



## Toxic strains of the *Alexandrium ostenfeldii* complex in southern South America (Beagle Channel, Argentina)



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### ABSTRACT

During phytoplankton monitoring in the Beagle Channel ( $\approx 54^{\circ}52' S$ ,  $67^{\circ}32' W$ ) a previously undetected *Alexandrium* species was observed in coincidence with mouse bioassay toxicity. Detailed thecal plates analysis using epifluorescence and scanning electron microscopy revealed the presence of the *Alexandrium ostenfeldii* species complex, showing a mixture of the diagnostic features usually used to discriminate between the morphospecies *A. ostenfeldii* and *A. peruvianum*. Cells of the *A. ostenfeldii* complex were commonly observed during spring after the main annual diatom bloom, when temperatures and salinities were respectively around  $7.5\text{--}10^{\circ}C$  and  $30\text{--}30.5$  psu, and nutrients showed a seasonal decrease. Toxin analysis by liquid chromatography–mass spectrometry revealed the production of 13-desmethyl spirolide C and 20-methyl spirolide G in cell cultures. The cellular content of spirolides during exponential phase growth was  $0.5906 \pm 0.0032$  and  $0.1577 \pm 0.0023$  pg cell<sup>-1</sup> for 13-desMe-C and 20-Me-G, respectively. A third unknown compound, with a structure resembling that of spirolides was also detected in culture. Moreover, an additional compound with a similar  $m/z$  (692) than that of 13-desMe-C but presenting a higher retention time ( $R_t = 40.5$  min) was found in high proportions in mussel samples. PSP toxins were present at low concentration in mussels but were not detected in cultures. These results extend the world-wide distribution of toxic strains of the *A. ostenfeldii* complex to the Beagle Channel (southern South America), where toxic events have been traditionally linked to the presence of *Alexandrium catenella*. This is the first confirmed occurrence of spirolides in mussels and plankton from Argentina, which highlights the importance of monitoring these toxins and their producing organisms to protect public health and improve the management of shellfish resources.

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## 1. Introduction

The occurrence of some species of the marine dinoflagellate genus *Alexandrium* Halim has been usually related to harmful algal bloom events in many parts of the world (Anderson et al., 2012). Most of the harmful species of this genus produces paralytic shellfish poisoning (PSP) toxins, which have shown a high diversity in cultured and natural populations of *Alexandrium* from southern South America (Persich et al., 2006; Montoya et al., 2010). Moreover, the presence of other toxin families has been also

recorded (Hu et al., 1996; Cembella et al., 2000; Hsia et al., 2006; Van Wagoner et al., 2011). From the more than 30 described *Alexandrium* species, only *A. ostenfeldii* and *A. peruvianum* have been found to produce cyclic imine neurotoxins called spirolides (SPXs) (Cembella et al., 2000; Franco et al., 2006). In the last years, there have been several reports on the production of spirolides by these two *Alexandrium* species in North America and Europe (e.g. Aasen et al., 2005; MacKinnon et al., 2006; Ciminiello et al., 2007; Borkman et al., 2012; Tomas et al., 2012). Likewise, the first record on the presence of spirolides in the Southern Hemisphere has been recently observed in shellfish from northern Chile (Alvarez et al., 2010), suggesting a more widespread distribution than previously thought.

*Alexandrium ostenfeldii* and *Alexandrium peruvianum* are morphologically very similar species that have been originally

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distinguished mainly by the shape of the 1' and the s.a. plates (Balech and Tangen, 1985). However, a high variability in the shape of these two diagnostic plates has been recurrently observed in natural populations or strains from several locations (e.g. Lim et al., 2005; Touzet et al., 2008; Gu, 2011), making difficult to unambiguously assign specimens to one of the two species. Moreover, molecular studies suggest that *A. ostenfeldii* and *A. peruvianum* could represent a species complex with a considerable cryptic diversity or a single genetically structured species with high morphological variation (Kremp et al., 2009, 2014).

In temperate-cold waters of the Beagle Channel the presence of *Alexandrium catenella* has been linked to one of the most important PSP event observed worldwide (Carreto and Benavides, 1993; Benavides et al., 1995). Since that, the occurrence of such toxic events related to *A. catenella* has been recurrently reported (Goya and Maldonado, 2014). During a HAB monitoring programme carried out in a mussel harvesting area of the Beagle Channel since 2005–2007, the results of toxicity bioassays showed values above the accepted limits when the presence of *A. catenella* in plankton samples was extremely low or undetectable (Hernando et al., 2007; Goya and Maldonado, 2014). By contrast, the presence of solitary cells, usually larger and more globose in shape than *A. catenella* was observed. This fact promoted further research efforts to elucidate the source of toxicity, including cell culture establishment and toxin analysis.

