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Harmful phytoplankton in the Beagle Channel (South America) as a potential threat to aquaculture activities

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ABSTRACT

The Beagle Channel is a remote subantarctic environment where mussel aquaculture initiatives have existed since the early 1990s. Here we analyze phytoplankton biomass and composition, and the occurrence of harmful microalgae species and their toxins at three sites during the period 2015–2016. The occurrence of potentially harmful algae was observed throughout the study period, including toxigenic dinoflagellates such as *Alexandrium catenella* (Group I of the *A. tamarense* complex), *A. ostenfeldii*, *Dinophysis acuminata*, *Gonyaulax spinifera*, *Azadinium* sp., and the diatoms *Pseudo-nitzschia australis* and *P. fraudulenta*. Toxic dinoflagellates were detected in low densities whereas a *Pseudo-nitzschia* bloom was observed in late February. Isolates of *A. catenella* and *P. delicatissima* sensu stricto were phylogenetically characterized. The toxin profile of *A. catenella* was dominated by GTX4, while *P. delicatissima* sensu stricto showed no production of the neurotoxin domoic acid in culture conditions. The results provide base-line information for the management of harmful algal blooms in this little explored subantarctic area.

1. Introduction

Phytoplankton is an essential component of aquatic marine ecosystems, forming the base of food webs and responsible for most of the coastal waters primary production (Graham and Wilcox, 2000). However, a low percentage of species has the ability to produce toxic compounds and/or exert harmful effects that have negative impacts on the environment, causing severe economic losses to aquaculture, damaging tourism and causing episodes of mortality of natural populations of fish, birds and marine mammals (Hallegraeff, 2004). Phycotoxins can also affect humans through the food chain inducing lethal or sublethal responses, such as gastrointestinal or neurological disorders (Fire and Van Dolah, 2012). The number of known toxigenic species is increasing (Moestrup et al., 2018), and almost all coastal areas around the world can potentially be affected by harmful algae (Lassus et al., 2016). Apart from producing toxins, phytoplankton species can cause harm by seriously damaging fish gills either mechanically or through the production of hemolytic substances, or by reaching very high biomass levels causing oxygen depletion, killing fish and invertebrates (Hallegraeff, 2004).

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The occurrence of toxigenic algal blooms in the Beagle Channel was reported for the first time in 1886, when aboriginal inhabitants near Ushuaia (Argentina) were severely poisoned and some of them died after ingestion of mussels (Montoya et al., 2018, and reference therein). More recently, in summer 1992, an intense toxic bloom of *Alexandrium catenella* was recorded with toxicity values reaching up to 127,000 μg STX eq. 100 g^{-1} in mussels (Benavides et al., 1995). The outbreak represented the highest toxicity values recorded in the world during a paralytic shellfish poisoning (PSP) event and resulted in a high number of human intoxications (some people died), and high mortality of fish and marine birds (Carreto and Benavides, 1993). Since then, *A. catenella* blooms of lower intensity have been observed recurrently in the region (Goya and Maldonado, 2014). Usually *A. catenella* and *A. ostenfeldii* are co-occurring, but the latter has only been shown to produce spirolides, not PSP toxins, in culture (Almandoz et al., 2014). By contrast, little is known about the occurrence of other toxigenic species of dinoflagellates and diatoms, which have recently been found in northern waters of the Argentine Sea (e.g. Akselman et al., 2015; Fabro et al., 2016; Tillmann et al., 2016; Almandoz et al., 2017).

Mussel production and artisanal harvesting in the eastern sector of the Beagle Channel (i.e. Brown Bay) are both a food item and a commercial resource for the local population. A project to implement a multitrophic aquaculture system in this area has recently been proposed with economic support from the Argentinean national and provincial governmental sectors. The seasonal distribution of phytoplankton biomass and the general phytoplankton composition has been described in a recent study, also briefly mentioning the presence of potentially toxic algae (Almandoz et al., 2011). Here we provide the first study focusing on the occurrence of harmful microalgae during an annual cycle, together with toxinological analyses. This contributes as baseline information before the implementation of large scale aquaculture farming activities in this little explored subantarctic area.

2. Material and methods

2.1. Field sampling

The sampling area is located in the northeastern sector of the Beagle Channel (Tierra del Fuego archipelago), at the southernmost tip of South America. Sampling was carried out over a whole annual cycle from September 2015 to August 2016 at three coastal sites in Brown Bay where small-scale mussel aquaculture of *Mytilus chilensis* and *Aulacomya atra* is practiced. These sites correspond to inner (A: 54°51′ 32,6″S–67°30′26,4″W), intermediate (B: 54°52′21,3″S–67°32′12,2″W) and outer (C: 54°52′ 54,5″S–67°35′48,9″W) waters of Brown Bay in the northern margin of the Beagle Channel (Fig. 1). Sampling frequency was monthly during most of the sampling period.

Sea water was collected with a 5 L Niskin bottle at 2 m depth from a Zodiac boat for the analysis of chlorophyll *a* (Chl-a) as a proxy of phytoplankton biomass, nutrients (dissolved inorganic nitrogen, phosphate and silicate), particulate organic matter (POM), and suspended particulate matter (SPM). Subsurface water temperature was measured in situ with a mercury thermometer. Aliquots of 250 mL were preserved with acidic Lugol's solution (2% final concentration) for quantitative phytoplankton analyses. In addition, qualitative phytoplankton samples were collected by vertical net tows with a 20 μm net through the upper 5–10 m of the water column and fixed with Lugol's solution. Live aliquots of net and bottle samples collected on 25 September 2015 and 28 April 2016 were kept cooled for cell culture establishment.

2.2. Water quality analyses

Water samples for quality analyses were kept refrigerated (4 °C) and processed immediately after sampling. For dissolved nutrients and POM analyses, aliquots of 1–2 L were filtered onto Whatman GF/C filters and kept frozen (− 20 °C) until analysis. POM was determined following the method described by Strickland and Parsons (1972). Dissolved inorganic nitrogen (DIN) concentration was determined by the micro-Kjeldahl method (APHA-AWWA-WEF, 2005). Phosphate concentration was analyzed with a Perkin Elmer Lambda 25 UV–visible spectrometer, following the ascorbic acid method (APHA-AWWA-WEF, 2005). Silicate concentration was determined with the Macherey-Nagel test kits, approved by the Environmental Protection Agency, which are based on the formation of silicomolybdenum blue (APHA-AWWA-WEF, 2005). For Chl-a analyses, samples of 1 L were filtered through GF/F filters, pigments were extracted in 90% acetone, analyzed using the above-mentioned spectrometer and corrected for phaeopigment content following the method of Lorenzen (1967). For SPM determination, aliquots of 0.5–1 L were filtered through 0.45 μm nitrocellulose membranes and the gravimetric method was applied to determine the final values (Strickland and Parsons, 1972).

2.3. Microscopic analyses

Quantitative and qualitative phytoplankton samples were analyzed by a combination of light and electron microscopy. The abundance (cells L^{-1}) of main phytoplankton taxa in samples collected by Niskin bottles was determined with a phase-contrast Leica DMIL LED inverted microscope according to the procedures described by Utermöhl (1958). Before counting, subsamples of 50 mL were settled for 24 h in a composite sedimentation chamber. The organisms were counted in two stages; at least 300 cells of the dominant taxa were counted in random fields of the chamber to estimate general phytoplankton composition, whereas the whole chamber bottom was scanned to count toxigenic and sparse species. Phytoplankton taxa were identified to the lowest possible taxonomic level. However, some small phytoflagellates which lost their flagella during fixation were included in a single group as “phytoflagellates < 10 μm”.

Further qualitative observations were done with phase contrast, differential interference contrast (DIC) and UV epifluorescence microscopy with two Leica DM2500 microscopes. Dinoflagellate cells were stained with the optical brightener calcofluor-white for epifluorescence microscopic observations of the thecal plates (Fritz and Triemer, 1985).

