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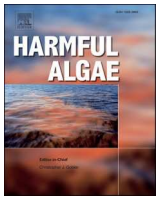

Synchronic distribution of the dinoflagellate *Protoceratium reticulatum* and yessotoxins in a high stratified fjord system: Tidal or light modulation?

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ABSTRACT

Protoceratium reticulatum is the main yessotoxin-producer along the Chilean coast. Thus far, the yessotoxin levels recorded in this region have not posed a serious threat to human health. However, a bloom of *P. reticulatum* during the austral summer of 2022 caused the first ban of shellfish collection, due to the high toxin levels. A bloom of *P. reticulatum* during the austral summer of 2020 allowed an evaluation of the fine-scale distribution of the dinoflagellate during a tidal cycle. High-resolution measurements of biophysical properties were carried out in mid-summer (February 18–19) at a fixed sampling station in Puyuhuapi Fjord, Chilean Patagonia, as part of an intensive 24-h biophysical experiment to monitor the circadian distributions of *P. reticulatum* vegetative cells and yessotoxins. High *P. reticulatum* cell densities ($>20 \times 10^3$ cells L^{-1}) were found in association with a warmer (14.5–15 °C) and estuarine (23.5–24.5 g kg^{-1}) sub-surface water layer (6–8 m). *P. reticulatum* cell numbers and yessotoxins followed a synchronic distribution pattern consistent with the excursions of the pycnocline. Nevertheless, the surface aggregation of the cells was modulated by the light cycle, suggesting daily vertical migration. The yessotoxin content per *P. reticulatum* cell ranged from 9.4 to 52.2 pg. This study demonstrates both the value of fine-scale resolution measurements of biophysical properties in a highly stratified system and the potential ecosystem impact of *P. reticulatum* strains producing high levels of yessotoxins.

1. Introduction

Protoceratium reticulatum (Bütschli., 1885) is a dinoflagellate with a wide geographical distribution (Paz et al., 2008, 2004). Its yessotoxin-producing ability was initially identified in strains from New Zealand (Satake et al., 1997) and later in those from Spain (Paz et al., 2004), the United States (Paz et al., 2007), Japan (Satake et al., 1999), Norway (Ramstad et al., 2001), Greenland (Sala-Pérez et al., 2016), Italy (Ciminiello et al., 2003), Canada and the United Kingdom (Stobo et al., 2003), Argentina (Akselman et al., 2015), and Chile (Álvarez et al., 2011; Díaz et al., 2022; Yasumoto and Takizawa, 1997), among others.

Yessotoxins (YTXs) comprise a group of ~100 lipophilic toxin analogues (Miles et al., 2005). These marine polyether were first isolated in 1986 in Japan, from the scallop *Pactinopecten yessoensis* (Murata et al.,

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1987). They are potent cytotoxins (Pérez-Gómez et al., 2006) with cardiotoxic effects when injected intraperitoneally in mice (Aune et al., 2002; Terao et al., 1990); their oral toxicity, by contrast, is low. Although there are no reports of intoxications in humans (Munday et al., 2008), the known toxic effects of YTXs have led European authorities to stipulate a maximum permitted limit in shellfish of 3.75 mg YTX equivalents kg^{-1} (European Commission, 2013).

Most of the YTX analogues found so far are produced by *Protoceratium reticulatum* (Paz et al., 2008), which releases them into the surrounding aqueous environment (Hess and Aasen, 2007). However, other dinoflagellate species also produce YTXs, including *Lingulaulax polyedra* (Stein) Dodge (Paz et al., 2004), *Gonyaulax spinifera* (Claparede & Lachmann) Diesing (Rhodes et al., 2006; Riccardi et al., 2009), and *Gonyaulax taylorii* (Álvarez et al., 2016).

YTXs have been associated with marine invertebrate mortalities in different parts of the world. Mass mortalities of clams (*Donax serra*) and mussels (*Choromytilus meridionalis* and *Aulacomya atra*) off the coast of South Africa were recorded during a bloom of *P. reticulatum* (Grindley and Nel, 1968; Horstman, 1981). Pitcher et al. (2019) reported the death of millions of farmed abalone in South Africa during a bloom of the dinoflagellates *G. spinifera* and L. *polyedrum*. Mass mortalities of abalone (*Haliotis rufescens*) have also been identified in association with the presence of YTXs produced by *G. spinifera* (Rogers-Bennett et al., 2012). Jurgens et al. (2015) documented a severe mortality event, possibly attributable to a YTX bloom, affecting the urchin *Strongylocentrotus purpuratus* and the starfish *Leptaterias* sp., with a mortality rate of nearly 99 % over ~100 linear km of coastline of California (United States). The Chilean coast has also not been spared, as a mass mortality of invertebrates (sea urchins, cuttlefish and sea stars) in northern Chile during the austral summer of 2019 was shown to be associated with the presence of YTXs Álvarez et al. (2020). In 2022, the first preventive closure due to YTXs was declared in Chile, based on concern regarding the apparently increasing toxicity of these previously unconsidered events.

