FIRST *OSTREOPSIS* CF. *SIAMENSIS* BLOOM DETECTED IN LISBON BAY, PORTUGAL

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Abstract
During late September 2017 samples of macroalgae and surface waters were collected from rocky pools in the bay of Lisbon, Cascais (Western Iberian Peninsula), along a geographical gradient (E-W). The macroalgae communities were characterised by small, mostly finely branched and foliose thalli, forming a diverse cespitose assemblage. Analysis of benthic harmful algal species (BHABs) revealed high concentrations of potentially toxic microalgae of the genus *Ostreopsis*, not only in the benthos but also in the water column. The geographical distribution of samples allowed the identification of a concentration gradient of *Ostreopsis* spp. radiating from a well-defined hotspot (concentrations up to 1.04×10^5 cells g^{-1} dry weight of macroalgae turf). Five cultures were established from single-cell isolation and molecular identification of the ITS rDNA region revealed that all cultures belonged to the *O.* cf. *siamensis* clade. Although *Ostreospsis* cf. *siamensis* has been previously reported from Lisbon bay, this is the first time bloom concentrations have been recorded.

Keywords: Bay of Cascais, Benthic HAB, Dinoflagellates, *Ostreopsis*, Portugal

Introduction

Events of benthic HABs have been increasing during the last decades in temperate regions. These have been associated with the proliferation of toxic dinoflagellates, specifically species of the genus *Ostreopsis*. The Mediterranean coast of the Iberian Peninsula has regularly been affected by blooms of the highly toxic species *O.* cf. *ovata* (Vila et al. 2016). This species produces potent toxins of the palytoxin group and has been responsible for several outbreaks leading to the mortality of marine organisms, human health problems and economic losses due to beach closures in touristic areas (Vila et al. 2006). In the Atlantic South Iberian coast (D. Ana beach, Lagos, Portugal) a bloom of *O.* cf. *ovata* was recorded in 2011, with no significant harmful effects (David et al. 2012). So far, this is the only area, from where *O.* cf. *ovata* has been reported in the Atlantic Iberian coast (David et al. 2012, Ramos et al. 2015). Another *Ostreopsis* species, *O.* cf *siamensis*, is also present in the Atlantic Iberian coast and in the Mediterranean Sea. While in the Mediterranean Sea it has a more restricted distribution, when compared to *O.* cf. *ovata*, in the Atlantic coast the opposite is observed: *O.* cf. *ovata* is only known to be present in the south of Portugal, while *O.* cf. *siamensis* presents a wide, albeit fragmented, distribution from the south of Portugal to the south of France (Bay of Biscay) (David et al. 2013). Since 2010, Lisbon bay has been sampled every year in late Summer-early Autumn, and cells of *O.* cf. *siamensis* are regularly found, both in the benthos and in the water column, although in very low concentrations.

Material and Methods

In September 2017, a sampling survey took place in Lisbon bay to study the occurrence of *Ostreopsis* spp.. The area is characterized by a succession of sandy and rocky beaches and sampling took place in intertidal pools. Samples of macroalgae with the surrounding water were collected with plastic bags in 11 sites along an E-W transect (Fig. 1). Samples were shaken, sieved and stored in collecting bottles. To estimate the abundances of the epiphytic community samples were measured to a known volume and subsamples were fixed in 2% neutral Lugol. The remaining live sample was used for observation, isolation and establishment of monoclonal cultures. Single cells were isolated using a glass micropipette. Each cell was washed three times in filter-sterilized seawater, transferred into a 24-multiwell plate with f/20-si medium and kept under 12:12 L/D cycle with a photon flux density of 80 µmol photons$\cdot m^{-2}\cdot s^{-1}$. The fresh (FW) and dry weight (DW) of the distinct macroalgae was determined. The concentration of epiphytic *Ostreopsis* spp. was estimated using a Sedgwick-Rafter chamber and the results were expressed as cells g^{-1} FW and cells g^{-1} DW of macroalgae. The planktonic concentration of *Ostreopsis* cells (cells L^{-1}), was determined using the Utermöhl method. Amplification of the ITS1-5.8S-ITS2 rDNA, was performed with the ITSA and ITSB primers (Adachi et al. 1994) for identification of the established *Ostreopsis* cultures. Of each culture, 30 mL were centrifuged into a pellet and the rDNA was extracted, amplified and sequenced. Phylogenetic analyses were performed with maximum likelihood (ML), neighbor joining (NJ), and maximum parsimony (MP), using *Coolia* sequences as outgroup.

