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Corresponding Author: Dr Luisa Mangialajo

First Author: Luisa Mangialajo

Order of Authors: Luisa Mangialajo; Nicolas Ganzin; Stefano Accoroni; Valentina Asnaghi; Aurelie Blanfuné; Marina Cabrini; Riccardo Cattaneo-Vietti; Fabienne Chavanon; Mariachiara Chiantore; Stephanie Cohu; Eleonora Costa; Daniela Fornasaro; Hubert Grossel; Francoise Marco-Mirailles; Mercedes Maso; Albert Rene; Anna Maria Rossi; Montserat M Sala; Thierry Thibaut; Cecilia Totti; Magda Vila; Rodolphe Lemee

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Trends in Ostreopsis proliferation along the Northern Mediterranean coasts

Luisa Mangialajo1*, Nicolas Ganzin2, Stefano Accoroni3, Valentina Asnaghi4, Aurélie Blanfuné5,1, Marina Cabrini6, Riccardo Cattaneo-Vietti4, Fabienne Chavanon2, Mariachiara Chiantore5, Stéphanie Cohu1,5, Eleonora Costa4, Daniela Fornasaro6, Hubert Grossel2, Françoise Marco-Miralles2, Mercedes Masó7, Albert Reñé7, Anna Maria Rossi4, M. Montserrat Sala7, Thierry Thibaut1, Cecilia Totti3, Magda Vila7, Rodolphe Lemée5.

1Université de Nice-Sophia Antipolis, EA ECOMERS, Parc Valrose, BP 71 06108 Nice cedex 2, France. E-mail: luisa.mangialajo@unice.fr
2Ifremer – Institut français de recherche pour l’exploitation de la mer – Centre de Méditerranée, Laboratoire Environnement / Ressources. ZP de Brégaillon, BP 330, 83507 La Seyne sur mer, France
3Università Politecnica delle Marche, Dipartimento di Scienze del Mare, via Brecce Bianche, 60131 Ancona, Italy
4Università degli Studi di Genova, Dip.Te.Ris., C.so Europa 26, 16132 Genova, Italy
5Université Pierre et Marie Curie – Paris 6, CNRS, Marine Microbial Ecology Group, Laboratoire d’Océanographie de Villefranche, BP 28, 06234 Villefranche-sur-Mer, France
6Istituto Nazionale di Oceanografia e Geofisica Sperimentale, Dipartimento di Oceanografi Biologica, Via Auguste Piccard, 54, 34014 Trieste, Italy
7Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37-42, 08003 Barcelona, Spain

Corresponding author: Luisa Mangialajo, ¹Université de Nice-Sophia Antipolis, EA ECOMERS, Parc Valrose, BP 71 06108 Nice cedex 2, France. Phone: +33 (0) 4 92076883; fax : +33 (0) 4 92076849; e-mail: luisa.mangialajo@unice.fr
Abstract

Harmful benthic microalgae blooms represent an emergent phenomenon in temperate zones, causing health, ecological and economic concern. The main goal of this work is to compile records of *Ostreopsis* at large temporal and spatial scales, in order to study the relationship between cell abundances, the periodicity and intensity of the blooms and the role of sea water temperature in 14 Spanish, French, Monegasque and Italian sites located along the northern limits of the Mediterranean Sea. General trends are observed in the two considered basins: the north-western Mediterranean Sea, in which higher cell abundances are mostly recorded in mid-summer (end of July), and the northern Adriatic Sea where they occur in early fall (end of September). The sea water temperature does not seem to be a primary driver, and the maximal abundance periods were site and year specific. Such results represent an important step in the understanding of harmful benthic microalgae blooms in temperate areas, and provide a good base for managers in the attempt to monitor and forecast benthic harmful microalgae blooms.

