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Occurrence of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation with environmental factors in Monaco (NW Mediterranean)

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ABSTRACT

To study environment characteristics favoring the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*, a survey was conducted in Monaco (NW Mediterranean Sea), in summers 2007 and 2008. Epiphytic and planktonic blooms occurred almost simultaneously and a high variation of abundances at low spatial scales was observed. An early and very marked bloom occurred in 2007, compared to a later and less abundant development in 2008. These distinct patterns in bloom timing corresponded with very different hydroclimatic scenarios in 2007 (hot spring and relatively cold summer) and 2008 (standard year compared to the median year profile estimated with data from 1995 to 2008). No clear impacts of summer seawater temperature, rainfall or nutrient concentrations were evident. Strong wind may favor the dispersal of benthic and planktonic cells. Our study suggests that further investigations are needed to examine the potential role of *Ostreopsis* nutritional mode (i.e. autotrophy vs. mixotrophy).

1. Introduction

Events of Harmful Algal Blooms (HABs) have increased all around the world over the past several decades, with an increase in the diversity of the harmful species and the number of areas affected (Anderson, 1995; Van Dolah, 2000; Cheng et al., 2005; Maso and Garcés, 2006). For instance, worldwide occurrence of the toxic benthic dinoflagellates genus *Ostreopsis* Schmidt has increased during the last 15 years (Rhodes, 2011). Previously, species of this genus were frequently observed in tropical and subtropical areas (Ballantine et al., 1988; Morton et al., 1992; Grzebyk et al., 1994; Parsons and Preskitt, 2007) and were found generally between 35° North and 35° South latitude, but blooms in the Mediterranean Sea and in New Zealand have become frequent (Chang et al., 2000; Rhodes et al., 2000; Vila et al., 2001a; Maso and Garcés, 2006; Spatharis et al., 2009; Rhodes, 2011). While this genus was first reported in 1972 for the French Riviera (Taylor, 1979), the first known bloom in the Mediterranean Sea of *Ostreopsis* cf. *ovata* Fukuyo was recorded in 1998 along the Tuscany coasts (Sansoni et al., 2003). Since then this species has been found in the Balearic Sea in 2001 (Penna et al., 2005), in the Ligurian Sea from 2005 (Ciminiello et al., 2006; Mangialajo et al., 2008), in the Tyrrhenian Sea from 1994 (Tognetto et al., 1995; Simoni et al., 2003; Penna et al., 2005; Zingone et al., 2006; Guerrini et al., 2010), in the Adriatic Sea in 2006 (Monti et al., 2007; Battocchi et al., 2010; Totti et al., 2010) and in the North Aegean Sea from 2003 (Aligizaki and Nikolaidis, 2006; Aligizaki et al., 2008). *Ostreopsis* sp. has been observed along the Catalan coast from 1995 (Vila et al., 2001a,b; Battocchi et al., 2010). Another species, *Ostreopsis* cf. *siamensis* Schmidt, was first recorded in 1979 in the Eastern Mediterranean Sea, along the Lebanese coast (Abboud-Abi Saab, 1989) and since has been frequently observed in the Tunisian and the Greek waters (Turki, 2005; Aligizaki and Nikolaidis, 2006). The origin of *Ostreopsis* species in the Mediterranean is yet uncertain, but the similar phylogenetic traits of NW Mediterranean and Brazilian strains of *O.* cf. *ovata* (Penna et al., 2005) suggest a great increase in distribution. This hypothesis seems to be confirmed by the recent biogeographical analysis of Penna et al. (2010), who shows that *Ostreopsis* samples from the Mediterranean basin and Atlantic (West and East coasts) belong to a single clade.

Species of the genus *Ostreopsis* are preferentially epiphytic/epibenthic and grow in shallow waters, on macrophytes or directly on the abiotic substrates (Ballantine et al., 1985; Faust and Morton, 1995; Vila et al., 2001b; Simoni et al., 2003; Totti et al., 2010). But with rapid proliferation, cells may detach from the substrate

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and are found in the water column (Fukuyo, 1981; Totti et al., 2010). Cells in the water column can form dense aggregates occurring as mucilage on the surface (Vila et al., 2001b).

Ostreopsis is known to produce palytoxin (PTX) and analogues; PTX is one of the most potent phycotoxins in tropical seafood intoxications (Ito et al., 1996; Yasumoto, 1998; Onuma et al., 1999; Taniyama et al., 2003; Lenoir et al., 2004; Riobo et al., 2006; Deeds and Schwartz, 2010). In the Mediterranean Sea, neurotoxic effects due to toxin accumulation in food web have not yet been reported and *Ostreopsis* species are implicated thus far only in respiratory affections and skin or eyes irritations, in events in Italy and Spain (Simoni et al., 2003; Brescianini et al., 2006; Ciminiello et al., 2006; Barroso Garcia et al., 2008; Tichadou et al., 2010). These syndromes may be caused by simple contact and/or inhalation of cells (or toxins), and can affect people near the shore exposed to marine aerosols during *Ostreopsis* bloom events (Gallitelli et al., 2005; Kermarec et al., 2008; Tubaro et al., 2011).

Concerning possible impacts on marine organisms, mass mortalities of urchins and shellfishes have been observed during *Ostreopsis* blooms in Italy (Simoni et al., 2003; Totti et al., 2010) and New Zealand (Shears and Ross, 2009). While *Ostreopsis* probably had an important part in these mortality events, the impact of others environmental factors, such as temperature increase or oxygen depletion, need to be further studied.

To predict blooms of *Ostreopsis*, it is essential to determine environmental conditions which favor this microalga. In tropical areas, despite the relative stability of environmental parameters, some seasonal patterns of *Ostreopsis* spp. abundances have been reported. A few studies have tried to highlight the causes of these variations, and have advanced opposing trends: in Puerto Rico no link between *Ostreopsis lenticularis* blooms and temperature (always above 25 °C) was observed (Ballantine et al., 1988), while in Florida, Morton et al. (1992) showed that *Ostreopsis siamensis* and *Ostreopsis heptagona* were negatively correlated with a similar range of temperatures. In the Gulf of Mexico, Okolodkov et al. (2007) concluded that the observed seasonal trends of *O. heptagona* could not be explained by temperature. In Hawaii, *O. ovata* abundances seemed to be positively correlated with some nutrient parameters (Parsons and Preskitt, 2007). Anthropogenic nutrient enrichment, resulting in the increase of macroalgae cover, also seems to have a positive impact on *Ostreopsis* spp. growth in Florida Keys (Lapointe et al., 2004) and in Christmas Island, SE Indian Ocean (Briggs and Leff, 2007). Other environmental parameters, such as wind (Okolodkov et al., 2007) and rainfall (Morton et al., 1992), could have a significant impact on *Ostreopsis* blooms occurrence, but their roles remain controversial.

