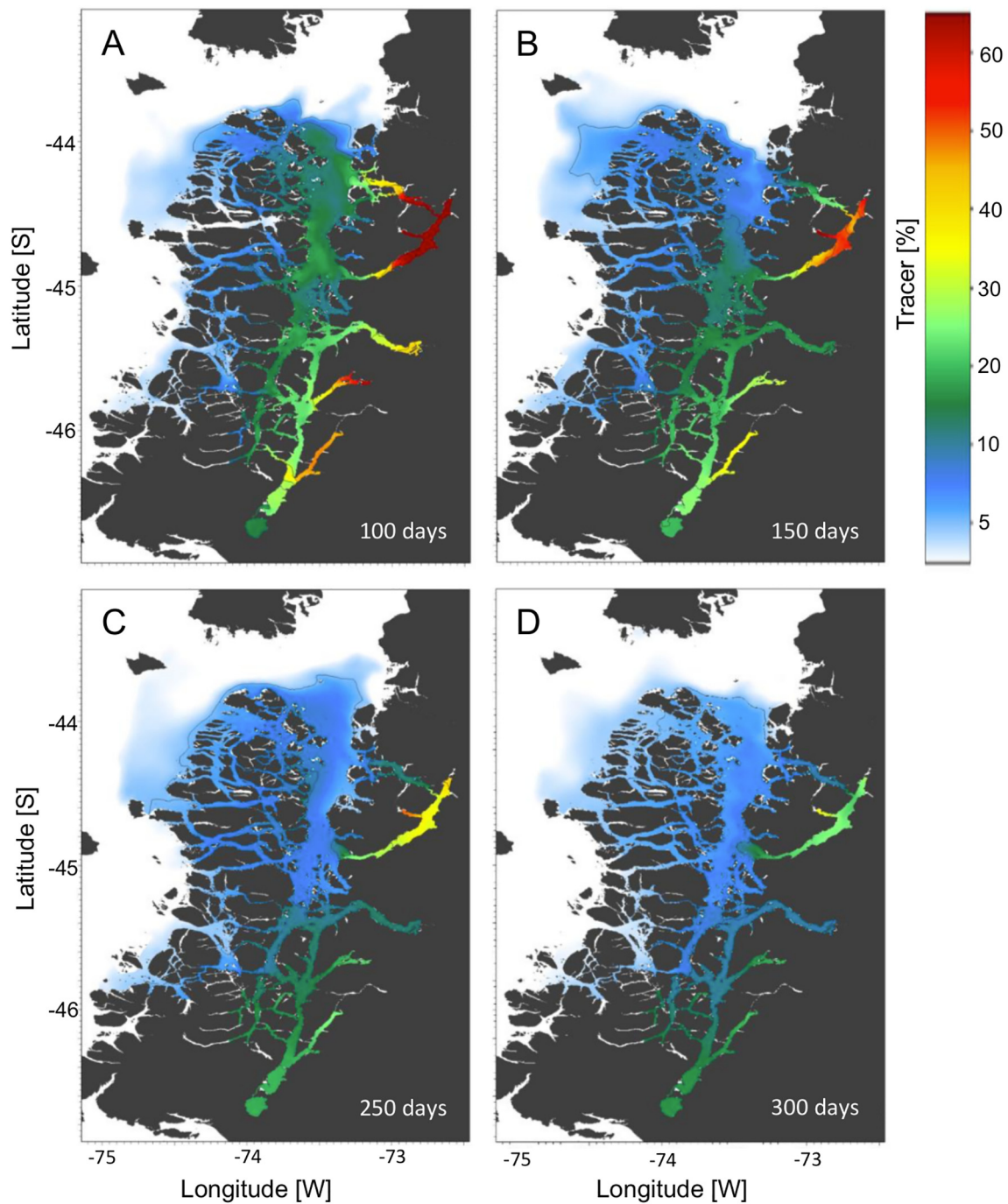
Dry conditions in summer 2018 were consistent with the positive phase of SAM (Fig. 2D) when its index reached +1.1 hPa, well into the upper quartile of the historical distribution (Fig. 10B). The summer drought was interrupted twice by moderate rainfall in December 2017, but then became firmly established and also brought warmer than normal conditions during February 2018. The SAM impact was strong enough to cancel the expectation of a wetter than average summer linked to moderate La Niña conditions in that period (Montecinos and Aceituno, 2003).