For diatom identification, an aliquot of each net sample was washed with distilled water several times and then treated to remove organic material (Prygiel and Coste, 2000). Raw (untreated) and cleaned material was mounted on permanent slides with Naphrax mounting medium according to Ferrario et al. (1995). Slides were deposited in the Diatom Collection (LPC) of the Herbarium of the División Científica Ficología, Facultad de Ciencias Naturales y Museo (Universidad Nacional de La Plata). In order to estimate the relative cell abundance of *Pseudo-nitzschia* species during the peak abundance on February 25, all valves present on each of the three treated slides (average of 155 *Pseudo-nitzschia* valves per slide) were enumerated at 1000×. For more in-depth studies, the raw and cleaned materials were mounted onto glass stubs, sputter coated with Au−Pd, and examined using a JEOL JSM-6360 LV (JEOL Ltd., Tokyo, Japan) scanning electron microscope (SEM). In addition, drops of cleaned material were placed on Formvar-coated copper grids, dried, and studied in a JEM 1200 EX II (JEOL Ltd., Tokyo, Japan) transmission electron microscope (TEM).

2.4. Cell cultures

Single cells of *Pseudo-nitzschia* and *Alexandrium* were isolated by micropipette and placed into 96-well plates containing natural seawater filtered by 0.2 μm and f/2 medium enriched with silicate (*Pseudo-nitzschia*) or L1 without silicate (*Alexandrium*). Cultures were maintained at 10 °C (± 0.5), under a 12:12 h light:dark photoperiod cycle. Successful isolates were transferred into 100 and 250 mL Erlenmeyer flasks and maintained under the above growth conditions.

Fig. 1. Map of the study area showing the location of sampling sites (A–C) in the Beagle Channel.

2.5. DNA extraction, amplification, and sequencing

Culture material of a *Pseudo-nitzschia* strain (BC_Psn1) was harvested by centrifugation and frozen at −20 °C. DNA extraction, PCR amplification of the ITS regions of the nuclear rRNA using the primers ITS1 and ITS4 (White et al., 1990), purification and sequencing followed Gai et al. (2018). The sequence was aligned with sequences of other closely related *Pseudo-nitzschia* species. Twenty sequences were included in the alignment comprising 847 base pairs. After excluding ambiguously aligned positions, the remaining 753 base pairs were used in the phylogenetic analyses conducted in PAUP (Version 4.0, Swofford, 2002). Maximum likelihood (ML) analyses were performed using the optimal model found with a 99% level of significance using Modeltest (Posada and Crandall, 1998), and 100 bootstrap analyses were performed to determine the robustness of the tree.

Genomic DNA of an *Alexandrium* strain (CB_Alex1) was isolated with the E.Z.N.A Plant DNA Kit (Omega Bio-Tek Inc., Norcross, USA). The original isolation protocol was modified by insertion of an additional washing step using buffer "SPW wash buffer". A NanoDrop ND-1000 system (Peqlab, Erlangen, Germany) was used to determine the concentration and the purity of the genomic DNA. Phylogenetic inferences were based on analyses of the D1/D2 region of the 28S large subunit (LSU). The fragment of the LSU was amplified with the universal primer set D1R-F (5′ACC CGC TGA ATT TAA GCA TA3′) and D2C-R (5′CCT TGG TCC GTG TTT CAA GA3′) (John et al., 2014). The PCR cocktail of a final volume of 50 µl contained ~20 ng genomic DNA as template, 1× amplification buffer (5 Prime, Hamburg, Germany), 0,1 mM dNTPs (5 Prime, Hamburg, Germany), 0,1 µM of each primer, and 0,05 U of HotMaterTaq DNA polymerase (5 Prime, Hamburg, Germany). The amplification protocol was 2 min at 94 °C, 30 s at 94 °C, 30 s at 55 °C, and 2 min at 68 °C for 35 cycles and an extension for 10 min at 68 °C. The correct size of the amplified PCR-fragment was determined on a 1.5% agarose gel. The PCR-product was purified with the Nucleospin Gel and PCR clean-up kit (Machery und Nagel, Germany) according the manufacturer's protocol and subjected to cloning into a PCR2.1-TOPO vector according to the protocol of the TOPO TA Cloning kit (Invitrogen, USA). Three to five clones per PCR amplicon were picked and sequenced via standard Big Dye Terminator v3.1 cycle sequencing chemistry (Applied Biosystems, Darmstadt, Germany). The resulting sequences were assembled using the DNAStar software package (Lasergene, USA). The LSU sequences obtained in this study were deposited in the GenBank database (Accession numbers will be provided subsequent to acceptance of the manuscript). The sequence of the *Alexandrium* strain CB_Alex1 from the Beagle Channel was compared with the LSU sequence assemblage used to formally revise the *Alexandrium tamarense* species complex taxonomy (John et al., 2014), and other available sequences from Argentine and Chilean waters. MEGA7 (Kumar et al., 2016) was used for sequence alignment. The LSU sequences were aligned using ClustalW. The final alignment for the LSU phylogeny was based on 62 sequences and consisted of 553 positions. The phylogenetic model was selected using the MEGA7 software package. A phylogenetic consensus tree was inferred using the Maximum Likelihood method (ML) based on the Tamura 3-parameter model (Tamura, 1992), with bootstrap values from the ML method (n = 1000 replicates) (Felsenstein, 1985). Branches corresponding to partitions reproduced in < 60% bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0,5849)). The analysis involved 47 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions with < 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position.

2.6. Toxin analyses

Alexandrium (CB_Alex1) and *Pseudo-nitzschia* (BC_Psn1) strains were harvested for toxin analysis during the mid-exponential growth phase and the stationary growth phase, respectively. Cells were concentrated

on Whatman GF/F filters. The analysis of amnesic shellfish toxins (domoic acid) in *Pseudo-nitzschia* was carried out by two analytical methods, the UV detection method (Wright et al., 1995) and the higher sensitivity method, based on the derivatization and fluorescent detection technique (Pocklington et al., 1990). For PSP toxins detection, filters were extracted with acetic acid 0.5 M and analyzed by the post-column derivatization HPLC method of Oshima (1995). The system was calibrated using a toxin standard from the CNRC-NRC National Research Council, Canada.

2.7. Data analysis

Non-parametric Kruskal-Wallis tests were employed to determine differences between water quality parameters (temperature, nutrients, POM and SPM), Chl-a, total phytoplankton and total diatom abundance among sites A, B and C (p critical = 0.05 in all cases).

3. Results and discussion

3.1. Water quality

Sea water temperature ranged between 4.7 (July) and 9.0 °C (February), following a predictable seasonal pattern, with values similar to those reported for Brown Bay for the 2005–2011 period (Gordillo et al., 2015). Temperature decreased from sites A to C (Table 1). DIN values, which varied from 0.2 to 5.5 μM, increased in all sites during spring-summer and were lower than previously reported for the area (Cardona Garzón et al., 2016; Barrera et al., 2017). Phosphate concentrations varied from 0.3 to 1.7 μM, with higher values detected at site C, especially between December and March, while silicate concentrations varied from 2.9 to 6.8 μM, the lower values corresponding to the spring-summer period, in coincidence with diatom abundance peaks (see below). Both these values were similar to earlier descriptions (Cardona Garzón et al., 2016; Barrera et al., 2017). Irregular peaks of inorganic nitrogen (mainly ammonia) and phosphate in Brown Bay have been previously associated with aquaculture activities, including the degradation of faeces of high nutritional value excreted by mussels (Giarratano and Amin, 2010). POM and SPM showed peaks values of 3.2 mgC L^{-1} and 38.2 mg L^{-1} respectively, at site C during the end of April, but showed no clear seasonal patterns. In this case, only 8% of SPM was organic, suggesting that the contribution of inorganic substances could be linked to terrestrial runoff. No significant differences in any water quality variable were detected among sites.

