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Relationships between the presence of *Ostreopsis* (Dinophyceae) in the Atlantic coast of the Iberian Peninsula and sea-surface temperature

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Abstract – An extensive sampling program was performed in the Atlantic side of the Iberian Peninsula on 17 sites during 2010 and 2011 summers, in order to characterize the distributional pattern of potential toxic dinoflagellates of the genus *Ostreopsis*. The study area presents a discontinuity in the species distribution in a pattern that parallels that of summer water temperature. *Ostreopsis* was not found in a relatively wide fringe of the Northwest side of the Peninsula, as water temperature is markedly lower than that in the Northeast and Southwest extremes where *Ostreopsis* has been found. Comparing the observed distribution of *Ostreopsis* on the Atlantic coast of the Peninsula with different sea surface temperature (SST) percentiles, it is notable that neither minimum nor maximum temperatures observed in the range of the study area can explain the species distribution but presumably is the length of the warm period what limits the genus presence. Thus we hypothesize that for *Ostreopsis* to be present in a certain area three continuous months with SST above 19.5°C may be necessary.

Atlantic coast / benthic dinoflagellates / distribution / Iberian Peninsula / *Ostreopsis* / sea surface temperature

INTRODUCTION

The assemblage of potentially toxic dinoflagellates of the *genera Coolia* Meunier, *Gambierdiscus* Adachi et Fukuyo, *Ostreopsis* Schmidt and *Prorocentrum* Ehrenberg have been profusely investigated during the last years not only in tropical and subtropical areas but also in temperate places. It has been observed that the genera *Coolia* and *Prorocentrum* are widely distributed (Penna *et al.,* 2005; Nagahama *et al.,* 2011), whereas *Gambierdiscus* and *Ostreopsis* have a more restricted distribution limited to warmer waters including the Mediterranean Sea (Aligizaki & Nikolaidis, 2006, 2008; Mangialajo *et al*., 2011). The taxonomy of these genera is currently under revision due to the presence of cryptic or semicryiptic species within each genus. The use of molecular methods as complementary tools to morphological observations is thus necessary (Scholin *et al.,* 1994). By using both approaches, new species are being described and their capacity to produce toxins analyzed (e. g. Penna *et al*., 2005; Fraga *et al*., 2008; Litaker *et al.,* 2009).

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There are a few references on the presence of *Ostreopsis* in temperate areas (Shears & Ross, 2009; Selina & Orlova, 2010; Silva *et al*., 2010) probably due to the fact that individuals of this genus grow optimally to bloom proportions mostly in warm waters. This is why in the case of warm-temperate areas with marked seasonality, such as the Mediterranean Sea, the species proliferate in summer and early autumn (Aligizaki & Nikolaidis, 2006; Zingone *et al.,* 2006). Regarding the Mediterranean Sea and the adjacent Atlantic areas, two species of *Ostreopsis*, *O.* cf. *ovata* Fukuyo and *O.* cf. *siamensis* Schmidt, are commonly found. Additionally, a third lineage from the Canary Islands has been recorded (Penna *et al*., 2010). The growth of *O.* cf. *ovata* has been observed to be stimulated at higher temperatures (Granéli *et al*., 2011), although the minimum threshold for *Ostreopsis* to proliferate in the field is still unclear (Mangialajo *et al*., 2011).

While the presence of *Ostreopsis* in the Mediterranean Sea is widely documented, its presence in the Atlantic coast of the Iberian Peninsula has only been recently reported. *O.* cf. *siamensis* has been found in the Portuguese west coast, firstly in the locality of Sines (Amorim *et al.*, 2010) and later in Cascais (Amorim *et al.*, 2010). This species has also been collected in the southeastern coast of the Bay of Biscay (Laza-Martínez *et al.,* 2011). The later report constitutes the northernmost observation of *Ostreopsis* in the Northern Atlantic Ocean. The study was performed in a coast stripe of about 75 km where *Ostreopsis* was detected in all the sampled sites. Taking into account that during summer water temperature decreases westwards along the coast, we hypothesized that *Ostreopsis* presence would be interrupted at a certain point in the Cantabrian coast. A similar temperature gradient can be seen along the Portuguese coast in a northward direction. During summer, the two extremes of the Atlantic coast of the Iberian Peninsula remain warmer than its northwest coast due to hydrographic and climatic features of the area (Peliz *et al.,* 2005; Lima *et al.,* 2006). Consequently, this area is particularly well suited to study the effect of temperature on the distribution of a particular taxon. The present study aimed to know the distributional pattern of dinoflagellates of the genus *Ostreopsis* along the Atlantic coast of the Iberian Peninsula in relationship with seawater temperature.