In the Chilean fjord system, low salinities generate sharp/pronounced haloclines and pycnoclines that hinder vertical mixing between surface and very deep water layers. The physical barriers created by the strong haline stratification directly affect the distribution of planktonic populations, including those of harmful algal bloom (HAB)-forming species (GEOHAB, 2010). Preferred sites of aggregation for HAB-forming dinoflagellates are the "thin layers" of the water column, where the physiological parameters greatly differ from those at other depths (GEOHAB, 2008). Nonetheless, these layers may escape detection by conventional sampling methods (Escalera et al., 2012). While aggregates of *P. reticulatum* in this type of vertical structure has been observed in the Chilean fjord system (Alves de Souza et al., 2014), the spatiotemporal variations of vegetative cells of this species and YTX concentrations during intensive tidal cycles have yet to be studied at the same scale. Thus, in this work we evaluated the fine-scale distribution of *P. reticulatum* vegetative cells and YTXs released by the dinoflagellate during a tidal cycle at a fixed sampling station in Puyuhuapi Fjord, Chilean Patagonia.

2. Material and methods

2.1. Study area

The southern coast of Chile, from 41 to 55 ° S, constitutes one of the most extensive fjord and channel systems in the world (Fig. 1). This highly stratified system, due to heavy freshwater inflow from rivers and glacier melting, has a rugged bathymetry and a highly dissected coastline. In addition, the area is subject to heavy rainfall exhibiting strong seasonal and latitudinal patterns, with an average of 2700 mm $year^{-1}$ and up to 5000 mm in exceptional years (Pickard, 1971; Sauter, 2020). Water column stratification in the fjord is extremely variable and is maximal in the innermost areas subject to tidal energy perturbations (Valle-Levinson, 2010). These features make the Chilean fjords region a unique system for the study of HABs and their physical-biological interactions at different spatial and temporal scales.

The 100-km long Puyuhuapi Fjord, located in the Aysén region (northern Patagonia), forms part of the fjord system. It has two connections with oceanic waters, through the Moraleda Channel at the mouth and the Jacaf Channel close to the head (Schneider et al., 2014). The main freshwater inputs are from riverine inflows and rainfall. The mouth of the main river flowing into Puyuhupi Fjord (Cisnes River, average discharge 218 m^3s^{-1}) is located in the middle reaches of the fjord. The characteristics of the river flow affect the hydrodynamic conditions within the fjord system, including stratification and the water residence time, which in Puyuhuapi Fjord is up to ~250 days (Pinilla, 2018; Pinilla et al., 2019), and directly promote phytoplankton

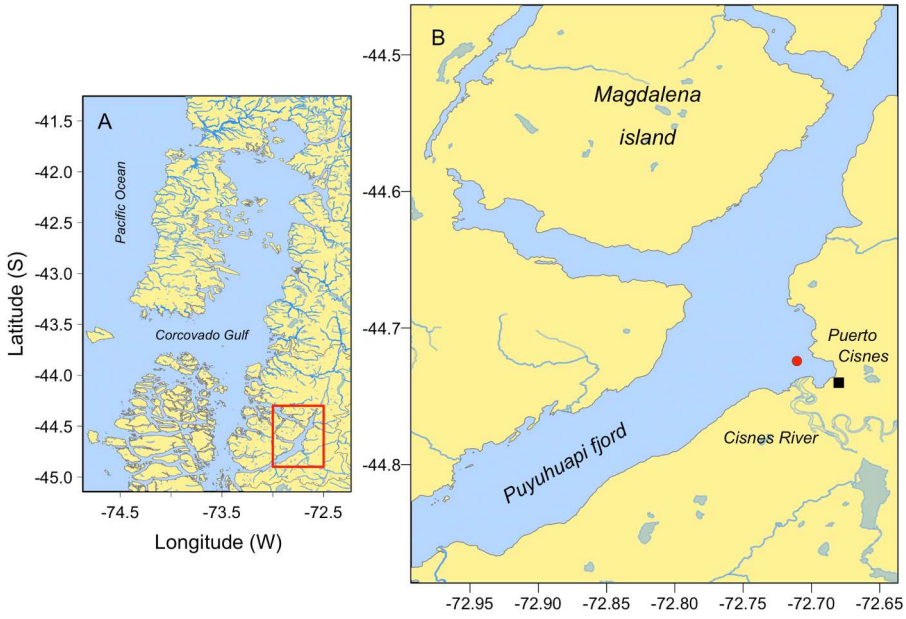

Fig. 1. Map of the study area showing A) the northern Chilean Patagonia fjord system (the box delimits the Puyuhuapi Fjord) and B) Puyuhuapi Fjord, showing the sampling station (full red circle) where our 24-h biophysical experiment was carried out in February 2020.

retention and HAB development (Díaz et al., 2023a, 2021).

The Patagonian fjords are of the two-layered estuarine-type. The two well-defined layers are formed by a superficial and more variable layer, the estuarine water (EW), and a more uniform saltier lower layer, the sub-Antarctic water (SAAW), with salinity > 33 at 150 m depth. An intermediate layer, the modified sub-Antarctic water (MSAAW), with salinities between 31 and 33, is formed by the mixture of the EW and SAAW layers. Furthermore, depending on freshwater inputs, different water masses can be identified within the estuarine surface water: freshwater (FW, salinity < 11), estuarine freshwater (EFW, salinity ranging from 11 to 21), and estuarine saline water (ESW, salinity ranging from 21 to 31) (Pérez-Santos et al., 2014; Schneider et al., 2014).