Results and Discussion

When sampling a few of the tidal pools (Fig. 1, site G), the presence of mucilaginous threads was immediately apparent (Fig. 2). A thin yellow/brownish pellicle covered the substrate, which was dominated by small, mostly finely branched and foliose thalli, forming a diverse cespitose assemblage, although some specimens of *Dictyota dichotoma*, *Codium* sp., and *Halopteris scoparia*, among others, were also present (Table 1). The epiphytic community was checked under the microscope which confirmed the occurrence of an *Ostreopsis* sp. bloom (Fig. 2). From the isolated cells, 5 cultures were established and are kept in the Algal culture collection of the University of Lisbon (ALISU). Molecular analyses revealed that all sequences from this study grouped in the *Ostreopsis* cf. *siamensis* clade (Fig. 3). Regarding abundances, the highest cell concentration was found in station G with $1.0x10^5$ cells g^{-1} DW ($3.4x10^4$ cells g^{-1} FW). The geographical spread of sampling allowed the identification of this site as a hotspot, with concentrations of the epiphytic *O.* cf. *siamensis* decreasing to the east and to the west of station G (Fig. 4). At this site the concentration of planktonic cells was $2.1x10^3$ cells L^{-1}. The concentration values reported here are low when compared to *O.* cf. *ovata* in the Mediterranean Sea ($6.8x10^4$ cells L^{-1}; $8.5x10^6$ cells g^{-1} FW (Cohu et al. 2013); $1.3x10^6$ cells g^{-1} FW and $1.6x10^7$ cells g^{-1} DW (Accoroni et al. 2011). However, reports of blooms of *Ostreopsis* cf. *siamensis* in the Atlantic and Mediterranean Sea are rare. To our knowledge only two reports have previously been published. One was from the Moroccan Atlantic coast ($3.7x10^3$ – 10^{-5} cells L^{-1}) (Bennouna et al. 2010) and one from Tunisia ($3.7x10^4$ cells L^{-1}), in the Mediterranean Sea (Turki et al. 2010). Work on the toxicity of *O.* cf. *siamensis* has shown that it produces palytoxin in almost undetectable concentrations (Ciminiello et al. 2013) representing a low risk for the environment and human health. The lack of nuisance impacts may contribute for blooms going unnoticed.

O. cf. *siamensis* was first detected in the West coast of Iberia in 2008 (Amorim et al. 2010) and has since been regularly detected at very low concentrations. In the present work, we report for the first time bloom concentrations of *Ostreopsis* cf. *siamensis* in the West coast of Iberia where no human health issues have been reported. Nevertheless, this raises questions regarding the environmental conditions that may trigger blooms and how these may be related to the warming trend of present day oceans. Very little is known on the ecology of *O.* cf. *siamensis* and more work is needed to better understand the distribution and bloom dynamics of this species in temperate waters.

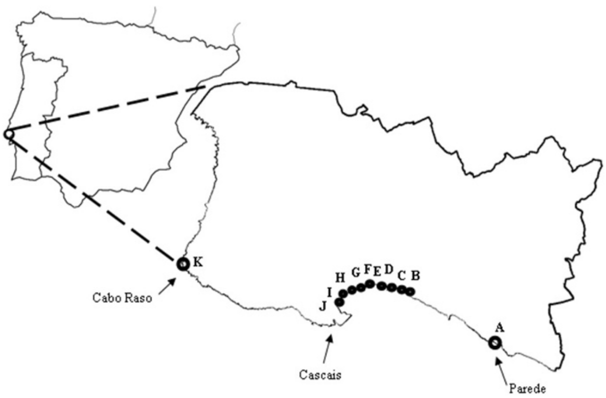

Fig.1. Sampling sites in the Bay of Lisbon.