3.2. Phytoplankton biomass, cell abundance and community composition

Chl-a values ranged from 0.32 to 5.55 μg L^{-1}, with the lowest values in autumn-winter (average 0.92 μg L^{-1}) and the highest in spring-summer (average 2.36 μg L^{-1}). This marked seasonal phytoplankton cycle, with higher biomass accumulation between October and March, and lower values during the rest of the year has previously been observed in nearby waters of Punta Paraná, i.e. close to site C, during 2006–2007 (Almandoz et al., 2011). Chl-a peaks were mainly observed at sites A or C, although no significant differences in Chl-a values were observed among sites (Kruskal-Wallis H = 3.06; p = 0.22).

The highest Chl-a peaks (> 5 μg Chl-a L^{-1}) in spring (October 7) and summer (February 25) at sites A and C respectively were associated with diatom blooms, mainly represented by species of *Thalassiosira* and *Chaetoceros*. The occurrence of diatom blooms of the genus *Chaetoceros* and to a lesser extent *Thalassiosira* seems to be a recurrent phenomenon in the Beagle Channel, as also revealed by previous studies (Hamamé and Antezana, 1999; Avaria et al., 2003; Pizarro et al., 2005; Almandoz et al., 2011). A significant and positive correlation was found between diatom density and Chl-a values (Spearman correlation $r_s = 0.58$; $p < 0.05$).

Total phytoplankton cell abundance showed a marked peak at the end of October, reaching up to 15–17 × 10^6 cells L^{-1} in all three sampling sites. The bloom was dominated by small (< 5 μm) unidentified phytoflagellates, observed both solitary or in colonies resembling *Phaeocystis*. High-density blooms of *Phaeocystis* may affect fisheries through alteration of fish taste, fish mortality, and may have negative impacts on shellfish growth and reproduction (Schoemann et al., 2005 and references therein). The highest cell densities of the *Phaeocystis*-like phytoflagellates were usually detected at site A throughout the year, but no significant differences were observed among sites (Kruskal-Wallis H = 2.96; p = 0.23). As far as we know there are no previous reports of blooms of small phytoflagellates with such high densities (1.7 × 10^7 cells L^{-1}) for the area.

The most abundant phytoplankton groups were, in decreasing order, unidentified small phytoflagellates (< 10 μm), diatoms and cryptophytes, which together accounted for > 90% of cell abundance in all three sites throughout the study. Dinoflagellates, prasinophytes, euglenophytes and silicoflagellates were detected in lower abundances.

Diatom densities varied from 1.3 × 10^3 (site B, July 11) to 2.1 × 10^6 cells L^{-1} (site A, November 23). Higher densities were usually found at site A, although no significant differences were observed among sites (Kruskal-Wallis H = 4.95; p = 0.08). The diatom genera *Chaetoceros*, *Thalassiosira*, *Skeletonema*, *Guinardia* and *Pseudo-nitzschia* were the most important in terms of relative cell abundance. Their relative contribution showed considerable variation among sites. For example, the genus *Chaetoceros* dominated diatom assemblages during the first three sampling dates at site C, whereas it was mainly the genus *Thalassiosira* that dominated during the same period at sites A and B. Likewise, a marked predominance of *Guinardia delicatula* was found only at site C, whereas *Chaetoceros* and *Pseudo-nitzschia* species dominated at sites A and B on December 16.

Several *Chaetoceros* species usually co-occurred, with the most abundant being *C.* aff. *socialis*, reaching a maximum concentration of 1.8 × 10^6 cells L^{-1} at site A on February 16. Other important species in terms of abundance were *C.* aff. *debilis* and *C.* aff. *neglectus* (max. around 3.2 × 10^5 cells L^{-1}) whereas the harmful species *Chaetoceros convolutus* was occasionally found and in low densities (max. 6.2 × 10^3 cells L^{-1}). *Chaetoceros* species are characterized by the presence of hair-like extensions called setae, which can cause damage to fish gills, such as

Table 1
Range and average values of temperature, dissolved inorganic nitrogen (DIN), phosphate, silicate, particulate organic matter (POM), and suspended particulate matter (SPM) recorded at sampling sites (A–C) in the Beagle Channel.

	Site A		Site B		Site C	
	Range	Average	Range	Average	Range	Average
Temperature (°C)	5.3–9.0	7.0	5.1–8.7	6.8	4.7–8.0	6.3
DIN (μM)	0.25–5.00	2.7	0.50–4.50	2.7	0.25–5.5	2.6
Phosphate (μM)	0.31–1.30	0.7	0.52–1.04	0.8	0.44–1.69	1.0
Silicate (μM)	3.01–6.65	4.6	3.13–6.75	4.8	2.87–6.47	4.9
POM (mgC L^{-1})	0.4–2.3	1.0	0.3–1.2	0.7	0.3–3.2	1.0
SPM (mg L^{-1})	7.4–15.5	10.1	6.4–2.1	11.6	6.6–38.2	12.0

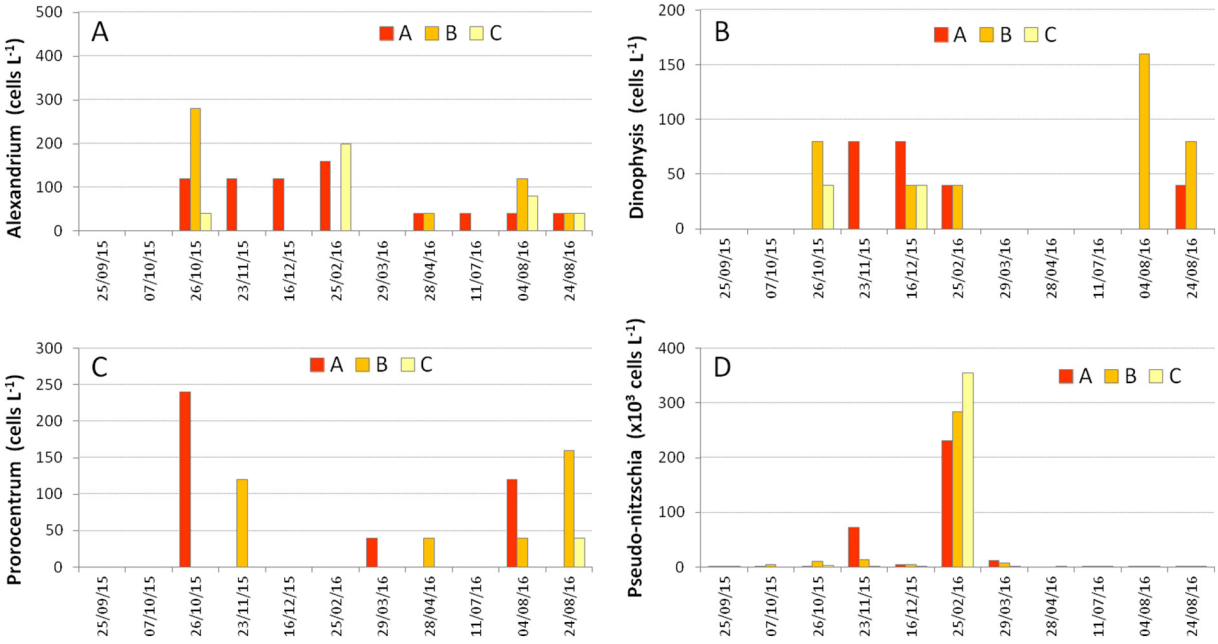

Fig. 2. Cell abundance of main toxigenic taxa observed in the three sampling sites in the Beagle Channel. A) *Alexandrium*; B) *Dinophysis*; C) *Prorocentrum*, and D) *Pseudo-nitzschia*.