2.2. Field sampling

High-resolution measurements of physical and biological variables were carried out during a 24-h cycle at a fixed sampling station in Puyuhuapi Fjord, NW Patagonia, to study the small-scale interactions that modulate the distribution patterns of *P. reticulatum* and associated YTXs (Fig. 1B). The 24-h biophysical cycle was carried out from 18 to 19 February 2020.

2.2.1. Hydrographic measurements

Vertical profiles of temperature and salinity were obtained with an RBR Oceanographic CTD profiler, model Concerto 3 (http://www.rbr-global.com). The CTD probe was cast hourly to 50 m depth with a sampling rate of 8 Hz (8 measurements per second). CTD data were processed using the software provided by the manufacturer and depicted using Ocean Data View software version 5.1 (Schlitzer, 2015).

2.2.2. Phytoplankton and yessotoxins

Unconcentrated seawater samples (125 mL) for quantitative analyses of microphytoplankton were collected every 2 h at 2-m intervals from the surface to 20 m depth, using 5-L Niskin bottles, and immediately fixed with neutral Lugol's iodine solution (Lovegrove, 1960).

For YTX analyses, bottle samples were collected every 2 h at 4-m intervals from the surface to 20 m (6 fixed depths); 1-L aliquots were filtered through Whatman GF/F fiberglass filters (25 mm Ø, 0.7 μm pore size; Whatman, Maidstone, England). The filters and filtered material were placed in a cryotube, mixed with 1 mL of analytical grade methanol, and stored in the laboratory at −20 °C until the analysis.

Vertical hauls (0–20 m) with a 20-μm mesh net were also collected every hour for YTX analyses. The entire content of the net was filtered through Whatman GF/F fiberglass filters (25 mm Ø, 0.7 μm pore size). The filters and filtered material were placed in a cryotube, mixed with 1 mL of analytical grade methanol, and stored in the laboratory at −20 °C until the analysis.

For quantitative analyses of microphytoplankton, 10-mL aliquots of unconcentrated, acidic Lugol's-fixed samples were left to sediment for 24 h and then observed under an inverted microscope (Olympus CKX41) using the method described in Utermöhl (1958). To enumerate large species such as *P. reticulatum*, the entire surface of the chamber was scanned at a magnification of × 100, resulting in a detection limit of 100 cells L^{-1}.

2.2.3. Sample preparation and toxin analysis

To extract toxins from the bottle and net-tow samples, the cryotubes were centrifuged (4000 g; 10 min) and the obtained pellet was resuspended in 1 mL of methanol (100 %). Cells in the suspension were disrupted with a Branson Ultrasonic 250 sonifier (Danbury, CT, USA). The extract was clarified by centrifugation (20,000 g; 20 min), filtered through 0.22-μm Clarinert nylon syringe filters (13 mm diameter) (Bonna–Agela Technologies, Torrance, CA, USA), and stored in an autosampler vial at –20 °C until the analysis.

The presence of YTXs and homo-yessotoxins (homo-YTXs) in the extracts was detected by liquid chromatography/high resolution mass spectrometry (LC-HRMS) following the method described by Regueiro et al. (2011) with slight modifications. The instrumental analysis was developed using a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Sunnyvale, CA, USA). A reversed-phase HPLC column Gemini NX-C18 (50 mm × 2 mm; 3 μm) with an Ultra Guard C18 column (Phenomenex, Torrance, CA, USA) was used. The flow rate was set to 0.35 mL min^{-1} and the injection volume was 10 μL. The mobile phase was used in gradient mode as follows: 81 % of eluent A (100 % water containing 6.7 mM NH4OH) and 19 % of eluent B (90 % acetonitrile: 10 % water with 6.7 mM NH4OH) held for 1 min, followed by a linear increase to 95 % B for 5 min, a 2-min hold, and then a return to the initial condition of 19 % B. The column was re-equilibrated for 5 min.

YTXs and homo-YTX were detected using a HRMS system (Q Exactive Focus) equipped with an electrospray interphase HESI II (Thermo Fisher Scientific, Sunnyvale, CA, USA) operated in negative ionization mode with a spray voltage of 3 kV and in positive ionization mode with a spray voltage of 3.5 kV. The temperature of the ion transfer tube and the HESI vaporizer was set at 200 °C and 350 °C, respectively. Nitrogen (>99.98 %) was employed as the sheath gas and the auxiliary gas, at pressures of 30 and 4 arbitrary units, respectively. Data were acquired in selected ion monitoring (SIM) and data-dependent ($ddMS^2$) acquisition modes (for quantification and confirmation, respectively). All analyses were performed with a mass inclusion list, including the precursor ion masses, expected retention time window, and collision energy for each toxin. In SIM mode, the mass was set to 570.2330 *m/z* and 577.2396 *m/z* for YTX and homo-YTX, respectively. The scan mass range was set at *m/z* 100–1000, with a mass resolution of 35,000, an automatic gain control (AGC) of 2×10^5, and a maximum injection time (IT) of 3000 ms. For $ddMS^2$ the mass resolution was set at 70,000, the AGC at 2×10^5, and the IT at 3000 ms. In both cases, the isolation windows were 2 *m/z*. The toxin concentration in the extracts was quantified by comparing the areas or the peaks obtained in the chromatograms with those of certified reference materials obtained from the NCR, Canada. The method's quantification limit was 8 ng mL^{-1} for YTX and 5 ng mL^{-1} for homo-YTX.