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1 Introduction

*Ostreopsis* species are benthic dinoflagellates generally recorded in tropical and subtropical seas, but the worldwide occurrence of this genus increased markedly in the last decade and this trend is likely to continue (Rhodes, 2010). In particular, during the last 15 years, *Ostreopsis cf. ovata* and/or *Ostreopsis cf. siamensis* were found in high concentrations during the summer in temperate regions such as New-Zealand (Chang et al., 2000; Rhodes et al., 2000, Shears & Ross, 2009) and the Mediterranean Sea (Vila et al., 2001; Penna et al., 2005; Mangialajo et al., 2008; Totti et al., 2010); high concentrations of *Ostreopsis* were also observed in the waters of Japan, witnessed by the presence of a considerable number of microalgae cells in parrotfishes (Taniyama et al., 2003), and of Russia (Selina & Orlova, 2010). Those observations imply an extension of the area of distribution of *Ostreopsis* in both the northern and southern hemispheres (Shears and Ross, 2009). As suggested by Miraglia et al. (2009), the changing global climate could affect the distribution of Harmful Algal Blooms (HABs) and the related sanitary problems. Whether this extension represents a new introduction supported by climate change or represents the proliferation of species that have already been present for a long time at low concentrations (Tognetto et al., 1995) is still questionable. In fact *Ostreopsis* sp. (probably *O. ovata*) was already observed during the seventies in the bay of Villefranche (northern Mediterranean; Taylor, 1979).

In tropical zones, several species of *Ostreopsis* are commonly associated with *Prorocentrum* and *Gambierdiscus* and therefore were suspected to be involved in ciguatera fish poisoning (Tosteson et al., 1989; Tindall & Morton, 1998). Now it has been clarified that *Ostreopsis* species produce palytoxin (PTX) and analogs (Riobó et al., 2006; Ciminiello et al., 2008) and a number of deaths directly associated with the ingestion of PTX contaminated seafood, i.e. crustacean (Alcala et al., 1988) or fish (Onuma et al., 1999; Taniyama et al., 2003), were reported.

In temperate zones, *Ostreopsis* blooms were mainly involved in i) intoxications by inhalation (Gallitelli et al., 2005; Durando et al., 2007), ii) irritations by contact and iii) mass mortalities of invertebrates. Intoxications by ingestion have not been reported, even if PTX and/or analogs have already been found in seafood by chemical or toxicological analysis (Taniyama et al., 2003; Aligizaki et al., 2008; Amzyl, pers. comm.).

The most important inhalation intoxications were observed in Italy in 2001 (Casavola et al., 2005) and in 2005 (Brescianini et al., 2006) and in Spain in 2004 and 2006 (Alvarez et al., 2005; Barroso Garcia et al., 2008; Vila et al., 2008), concerning more than 200 persons each time (from swimmers to beach walkers or local residents). During the summer of 2009, local newspapers reported the intoxication of several hundreds of persons in Algeria, related to *Ostreopsis* blooms (Illoul, pers.
comm.). Respiratory intoxications are due to inhalation of seawater droplets containing *Ostreopsis* cells or fragments of cells and/or aerosolized toxins.

Irritations due to contact (mainly skin irritations) have also been reported (Tichadou et al., in press), but generally the extent of such events can be underestimated due to the non-specificity of the symptoms and to the fact that medical support is generally not necessary and affections are therefore not recorded.

Invertebrate mass mortalities linked to *Ostreopsis* blooms have been observed by several authors in the Mediterranean Sea (Sansoni, 2003; Simoni et al., 2003; Totti et al., 2010), in Brazil (Granéli et al., 2002) and in New-Zealand (Shears & Ross, 2009). Even if oxygen depletion or high seawater temperature (often associated to HAB blooms) could also be involved in those mortalities, the role of the toxic microalgae seems to be substantial (Shears & Ross, 2009).