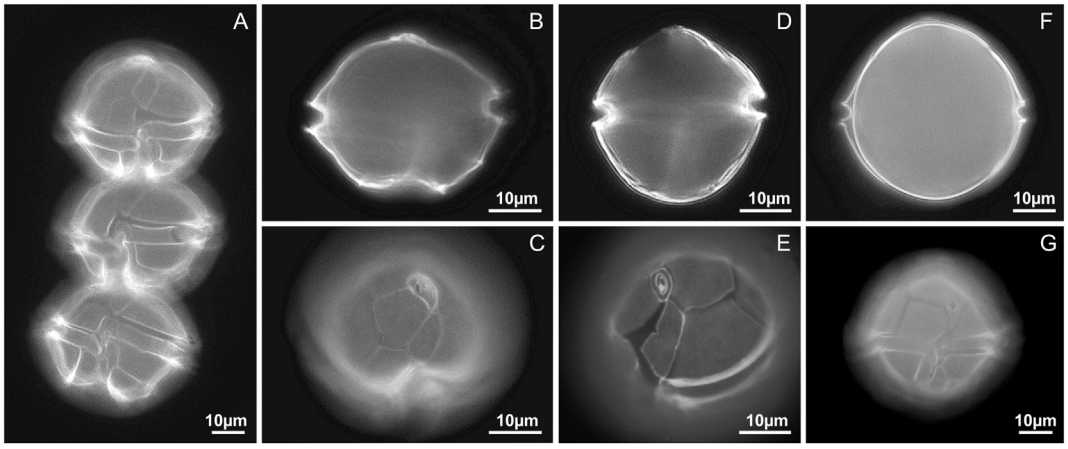

Fig. 3. Fluorescence micrographs of *Alexandrium* specimens from the Beagle Channel. A–E) *Alexandrium tamarense* species complex. Note the differences between the most commonly observed cells, i.e. flattened anterior-posteriorly and without a ventral pore in the plate 1′ (A–C) and the more rare isodiametric cells with a ventral pore in the plate 1′ (D–E). F–G) *Alexandrium ostenfeldii*.

capillary hemorrhage, lack of proper gas exchange, mucus secretion, and secondary infections (Lassus et al., 2016). Massive deaths of several commercial fish have been reported associated with *Chaetoceros* blooms, and laboratory exposure of juvenile Chinook and Coho salmon to *C. convolutus* caused mortality at concentrations as low as 1.5×10^3 cells L^{-1} (Landsberg, 2002 and references therein).

Dinoflagellate abundance ranged from 80 to 7.2×10^4 cells L^{-1}, representing an average of 2% of total phytoplankton, with a maximum of 6.3% on July 11 at site C. Small (< 20 µm) naked specimens were the most abundant dinoflagellates, followed by unidentified species of *Gyrodinium*, *Katodinium*, *Protoperidinium* and *Tripos*. Low relative abundances of dinoflagellates have previously been observed in the study area (Almandoz et al., 2011).

3.3. Occurrence of toxigenic species

3.3.1. Alexandrium species

The genus *Alexandrium* was observed in at least one sampling site in eight of the eleven sampling dates. The highest density, i.e. 280 cells L^{-1}, was recorded on October 26 in site B (Fig. 2a). PSP events worldwide are usually associated with densities of toxigenic *Alexandrium* species from values in the order of 10^3–10^4 cells L^{-1} (Reguera, 2002), and blooms of up to 8.2×10^5 cells L^{-1} have previously been observed in the Beagle Channel (Benavides et al., 1995).

Detailed plate examination of *Alexandrium* specimens with fluorescence microscopy revealed the occurrence of three different morphospecies, which conformed to classical species descriptions (i.e., Balech, 1995) of *A. catenella* (Fig. 3 a–c), *A. tamarense* (Fig. 3 d–e) and *A. ostenfeldii* (Fig. 3 f–g). Traditionally, the cell shape, the presence of a ventral pore in the plate 1′ and the ability to form long chains were considered as the main characters to distinguish the morphologically defined species *A. catenella, A. tamarense* and *A. fundyense* (Balech, 1995), which conform the *A. tamarense* species complex. But more recent molecular studies have revealed that these morphological characters do not reflect genetic differences and demonstrated that the *A. tamarense* species complex is composed of five groups/clades of cryptic

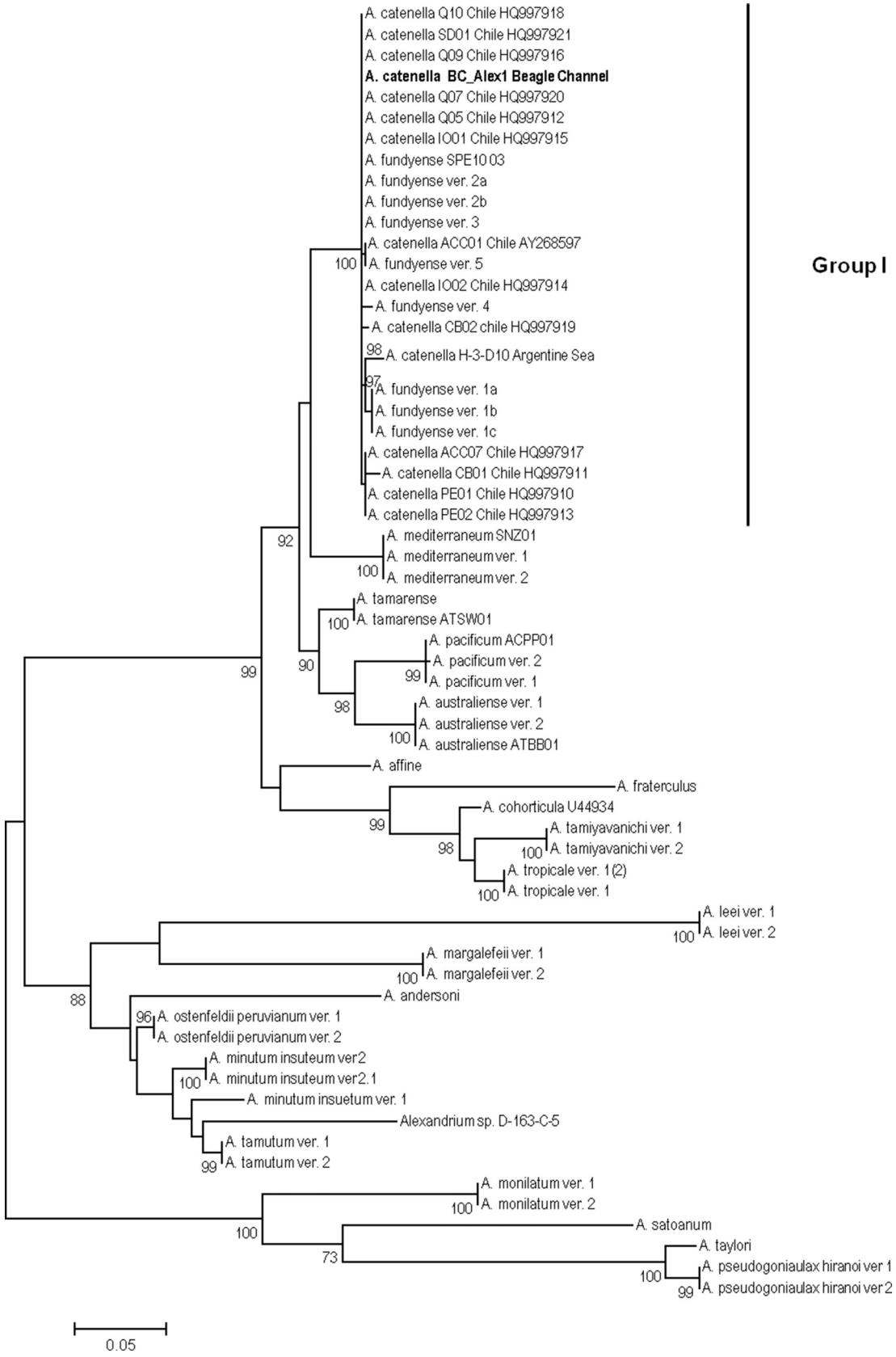

Fig. 4. Placement of *A. catenella* strain CB_Alex1 from the Beagle Channel within the phylogenetic tree of the *A. tamarense* species complex based on the D1-D2 LSU rDNA sequences obtained in this study and published previously (Varela et al., 2012; John et al., 2014). The phylogenetic tree was constructed using the maximum-likelihood method. Numbers at the branches indicate the percentage of bootstrap support (n = 1000).