3. Results

3.1. Hydrographic conditions

The hydrographic measurements revealed a strong thermohaline stratification during the 24-h experiment (Fig. 2). A thermal inversion throughout the study cycle was observed, attributable to the proximity of the fixed sampling station to the mouth of the Cisnes River. Thus, a four-layer structure was clearly evidenced: a colder (12.5–14.5 °C) and fresher (salinity < 11 g kg^{-1}) water layer < 2 m thick; a warmer (14.5–15 °C) and saltier (11–21 g kg^{-1}) sub-surface (2–5 m) water layer, the EFW (Fig. 2A, B); from 5 to 25 m, a third, colder (12–14 °C) and saltier (21–31 g kg^{-1}) layer, the ESW; and at depths below 25 m, the MSAAW (salinity 31–33 g kg^{-1}; Fig. 2A, B).

The thermohaline gradient in the surface layer contributed to the strong stratification in the top 5 m, which persisted throughout the experiment (Fig. 2C) and had a maximal buoyancy frequency of up to 120 cycles h^{-1} (Fig. 2D). Below 20 m depth, the buoyancy frequency did not exceed 20 cycles h^{-1}. The isopycnals followed a temporal pattern, with a clear semidiurnal tidal signal.

3.2. Distribution of P. reticulatum and yessotoxins

Both *P. reticulatum* and YTXs were detected throughout the 24-h experiment (Fig. 3). All of the analyzed samples collected at discrete depths (each 2 m) contained evidence of the daily vertical migration (DVM) of *P. reticulatum* during the 24 h biophysical experiment, with cell maxima remaining associated with a warmer (14.5–15 °C) and estuarine (23.5–24.5 g kg^{-1}) sub-surface water layer (6–8 m).

Maximum cells densities of *P. reticulatum* (>10,000 cells L^{-1}) were

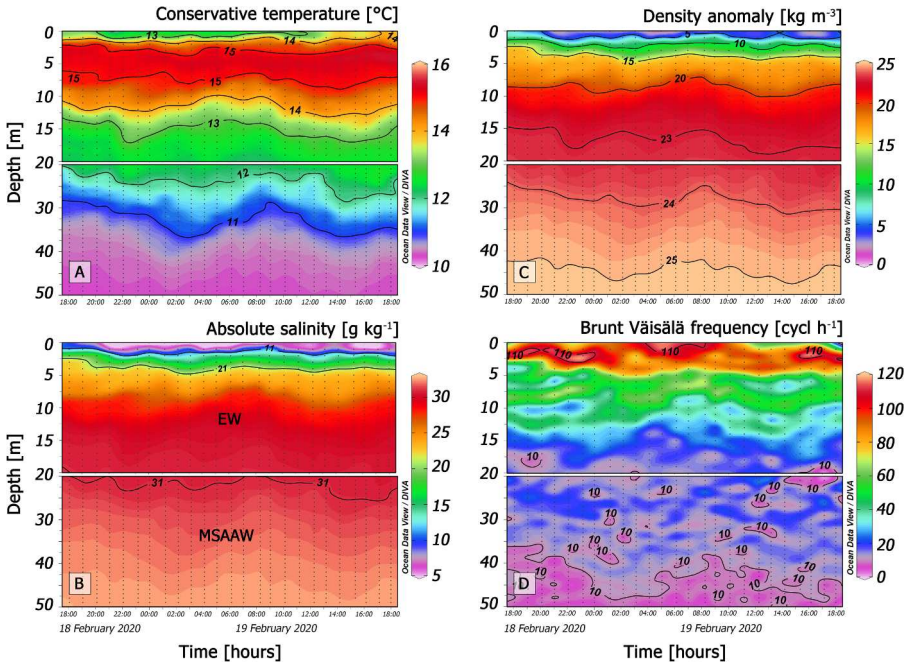

Fig. 2. Vertical distribution (0–20 m) of A) conservative temperature (°C); B) absolute salinity (g kg^{-1}); C) density anomaly (kg m^{-3}); and D) the Brunt–Väisällä frequency (cycles h^{-1}) determined hourly during a 24-h biophysical experiment carried out from February 18th to 19th, 2020 at Puyuhuapi Fjord.