In temperate zones, the most studied areas are the Mediterranean Sea and the New Zealand coasts. In these areas, medium to elevated temperatures (from 22 to 30 °C) seem to favor *Ostreopsis* spp. blooms (Chang et al., 1997; Simoni et al., 2003; Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2008), but the link between temperature and *Ostreopsis* growth is still not clear (Mangialajo et al., 2011). The potential role of other physical or chemical parameters has yet to be demonstrated, because of the lack of *in situ* studies.

To manage and prevent economic and health risks related to *Ostreopsis*, an *in situ* monitoring of *O.* cf. *ovata* development was carried out in Monaco, NW Mediterranean Sea, during the summers 2007 and 2008. This very small country (2 km^2) has the highest density of population recorded in the world, with 22,083 inhabitants per sq. km predicted for 2010 (United Nations, 2009). The high density of seaside inhabitants could represent a large population potentially at risk through exposure to aerosols which can transport *Ostreopsis* toxins and/or cells, as during the intoxication event in Pozo del Esparto (Spain, Andalousia) in August 2006 (Barroso Garcia et al., 2008). A large part of the seaside activities in Monaco are centralized on the only large beach of the city (Larvotto), which could increase the health risk in case of important *Ostreopsis* bloom. In this study, temporal changes in the abundances of epiphytic and planktonic *Ostreopsis* cells were monitored during summers 2007 and 2008 along the Larvotto beach of Monaco and related to different environmental factors, in an attempt to study conditions which favor blooms.

2. Materials and methods

2.1. Molecular analysis

In order to confirm *O.* cf. *ovata* identification, molecular analysis was applied on live samples collected on macroalgae in 2007 on the Larvotto beach. Without any DNA extraction and purification steps (not necessary), we use the single cell amplification method (Takano and Horiguchi, 2005) and amplified the ITS sequences of at least 10 cells. The PCR was performed using the GoTaq® DNA polymerase (Promega), the buffer 5X Green GoTaq® Flexi and the ITS A and ITS B primers used by Adachi et al. (1994). The amplification included a first denaturing step (5 min at 94 °C), 35 cycles composed of one denaturing step (45 s at 94 °C), one annealing step (45 s at 53 °C) and one elongation step (45 s at 72 °C), and a final extension step (7 min at 72 °C). The purification of the PCR products was done with the QIAquick PCR purification kit (Quiagen). Sequencing was performed by the OUEST-Genopole sequencing platform of the Roscoff Biological Station. The obtained sequences were realigned and corrected using the BioEdit software, and compared to GenBank *Ostreopsis* sequences.

2.2. Sampling and quantification of cells abundances

During July and August 2007 and 2008, samples were collected by snorkeling once a week (on Monday morning, before 11:00 am) in eight sites at the Larvotto beach of Monaco (Fig. 1). Sites numbered 1–5 were on artificial rocks, while sites A, B, and C were located on sandy beach. To quantify planktonic concentrations of *O.* cf. *ovata*, 250 ml of seawater were sampled in a plastic flask at about 20 cm above a specific macroalga located at 50 cm depth on rocky substrate (sites 1–5; one sample per week in each site). We sampled seawater before macroalgae in order to avoid any resuspension of epiphytic *Ostreopsis* cells in the water column. Then, to determine epiphytic quantities of *O.* cf. *ovata*, the specific macroalga was sampled, avoiding as much

Fig. 1. The Mediterranean Sea with the position of Monaco in the NW part and map (Google Earth) showing location of sampling sites on the Larvotto beach (Monaco). Sites A, B and C: sandy beach. Sites 1–5: artificial rocks.

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as possible any loss of microalgae by putting it directly and carefully in a 250 ml plastic flask with the surrounding seawater (sites 1–5; one sample per week in each site). The macroalgae species was mainly *Stypocaulon scoparium* (Linnaeus) Kützing, except in site 2 where *Corallina elongata* (Ellis and Solander) was the only species easily available. For beach sites (A, B and C), only one seawater sample was collected each week, at 30 cm depth and about 20 cm above the sand.

O. cf. *ovata* cells were fixed by adding acidic Lugol at 1% (vol./vol.) for planktonic samples or 2% (vol./vol.) for epiphytic samples. Samples were kept at 4 °C in dark. Concentration of planktonic *Ostreopsis* was determined with an inverted microscope (Utermöhl method, 1958) using 50 ml water subsamples. To determine the abundance of epiphytic cells, macroalgae samples were vigorously shaken and wash water was filtered through a 500 μm meshed filter to separate macroalgae and water containing microalgae. Macroalgae were rinsed twice with 100 ml of 0.2 μm filtered seawater to recover a maximum of microalgae. Cells abundances in the filtered water were evaluated with a standard microscope using standard volume chambers (1 ml; Sedgwick Rafter©). The weight of macroalgae was measured to relate the abundance of *Ostreopsis* to macroalgal biomass as number of cells per gram of fresh weight macroalgae (cells g^{-1} FW).

2.3. Environmental factors

2.3.1. Nutrient and Chlorophyll a determinations

Two samples of seawater were collected at each sites (1–5) to determine nutrient and Chlorophyll a (Chl a) concentrations. Water samples for nutrient determinations were fixed with 0.06% of $HgCl_2$ solution and kept in dark at 4 °C. Concentrations of NO_2, NO_3, PO_4 and $Si(OH)_4$ were measured using an automatic analysis chain (EV2-Alliance Instrument) according to Tréguer and Corre (1975). Water samples for Chl a quantification were filtered (1 l per sample) on a Whatman GF/F. The filters were kept frozen (−20 °C) before extraction in a 90% acetone solution. Chl a concentration was measured using fluorometry, following Lorenzen and Downs (1986).

2.3.2. Hydrology and meteorology

To compare 2007 and 2008 hydroclimatic conditions, hydrological data were taken from Point B in Villefranche Bay (one of the French coastal monitoring site studied by the SOMLIT network) and meteorological records were obtained at Saint Jean Cap Ferrat station (Météo France). Both Villefranche Bay and Cap Ferrat were located at less than 10 km west of Monaco and can be considered as reference areas for the nearby zone studied. Temperature

Table 1
Modalities number and classes bounds of active and illustrative variables used for Multiple Correspondence Analysis (MCA). Inferior bunds included, superior bunds excluded.