Therefore, from the management perspective, the *Ostreopsis* bloom phenomenon is complex and shows several intermingled facets, from human intoxication to potential ecosystemic modifications and loss of resources. While intoxications by inhalation are mostly linked to the cell concentrations in the water (planktonic cells), the irritations by contact are potentially mostly linked to the abundance of benthic cells. The mass mortalities are potentially linked to both planktonic (*via* the filter-feeders) and benthic (*via* herbivores) cells. The link between planktonic and benthic abundances is still unclear: Mangialajo et al. (2008) and Vila et al. (2001) found a simultaneous increase of abundances in both matrices, while Totti et al. (2010) mostly observed, at a small temporal scale, increases of cell concentrations in the water column closely related to hydrodynamic events. Unfortunately, the dynamics of benthic and planktonic *Ostreopsis* cells are still poorly known, as are the ecological factors involved in the blooms. The temperature seems to be a crucial parameter, as already reported following field observations (Sansoni et al., 2003; Simoni et al., 2003; Mangialajo et al., 2008; Cabrini et al., 2010) and *in vitro* experiments (Granéli et al., in press), but the role of such a parameter is still being debated (Vila et al., 2001; Selina & Orlova, 2010, Totti et al., 2010). The evaluation of the effect of seawater temperature on the development of *Ostreopsis* species at their biogeographical limits is therefore of great interest, especially with regard to global warming.

The main goal of this work is to compile records of *Ostreopsis* abundances at large temporal and spatial scales in order to study the relationship between benthic and planktonic abundances, the periodicity and intensity of the blooms and the role of sea water temperature in the distribution of *Ostreopsis* at its Mediterranean northern limits (Spain, France, Monaco and Italy).

2 Materials and methods
2.1 Benthic and planktonic abundances

A total of 14 sites were considered for this study. From West to East (Fig. 1), 1 site was sampled in Spain (Sant Andreu de Llavaneres), 6 in France (Morgiret, Endoume, Méjean, Rochambeau, Marinières and Lido), 1 in Monaco (Larvotto) and 6 in Italy (Quarto, Pontetto, Portofino, Passetto, Portonovo and Santa Croce). Wave exposure, substrate type, geographic coordinates and the research group that carried out the sampling are listed in Table 1.

Two species have been identified in the western Mediterranean Sea, Ostreopsis cf. ovata and O. cf. siamensis (Penna et al., 2005; 2010). In all the sites considered in this study, taxonomic and/or genetic analysis highlighted the single presence of O. cf. ovata. Nevertheless we cannot exclude the sporadic presence of O. cf. siamensis.

In each site, abundances of epiphytic and planktonic Ostreopsis cells were recorded. Seawater samples for estimating abundances of planktonic cells were collected at shallow depth (approximately 0.5 m depth) using a plastic flask (from 250 ml to 1 l, according to the research group sampling protocols) and fixed with 2 - 4% neutralized formaldehyde or a 1 - 2 % Lugol’s solution (and in this case kept in the dark at 4°C before counting). Macroalgal samples for estimating abundances of benthic cells were sampled opening a plastic flask or bag on the chosen macroalgae in shallow waters (0.5 - 1 m for all the research groups except Trieste groups that performed the sampling at 2 m depth). The macroalgae were then delicately torn away and kept in the flask or bag. Different species of macroalgae (Table 2) were collected according to the site and the period; sampled macroalgae generally corresponded to the most abundant species in each site. Benthic samples were fixed with 2 - 4 % neutralized formaldehyde or a 1 - 2 % Lugol’s solution (and in this case kept in the dark at 4°C before counting).

Concentrations of Ostreopsis in the water samples were evaluated with an inverted microscope using the Utermöhl method (settling a minimum of 50 ml of sea water). The abundance of benthic cells was evaluated after a vigorous shake of the macroalgae samples, followed by the separation of macro and micro algae. Ostreopsis cells were then counted following the Utermöhl method, or using a direct microscope with calibrated slides. Results were expressed as the abundance of planktonic cells per liter and of benthic cells per gram of fresh weight of macroalgae (g FW).