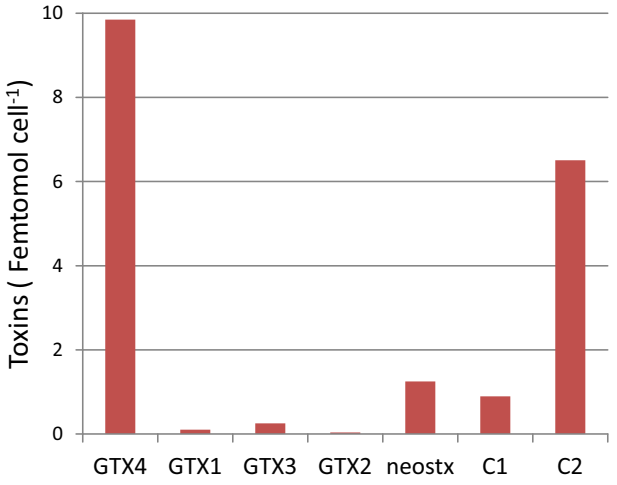

Fig. 5. Cellular content of Paralytic Shellfish Toxins in the strain of *A. catenella* isolated from the Beagle Channel.

species (John et al., 2003, 2014; Lilly et al., 2007; Anderson et al., 2012; Wang et al., 2014).

After alignment and phylogenetic placement of the *Alexandrium* sequence CB_Alex1 isolated in this study with other *Alexandrium* taxa (e.g. Varela et al., 2012; John et al., 2014) the D1-D2 LSU rDNA clustered within *Alexandrium* ribotype Group I, consistent with *A. catenella* (Fig. 4). The cluster contained also sequences of *A. catenella* strains isolated in Argentinean and Chilean waters. Genetic distances in this cluster were low (< 0.02) in general and the sequence of *A. catenella* CB_Alex1 displayed highest similarities to *A. catenella* sequences isolated in more northern areas along the Chilean coast. Previous molecular studies have shown that strains of *A. catenella* from the Beagle Channel and Chilean waters from the southeast Pacific coast clustered in Group I of the *A. tamarense* species complex (Aguilera-Belmonte et al., 2011; Varela et al., 2012), as well as strains from Argentine waters from the Northern Patagonian Shelf, in the southwestern Atlantic (Penna et al., 2008; Fabro et al., 2017; Guinder et al., 2018). However, the sequence of *A. catenella* H-3-D10, isolated in the Argentinean Sea segregated in a cluster with *A. fundyense* ver. 1a-1c (=*A. catenella*) that was well supported by a bootstrap-value of 97%, while the remaining sequences from the Chilean coast (Puerto Eden) and the Beagle Channel segregated in a cluster with relatively low bootstrap support (> 67%). This segregation might suggest regional variability within *Alexandrium* ribotype group I, in accordance with Varela et al. (2012), which has to be further elucidated and specified by assessing sequence variability of significantly more strains of *Alexandrium* ribotype group I isolated in South American waters, and adding phylogenetic analyses of the more variable ITS.

Most field specimens of the *A. tamarense* species complex observed in this study were somewhat flattened anterior-posteriorly and without a ventral pore in the plate 1′, sometimes grouped in short chains (Fig. 3a–c). These morphological characteristics are in accordance with the morphological description of *A. tamarense* species complex group I provided by John et al. (2014), i.e. *A. catenella* according to Prud'homme van Reine (2017). However, more isodiametric cells with a ventral pore in the plate 1′ (Fig. 3d–e) were also occasionally detected. This last morphotype seems to be the most common in Argentine waters from the southwestern Atlantic (Fabro et al., 2017), in contrast with that observed in Chilean waters from the southeast Pacific coast (Aguilera-Belmonte et al., 2011).

Almost all strains from Group I of the *A. tamarense* species complex tested worldwide for the production of PSP toxins (i.e. saxitoxins and derivatives) have been found to be toxic, but their toxin profiles are variable (John et al., 2014 and references therein). Analysis of the toxin composition of the *A. catenella* strain CB_Alex1 showed a toxic profile dominated by GTX4 (Fig. 5), in agreement with strain CB02 from the Beagle Channel (Varela et al., 2012). By contrast, strains of *A. catenella* from southern waters of Argentina and Chile are usually dominated by the less toxic N-sulfocarbamoyl derivatives C1 + C2 (Montoya et al., 2010; Varela et al., 2012), which were less important in the Beagle Channel. In addition, the strain from the Beagle Chanel contained a

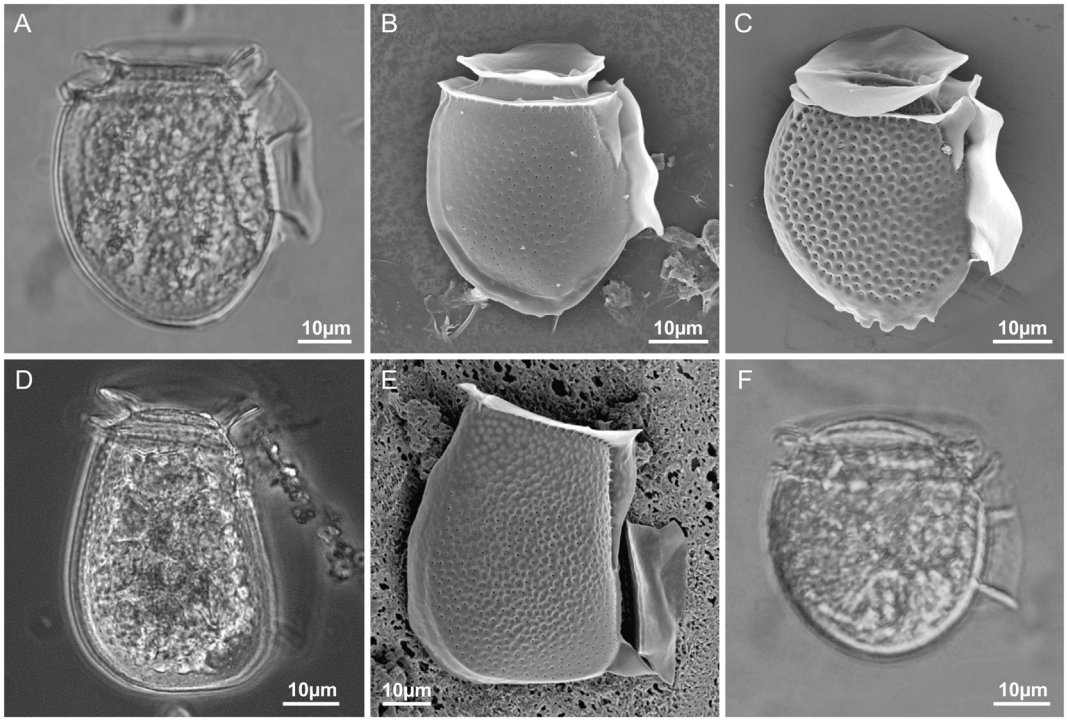

Fig. 6. Light and electron micrographs of *Dinophysis* and *Phalacroma* specimens from the Beagle Channel. A–C) *Dinophysis acuminata*; D–E) *Dinophysis truncata*; F) *Phalacroma rotundatum.*