	Variables	Number of modalities	Bounds
Active variables	Epiphytic *Ostreopsis* (OFW = *Ostreopsis* cells g^{-1} fresh weight)	5	OFW1: 0–10,000 cells g^{-1} FW OFW2: 10,000–20,000 cells g^{-1} FW OFW3: 20,000–100,000 cells g^{-1} FW OFW4: 100,000–200,000 cells g^{-1} FW OFW5: >200,000 cells g^{-1} FW
	Planktonic *Ostreopsis* (OL = *Ostreopsis* cells l^{-1})	5	OL1: 0–500 cells l^{-1} OL2: 500–1000 cells l^{-1} OL3: 1000–4000 cells l^{-1} OL4: 4000–30,000 cells l^{-1} OL5: >30,000 cells l^{-1}
Illustrative variables	Temperature (*T*)	5	*T* < 22 °C *T*22–23 °C *T*23–24 °C *T*24–25 °C *T* > 25 °C
	Precipitations (PR)	4	PR1: precipitations of 0 mm $week^{-1}$ PR2: precipitations of 0–1 mm $week^{-1}$ PR3: precipitations of 1–5 mm $week^{-1}$ PR4: precipitations > 5 mm $week^{-1}$
	East wind (>15 kt) (WE)	2	WE0: no windy day $week^{-1}$ WE+: at least 1 windy day.$week^{-1}$
	West wind (>15 kt) (WW)	2	WW0: no windy day $week^{-1}$ WW+: at least 1 windy day $week^{-1}$
	$SiOH_4$	4	$SiOH_4$ 1: 0.48–0.85 μM $SiOH_4$ 2: 0.85–1.08 μM $SiOH_4$ 3: 1.08–1.43 μM $SiOH_4$ 4: 1.43–4.01 μM
	$[PO_4]$	4	PO_4 1: 0–0.02 μM PO_4 2: 0.02–0.06 μM PO_4 3: 0.06–0.09 μM PO_4 4: 0.09–0.60 μM
	$[NO_3]$	4	NO_3 1: 0.36–0.96 μM NO_3 2: 0.96–1.30 μM NO_3 3: 1.30–1.83 μM NO_3 4: 1.83–5.29 μM
	$[NO_2]$	4	NO_2 1: 0–0.05 μM NO_2 2: 0.05–0.06 μM NO_2 3: 0.06–0.10 μM NO_2 4: 0.10–0.16 μM
	[Chlorophyll a]	5	Chl 1: 0.12–0.31 μg l^{-1} Chl 2: 0.31–0.48 μg l^{-1} Chl 3: 0.48–0.60 μg l^{-1} Chl 4: 0.60–0.95 μg l^{-1} Chl 5: 0.95–3.96 μg l^{-1}

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through the water column, rainfall, as well as orientation, intensity and frequencies of wind were used as ecological factors in order to potentially explain *Ostreopsis* development. Seawater temperature was also measured (±0.1 °C) each week during the sampling at Larvotto beach.

2.4. Statistical analysis

As variances of *O.* cf. *ovata* abundances were not homogeneous, the non-parametric Friedman ANOVA was chosen to test temporal variability (repeated measures test) and a non-parametric Kruskal–Wallis ANOVA used to assess spatial variability (independent measures test).

Pearson (parametric) and Spearman (nonparametric) correlation tests were applied on rocky sites data of 2007 and 2008, to determine the relationship tendency between epiphytic and planktonic abundances. The same tests were also used to examine relationships between temperature and concentrations of epiphytic and planktonic cells.

To highlight potential effect of the different environmental parameters on the *O.* cf. *ovata* abundance, a Multiple Correspondence Analysis (MCA) was applied on data obtained from sites 1 on data from sites 1 to 5. This analysis describes the total inertia of a multidimensional set of data in a space of fewer dimensions. This method is based on the principle of correspondence analysis, but is applied to disjunctive tables and uses a chi-squared metric. Data of all parameters, excluding the variable "sites" numbered from 1 to 5, were encoded in classes as shown in the Table 1. Epiphytic and planktonic *Ostreopsis* cells abundances represent active variables, contributing to the construction of the factorial space. In order to determine an ecological explanation for the axes, data set of temperature, wind, nutrients and "Chlorophyll a" concentrations, corresponding to illustrative variables, were plotted in the obtained factorial space.

3. Results

3.1. Molecular analysis

ITS and 5.8S rDNA obtained sequences completely aligned with the *O.* cf. *ovata* sequence from Penna et al. (2005) in GenBank. Molecular identification of *O.* cf. *ovata* agreed with morphological identification (tabulation of cells identical to the description of Penna et al., 2005).

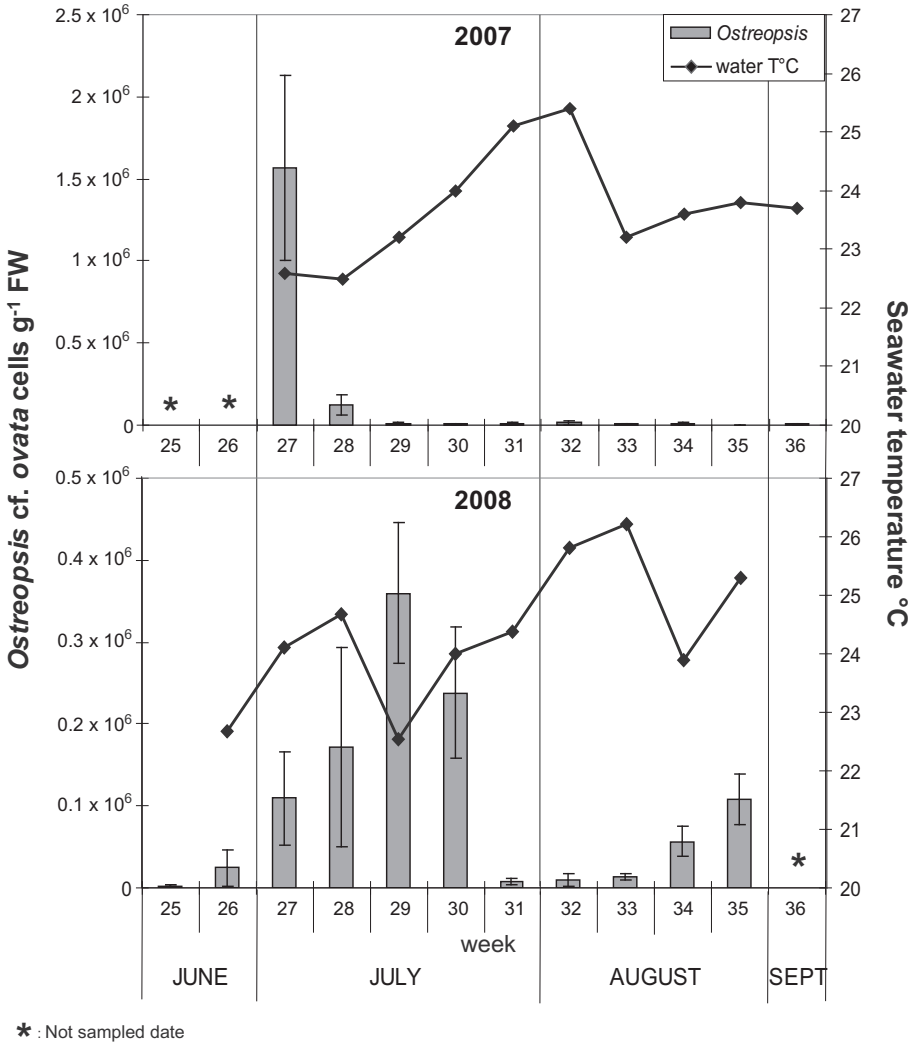

Fig. 2. *Ostreopsis* cf. *ovata* mean epiphytic cells abundances (±SE) observed at the sites 1–5 and water temperature measured during the summers 2007 and 2008 on the Larvotto beach (Monaco).