2.3 Sea water temperature

In order to study the role of sea water temperature (SWT) on the dynamics of blooms, we used, when available, the temperature values measured in situ by each group during the sampling. In order to give a general seasonal characterization of the temperature of the surface waters in the
study area, satellite derived Sea Surface Temperatures (SST) were also used to generate monthly or seasonal average temperature maps. Satellite derived SST images are computed from the Thermal Infra-Red bands of coarse resolution satellite sensors (see for example Barton (2001) for a review on the data and methods). They are nowadays available globally, thanks to international collaboration projects such as the Global Ocean Data Assimilation Experiment (GODAE, Donlon et al. 2007). The data used in this study, kindly provided by the CERSAT (Centre of Research and Satellite exploitation of IFREMER, French National Oceanography Research Institute), were produced by Meteo-France for the Ocean & Sea-Ice Satellite Application Facility (Copyright 2010 EUMETSAT; see SAFOSI (2009) for details on the product). The sensor used in this case was NOAA/AVHRR (the Advanced Very High Resolution Radiometer of the National Oceanographic and Atmospheric Administration, USA), the image resolution being of approximately 1 km. With several AVHRR sensors operational at the present time, several images were available each day. Images of June to October for the years 2007, 2008 and 2009 were processed into monthly and summer averages.

3 Results

3.1 Benthic and planktonic Ostreopsis abundances
A total of more than 600 samples was analyzed in order to assess abundances of both benthic and planktonic Ostreopsis cells. Data relative to monthly maximal values of cell abundances (EMV: epiphytic maximal value; PMV: planktonic maximal value) for each considered site and year are reported in Table 2, together with the sampling dates and the in situ measured sea water temperature (SWT).

Although the distribution of points seems quite scattered (Figure 2), the correlation between EMV and PMV in a logarithmic scale is significant (n = 124; r = 0.7246; p < 0.01) and the percentage of variation explained is not negligible (R² = 0.5251). Benthic cell abundances are more conservative, showing more regular increase and decrease during the considered period, while planktonic cell abundances can show abrupt changes and a less regular trend over the summer period, as the concentrations in the water may change drastically due to sea conditions. For these reasons, the quantification of benthic cell abundance is a better indicator of the occurrence of blooms and we focus our synthesis mostly on the dynamics of EMV, which we consider more informative in the attempt to forecast Ostreopsis blooms that may represent a real health risk.
3.2 Temporal and spatial variability

As reported in the Materials and methods section, each research group involved in this study, although using a similar method of sample collection and treatment, collected different macroalgal species, in function of the most abundant species found in the benthic vegetation of the study sites. The Barcelona group always collected *Corallina elongata* or *Stypocaulon scoparium*, choosing the most abundant when they co-occurred; the Toulon group collected a mix of ribbon-like *Dictyota* and thin articulated *Corallinales* (mostly *Jania rubens* and *Haliptilon virgatum*), the Nice, Villefranche and Genoa groups *Stypocaulon scoparium*, the Trieste group *Dictyota dichotoma* and the Ancona group a different macroalgal species for each year/site (*Hypnea musciformis*, *Dictyota dichotoma*, *Polysiphonia* sp. and *Ulva* cf. *laetevirens*), as specified in Table 2. It is therefore very difficult to compare the different sites on an absolute quantitative basis (Lobel et al., 1988); nevertheless, when comparing sites sampled by the same group, or sites with the same macroalgal species, some sites consistently appear to have lower EMV values (e.g. Lido compared to Larvotto, Marinières and Rochambeau; Portofino compared to Pontetto and Quarto; Endoume compared to Méjean and Morgiret).

The periods of occurrence of relative maximal EMV values for each year (Y-EMV) were site and year specific. In the NW Mediterranean, most of Y-EMVs occurred in July (15 out of 24 records) and especially in the second half of the month (12 records). Y-EMVs in August are common (6 records), although two of them correspond to the first day of the month and can be included in the end-of-July group. Maximal values were recorded only once in June, September and October, while maximal values in November were never recorded. Nevertheless, it should be underlined that the sampling is not balanced and especially that June, October and November data are less abundant, due to the lower health risk linked to lower beach attendance. Most of the late-in-the-season Y-EMVs occurred in the N Adriatic Sea, where the highest concentrations are generally recorded at the end of September or at the beginning of October (4 records out of 5). In Passetto in 2009 the Y-EMV was recorded at the end of August, but EMV values were still high (same order of magnitude) in the first days of September. Benthic cell abundances seem to be very low in early summer, increasing rapidly in late summer and autumn, as in S. Croce where the maximum abundance was recorded at the end of September 2009.