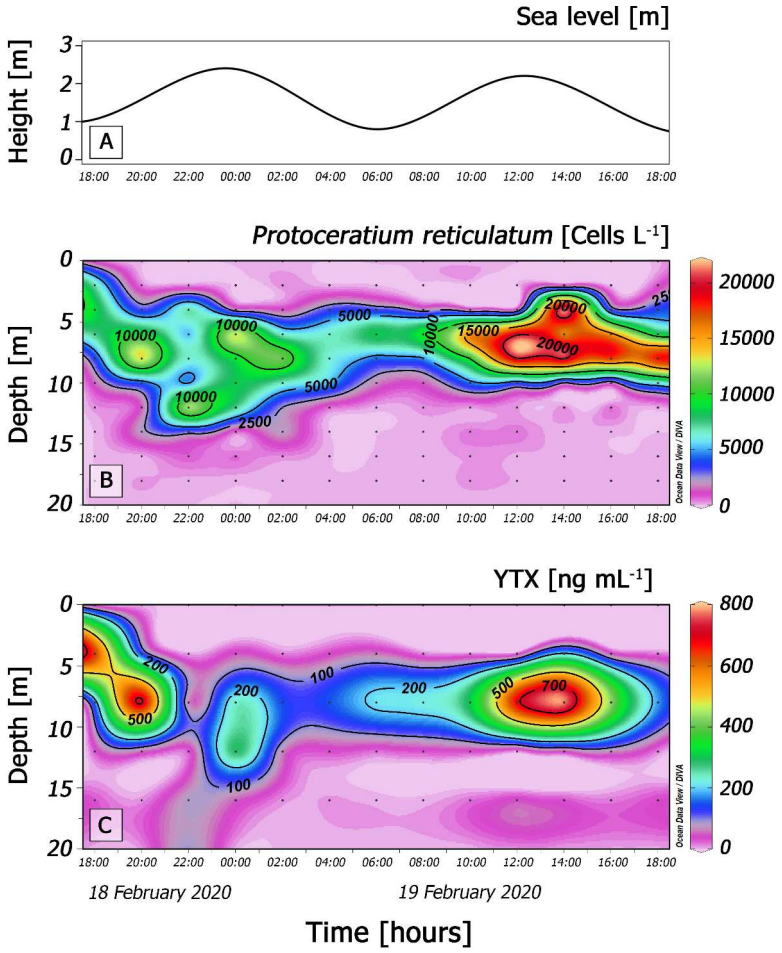

Fig. 3. A) Sea level (m) recorded hourly; B) vertical distribution of *Protoceratium reticulatum* determined every 2 h; and C) yessotoxins from the surface to 20 m depth during a 24-h cell-cycle study carried out from February 18th to 19th, 2020.

concentrated in the sub-surface layer, with a vertical distribution pattern that followed the excursions of the pycnocline (Fig. 3A). Despite this clear pattern, densities of 7000 cells L^{-1} were also recorded very close to the surface (2 m) at the beginning of the cycle (Fig. 3A), associated with a temperature of 15.5 °C and a salinity of 18.8 g kg^{-1} (Fig. 2A-B). By the end of the cycle there was an increase in the cell density, with a maximum $> 20 \times 10^3$ cells L^{-1} (Fig. 3A), suggesting DVM induced by the light cycle and modulated by the undulations of the pycnocline.

The distribution of YTXs followed a spatiotemporal pattern very similar to that of *P. reticulatum* cells, with a significant increase in the concentration of YTXs by the end of the cycle (Fig. 3B). The absence of other YTX-producing phytoplankton species, such as *Lingulaulax polyedra, Gonyaulax spinifera*, and *Gonyaulax taylorii*, in the samples from either the net or the bottles points to *P. reticulatum* as the main, or perhaps the only, causative agent of the detected YTX. Estimates of the YTX content per cell at the layer (8 m) where the cell maximum was recorded ranged from 9.4 to 52.2 pg (Fig. 3B). Likewise, YTXs were detected in 100 % of the net-towed samples, with concentration maxima occurring at 19:00 h, 11:00 h, 13:00 h, and 14:00 h (147.5, 119.7, 138.9, 413.5 ng YTX mL^{-1}, respectively) (Fig. 4).

The LC-HRMS analysis revealed the presence of YTX in all plankton samples in which *P. reticulatum* was present (Fig. 4). The analysis showed a first chromatography peak characterized by a retention time of 4.51 min and with a doubly charged ion $[M + 2H]2-$ at m/z 570.2323. The MS/MS fragmentation mass spectrum of the parent mass at 570.2323 m/z confirmed the identification of YTX, as the characteristic fragment ions at m/z 467.1679 396.1355 m/z were detected (Fig. 5). No homo-YTX was found in the plankton samples.

4. Discussion

Although *P. reticulatum* and YTXs were first detected in the Chilean fjords systems more than 25 years ago (Lembeye, 2004; Yasumoto and Takizawa, 1997), the physiological conditions, ecology, and environmental forcing that modulate its spatiotemporal distribution patterns in these types of systems are still poorly understood. In 2022, high levels of YTXs caused the first preventive closure of the shellfish harvest in Chile, but whether the high toxicity was part of an increasing trend of *P. reticulatum* in this area or merely a sporadic event remains to be determined. Our study is the first in Chile, and, to our knowledge, in the world, to have examined the 24-h vertical distribution patterns of vegetative cells of *P. reticulatum* and of YTXs in the water column at the same scale during a high-resolution field experiment.