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3.2. Ostreopsis development

On macroalgae (sites 1–5), *O.* cf. *ovata* development was very different during the 2 years of the study (Fig. 2) and in 2007, maximum abundances attained almost five times the values estimated for summer 2008. During the summer of 2007, the highest abundance of *Ostreopsis* was observed during the first sampling week (2nd July, week 27) when the mean abundance reached value of about $1.57 \pm 0.56 \times 10^6$ cells g^{-1} FW (mean ± SE). This was the only sampling date that significantly differed from the others (Friedman ANOVA, $p < 0.01$; $N = 50$; Fc = 21.9; df = 9). The following week, the amount of epiphytic microalgae decreased and the mean abundance reached only $0.09 \pm 0.05 \times 10^6$ cells g^{-1} FW. During the rest of the summer, abundances of epiphytic cells remained low. During summer 2008, Friedman ANOVA highlighted a significant temporal variation among sampling dates ($p < 0.01$; $N = 55$; Fc = 36.4; df = 10). Compared to the previous summer, the highest abundances occurred later, on the 15th of July (week 29) with a maximum mean abundance of about $0.36 \pm 0.09 \times 10^6$ cells g^{-1} FW. During the following weeks, abundances quickly dropped to less than 0.01×10^6 cells g^{-1} FW. A second bloom began mid-August and reached $0.11 \pm 0.03 \times 10^6$ cells g^{-1} FW during the last sampling date (28th August, week 35).

Unlike epiphytic abundances, *O.* cf. *ovata* planktonic concentrations above the rocky substrate on sites 1–5 (Fig. 3), were slightly higher in 2008 than in 2007. During summer 2007, the planktonic concentrations followed the same trend as epiphytic abundances, Friedman ANOVA indicated significant temporal variation ($p < 0.01$; $N = 50$; Fc = 34.9; df = 9). The maximum mean concentration ($20.9 \pm 7.8 \times 10^3$ cells l^{-1}) occurred during the first sampling date (2nd July, week 27) and was followed by a rapid fall of concentrations in the next weeks, which never exceeded 4×10^3 cells l^{-1}. A slight peak of planktonic cells was observed the 6th August (week 32), with a mean concentration reaching $1.2 \pm 0.5 \times 10^3$ cells l^{-1}. The non-parametric Spearman test showed a significant correlation between planktonic and epiphytic cell abundances ($p < 0.01$, $N = 50$, $r_s = 0.57$). These two parameters followed a linear regression with a high explained percentage of variation ($R^2 = 0.82$, graph not shown). During summer 2008, temporal variation was again significant (Friedman ANOVA, $p < 0.01$; $N = 55$; Fc = 42.2; df = 10). Compared to the epiphytic *O.* cf. *ovata* bloom, the planktonic cells peak occurred one week earlier (7th July, week 28), with a maximum mean concentration of $59.1 \pm 43.7 \times 10^3$ cells l^{-1}. During the following weeks, concentrations decreased to less than 0.5×10^3 cells l^{-1}. In contrast to the pattern in epiphytic cells, no significant increase of planktonic cells

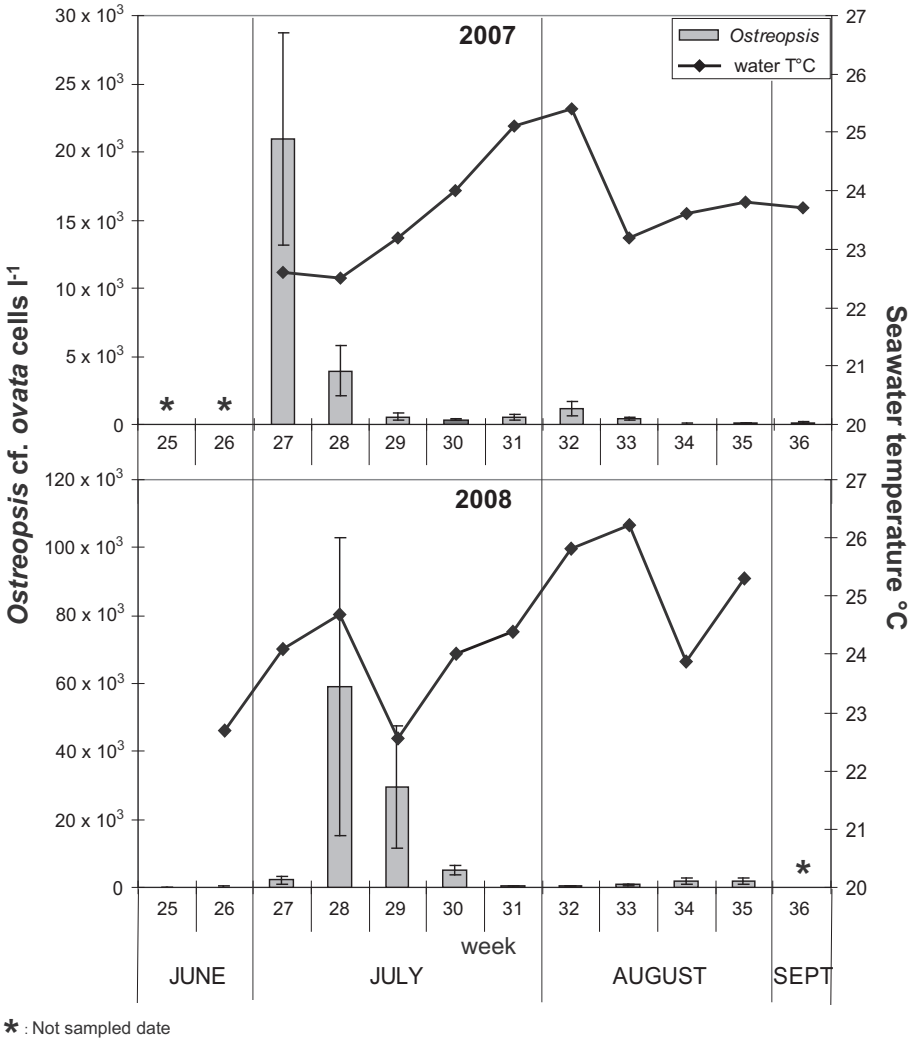

Fig. 3. *Ostreopsis* cf. *ovata* mean planktonic cells concentrations (±SE) observed on the sites 1–5 and *in situ* temperature measured during the summers 2007 and 2008 on the Larvotto beach (Monaco).