MATERIALS AND METHODS

Study area and sampling

In order to establish the spatial distribution of the genus *Ostreopsis*, 17 sampling sites spread out along the Atlantic coast of the Iberian Peninsula (36°86'-43°47' N and 9°53-1.35° W) were selected (Fig. 1). The area between sites 13 and 14 was not sampled because *Ostreopsis* was already known to be present there (Laza-Martínez *et al*., 2011). Since *Ostreopsis* can be found mostly living epiphytically on macroalgae, rocky shores were selected as sampling sites. As no rocky shores were present in site 17 and in the surroundings, the seagrass *Zostera marina* Linnaeus growing in a soft bottom embayment was sampled instead. All sites were sampled once from August 2010 to October 2010 and samples were also collected in September 2011 at sites 1, 2, 13 and 14. Macrophytes were collected from shallow depths during low tides in half a litre plastic bottles with seawater.

Fig. 1. Study area and sampling sites (1-17) along the Atlantic coast of the Iberian Peninsula. Additionally two sites from the Mediterranean coast of the Iberian Peninsula (A and B) were used for comparison purposes.

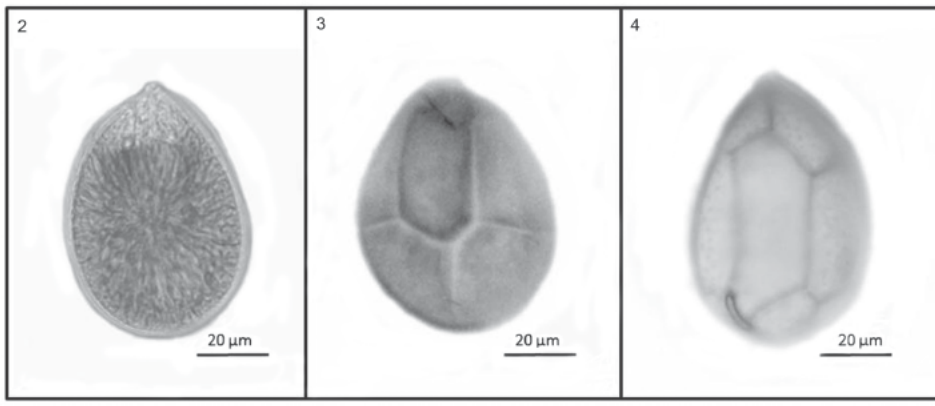
The bottles were vigorously shaken for about one minute in order to detach the epiphytic algae and the material was then filtered through a 200 µm sieve net to remove larger particles.

Sea surface temperature (SST)

Monthly average (1985-2009) sea surface temperature with a resolution of 4×4 km was obtained from satellite images (AVHRR Pathfinder V5 product, NASA PO.DAAC). To understand the spatial and temporal behavior of the SST of the Iberian Peninsula, different percentiles were obtained for each 4×4 km parcel and the resulting maps were compared with the distribution of *Ostreopsis* spp. The percentiles were calculated by analyzing all the temperature data. A percentile of a variable is the value where under that point, a certain percent of observations fall. For example, the 75^{th} percentile, also known as the third quartile, is the value or score below which 75 percent of the observations may be found. In addition, a set of daily data corresponding to 2010 and 2011 SST with a spatial resolution of 6 km were obtained from Operational Sea Surface Temperature and Sea Ice Analysis (OSTIA, Met Office Hadley Centre observations datasets) (Martin *et al.,* 2007; Stark, 2007).