4.1. Synchronic distribution pattern and diel modulation

Microscale physical-biological interactions in fjords and semi-enclosed systems are modulated both spatially and temporally by processes such as turbulence, tidal cycles, and circadian rhythms (GEOHAB, 2010). In this study, the spatiotemporal distribution of *P. reticulatum* cells and YTXs showed a perfect synchrony, thus implicating the dinoflagellate as the main producer of YTX during the study period, since other species known to produce YTXs, such as *Lingulaulax polyedra* (Stein) Dodge (Paz et al., 2004), *Gonyaulax spinifera* (Claparede & Lachmann) Diesing (Rhodes et al., 2006; Riccardi et al., 2009), and *Gonyaulax taylorii* (Álvarez et al., 2016), were not detected.

Erga et al. (2015) investigated a Norwegian strain of *P. reticulatum* and found a DVM-type pattern modulated by positive phototaxis. In a laboratory experiment, the authors simulated stratified marine conditions and observed dense surface patches of *P. reticulatum* cells after 1–2 h of light exposure. In that experiment, the cells needed 4 days to cross a weak (4.5 g kg^{-1}) halocline and 8–10 days to cross a stronger (14.1 g kg^{-1}) halocline. The cell aggregations at the surface observed by Erga et al. (2015) are consistent with the results of our 24-h field experiment, in which cell aggregations were observed between 08:00 and 16:00 h, with a maximum at 12:00 h (Fig. 3A). DVM has also been obsserved for other red-tide microalgae, such as *Dinophysis acuta* (Baldrich et al., 2021), *Chattonella antiqua*, and *Karenia mikimotoi* (Shitaka et al., 2014). Baldrich et al. (2021) during a field experiment of 48 h carried out in summer 2019 at the same fjord. *D. acuta* followed an inverse DVM pattern, with maximum cell densities closer to the surface at night. In that study, carried out at an oceanographic buoy platform located 17 km from the mouth of Cisnes River, the DVM of *D. acuta* was between 4 and 8 m over 12 h and was modulated by prey availability. By contrast, our study was conducted much closer to the river's mouth (2.5 km), where the influence of freshwater is greater. The strong physical barrier generated by the intense halocline during the 24-h cycle (up to 25.6 g kg^{-1} between the surface and 10 m depth) in Puyuhuapi Fjord, attributable to the large freshwater supply (mean 218 $m^3 s^{-1}$) from the Cisnes River, significantly restricted the DVM of *P. reticulatum*.

Guerrini et al. (2007) showed that *P. reticulatum* can grow in over a wide salinity range of 20–44 g kg^{-1}, although the highest growth rate (μ = 0.5) was recorded at a salinity of 22 g kg^{-1}. The authors also determined that cell toxicity changed with changes in salinity and was greater at a salinity of 32, at which YTX production was 21.2 ± 2.6 pg $cell^{-1}$. Our results similarly showed the presence of cells across a wide salinity range, from 3.5 to 32 g kg^{-1}, although the highest cell densities occurred within a narrower range of 20–30 g kg^{-1} (Fig. 6).

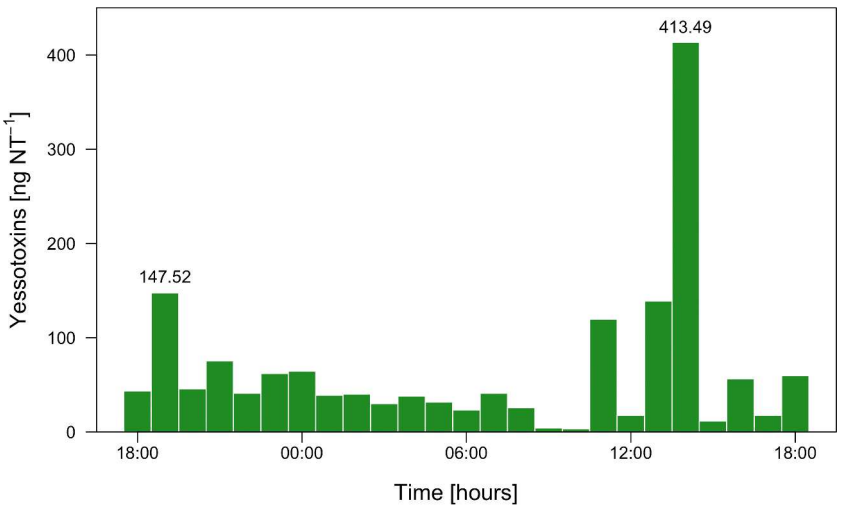

Fig. 4. Hourly distribution of yessotoxin in vertical (0–20 m) plankton net (20 μm) tows (NT) at a fixed station in Puyuhuapi Fjord during a 24-h cell-cycle study carried out from February 18th to 19th, 2020.