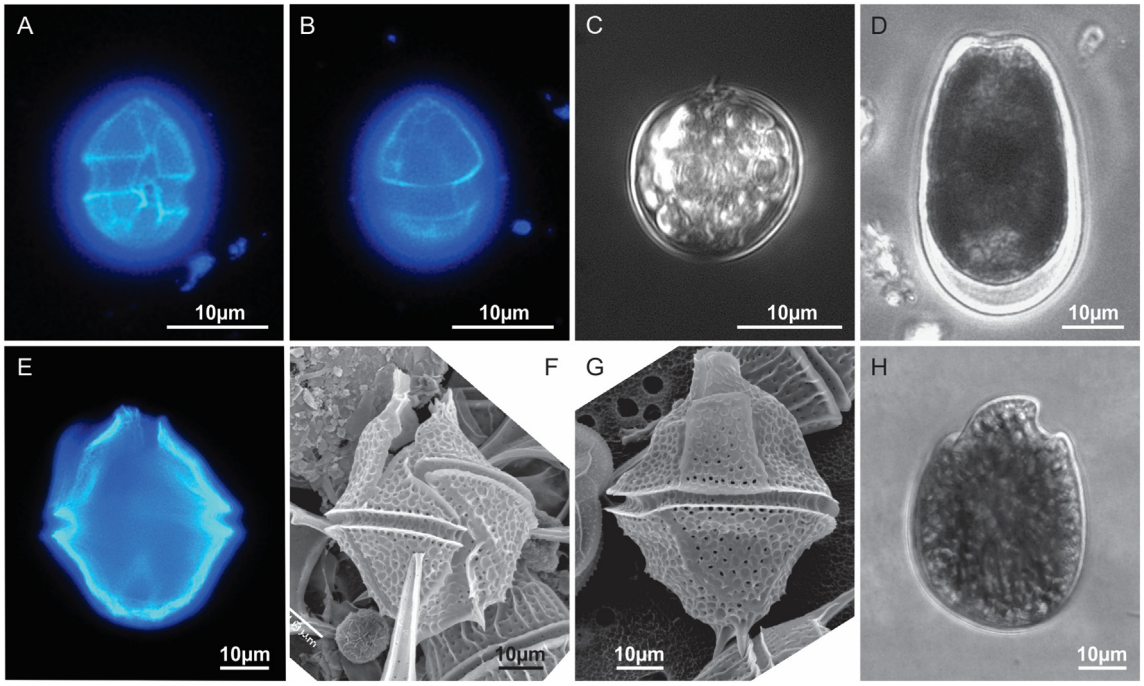

Fig. 7. Light and electron micrographs of potentially toxigenic dinoflagellates from the Beagle Channel. A–B) *Azadinium* sp.; C) *Prorocentrum* aff. cordatum; D) *Prorocentrum* aff. *lima*; E–G) *Gonyaulax spinifera* complex; H) *Amphidinium* sp.

considerable amount of neoSTX, C1 and a lesser amount of gonyautoxins GTX3,1,2, while lacking other common toxins of *A. catenella* strains isolated from southern Chile, such as GTX5 and GTX6 (Carreto et al., 2001; Krock et al., 2007; Montoya et al., 2010).

The production of PSP toxins in *A. ostenfeldii*, the other *Alexandrium* species observed in this study, seems to be confined only to some strains (Salgado et al., 2015). Particularly, a strain of *A. ostenfeldii* isolated from the Beagle Channel produced spirolides but not PSP toxins (Almandoz et al., 2014), whereas a strain isolated in southern Chile produced only PSP toxins (Salgado et al., 2015) and strains from northern Argentine shelf waters produced both PSP and spirolides (Guinder et al., 2018). According to Suikkanen et al. (2013), the ability of different strains of *A. ostenfeldii* to produce either PSP toxins or spirolides could be related to their phylogeny and biogeographic origin.

3.3.2. Dinophysis and Phalacroma species

The genus *Dinophysis/Phalacroma* was observed in six of the eleven sampling dates, with densities usually lower than 100 cells L^{-1}. The highest density, i.e. 160 cells L^{-1}, was recorded on August 4 in site B (Fig. 2b). Two species linked to production of diarrheic toxins were detected, *Dinophysis acuminata* and *Phalacroma rotundatum*, together with the sporadic occurrence of the non-toxigenic species *D. truncata* (Fig. 6). *D. acuminata* produces okadaic acid and derivatives, which are responsible of diarrheic shellfish poisonings (DSP) in temperate waters around the world (Reguera and Pizarro, 2008). This species can cause shellfish toxicity at cell concentrations as low as 200 cells L^{-1} (Faust and Gulledge, 2002). By contrast, there is controversy about the toxigenic nature of *P. rotundatum*. Despite the production of phycotoxins by *P. rotundatum* has not been confirmed, it has been suggested that it may act as a vector for toxins taken up by ciliate prey that have previously fed on co-occurring toxic *Dinophysis* sp. (González-Gil et al., 2011).

The occurrence of pectenotoxins (PTX-2), another group of phycotoxins linked to the genus *Dinophysis*, has recently been detected in the Beagle Channel, without information on phytoplankton composition (Krock et al., 2015). These toxins have presently not been related to human intoxications, but PTX-1 is highly hepatotoxic (Terao et al., 1986) and induces apoptosis in rat and salmon hepatocytes (Dominguez et al., 2010).

3.3.3. Azadinium species

Small dinoflagellates resembling the genus *Azadinium*, with an elliptical theca of approximately 15 μm length and 11 μm width, were occasionally detected in bottle samples during cell counts. Picked *Azadinium*-like cells observed with fluorescence microscopy revealed the presence of a broad cingulum, an episome higher than the hyposome and an arrangement of thecal plates (Fig. 7a–b) consistent with the genus *Azadinium* (Tillmann et al., 2009).

Azadinium is a recently described genus that presently comprises 13 species, of which three are capable of producing > 50 analogs of azaspiracid toxins (Tillmann, 2018a, 2018b). Intense blooms of the genus *Azadinium* have been observed in shelf water from the northern Argentine Sea (Akselman and Negri, 2012), a region characterized by its notable species diversity (Tillmann, 2018b). Further molecular and ultrastructural studies are needed in order to confirm the specific identity of *Azadinium*-like specimens observed in the Beagle Channel.

3.3.4. Other potentially toxigenic dinoflagellates

Species of the genus *Prorocentrum* were only occasionally found, with highest densities (i.e. 240 cells L^{-1}) detected on October 26 in site A (Fig. 2c). Planktonic species such as *P.* aff. *cordatum* (Fig. 7c) and *P. micans* were the most commonly observed, while other epiphytic or benthic *Prorocentrum* species such as *P.* aff. *lima* (Fig. 7d) and other unidentified specimens were only occasionally found in net samples. The production of okadaic acid and analogues has been associated with several epiphytic species of *Prorocentrum*, such as those of the *P. lima* species complex (Aligizaki et al., 2009). In Argentina, human intoxication by DSP associated with the occurrence of *P. lima* was recorded in 1999, in the coast of Chubut Province (Gayoso et al., 2002).