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Table 2
Maximum planktonic cell concentrations observed at the sites A, B and C, and dates of occurrences during the summers 2007 and 2008 on the Larvotto beach (Monaco).

	Site A	Site B	Site C
2007	Week 27 (2nd July 2007): 3980 cells l^{-1}	Week 27 (2nd July 2007): 8220 cells l^{-1}	Week 27 (2nd July 2007): 11,160 cells l^{-1}
2008	Weeks 27 and 28 (1st and 7th July 2008): 10,340 cells l^{-1}	Week 28 (7th July 2008): 6680 cells l^{-1}	Week 34 (25th August 2008): 8520 cells l^{-1}

occurred during the rest of the season. The Pearson linear correlation between planktonic and epiphytic log-transformed concentrations was significant ($p < 0.01$, $N = 55$, $r_s = 0.71$), with an explained percentage of variation of $R^2 = 0.50$ (data not shown).

Near the beach, the three sandy sites A, B and C showed very different results comparing summers 2007 and 2008 (Table 2). In 2007, the highest planktonic concentrations occurred in the three sites on the same date (2nd July, week 27). After this peak, concentrations rapidly drop to less than 300 cells l^{-1} in all sites and did not increase during the summer. In 2008, the highest planktonic concentrations on sandy beach sites were observed the 1st and 7th July (weeks 27 and 28) in sites A and B, during the period of the first bloom on rocky sites. However, maximum concentrations peak in site C occurred much later, the 18th August (week 34), along with the second bloom on rocky sites.

In addition to the significant temporal variation of *O.* cf. *ovata* abundances, an important variability at small spatial scale (i.e. between sites 1 to 5) was also observed at all the sampling dates, as shown by the large standard errors (SE) associated with both epiphytic and planktonic mean abundances (Figs. 2 and 3). The distribution of maximal and minimal abundances among the five sites was not similar from one week to the next (cf. epiphytic abundances in 2008, Fig. 4). Indeed, no site differed statistically from the others in 2007 as in 2008, neither for epiphytic nor for planktonic abundances (Kruskal–Wallis ANOVAs (df = 4): $p = 0.17$, $H = 6.48$ for epiphytic data and $p = 0.93$, $H = 0.87$ for planktonic data in 2007 ($N = 50$); $p = 0.64$, $H = 2.55$ for epiphytic data and $p = 0.84$, $H = 1.42$ for planktonic data in 2008 ($N = 55$)).

3.3. Influence of environmental factors

From 2007 data, a weak linear negative correlation between *in situ* summer temperatures and epiphytic *O.* cf. *ovata* abundances (log-transformed) was observed (Pearson test, $p < 0.01$, $N = 50$, $r = -0.47$), but with a low explained percentage of correlation ($R^2 = 0.22$). This result can be connected to the fact that the main 2007 bloom event (2nd July, week 27) occurred during the coldest summer period, with temperature not exceeding 22.5 °C. In contrast, no relationship between planktonic abundances and temperatures was observed (Spearman test: $p > 0.05$, $N = 50$, $r_s = -0.28$). Furthermore in 2008, epiphytic and planktonic cell abundances were not relatable to temperature (Spearman test: $p > 0.4$, $N = 55$, r_s = -0.08 and 0.11 respectively). The minimal summer temperature (22.6 °C, recorded the 15th July, week 29) coincided with the maximum development of *Ostreopsis*, as observed in 2007. At the end of August 2008, the second bloom seemed to begin after a marked drop in temperature.

In order to evaluate the potential impact of temperature throughout the whole year on the summer development of *O.* cf. *ovata* and the variability observed during the two sampling seasons, annual temperatures and temperature anomalies were examined with Villefranche Bay data, from surface to 40 m depth (Fig. 5). Temperature anomalies were calculated comparing temperature profiles of 2007 and 2008 with the median year profile estimated with data from 1995 to 2008. Year 2007 showed a particular profile, marked by hot spring temperatures. Positive anomalies were evident from early April to mid-May and reached from +1 to +3 °C at the surface. This period was followed by a relatively cool summer, with null and negative anomalies at the surface, from early June to late September. Maximum negative anomalies reaching −3 °C were estimated principally for late June and late July. The year 2008 corresponded with the standard temperature profile showing then no extreme anomalies. Some short terms events of cooling occurred during the summer, negative anomalies once reached −2 °C at the surface, but summer 2008 was globally a median season. Compared to the year 2007, spring 2008 was significantly colder, but summer 2008 was warmer.

The results of the MCA analysis are shown in the Fig. 6 (a and b) for the two sampling years, with the representation of physical parameters (temperature, precipitation, wind and sites) separated from chemical parameters (nutrients and Chlorophyll a) for clarity. For summer 2007, the two axes forming the first factorial space have a quite important percentage of inertia for an MCA, respectively 26.46% and 19.85%, showing a good explanation of the projected variables by the plane. Similarly, for summer 2008, the two first axes represent the quite good percentages 21.71% and 17.66% of the total inertia. Epiphytic *Ostreopsis* cells abundances were situated near the planktonic *Ostreopsis* cells concentrations

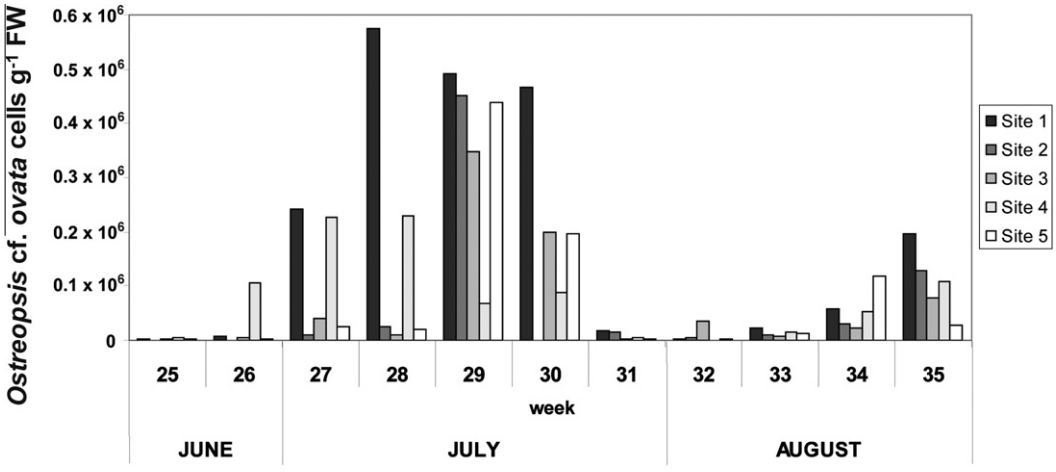

Fig. 4. Illustration of *Ostreopsis* cf. *ovata* spatial variation: epiphytic cells abundances at sites 1–5 during summer 2008 on the Larvotto beach (Monaco).