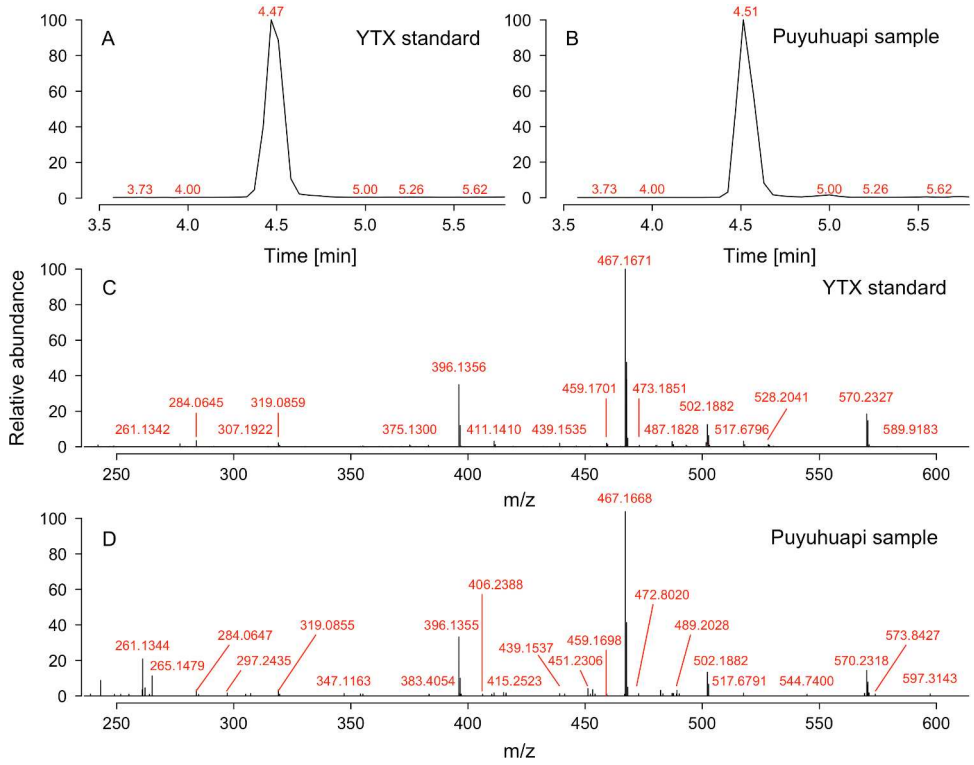

Fig. 5. Selected ion monitoring (SIM) chromatogram of: (A) yessotoxin standard and (B) Puyuhuapi Fjord water sample. Selected product ion spectrum of: (C) yessotoxin standard; (D) Puyuhuapi Fjord water sample.

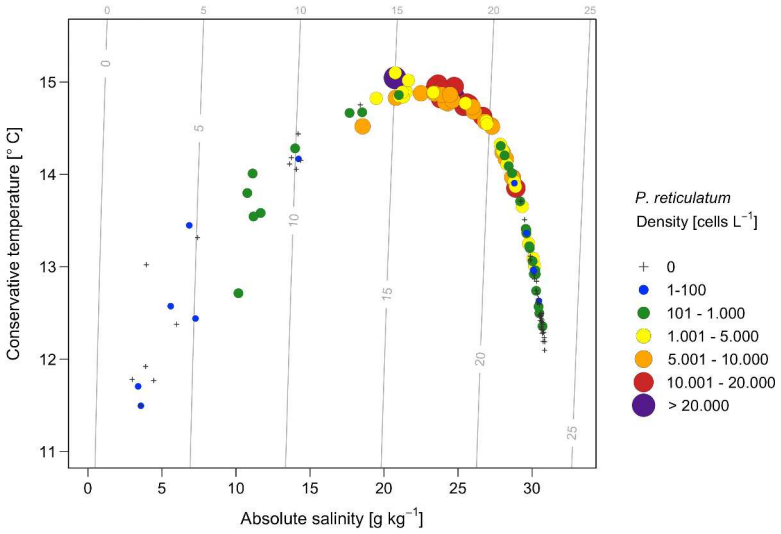

Fig. 6. Cells densities (cells L^{-1}) of *P. reticulatum* plotted over a TS diagram during the 24-h cell-cycle study. Contour lines (gray) represent isopycnals spaced at intervals of 5 σ.

4.2. Toxicity of a local strain and potential implications

In Chilean Patagonia, the presence of YTX in plankton samples from Reloncaví Fjord (Los Lagos region) was reported by Alves de Souza et al. (2014), who measured values of ~0.0032 ng mL^{-1} in association with a *P. reticulatum* bloom (ca. 2.2 × 10^3 cell L^{-1}). The concentration of YTX (maximum of 420 ng mL^{-1}) was lower than that reported in Puyuhuapi Fjord but the cell density was higher (>10,000 cells L^{-1}), suggesting that the Reloncavi Fjord population was less toxic than that from Puyuhuapi Fjord. In samples from the latter, YTX production ranged from 9.4 to 52.2 pg YTX $cell^{-1}$, which was substantially higher than that determined by Álvarez et al. (2011) during a *P. reticulatum* bloom in northern Chile, where production ranged from 0.2 to 0.4 pg $cell^{-1}$. However, in both cases, these values may be inaccurate, because the determinations were made in phytoplankton samples. A precise analysis of the toxin content of *P. reticulatum* strains requires a laboratory study of cultures established from cells isolated along the Chilean coast.