Blooms of *P. cordatum* have been associated with diarrheic shellfish poisoning (DSP) in Europe, Asia and North America, including human deaths (Lassus et al., 2016). In the Argentine Sea, where spring blooms of up to 1×10^7 cells L^{-1} of *P. cordatum* (as *P. minimum*) have been recorded (Sabatini et al., 2012; Carreto et al., 2018), little is known about its toxinology. However, a bloom in the north of Buenos Aires

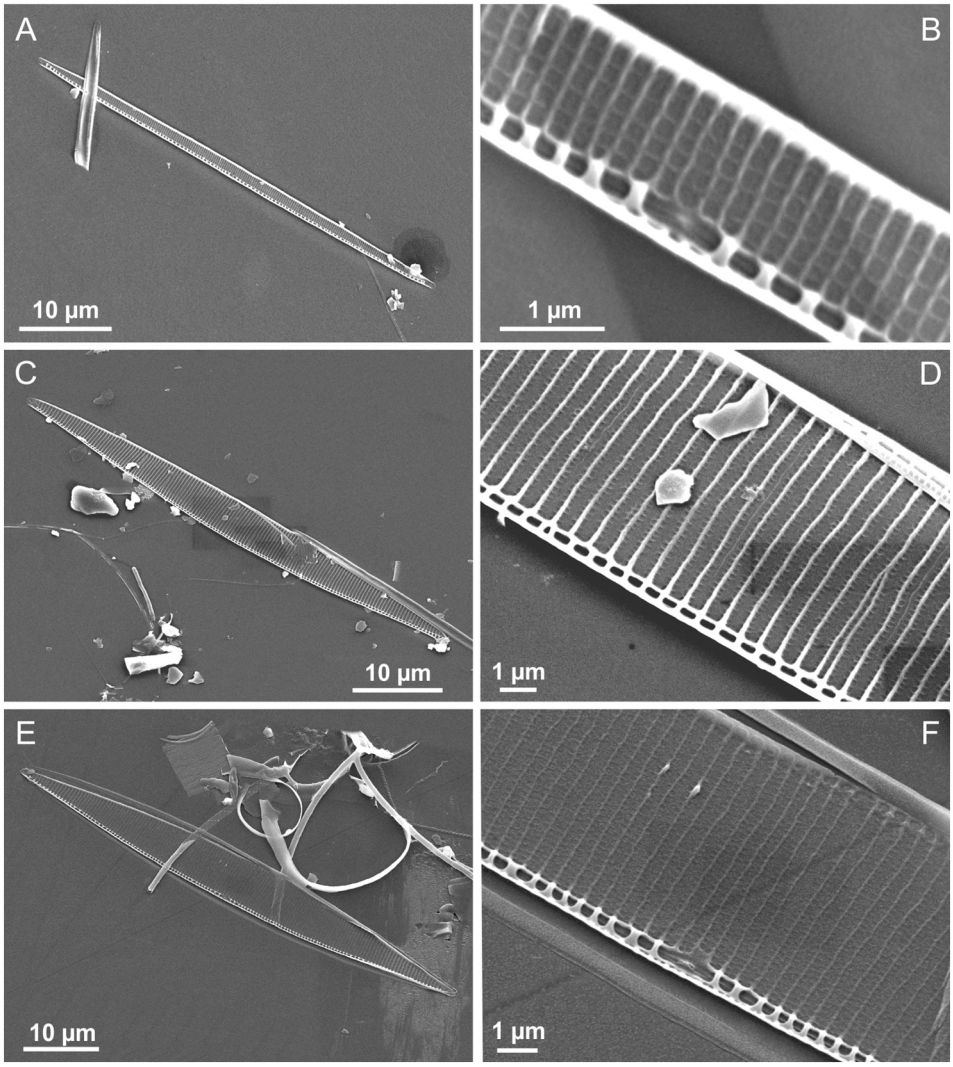

Fig. 8. SEM micrographs of specimens of *Pseudo-nitzschia* from the Beagle Channel. A–B) *Pseudo-nitzschia pseudodelicatissima* complex; C–D) *P. australis*, and E–F) *P. fraudulenta*.

Province was associated with fish mortality (Carreto et al., 2008).

A few specimens of the *Gonyaulax spinifera* complex (Fig. 7e–g) were observed only in net samples from site C. Representatives of this species complex have been associated with yessotoxin (YTX) production in the Adriatic Sea (Riccardi et al., 2009) and New Zealand (Rhodes et al., 2006). By contrast, YTX production in the Argentine Sea has been mainly associated with the species *Prorocentrum reticulatum,* not *G. spinifera* (Akselman et al., 2015; Fabro et al., 2018), a species that was not detected during the present study.

Finally, a single specimen of *Amphidinium* with an oval shape, 49 µm in length 37 µm in width, with a minute and left deflected epicone (Fig. 7h) was detected at site C. The genus *Amphidinium* comprises several species of athecate benthic or endosymbiotic dinoflagellates, whose specific identification based on morphological characters is usually uncertain and require molecular studies (Jørgensen et al., 2004; Karafas et al., 2017). Some *Amphidium* species produce ichthyotoxic compounds (Lassus et al., 2016).

3.3.5. *Pseudo-nitzschia species*

Several species of the diatom genus *Pseudo-nitzschia* are known to produce the neurotoxin domoic acid (DA) which is known to accumulate in organisms in the marine food web and cause harm to birds, mammals and humans (Lelong et al., 2012; Bates et al., 2018). *Pseudo-nitzschia* cells were observed on all sampling dates and usually co-occurring in all three sampling sites. A peak abundance of *Pseudo-nitzschia* species was recorded on February 25, when they reached up to $\approx 3.5 \times 10^5$ cells L^{-1} at site C (Fig. 2d), a density similar to what was found in Danish and Canadian waters when DA concentrations exceeded the international acceptance limit (Lundholm et al., 2005). An increase in the abundance of *Pseudo-nitzschia* cells during the end of summer has previously been reported in the Beagle Channel (Almandoz et al., 2011). The observed bloom was mainly composed of unidentified specimens of the *Pseudo-nitzschia pseudodelicatissima* species complex (Fig. 8a–b), which represented 61–69% of total *Pseudo-nitzschia*, whereas *P. australis* and *P. fraudulenta* (Fig. 8c–f) were found in lower cell abundances (12–24% and 15–19%, respectively).

The morphology of *P. australis* and *P. fraudulenta* fitted well with the classical descriptions provided by Hasle (1965). Valves of *P. australis* were 64–107 µm long and 6.9–7.1 µm wide, with 17–18 striae and fibulae in 10 µm, without central interspace, and striae composed of two rows of poroids (4–6 in 1 µm). Valves of *P. fraudulenta* were 60–75 µm long and 5.0–7.5 µm wide, with 22–25 striae and fibulae in 10 µm, with a central interspace, and striae composed of two rows of poroids (6 in 1 µm).

Specimens of the *P. pseudodelicatissima* species complex found in this study had linear or slightly lanceolate valves that were 45–83 µm long and 1.5–1.7 µm wide, with a central interspace. The valves have 23–25 fibulae and 41–43 striae in 10 µm. Each stria consisted of one row of