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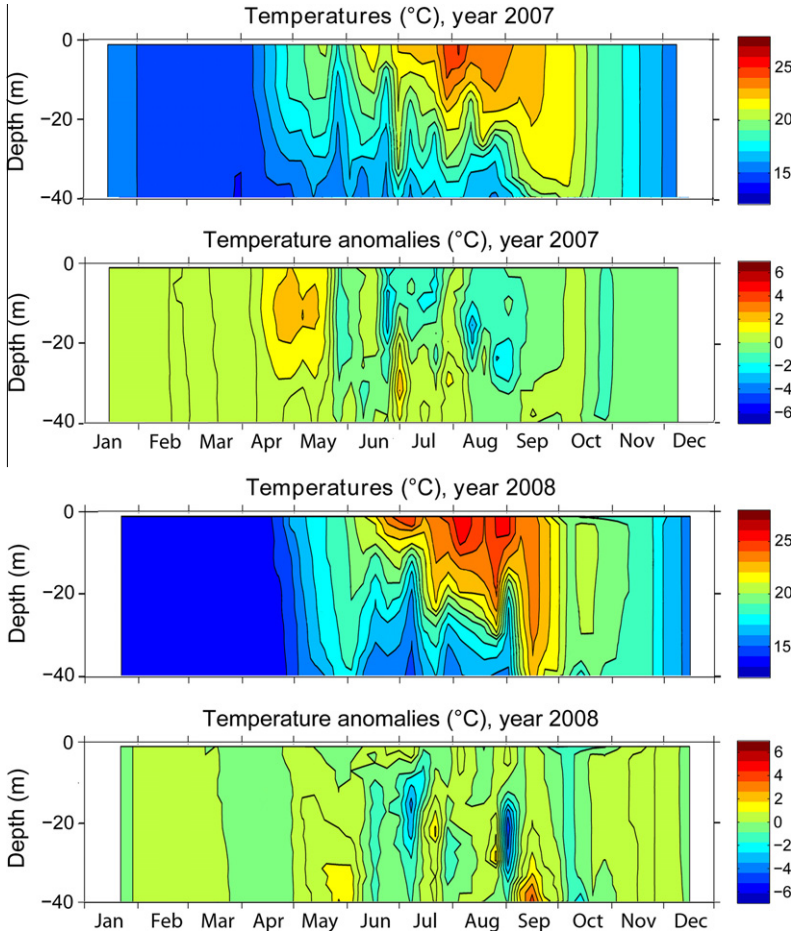

Fig. 5. Seawater temperatures and temperature anomalies measured during the years 2007 and 2008, from the surface to 40 m deep, in the Villefranche bay (France).

on the plane for each class of data (except for the class 4 in 2008), which indicates a good relation between the two parameters. Those abundances were located all along the horizontal axis, and extreme values were associated to high contribution on this axis (data not shown). During the two summers, all the sites (1–5) are grouped in the central part of the plot, implying no significant differences in *Ostreopsis* abundances between them. The MCA did not highlight a clear impact of rainfall on microalgae development for both summers.

Differences in other possible forcing factors were observed between the 2 years. In 2007, the temperature class 22–23 °C differed clearly from other and matches with the highest *Ostreopsis* cells abundances. Classes of 23–24 and 24–25 °C corresponded to the lowest abundances. The MCA highlighted a weak link between the presence of west wind and high *Ostreopsis* abundances. Regarding nutrients, a relation between high silicate concentrations and high *Ostreopsis* abundances was observed. Phosphates did not seem to have any impact on *Ostreopsis* abundances. Low nitrate concentrations showed a good link with high *Ostreopsis* abundances, nitrite having the same tendency but less evident. Finally, the analysis showed a positive relation between *Ostreopsis* and Chlorophyll a concentrations.

In 2008, classes of temperature lower than 22 °C and higher than 25 °C were strongly correlated with low *Ostreopsis* abundances, while high abundances seemed to match with the classes 22–23, 23–24 and 24–25 °C. Unlike 2007, west and east winds followed the same trend, weeks without wind strongly matching with highest *Ostreopsis* abundances. Concerning the impact of nutrient concentrations, high silicate concentrations had a good correlation with lowest *Ostreopsis* abundances, instead of 2007. All classes of phosphate concentrations were grouped in the central part of the plot and did not seem to have any impact on *Ostreopsis* abundances, as in 2007. Similarly, nitrate did not show any correlation with *Ostreopsis* abundances, while the class of lowest nitrite concentrations corresponds to the lowest *Ostreopsis* abundances. Finally and in contrast to 2007 results, we did not observe any significant correlation between *Ostreopsis* and Chlorophyll a concentrations.

4. Discussion

4.1. Ostreopsis development

The development of *Ostreopsis* species in the Mediterranean is well-documented and up to now the highest epiphytic abundances observed in the western part of the Mediterranean were 7.2×10^6 cells g^{-1} FW in Catalonia (Mangialajo et al., 2011), 2.5×10^6 cells g^{-1} FW on the Genoa coasts (Mangialajo et al., 2008) and 1.7×10^6 cells g^{-1} FW in the Adriatic Sea (Totti et al., 2010). In the eastern Mediterranean (Greece), a maximal abundance of 0.41×10^6 cells g^{-1} FW was observed (Aligizaki and Nikolaidis, 2006). Therefore, the maximum cell abundance observed at Monaco the 2nd July 2007 (2.8×10^6 cells g^{-1} FW in the site 2) was one of the highest abundances of benthic *Ostreopsis* cells ever measured in the Mediterranean Sea.

Concerning planktonic concentrations, our maximum value occurred during the 2008 summer (7th July) and reached 213×10^3 cells l^{-1} in the site 5. This value is near those reported in Catalonia (386×10^3 cells l^{-1}) and in the Villefranche Bay