RESULTS AND DISCUSSION

Ostreopsis cells (Figs 2, 3 and 4) were found in two different coastal areas of the Atlantic Iberian Peninsula: stations 12-15 of the Northeastern side and stations 1-3 of the Southwestern side (Fig. 1). Clonal strains from all these stations



Figs 2-4. *Ostreopsis* cells from the South of the Bay of Biscay observed under Light Microscopy. **2.** Live cell. **3.** Hypotheca of a calcofluor stained cell. **4.** Epitheca of a calcofluor stained cell.

were isolated and its detailed morphological and molecular characterization is currently under analysis. The difficulty in finding rocky shores in the surroundings of site 17 and northwards, in addition to strong waves while sampling in site 16, impeded adequate macroalgae sampling in both places. We consider that the absence of *Ostreopsis* spp. in sites 16 and 17 could be due to sampling conditions and not the result of a distribution limit between sites 15 and 16. Although this study does not focus on the abundance of *Ostreopsis*, we consider of interest to note that during the sampling in Lagos (site 2) in September 2011, were observed in the water column and sea surface (David *et al.*, 2012).

The presence of two distribution limits for *Ostreopsis* spp. in the Iberian Peninsula – one westward in the northern coast and the other northward in the west coast – allowed us to search for temperature statistics that could explain the observed distribution by looking for coincidences in the statistics values of the two distributional limits. Neither minimum nor maximum temperatures in the range observed in the study area can explain *Ostreopsis* spp. distribution. Minimum temperatures in sites where *Ostreopsis* spp. were not found (sites 4-11) are higher or similar to those of the areas where it was present (around 11°C in the south-eastern Bay of Biscay). On the other hand, the maximum temperature in sites 8-10 (22.3-23.5°C) where *Ostreopsis* spp. were not found are similar or higher than those of sites 1-3 where *Ostreopsis* spp. is present (Fig. 5). With this in mind, we presume that the length of the warm period is what limits the presence of species of this genus in sites 8-10. To gain insight into the temporal behaviour of SST, we have studied different percentiles among which the 75^{th} is the one which better explains the observed distribution of *Ostreopsis* (Fig. 6). The temperatures shown in figure 6 represent that 75% of temperature data from a corresponding point will be below this temperature, or conversely, that this temperature is surpassed by the 25% of the data (*i.e.* 3 out of 12 months). In comparing the two distribution limits (sites 3 and 12) with SST, we observe that although site 3 shows lower maximum temperatures and higher minimum temperatures comparing to site 12 (Fig. 5), both present the same 75^{th} percentile of 19.5°C (*i.e.* in both sites SST is expected to be above 19.5°C during three months). This percentile is about one and two degrees lower in sites 5 and 7, respectively. Meanwhile it is about one degree higher in the Southwestern Iberian coast (sites 1 and 2). Temperature percentiles can be biologically more informative than maximum temperatures