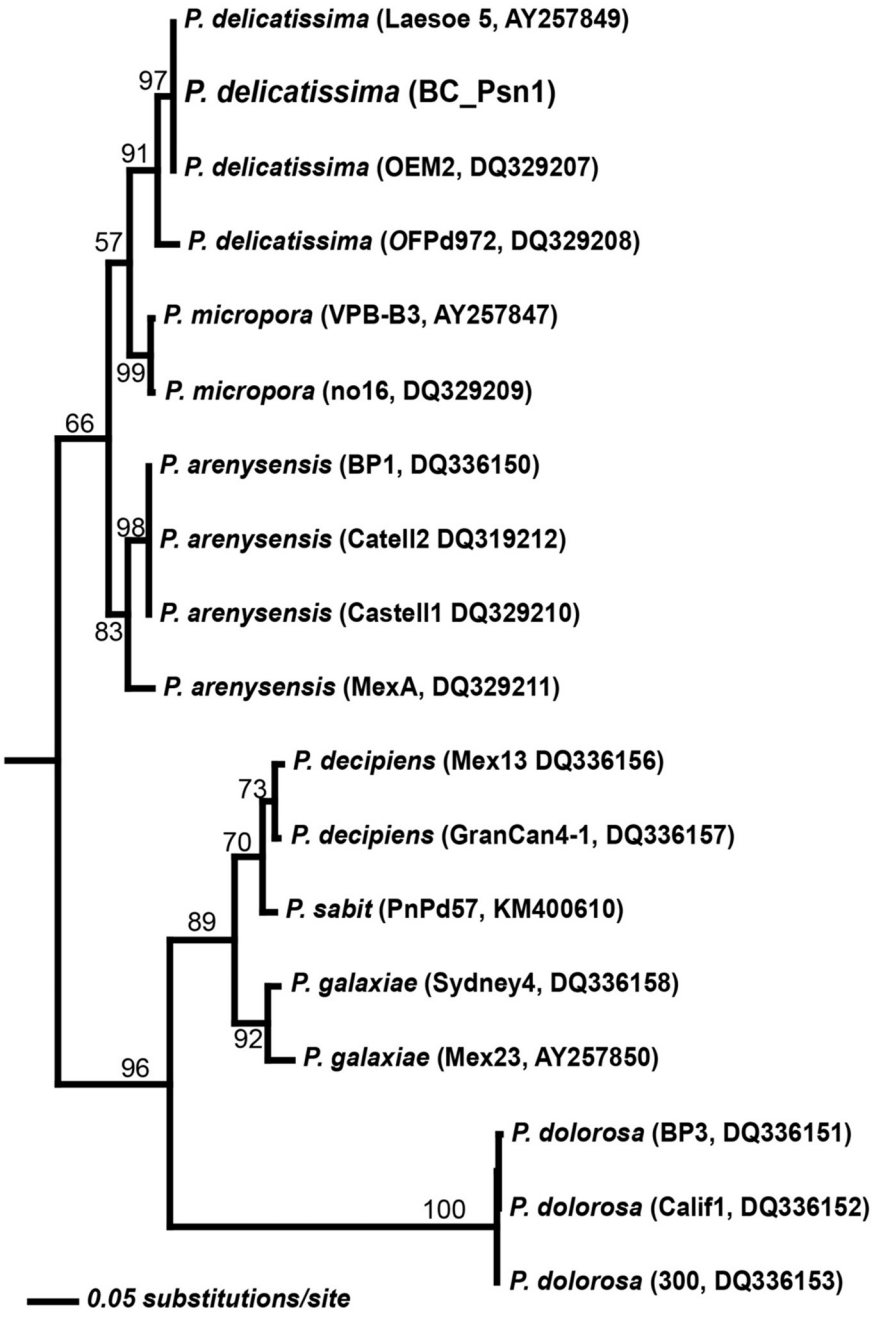

Fig. 9. Phylogenetic tree based on maximum likelihood analyses and bootstrap support based on 100 replicate analyses.

round to square poroids, with 5–6 poroids in 1 μm. The characters could e.g. agree with *P. cuspidata* and *P. pseudodelicatissima* (Lundholm et al., 2003). Nowadays the *P. pseudodelicatissima* species complex comprises about 23 species (Li et al., 2017), including several cryptic and pseudocryptic species. Thus, molecular studies are needed to clarify the identity of the observed specimens.

The species *P. australis* and *P. fraudulenta* are among several other *Pseudo-nitzschia* and *Nitzschia* species known as producers of DA worldwide (Bates et al., 2018 and references therein; Lundholm, 2018). *P. australis* is a globally distributed highly toxigenic species that has caused toxic blooms resulting in DA in the marine food web in many parts of the world, whereas *P. fraudulenta* is also globally distributed but only slightly toxigenic (Bates et al., 2018). Particularly, *P. australis* has been considered as the main source of DA in shelf waters from the Argentine Sea (Almandoz et al., 2017), and blooms of *P. australis* were associated with high levels of DA in southern right whales from Nuevo and San José Gulfs, and Peninsula Valdes (D'Agostino et al., 2017). Despite the lack of samples for DA analysis during our study, DA has previously been detected in net phytoplankton samples collected in the eastern sector of the Beagle Channel during late summer (Krock et al., 2015).

Molecular analyses revealed that the *Pseudo-nitzschia* strain BC_Psn1 from the Beagle Channel clustered in a clade comprising strains of *P. delicatissima* (Fig. 9). The sequence from the Beagle Channel is identical to strains of *P. delicatissima* sensu stricto (Lundholm et al., 2006). *P. delicatissima* is part of the *P. delicatissima* species complex comprising species that are cryptic or pseudocryptic and hence difficult or impossible to identify without molecular data. *P. delicatissima* is a globally distributed species, but has not previously been identified with certainty from South American waters (Lundholm et al., 2006). Electron microscopy analyses showed valves linear to slightly lanceolate in shape (Fig. 10a–b), 35–80 μm long and 1.8–2.1 μm wide, with a central

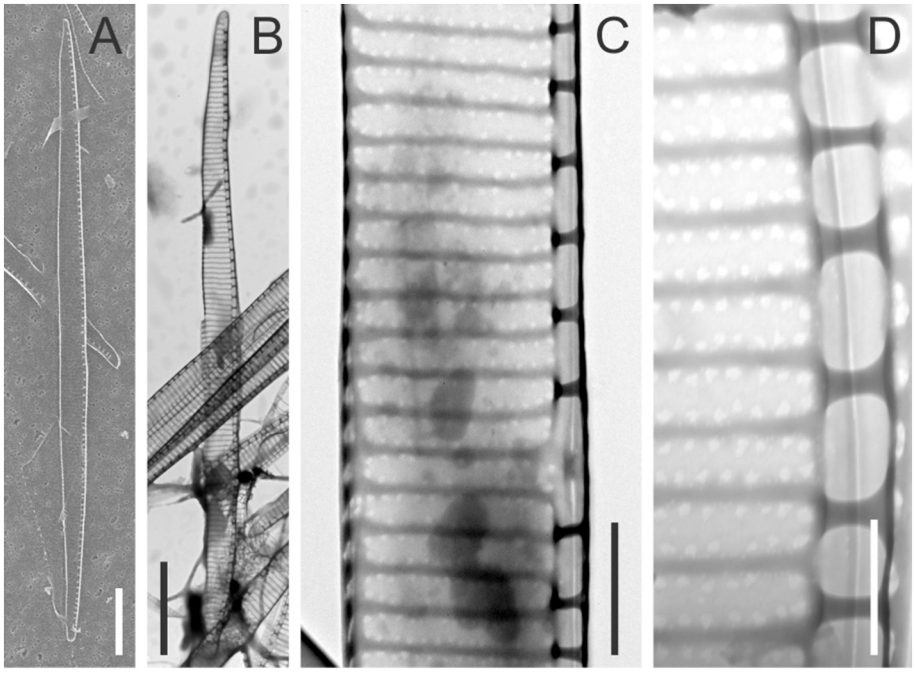

Fig. 10. SEM and TEM micrographs of specimens of *Pseudo-nitzschia delicatissima* sensu stricto from the Beagle Channel. A–B) General valve view; C) Detail of valve centre; D) Detail of poroids. Scale bar = 5 µm (A–B), 1 µm (C) and 0.5 µm (D).

interspace (Fig. 10c). They have 23–24 fibulae and 35–36 striae in 10 µm. Striae are composed of two rows of small poroids (Fig. 10d), with 9–12 poroids in 1 µm. In accordance with previous studies (Lundholm et al., 2006), *P. delicatissima* sensu stricto from the Beagle Channel tested negative for DA production, although a few studies report slight toxicity of *P. delicatissima* (see Bates et al., 2018).

4. Conclusions

Similar to many other parts of the world, the occurrence of harmful algae blooms is a potential threat for aquaculture activities in the Beagle Channel. No significant differences in water quality parameters and phytoplankton compositions were detected among sites. Previous records in this area revealed extremely high PSP toxicities resulting in human fatalities as well as in very long shellfish closures, as a consequence of *Alexandrium* blooms. In this study, we also detected other potential sources of toxicity, including species of *Dinophysis*, *Prorocentrum*, *Azadinium*, *Gonyaulax*, *Amphidinium* and *Pseudo-nitzschia*. Although they were usually observed in very low densities, further studies are needed to evaluate their toxicity and population dynamics in the Beagle Channel. High densities of other potential harmful species, such as *Phaeocystis*-like and *Chaetoceros* species is also highlighted, considering the future potential development of fish aquaculture in the study area.

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