At the mesoscale, meteorological conditions in 2017–2018 in the Aysén region were favourable for intensification of the haline stratification in spring and thermal stratification in summer. By mid-February 2018, a sea surface temperature (SST) maximum of 18 °C – an anomalous value for this area – was recorded during the 25 km sampling transect along PF. These temperatures and associated thermal stratification strengthened the permanent haline stratification of the fjord. Similar hydroclimatic conditions were observed during summer 2016, when major blooms of the phytoplankton *Pseudochattonella verruculosa* and the dinoflagellate *Alexandrium catenella* developed and led to large economic losses and social unrest in coastal areas of Los Lagos and Aysén

regions (Álvarez et al., 2019; Díaz et al., 2019a; León-Muñoz et al., 2018; Mascareño et al., 2018). Summer (DJF) rainfall values in Puerto Montt in 2016 were quite similar (ca. 130 mm, see Fig. 10A) to those observed in 2018, and both years were characterized by a positive SAM phase. Nevertheless, there were important differences between these two years. The summer 2016 drought was associated with a coincidence of positive SAM and strong El Niño conditions, affected all western Patagonia (from 40 to 55°S), and persisted well into autumn and early winter, thus leading to widespread environmental disruption (Garreaud, 2018). The *Pseudochattonella* bloom started in late February and peaked the first week of March. In contrast, in summer 2018, ENSO and SAM had opposite signs, and local hydroclimatic anomalies affected only the northern half of western Patagonia. The *D. acuta* bloom, slow growing compared with *Pseudochattonella*, grew exponentially in January and reached its maxima in retention areas in February.

#### 4.2. High water residence time selects hot spots for low biomass harmful algae blooms

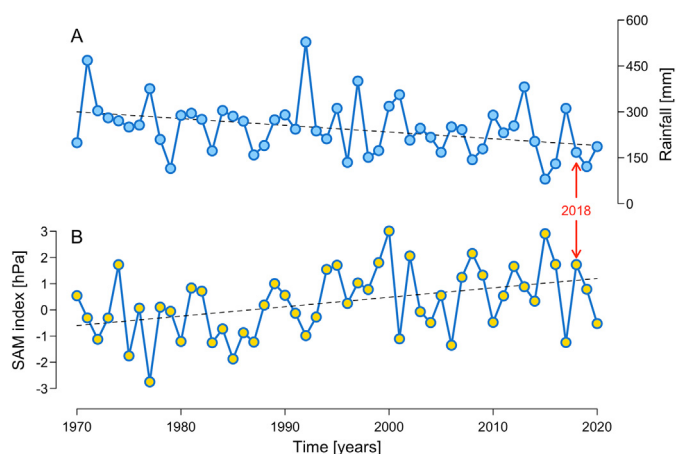
Water residence time in the system, water exchange with adjacent shelf waters and freshwater inputs from rivers, rainfall and glacier ice melt were identified as key issues to help explain development of HAB events in fjords and coastal embayments (Roy et al., 2018). Álvarez-Salgado et al. (2008) linked increased frequency of HABs in the Galician Rías, northwest Spain, with reduced upwelling and flushing rates (increased water residence time) of the Rías over a 40-year period.



**Fig. 9.** Percentage tracer for channels and fjords from Aysén Region obtained to estimate water renewal time for 1 year simulation: A) 100; B) 150; C) 250 and D) 300 days. Only values >5% are represented.

Weaker upwelling and reduced flushing favours thermal stratification and regenerated production leading to a decline in total centric diatoms abundance and an increase of dinoflagellates and pennate diatom *Pseudo-nitzschia* blooms (Pérez et al., 2010). A numerical model applied to all the fjords from the Aysén region showed that flushing rates in PF were two to three times lower than in the other fjords (Aysén, Quitralco and Culquelan fjords) (Pinilla et al., 2019). A complete renewal of PF was estimated to occur every 250 days, and every 300 days in Magdalena Sound. Distributions of *D. acuta* from monthly monitoring in the entire Aysén region confirmed PF and Jacaf Channel as epicentres of *D. acuta* bloom development. Thus, the areas with highest water residence time coincided with the stations where *D. acuta* cell maxima were detected.