Fig.2. Station G in the Bay of Lisbon. First image shows mucilaginous brown filaments in a water pool. Image to the right shows *Ostreopsis* cells under the optical microscope.

Table 1. Macroalgae community

Asparagopsis armata	*Corallina* sp.
Caulacanthus ustulatus	*Cystoseira* sp.
Ceramiacea	*Derbesia* sp
Ceramium spp.	*Dictyota dichotoma*
Champia sp.	*Gelidium pusillum*
Champia parvula	*Gracilaria foliifera*
Cladostephus spongiosus	*Halopteris scoparia*
Codium tomentosum	*Rhodomelaceae*
Colpomenia sp.	*Ulva* spp.

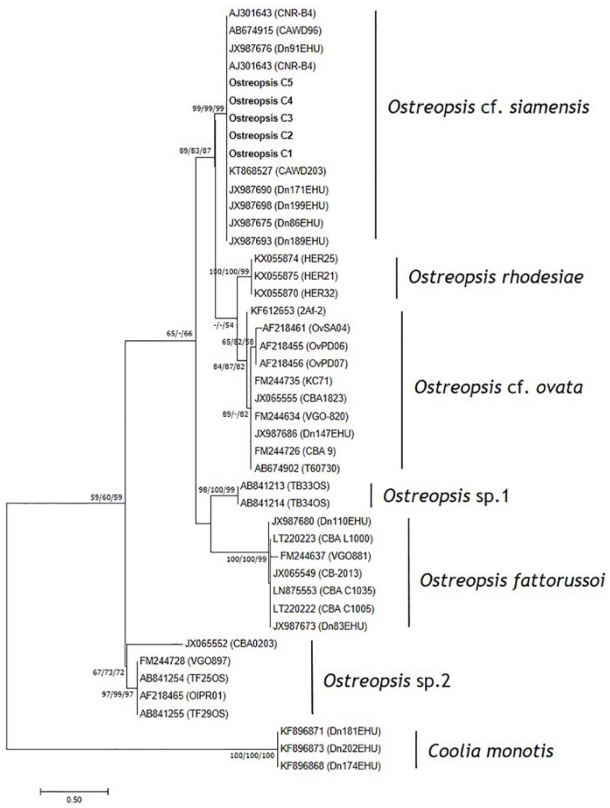

Fig.3. Maximum likelihood phylogenetic tree. Bootstrap values (NJ/MP/ML). Sequences from this study are in bold.

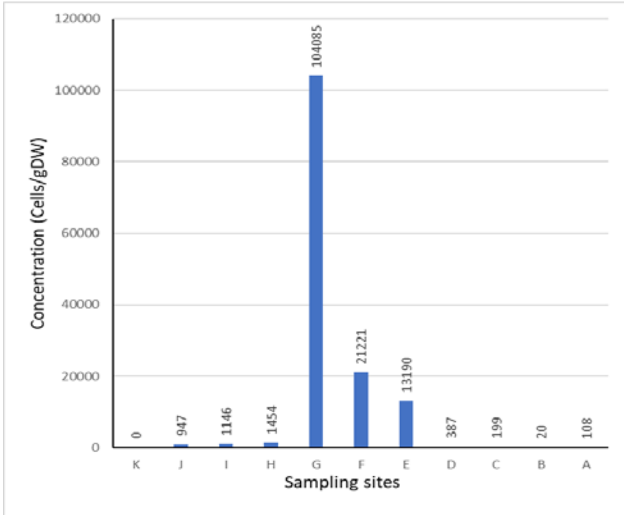

Fig.4. Concentration of epiphytic *Ostreopsis* cf. *siamensis* cells in grams per dry weight.

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