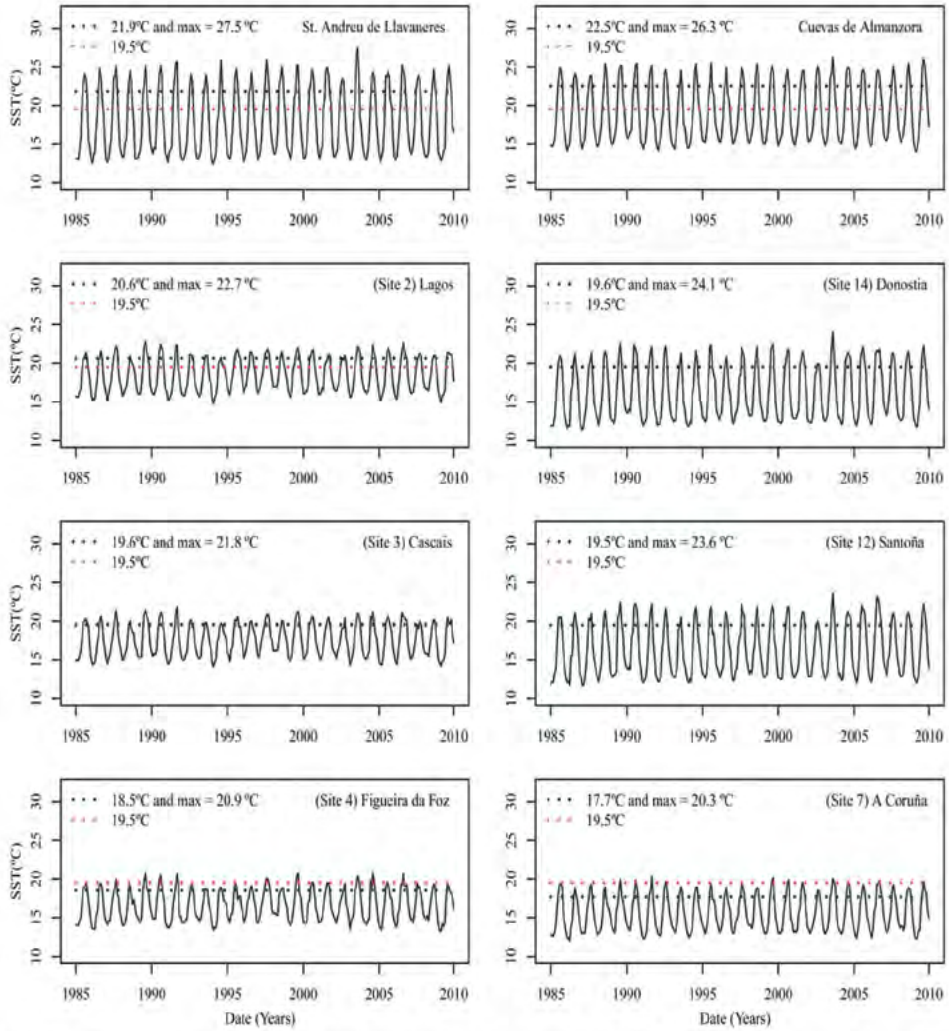


Fig. 5. Average monthly SST (1985 to 2009) from six sampling sites. These sites were selected as representative of different conditions in relation to the presence of *Ostreopsis*. It was chosen the two sites that marked the limit of the observed distribution of *Ostreopsis* (Cascais and Santoña), one site within each distribution area (Lagos and Donostia) and other two where it was not found (Figueira da Foz and A Coruña). Moreover, two sites from the Mediterranean side of the Peninsula, where nuisance blooms have been observed, are also included for comparative purposes: St. Andreu de Llavaneres from the north and Cuevas de Almanzora from the south. Dots in black show the 75th SST percentile for each locality. Temperature of 19.5°C (red dots –suggested value of the 75th SST percentile used to explain the distribution limit of *Ostreopsis*), has been included in every graphic, as a reference value to ease the comparison among different places.

concerning *Ostreopsis* presence and its blooming capacity. Note, for example, that maximum temperatures are higher in Donostia and Santoña compared to Lagos, but that the 75th SST percentile is higher in the later, where *Ostreopsis* has been observed to bloom in September 2011 (David *et al.*, 2012). SST was very similar