In this study, we provide the first report of spirolide detection in both mussels (*Mytilus edulis*) and cultures of the *Alexandrium ostenfeldii* complex from the Beagle Channel, together with a description of its morphology and occurrence in this sub-Antarctic area that sustains rich aquaculture activities.

## 2. Materials and methods

### 2.1. Phytoplankton monitoring programme

Field sampling was carried out between July 7, 2005 and December 6, 2007, at a fixed station (Almanza, Fig. 1) located in the Beagle Channel (54°52'22.85" S, 67°32'18.72" W). Sampling frequency was biweekly most of the year and weekly during the phytoplankton spring bloom. Water temperature and salinity were measured *in situ* with a Horiba U-10 multi-parametric sensor (Horiba Ltd., Kyoto, Japan). Seawater was sampled at 2 m depth using a 5 l Niskin bottle from a boat. Aliquots of 250 ml were preserved with 4% borax buffered formalin for quantitative phytoplankton analysis. In addition, sub-surface qualitative phytoplankton samples were taken using a 20 µm mesh net and fixed as previously described. All samples were kept in the dark at room temperature until analysis. For chlorophyll *a* (Chl *a*) determination, 0.5–1 l of seawater were filtered onto Whatman GF/F filters and kept frozen (−20 °C) until analysis, which was performed within one week of sampling. Pigment extracts were read in a Turner 450 Beckman spectrofluorometer and corrected for phaeopigments, following Holm-Hansen and Riemann (1978). Concentrations were calculated according to Holm-Hansen et al., 1965. Samples for nutrient analyses were filtered through Whatman GF/F filters and kept frozen (−20 °C) until analysis, which was done within three months after sampling. Nitrates, phosphates and silicates were measured with an automated analyzer (Autoanalyzer Technicon II), following the methods described in Grasshoff (1969), Grasshoff et al. (1983), Technicon (1977) and Eberlein and Kattner (1987).

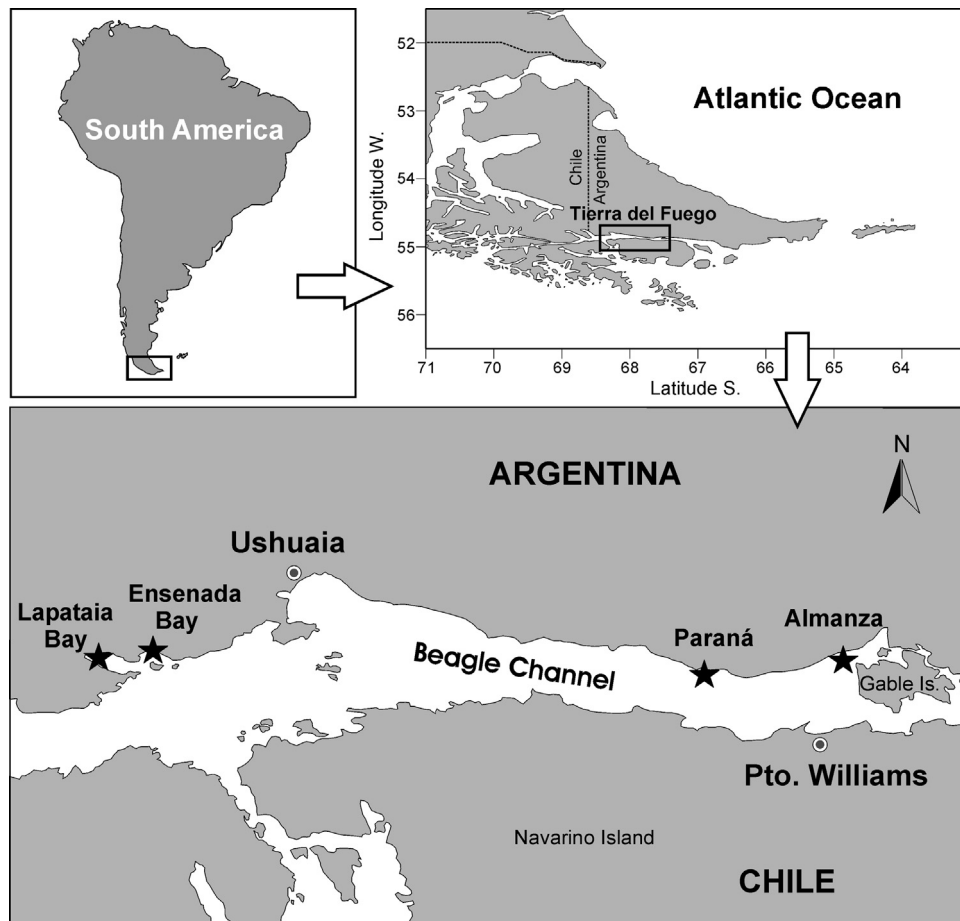


Fig. 1. Map of the study area and sampling sites in the Beagle Channel.

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