PF is the only fjord in the region connected with oceanic waters, through the Jacaf Channel. A crucial question in *Dinophysis* and other holoplanktonic dinoflagellate population dynamics is the location of the “pelagic seed banks” (sensu Smayda, 2002), i.e., overwintering cells that remain in the water column and act as inocula for bloom initiation. Off southwest Ireland, *D. acuta* populations develop initially in tidal fronts, with good in situ growth and feeding, as the water warms and a seasonal pycnocline is established (Raine et al., 2016). Late summer upwelling fronts have similarly been proposed as the initiation site of *D. acuta* populations in northwest Iberia (Díaz et al., 2019b; Moita et al., 2006). This species has a neritic distribution compared with the more coastal *D. acuminata* (Reguera et al., 2014). In the Aysén region, entry of oceanic waters into PF would provide *D. acuta* inocula from offshore populations.



**Fig. 10.** Austral summer (December–January–February) series of A) cumulative rainfall at Puerto Montt (El Tepual station: 41.4°S, 73.1°W, 85 m ASL) and B) Southern Annular Mode index.

#### 4.3. *Dinophysis acuta* bloom development and local environmental conditions

*Dinophysis* species are obligate mixotrophs that require specific ciliate prey to grow (Díaz et al., 2020; Hansen et al., 2013; Park et al., 2006). Field incubations with labelled nitrogen compounds in two upwelling areas (Seeyave et al., 2009, 2013) showed their preference for ammonia and organic nitrogen sources and identify *Dinophysis* as a high affinity strategist, adapted to nutrient-poor environments with regenerated nitrogen sources. These observations are supported by laboratory experiments (Hattenrath-Lehmann and Gobler, 2015; García-Portela et al., 2020) that showed no uptake of nitrate by these species. *Dinophysis acuminata* and *D. acuta* form low biomass HABs ( $10^3$ – $10^4$  cells  $L^{-1}$ ), and it is widely accepted that their bloom initiation and termination are driven by physical factors, i.e. the onset of water column stratification and turbulent mixing respectively (Maestrini, 1998; Reguera et al., 2012). *Dinophysis* blooms around southwestern Ireland occur from June to September when the water column is thermally stratified (Raine et al., 2010). In places where the pair *D. acuminata*/*D. acuta* is the main cause of toxic outbreaks, *D. acuminata* growth starts after the onset of a shallow thermohaline stratification in early spring, whereas *D. acuta* usually appears in late summer when thermoclines are deeper, and has a shorter growing season (Moita et al., 2006; Reguera et al., 1995). Therefore, although the initiation of the population growth for both species is associated with stratification, they have different adaptations to light (intensity and quality) and turbulence (García-Portela et al., 2018, 2019) and require different patterns in water column structure. High density blooms of *D. acuta* in NW Iberia have been related to hot dry summers allowing stable thermoclines, combined with moderate upwelling which ensures nutritional sources for their prey (Díaz et al., 2016; Escalera et al., 2010).

*Dinophysis acuta* strains in the Chilean fjords thrive with salinities much lower than those met by European strains in their shelf water habitats (>32 psu), but temperatures in the layers where their maxima are found are similar, in the 15.5–17 °C range. The exceptional *D. acuta* bloom described here has features in common with blooms in Western Europe, i.e. identification of positive SST anomalies and deeper pycnoclines, their location in relation to the pycnocline, and their growth season in late summer. These conditions should not be confused with those associated with advection of shelf populations into coastal embayments following wind reversals in southwestern Ireland (Raine et al., 2010), Scotland (Whyte et al., 2014) and northwestern Iberia (Escalera et al., 2010).

#### 4.4. Lipophilic toxins profiles during *D. acuta* blooms

Different species/strains of *Dinophysis* produce different proportions of lipophilic toxins and thus, their effect on shellfish resource is very variable. Toxin accumulation is also dependent on shellfish species uptake and biotransformation rates (Reguera et al., 2011). Prediction of the impact of *Dinophysis* blooms in a given area requires knowledge of the local strains' toxin profiles and ranges of variability in cell contents. Unambiguous results require analyses of individually picked cells of *Dinophysis* from field populations or even better, establishment of mixotrophic cultures of local strains and their analyses. Until present, cultures of *Dinophysis* have not been established in Chile, but analyses of picked cells of *D. acuminata* from north (Coquimbo) (Blanco et al., 2007) and south (Los Lagos) (Blanco et al., 2018; Fux et al., 2011) provinces have been made. In all cases, LC-MS revealed only pectenotoxins in detectable amounts.

In the present study, two *Dinophysis* species co-occurred. Extracellular toxins attached to organic matter in plankton concentrates can give misleading results in relation to the species present (Pizarro et al., 2008; Pizarro et al., 2009). Nevertheless, the February 22<sup>nd</sup> toxin profile corresponded to a concentrated bottle sample in which *D. acuta* made up more than 99% of total *Dinophysis* numbers. Furthermore, the toxin profile of this sample coincided with that from a net-tow sample at the same station which resembled a monospecific culture of *D. acuta* (Fig. S3). It is thus clear that *D. acuta* was the source of the OA, DTX-1 and PTX-2 in that sample.