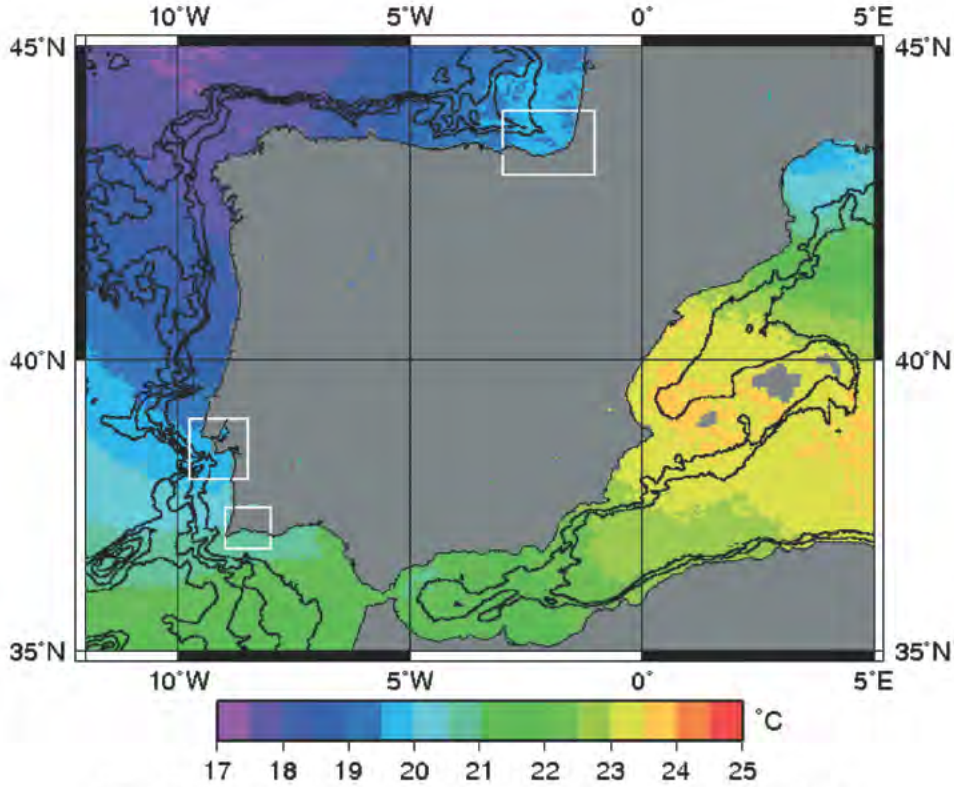


Fig. 6. 75th percentile of SST from 1985 to 2009 with a resolution of 4 × 4 km.

in the sampling days of both years in Lagos (20.9°C in 2010 and 20.5°C in 2011) (Fig. 7). Nevertheless, while maximum temperatures were higher in 2010 (22.6°C) when compared to 2011 (21.6°C), the 75th percentile was higher in 2011. For comparative purposes, two localities from the Mediterranean side of the Iberian Peninsula (St. Andreu de Llavaneres in the north (site A) and Cuevas de Almanzora in the south (site B)) (Fig. 1) where nuisance *Ostreopsis* blooms have been reported (Garcia *et al.*, 2008; Vila *et al.*, 2010) were also studied in terms of temporal SST behaviour (Fig. 5). It can be observed that SST 75th percentiles are markedly higher in the Mediterranean than in the Atlantic coast of the Iberian Peninsula.

In a study about SST between the years 1986 and 2008 performed close to site 14, Revilla *et al.* (2010) reported temperatures as high as 25.05°C and, moreover, they observed a slight but significant warming trend. This kind of trend in temperature has also been reported by Díez *et al.* (2012) in a study performed between sites 13 and 14 which showed temporal changes (from 1991 to 2008) in the sublittoral macrophyte community, with an increase in species of meridional affinity. On the other hand, it has been reported that warm-water macrophytes have expanded their distribution range to the north in Portuguese coasts (Lima *et al.*, 2007). Consequently, distribution shifts for *Ostreopsis* spp. can also be expected. Although this genus has not been found in high densities in the Bay of Biscay to date, considering the warming of the sea and specific annual