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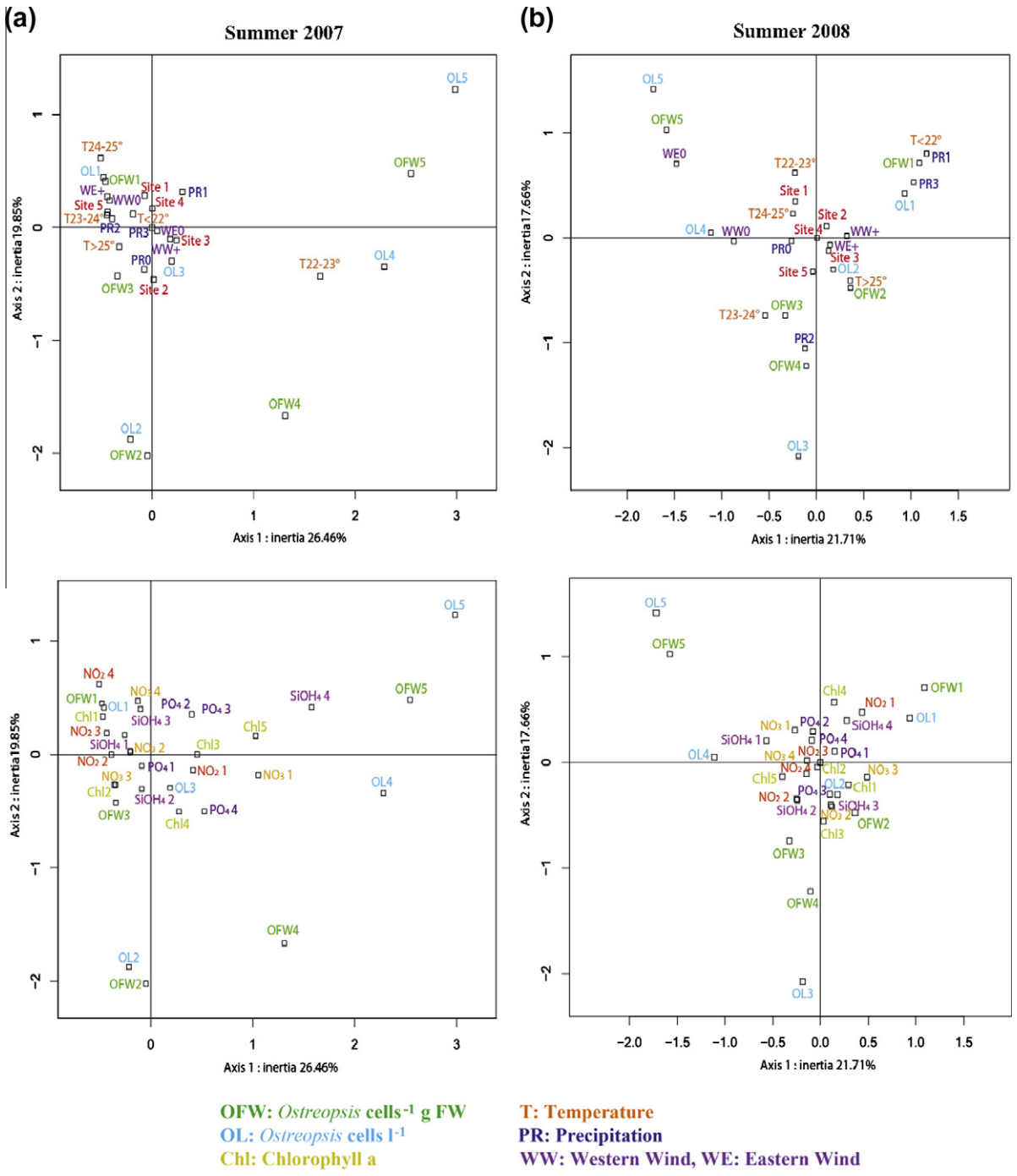

Fig. 6. Multiple Correspondence Analysis (MCA) applied on data obtained from sites 1 to 5 on the Larvotto beach (Monaco) during 2007 (a) and 2008 (b) summers. Data of all parameters, excluding the variable "sites" numbered from 1 to 5, are encoded in classes as shown in Table 1. For each year, physical parameters (temperature, precipitation, wind and sites) are separated from chemicals parameters (concentrations of nutrients and Chlorophyll a) for clarity.

(104×10^3 cells l^{-1}; Mangialajo et al., 2011), and is clearly higher than those found in Genoa coast (87×10^3 cells l^{-1}; Mangialajo et al., 2008), in the Adriatic Sea (25×10^3 cells l^{-1}; Totti et al., 2010) and in the Aegean Sea (16×10^3 cells l^{-1}; Aligizaki and Nikolaidis, 2006).

Ostreopsis spp. are considered to be epibenthic and their occurrence in the water column could be due to a resuspension of epiphyte cells, probably under the effect of hydrodynamic conditions, as suggested by Aligizaki and Nikolaidis (2006) and Totti et al. (2010). Following this hypothesis, planktonic *Ostreopsis* concentrations depend on the benthic stock. However, even with a relative low epiphytic abundance, a high hydrodynamism could increase the resuspension of cells and induce maximal concentrations in water. During summer 2007 in Monaco, the bloom occurred simultaneously on the macroalgae and in the water column. This phenomenon has been reported in several studies which have noted a temporal correspondence between the blooms of planktonic and benthic cells in the Mediterranean Sea (Vila et al., 2001b; Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2008, 2011). In contrast, in 2008 the peak of planktonic cells was recorded one week earlier than the maximum epiphytic abundance. This apparent asynchrony was probably related to different

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hydrodynamic conditions during the period, yielding more or less a resuspension of microalgae from the benthos. Unfortunately, no wave and current data were available to examine this hypothesis.

The absence of a statistically distinct site favoring *Ostreopsis* growth over summers 2007 and 2008 is likely due to the marked small scale variability (i.e. few tens of meters) of both epiphytic and planktonic cell concentration among the five sites observed during each sampling date. Various factors could explain this spatial variation, as diversity of biotic substrates (Totti et al., 2010) or waves and currents actions. Several reports hypothesized that hydrodynamic conditions have an important effect on the *Ostreopsis* development in the NW Mediterranean Sea, as Simoni et al. (2003) who observed intense blooms in breakwater areas with poor water circulation. In contrast, Vila et al. (2001b) found that epiphytic *Ostreopsis* abundances were highest in slightly turbulent sites, but this observation could be due to a relatively low presence of macroalgae in the sampled calm area. Some studies on the role of hydrodynamic conditions have been conducted in different temperate areas of New-Zealand, where high *Ostreopsis* spp. abundances were recorded in stable and less energetic environments (Chang et al., 1997; Shears and Ross, 2009). A significant negative linear relationship between the cover of *O. siamensis* and wind fetch has been also highlighted by Shears and Ross (2009). In the tropics (Mayotte Island, Indian Ocean), *Ostreopsis* spp. growth was favored in areas moderately exposed to wave action and dinoflagellates were never abundant in sites subject to high run-off (Grzebyk et al., 1994).

In our study, neither ANOVA nor MCA indicated a significant difference among site for *Ostreopsis* development. This was mainly due to the fact that maximum abundances values were not observed in the same site from one week to the other. However, these results do not mean that hydrodynamics are not important in spatial variation of *Ostreopsis* bloom: waves and coastal currents change according to global or local water circulation as well as wind intensity and direction. Moreover, those parameters are very difficult to evaluate and integrate, especially at the appropriate spatial and temporal scales (few tens of meters and few days).

4.2. Influence of environmental factors

4.2.1. Temperature

In spite of the inter-annual variations, a general scheme can be drawn, with a first and most important bloom in July and a possible second and less important one in August (year 2008). This pattern has already been described for the NW Mediterranean and especially the Ligurian Sea (Mangialajo et al., 2011). Our study was done in the framework of a public health monitoring program and was performed only during July and August. No data were then available before and after this period. It is possible that the maximum abundance of *Ostreopsis* was reached before the monitoring period and an important development of microalgae may have started several days before the end of June. This period of bloom (first week of July or last days of June) was very early and has never been observed before in the NW Mediterranean Sea (Mangialajo et al., 2011). Similarly, the second bloom observed during the last week of August 2008 may have reached its maximum value after the monitoring period. In particular, the presence of an eventual October second bloom, as already observed by Mangialajo et al. (2011) in the North Mediterranean Sea, could not been verified in the present study.