#### 4.5. Implications for research and monitoring of HAB events

Maximum cell densities of *D. acuta* on February 22<sup>nd</sup> were 70 times higher than the maximum value found in the IFOP monthly monitoring reports. The monitoring maximum appeared in an integrated (0–10 m) hose sample from a station at the head of PF on February 24<sup>th</sup>. At this point it is important to consider the patchy vertical distribution of dinoflagellate populations, in particular low biomass populations of *Dinophysis* species. This behaviour leads to large differences in *Dinophysis* counts depending on the sampling method. Differences between integrated and discrete-depth sampling of *Dinophysis* in an upwelling system with high recurrence of *D. acuminata* were described by Escalera et al. (2012). Hose samplers were recommended in 1986 as a safe method for monitoring purposes to avoid missing patchy populations of dinoflagellates (Lindahl, 1986). The main drawback of this method is that it can underestimate population numbers, in particular when they form thin layers. However, the results from the two sampling methods show similar trends. In the present study, sampling with both bottles and nets showed increased cell densities of *D. acuta* that reached record values at the confluence of the Jacaf Channel with Puyuguapi Fjord. The maximal density of *D. acuta* observed in the 10-y (2006–2016) time series of hose-sample analysis from the IFOP monitoring programme was 6000 cells  $L^{-1}$  in April 2008, at a station located in the Jacaf Channel, i.e., the same area where the monitoring maximum of 12,500 cells  $L^{-1}$ , was observed in summer 2018.

## 5. Conclusions

Between late spring 2017 and summer 2018, a high positive (+1.1 hPa) Southern Annular Mode (SAM) index was the overwhelming large scale source of climatic variability affecting the northern half of Chilean Patagonia. River discharge rates exceeding  $700 \text{ m}^3 \text{ s}^{-1}$  in the ice-melt season (November–December), and positive air temperature anomalies (+1 °C) and drought in summer (despite a moderate La Niña event), strengthened the spring haline stratification common in the fjords and promoted thermal stratification in summer (SST 18 °C). These conditions favoured the initiation (late spring) and exponential growth (summer) of *D. acuta* in Puyuhuapi Fjord, Aysén (XI Region). This population formed a thin layer in late February, the

highest cell density ( $664 \times 10^3$  cells  $L^{-1}$ ) ever recorded for this species, with cell numbers over 50 times higher than the maximum observed in integrated hose-samples from the IFOP monitoring. Lipophilic toxin profiles in net samples dominated by *D. acuta* and in filtered bottle samples from fixed depths with over 99% contribution of *D. acuta* were dominated by DSP toxins (OA + DTX-1) and PTX-2, and confirmed *D. acuta* as a main contributor to DSP events in the region. Model simulations showed PF was the fjord with the highest water residence time in Aysén. This result and the fact that Puyuhuapi's is the only fjord in Aysén with two connections with the open sea help to explain why this fjord, in particular the head of the fjord where it joins Jacaf Channel, is the hot spot of *D. acuta* blooms in the Aysén Region. Since the positive SAM trend in austral summers, and the consequent droughts in northern Patagonia are expected to continue throughout the 21st century, the potential for increased risk of *D. acuta* blooms and DSP events, should be considered when SAM in positive mode occurs combined with La Niña events in the Aysén region.

### CRediT authorship contribution statement

**Patricio A. Díaz:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Iván Pérez-Santos:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Gonzalo Álvarez:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Rene Garreaud:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Elías Pinilla:** Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization. **Manuel Díaz:** Software, Formal analysis, Investigation, Visualization. **Alondra Sandoval:** Software, Formal analysis, Investigation, Visualization. **Michael Araya:** Methodology, Validation, Formal analysis, Investigation, Visualization. **Francisco Álvarez:** Formal analysis, Investigation. **José Rengel:** Formal analysis, Investigation. **Paulina Montero:** Methodology, Formal analysis, Investigation, Writing – original draft. **Gemita Pizarro:** Methodology, Formal analysis, Investigation, Writing – original draft. **Loreto López:** Formal analysis, Investigation. **Luis Iriarte:** Formal analysis, Investigation. **Gabriela Igor:** Formal analysis, Investigation. **Beatriz Reguera:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision.

### 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.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.145621>.

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