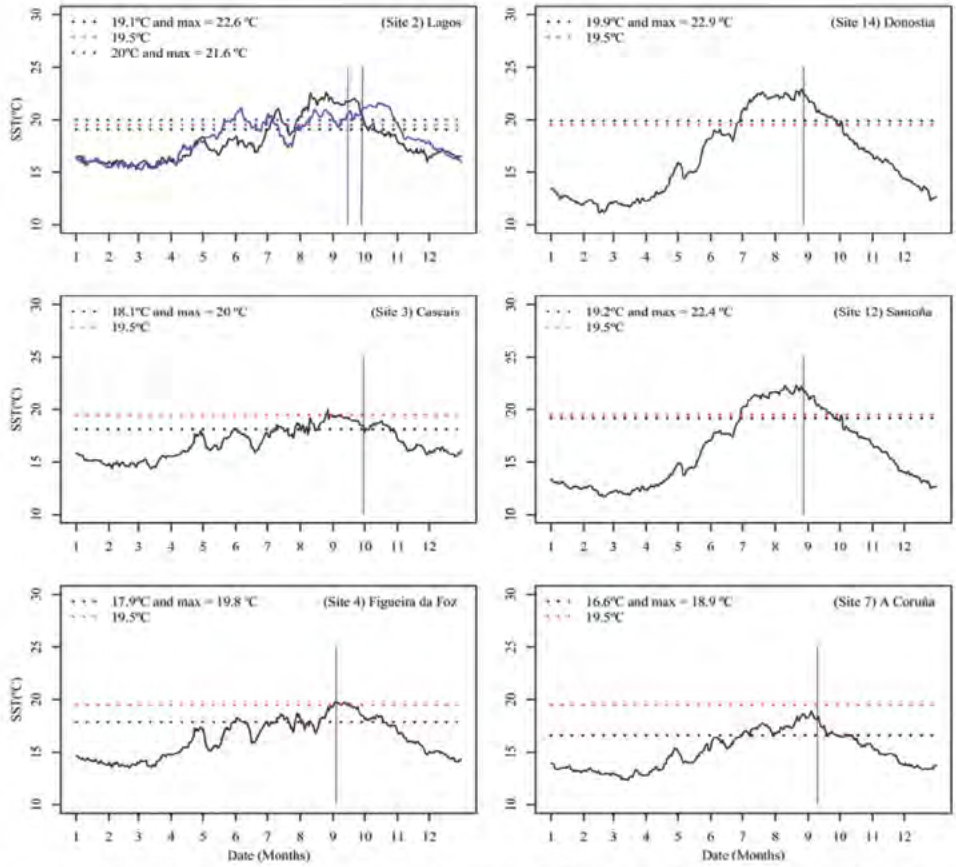


Fig. 7. Daily SST data during 2010 for selected sampling sites (legend of figure 3 for criteria of choice of selected sampling sites). Vertical lines represent sampling days. Black dots mark the 75th SST percentile of 2010. Red dots correspond to 19.5°C. For site 2, daily SST data during 2011 is also shown (in blue) and the day from 2011 when a bloom was observed is also marked (blue vertical line).

temperature anomalies, we presume that eventually, temperature would not be a limiting factor to the proliferation of this dinoflagellate in the area. For a genus that is considered to be composed of warm water species, its distribution limits can probably be explained by temperatures, whilst the factors enhancing its proliferation, leading to blooms can be more diverse and complex (Totti *et al.*, 2010). The accumulation of microalgae cells that are so weakly attached to the substrate is probably impeded in an area of high hydrodynamics such as the Cantabrian Sea where periods of calm water are short and protected sites such as embayments are scarce and mostly associated with estuarine areas.

We could expect that distribution limits of a genus as *Ostreopsis*, composed by warm water species, will be established by the species adapted to lower temperatures. According to Granéli *et al.* (2011), optimal growth temperatures for *Ostreopsis* cf. *ovata* in cultures are from 26 to 30°C and these results agree well with water temperature during proliferations of individuals of this species (Mangialajo *et al.,* 2008) in the Mediterranean Sea. However, peaks

of *Ostreopsis* cf. *ovata* have also been recorded with temperatures as low as 21.8°C and 16.8°C (Totti *et al.,* 2010) in the northern Adriatic Sea, which highlights the idea that the minimal temperature for *Ostreopsis* spp. to proliferate is still not clear (Mangialajo *et al.,* 2011). To our concern, the response to temperature of *O.* cf. *siamensis* has not been tested, but presumably, it could be adapted to lower temperatures since it was the only species found in the Bay of Biscay (Laza-Martínez *et al*., 2011).

In summary, comparing the observed distribution of *Ostreopsis* spp. in the Atlantic coast of the Iberian Peninsula with SST, we observed that the 75th SST percentile is the statistical parameter that better explains the current distribution. The 75th SST percentile of 19.5°C observed in both distribution limits leads us to hypothesize that, roughly, the *Ostreopsis* species present in the Iberian coasts, would need three continuous months with a SST within or above 19.5°C to be present in a certain area. Of course these results, based on low temporal replication (only two years are considered), need to be confirmed by future studies, in order to allow making predictions of future *Ostreopsis* spp. distributions in the Iberian Peninsula based on different seawater warming scenarios.

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