Most of the relative maximal PMV values for each year (Y-PMV) were also recorded in July (15 records out of 24), especially in the second half of the month (12 records). Nine Y-PMVs occurred in August, 3 in September and 1 in June and October. It is worth noting that Y-PMVs often occur in the same month as the Y-EMVs (15 records out of 24), and in most cases (11 records) in the very same week.
Whenever a long sampling season occurred (at least 4 months), it was quite common to observe a second *Ostreopsis* bloom following the first one (in 7 cases out of 14 selected sites/years). This is especially obvious in the NW Mediterranean sites showing a first bloom in early summer (e.g. St Andreu de Llavaneres 2009, Marinières 2008 and 2009, Rochambeau 2009, Quarto 2007 and Portofino 2007). In Sant Andreu de Llavaneres in 2008, the October bloom was even higher than the July one, while in the same site in 2007 only the July bloom was recorded, as in Morgiret and Lido in 2009. This phenomenon was not observed in the N Adriatic sites, although in Passetto in 2007 a decrease in benthic cell abundances is observed in September. A second increase of planktonic *Ostreopsis* cells was observed in 5 cases out of the 7 second benthic blooms, but, not surprisingly, a second increase of planktonic cells in the absence of second benthic bloom was never observed. Nevertheless the amount of data collected in this period of the year is lower than in the summer, and higher benthic and planktonic cell abundances may be more frequent than observed.

### 3.3 Role of sea water temperature

The correlation between EMV and PMV and the measured Sea Water Temperature (SWT) is reported on Fig. 3 and 4. The abundance of benthic cells was significantly correlated to the measured SWT \( (n = 108; r = 0.2837; p < 0.05) \), as much as the abundance of planktonic cells \( (n = 108; r = 0.2289; p < 0.05) \). In both cases the percentage of variation explained is negligible \( (EMV-R^2 = 0.0805; PMV-R^2 = 0.0524) \): less than 10% of the variation observed for EMV and PMV is explained by the sea water temperature.

The monthly evolution of SST in the considered summer seasons is reported on Fig. 5. The warmest month was consistently August, when the SST reached 28°C in most of the area. In June and October, SST never reached 24°C in the NW Mediterranean Sea, while it was higher in the N Adriatic Sea (more than 25°C). In the Gulf of Lyon the SST was consistently lower, mainly due to a high number of windy days, resulting in a drop of temperature due to the upwelling of cold deep seawater in several zones. Consequently, three zones can clearly be identified in the study area. The first zone (A), corresponding to Western Provence and the Gulf of Lyon, which includes the Morgiret, Endoume and Méjean sampling sites and is the coldest. The second zone (B), corresponding to the Balearic and Ligurian seas, includes the Sant Andreu de Llavaneres, Rochambeau, Marinières, Lido, Larvotto, Quarto, Pontetto and Portofino sampling sites and is characterized by intermediate summer temperatures. The third zone (C), corresponding to the N Adriatic Sea, includes the Passetto, Portonovo and Santa Croce sampling sites and always shows
higher SST than zones A and B. The differences between the 3 zones are very clear, independently from the considered month and year, especially in July and August, when the difference of SST between A and C can reach 8 °C. As it can be observed on Figure 5, inter-annual SST variability can be substantial, the SST values in the summers of 2007 and 2009 being higher than in 2008. Y-EMVs and Y-PMVs are shown superimposed to mean summer SST in Fig. 6. A general trend can be identified at basin scale: in the NW Mediterranean Sea, the maximal abundances of Ostreopsis were recorded in July-August, with the exception of Méjean in 2007 (Y-EMV in September) and Sant Andreu de Llavaneres in 2008 (Y-EMV in October). It is worth stressing that, in both cases, at the end of July cell abundances were very high, of the same order of magnitude as the registered Y-EMV. Although the mean summer SST was generally higher in the sites near Barcelona, Nice and Genova (Zone B) than in the sites sampled in the Gulf of Lyon (Zone A, and in particular Morgiret and Endoume), no particular difference in the Y-EMV and Y-PMV was observed, except in 2008 when a delay of two weeks to one month was observed. Conversely, in the N Adriatic Sea (Zone C) the Y-EMVs and Y-PMVs always occurred in late summer-early fall, the abundance of cells being negligible in early summer.