The high cellular YTX content estimated in the *P. reticulatum* population from Puyuhuapi Fjord is similar to that reported in cells selected from natural samples and in cultures from Italy (12 pg $cell^{-1}$) (Ciminiello et al., 2003), Norway (19–34 pg $cell^{-1}$) (Samdal et al., 2004), Spain (2.9–28.7 pg $cell^{-1}$) (Paz et al., 2007), Japan (47–59 pg $cell^{-1}$)

(Suzuki et al., 2007), and the United States (26.6 pg cell^{-1}) (King et al., 2021).

Given the high cellular YTX content and high cell densities of *P. reticulatum*, shellfish consumption may pose a health risk to local consumers. In Norway, a high toxin content (ca. 30 pg cell^{-1}) and low densities of *P. reticulatum* (2200 cell L^{-1}) were shown to be sufficient to contaminate blue mussels with YTX levels above those triggering harvest bans (Aasen et al., 2005). However, even YTX toxin values below regulatory limits could have a significant impact on the shellfish sector economy, by lowering larval survival and viability, as demonstrated for several mollusk species, including the commercially important scallop *Argopecten purpuratus* (Nieves et al., 2024, and references therein). Further research is needed to determine the relationship between *P. reticulatum* blooms, toxin accumulation, and the risk to shellfish banks in the Chilean fjord system.

4.3. Future perspectives

In recent years, blooms of YTX producers such as the dinoflagellate *P. reticulatum,* have threatened the mussel industry in the Los Lagos region. During the summer of 2009, a moderate *P. reticulatum* bloom (2.2×10^3 cell L^{-1}) correlated positively with the moderate to high concentrations of YTX in shellfish (51–496 ng g^{-1}) and in plankton concentrates (3.2 ng L^{-1}) in Reloncaví Fjord, Los Lagos (Alves de Souza et al., 2014). In the summer of 2015, densities of *P. reticulatum* of ~12×10^3 cell L^{-1} were reported at Bahía Huelmo—an important mussel cultivation area within Reloncaví Sound—associated with YTX concentrations in the mussel *Mytilus chilensis* as high as 2.65 mg YTX eq. kg^{-1}, which was close to the regulatory level (3.75 mg YTX eq. kg^{-1}), although a closure decree was not implemented. In the late summer of 2022, an intense bloom of *P. reticulatum* occurred in Reloncaví Sound, with YTX concentrations in the mussel *M. chilensis* reaching 17.02 mg kg^{-1} (Res. Ex. 4502/2022). This event was the first in Chile's history in which a closure decree was triggered due to YTX concentrations exceeding the maximum level permissible for seafood consumed by humans (3.75 mg YTX kg^{-1}). While *Gonyaulax taylorii* was identified as a YTX producer in northern Chile (Álvarez et al., 2016) and its presence in the Chilean fjord system cannot be ruled out, the *P. reticulatum* bloom that was the focus of our study proved to be almost monospecific.

What are the implications of our results regarding recent YTX bloom events in Chile? Our study demonstrated positive phototaxis by *P. reticulatum*, with high-density patches formed at certain hours of the day. This pattern can facilitate toxin accumulation and the transfer of toxins to higher trophic levels (Erga et al., 2015; Manfrin et al., 2012). The estuarine conditions and warmer water determined in our study may also have promoted the bloom initiation and development, as suggested in other studies of the Patagonian fjord system (Díaz and Figueroa, 2023; Díaz et al., 2023b). The relevance of these environmental factors was established in previous reports showing that low salinities and high temperatures are related to a higher cell toxin content, an effect that could be due to less release of YTXs into the extracellular medium, a higher rate of YTX production, or both (Guerrini et al., 2007).

5. Conclusions

A synchronic distribution pattern of the dinoflagellate *P. reticulatum* and YTXs in Puyuhuapi Fjord, a highly stratified system in northern Patagonia, was evidenced during a 24-h biophysical experiment. The absence of other YTX-producing species in the phytoplankton community, such as *Lingulaulax polyedra, Gonyaulax spinifera*, and *Gonyaulax taylorii*, allowed an estimation of the toxin content per *P. reticulatum* cell. Strains of this species from Puyuhuapi Fjord have a cellular YTX content (up to 50 pg per cell) comparable to that of highly toxic strains from Japan. The DVM of *P. reticulatum* was shown to be modulated by positive phototaxis rather than by the tidal signal, although it was significantly restricted by the strong physical barrier produced by the intense halocline (up to 25.6 g kg^{-1} between the surface and 10 m depth). Surface aggregations of *P. reticulatum* cells were recorded between the hours of 08:00 and 16:00, with a maximum at 12:00. In NW Patagonia, a future climate scenario characterized by a reduction in freshwater supplies and an increase in temperature could favor both DVM and surface aggregate formation. These events and their impacts should be the focus of in-depth studies, as both human health and shellfish production could be affected by the associated increases in YTX toxin levels.

CRediT authorship contribution statement

Patricio A. Díaz: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gonzalo Álvarez:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Camila Schwerter:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Ángela M. Baldrich:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Iván Pérez-Santos:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Manuel Díaz:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Michael Araya:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **María Gabriela Nieves:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Sergio A. Rosales:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation. **Guido Mancilla-Gutiérrez:** Writing – review & editing, Writing – original draft, Formal analysis. **Carla Arratia:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Rosa I. Figueroa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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