At least during the summer season (July and August), our results did not reveal any relationship between *Ostreopsis* development and seawater temperature, except a low negative correlation in 2007 for the epiphytic cells evolution. In 2007, the temperature of 22–23 °C seemed to favor microalgae growth, while in 2008 temperatures from 22 to 25 °C were well associated with high cell abundances. In both years, it is interesting to note that the most important blooms appeared during the lowest summer temperature periods. Then, *O.* cf. *ovata* seems to prefer temperatures from 22 to 25 °C and never proliferate at high temperatures (>25 °C). Relatively few studies in temperate areas have examined *in situ* abundances of *Ostreopsis* species variability with the temperature during the bloom season: results of our study differ from those of Mangialajo et al. (2008), who observed maximum *O. ovata* proliferation when temperature ranged from 26 to 30 °C on the Genoa coast. Similarly in Greece, Aligizaki and Nikolaidis (2006) observed peak abundances when temperature exceeded 26 °C. In the Tyrrhenian Sea, high *O. ovata* abundances were noted from 22 °C, but the author hypothesized that blooms were amplified by a thermophilic selective pressure when temperature reached 27–28 °C (Simoni et al., 2003). In contrast, along the Costa Brava (Spain), no link between *Ostreopsis* growth and temperature was found, blooms occuring at 18 °C in April, outside the usual period of *Ostreopsis* development (Vila et al., 2001b). Similarly, important blooms occurred at 19 °C near Marseille (SE France) during summer, in an area regularly affected by Mistral wind which induces up-wellings and decreases water temperature (Mangialajo et al., 2011). The direct relationship between *O.* cf. *ovata* development in the Mediterranean Sea and water temperature is then ambiguous, with possible occurrence of bloom from 18 to 30 °C.

In addition to the possible direct summer temperature effect, we considered the importance of annual hydroclimatic trends on *Ostreopsis* bloom occurrence. During summer 2007, the *Ostreopsis* bloom was earlier and much more important than in 2008. Hydroclimatic scenarios observed during those 2 years could explain this difference: year 2007 was characterized by a relatively cool summer preceded by a particular hot spring. Early high temperatures in April/May could be responsible for the early initiation of the bloom; then the fall of temperature during the summer could have restricted the development of the microalgae. This early bloom could have been favored because *Ostreopsis*, like many epibenthic dinoflagellates, follows the spring growth of macroalgae which depends mainly on the temperature and irradiance increases (Vila et al., 2001b). In contrast, 2008 was a year with a standard temperature profile associated with a mid-July peak of *Ostreopsis*, corresponding with the standard summer bloom period observed in the NW Mediterranean Sea (Mangialajo et al., 2011).

4.2.2. Rain, wind and nutrients

We found no clear impact of rainfall on *O.* cf. *ovata* abundances, similarly to the findings of Ballantine et al. (1988) on *O. lenticularis* in Puerto Rico. However, in Florida, Morton et al. (1992) assumed that rainfall could negatively impact the growth of *O. siamensis* and *O. heptagona via* a salinity decrease. But this hypothesis was controversial by the study of Parsons and Preskitt (2007) who reported an *in situ* negative relation between the *O. ovata* development and the salinity in the Hawaiian area. However, it should be noted that as the amount of rain never exceed few millimeters per week during summer 2007 and 2008 in our study area, this factor could not strongly influence seawater salinity and its impact on *Ostreopsis* development could not be really evaluated.

Relationships between *Ostreopsis* development and wind were different in 2007 and 2008. While the MCA did not show a significant impact of this parameter during summer 2007, the analysis highlighted a negative relation between *Ostreopsis* abundances and Western winds during 2008. The area was more affected by West wind (i.e. more frequent and intense) than by East wind during summer 2008 (data not shown). Part of the NW Mediterranean basin (Gulf of Lion and West part of the Ligurian Sea) is usually influenced by the Mistral (West wind), sometimes resulting in the drop of temperature due to the rising of cold (and nutrient rich) deep seawater during strong windy days. The association between

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West winds and relatively low *Ostreopsis* abundance during summer 2008 may result from the suspension of epiphytic cells and the dispersal of planktonic cells by the associated currents. This pattern is similar to that described by Okolodkov et al. (2007) who hypothesized that strong Northern winds could have contributed to the decrease of epiphytic dinoflagellate populations, including *O. heptagona*, in the Gulf of Mexico. Hydrodynamic level, including currents and waves, results from wind orientation, frequency and intensity. But bathymetry, substrate and coastline form also affect water dynamic energy at a specific point.

Nutrient concentrations measured during summers 2007 and 2008 in Monaco were of the same order of magnitude than those measured by Vila et al. (2001b) along the coasts of Catalonia and Majorca Island (Spain). No clear pattern between nutrients and *Ostreopsis* development were highlighted by the MCA. Phosphates and nitrogen (both nitrates and nitrites) seemed to have no impact and silicates were associated with opposite trends in 2007 and 2008.

Other studies have already mentioned that inorganic nitrogen, phosphorus and silicate concentrations appeared unassociated with benthic dinoflagellate distributions in tropical and Mediterranean areas (Grzebyk et al., 1994; Vila et al., 2001b). However, in Hawaii, *Ostreopsis* spp. abundance showed a positive correlation with nitrates, nitrites, phosphate and silicate concentrations (Parsons and Preskitt, 2007) and Burkholder et al. (2008) reported an indirect link between development of *Ostreopsis* species (*O. ovata*, *O. siamensis* and *O. lenticularis*) and anthropogenic nutrient inputs *via* the stimulation of macroalgal habitat. Therefore, the role of nutrients in the growth of dinoflagellates, including *Ostreopsis* spp., is still ambiguous. It appears worthwhile to investigate the role of mixotrophy in *Ostreopsis* spp. development, a nutritional mode suspected for these species (Burkholder et al., 2008).

5. Conclusion

To our knowledge, this is the first study of *O.* cf. *ovata* development in NW Mediterranean Sea taking into account diverse ecological factors: temperature (at year and seasonal scales), rain, winds and nutrients. No clear patterns were observed between the microalgae bloom and summer seawater temperature, rainfall or nutrients. In contrast, hydroclimatic conditions, and especially spring temperature anomalies, may have an important impact on the period of bloom appearance, while wind may favor the dispersal of benthic and planktonic cells. Further long-term studies are needed to confirm those results, including investigation of *Ostreopsis* mixotrophic nutrition, parameter potentially important in population dynamics.

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