4 Discussion

4.1 Benthic and planktonic Ostreopsis abundances
Harmful benthic microalgae development is an emergent phenomenon in temperate areas and the interest of scientists for Ostreopsis has recently increased (Shears & Ross, 2009). Nevertheless, the dynamics of benthic microalgae blooms are still mostly unknown. In the present study we found a relatively good correlation between epiphytic and planktonic cell abundances, as already observed by Mangialajo et al. (2008); this result, together with the relatively good correspondence between the dates of Y-EMV and Y-PMV, confirms that the benthic cells can be considered as the stock of available biomass. The delays observed between Y-EMV and Y-PMV can be related to the re-suspension of cells by wave action (e.g. Totti et al., 2010) and by vertical migration (e.g. Vila et al., 2001). In general we can consider that hydrodynamism (waves, boat traffic) and human trampling (especially in highly touristic zones) are important factors causing re-suspension of cells in shallow coastal areas at the local scale. Vertical migration of cells on a daily cycle (Vila et al., 2001) should also be considered, possibly linked to a potential critical density threshold (as observed for the migration of benthic diatoms up and down in the sediment, Guarini et al., 2008). More efforts are needed to disentangle the mechanism involved in the transfer of Ostreopsis cells from the benthos to the water column and vice versa.
High planktonic cell abundances represent a health risk for inhalation while benthic cell abundances are potentially mostly involved in irritations by contact. Both planktonic and benthic cells are involved in the invertebrates’ mass mortalities, with consequent potential modifications at the ecosystem level and loss of resources. No critical intoxication events have been recorded in the studied sites in the considered years. Nevertheless, some cases of inhalation and/or contact intoxication have been reported to the respective health vigilance systems in Sant Andreu de Llavaneres, Morgiret, Marinières, Larvotto, Quarto and Passetto. In August 1998, an event of invertebrates’ mass mortality was reported in Sant Andreu de Llavaneres (Vila et al, 2008) and in 2006 in Quarto (July) and in Morgiret (August) (author’s unpublished data), but no evident mass mortalities were recorded during the study period in the NW Mediterranean Sea, although some counts of limpets in Marinières showed a certain decrease, (author’s unpublished data). In the N Adriatic Sea, and in particular in Passetto, deaths of benthic invertebrates (echinoderms and molluscs) have been observed in circumscribed shallow areas in all the considered years (Totti et al., 2010).

Considering that planktonic abundances are highly variable (probably due to hydrodynamic events at local scale) and always occur concurrently to high benthic abundances, we recommend to managers to primarily monitor benthic abundances, which are more conservative and represent the stock of available biomass. For the monitoring of benthic Ostreopsis cells, it is important to deal with the variability linked to the macroalgal species sampled. Several options can be considered by managers: i) always sample the same macroalgal species (if the same species is common in all the sites and all along the year), ii) try to estimate conversion factors between different species, in order to be able to compare different sites and/or periods, iii) calculate the macroagal biomass for a surface unit of sea bottom and extrapolate the number of Ostreopsis cells per square meter of bottom (in this case, all the conspicuous macroalgal species should be considered in order to have cell abundances actually representative of the site).

4.2 Temporal and spatial variability

From a temporal point of view, in the NW Mediterranean Sea, the period when blooms can more likely occur is the end of July, while in the N Adriatic Sea blooms occur later in the season. We suggest to managers to increase the monitoring intensity in these periods. A second bloom, often of the same order of magnitude as the first one, can occur in late summer-fall, especially in the NW Mediterranean basin. Although this second bloom is less alarming in terms of beach attendance (the bathing season generally ends in September), managers should not neglect these blooms that may
represent a danger by potential contamination of seashells (Aligizaki et al., 2008) and cause benthic invertebrate mortalities.

From a spatial point of view, our results allowed to highlight that some sites seem to be more prone to *Ostreopsis* development than others. In Endoume, Lido and Portofino, benthic cell abundances were consistently lower than in neighboring sites, although the general characteristics of the sites (*e.g.* rock type, conformation of the coast, macroalgal communities, wave exposure, see M&M section) can be considered comparable. In these sites, planktonic abundances also are always low, never reaching 10 000 cells/l. As most episodes with health concern were associated with cell concentrations above 30 000 cells/l (Mangialajo et al., 2008; author’s unpublished data), we can consider that in some areas the risk is higher and managers should therefore focus their attention on monitoring sites where substantial blooms were observed in the past and are consequently more likely to occur.

### 4.3 Role of temperature

The potential role of sea water temperature (SWT) in regulating *Ostreopsis* blooms in temperate areas has been highlighted by previous studies (Mangialajo *et al*., 2008; Shears & Ross, 2009), although similar studies did not identify any direct correlation between cell abundances and SWT (Vila *et al*., 2001; Totti *et al*., 2010; Selina & Orlova, 2010). Granéli *et al.* (in press) found a strong relationship between temperature and *Ostreopsis* growth rate in culture and suggested that sea water temperature increase resulting from global warming could play a crucial role in inducing the geographical expansion and biomass accumulation by blooms of *Ostreopsis ovata*.

If sea water temperature is one of the major factors affecting *Ostreopsis* blooms, the Y-PMV and/or Y-EMV observed in this study should (1) occur in relation with thermal cycle of each defined zone (A, B and C) and (2) vary according to the interannual variability recorded during the study period. Our results highlighted a very low correlation between both EMV and PMV with SWT measured *in situ* and the shifts in periods of higher abundance of *Ostreopsis* between the 3 zones are not in agreement with the differences in SST. The delay observed between the two basins is the opposite of what one would have expected following the temperature differences (an early bloom in the N Adriatic Sea, where summer SST is higher, and a later bloom in the NW Mediterranean sites where summer SST is lower).

We cannot exclude that such differences may be explained with the occurrence of different strains having different temperature affinities. The Adriatic and Tyrrhenian strains investigated by Guerrini *et al.* (2010), presented significant differences in cell volumes (the Adriatic strain being nearly twice the size of the Tyrrhenian one), although in a recent biogeographical analysis, all the
Atlantic/Mediterranean *Ostreopsis* samples have been assessed to belong to a single clade (Penna et al., 2010).

In the NW Mediterranean Sea, obvious SST differences are noticed between zone A (Gulf of Lyon) and B (Balearic and Ligurian Sea), but no corresponding delay in *Ostreopsis* blooms is observed, except for the summer of 2008, where the blooms in the Gulf of Lyon were actually delayed by 2 to 4 weeks (especially in the Endoume and Morgiret sites). Although the sea water temperature may play a major role in a specific site, such a relationship is not visible any more when increasing the study scale to the basin level. It is likely that a minimum threshold temperature is needed for *Ostreopsis* proliferation: in a previous work, Mangialajo et al. (2008) hypothesized that *Ostreopsis* proliferation may be facilitated by a SWT above 26°C. Nevertheless, in this paper we report high benthic cell abundances at lower SWT levels (23°C in the NW Mediterranean and less than 20°C in the N Adriatic Sea). Unfortunately the data collected do not allow us to directly compare the intensity of blooms in the three considered zones.

The role of temperature requires further investigation and the impacts of other drivers have to be elucidated to better explain the bloom dynamics. Such drivers may be inorganic nutrients concentration and/or their ratio, substrate characteristics (*e.g.* composition of macroalgal communities), competitive and trophic interactions, or regulation of dinoflagellates by parasites (Chambouvet et al., 2008). Hydrodynamism due to waves and/or currents should also play an important role in regulating benthic dinoflagellate abundances, but the effect of this abiotic factor is very difficult to evaluate. Finally, human-induced drivers, such as eutrophication, which is known to facilitate microalgal blooms (Heisler et al., 2008), should also be taken into account. Human-induced impacts can act in a direct way (*e.g.* increased nutrients availability), but also indirectly, interacting with other factors (*e.g.* herbivory, Buskey, 2008; Smayda, 2008). In a changing world, in which human impacts are cumulated (Halpern et al., 2008) and interact in different ways (Crain et al., 2008), the mechanisms of facilitation of new phenomena should consider the interaction of human-driven and natural factors. This is particularly true in the investigated area (NW Mediterranean and N Adriatic Seas), where human pressure is high and historically rooted.

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Figure captions:

Fig. 1: Location of sampling sites along the North-Western Mediterranean and Northern Adriatic coasts.

Fig. 2: Correlation between Epiphytic Maximal Value (EMV) and Planktonic Maximal Value (PMV) for all the study sites, on a logarithmic scale.

Fig. 3: Correlation between Epiphytic Maximal Value (EMV) and measured Sea Water Temperature (SWT) for all the study sites; EMV is expressed in logarithmic scale.

Fig. 4: Correlation between Planktonic Maximal Value (EMV) and measured Sea Water Temperature (SWT) for all the study sites; PMV is expressed in logarithmic scale.

Fig. 5: Monthly average satellite derived Sea Surface Temperature maps for summer and early fall for 2007, 2008 and 2009. Zone A: Western Provence and the Gulf of Lyon; Zone B: Balearic and Ligurian seas; Zone C: Adriatic Sea.

Fig. 6: Maps of summer average of satellite derived Sea Surface Temperature together with dates (day/month) of the yearly Epiphytic Maximal Value (Y-EMV) and yearly Planktonic Maximal Value (Y-PMV).
Figure 2

\[ y = 0.7054x - 0.1691 \]

\[ R^2 = 0.5251 \]

**Ostreopsis cells / l of sea water**

**Ostreopsis cells / g of macroalgae (FW)**
Figure 3

The scatter plot shows the relationship between sea water temperature (°C) and the number of Ostreopsis cells per gram of macroalgae (FW). The linear regression equation is $y = 0.121x + 1.6394$ with a coefficient of determination $R^2 = 0.0805$. The data points are distributed across a wide range of sea water temperatures and cell counts, indicating a positive correlation between the two variables.
Figure 4

The graph shows a positive correlation between the sea water temperature (°C) and the number of Ostreopsis cells per liter of sea water. The linear regression equation is:

\[ y = 0.096x + 0.7182 \]

with \( R^2 = 0.0524 \).
Table 1. Sampling sites, research group that performed the sampling and the sample treatment, characteristics of the site and geographical coordinates.

<table>
<thead>
<tr>
<th>Site</th>
<th>Research group</th>
<th>Wave exposure</th>
<th>Substrate</th>
<th>Coordinates</th>
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<td>Natural rock</td>
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<td>Toulon</td>
<td>Exposed</td>
<td>Natural rock</td>
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<td>Toulon</td>
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<td>Natural rock</td>
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<td>Toulon</td>
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<td>Nice-Villefranche</td>
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Table 2: Synthesis of available data collected in the study sites in summer 2007, 2008 and 2009.

EMV: Epibenthic maximal value; PMV: Planktonic maximal value. In bold the maximal values for each year and site (Y-EMV; Y-PMV).

<table>
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<tr>
<th>Site (macroalgal species)</th>
<th>Year</th>
<th>Month</th>
<th>Sampling dates (day of the month)</th>
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<th>EMV date (Temp°C)</th>
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### Santa Croce (Dictyota dichotoma)

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|          |      | 21 (22.8) | 140 | 21 (22.1) | 5 (21.2) | 280 | 5 (21.2) |

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1. Sea water samples of dates 26/07/07, 11/08/07, 16/08/07, 07/09/07, 11/09/07, 19/06/0 are missing. Macroalgal samples of dates 11/10/07, 19/10/07, 29/11/07, 06/06/0, 29/07/09 are missing.

2. Sea water samples of dates : 01/07/07, 07/07/07, 15/07/07 are missing. Macroalgal samples of date : 30/07/08 is missing.

3. In 2007 macroalgal samples were collected in Quarto on fewer dates than seawater samples: 21- June; 9, 17, 20, 26- July; 1, 7, 13, 24, 29 August; 7 September; 23 October; 26 November. Seawater sample of date 23/10/2007 is missing.

4. Sea water samples of dates : 10/07/08 are missing.

5. Macroalgal samples of dates: 20/07/07, 11/06/08, 28/07/08 are missing.