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Ecology of *Ostreopsis* cf. *ovata* blooms in the northwestern Adriatic Sea

Stefano ACCORONI[a], Federica COLOMBO[a], Salvatore PICHIERRI[a], Tiziana ROMAGNOLI[a], Mauro MARINI[b], Cecilia BATTOCCHI[c], Antonella PENNA[c] & Cecilia TOTTI[a]*

[a]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy. Fax: +39 071 2204650; s.accoroni@univpm.it

[b]Istituto di Scienze Marine (CNR), Largo Fiera della Pesca, 60125 Ancona, Italy

[c]Dipartimento Scienze Biomolecolari, Università di Urbino, viale Trieste 296, 61100 Pesaro, Italy

Abstract – The ecology of *Ostreopsis* cf. *ovata* blooms was investigated to evaluate the role of environmental factors (temperature, hydrodynamism, nutrient concentrations, depth and substratum) on the bloom dynamics. This paper reports the present knowledge on *O.* cf. *ovata* blooms along the Conero Riviera (NW Adriatic Sea), on the basis of samplings carried out from 2006 to 2010. The annual maximum of benthic cell abundance was always observed in late-summer, reaching the order of magnitude of 10^6 cells g^{-1} fw (corresponding to 10^7 cells g^{-1} dw and 10^4 cells cm^{-2}) on macrophyte samples. Comparing the mean abundances settled on seaweeds with those growing on hard substrata, significantly higher abundances were observed on the latter. Hydrodynamism plays a major role in *Ostreopsis* blooms, as significantly higher abundances were observed in sheltered sites compared with exposed ones. The abundances of *O.* cf. *ovata* showed a marked decrease with depth. Temperature and nutrients do not seem to play an important effect on the *O.* cf. *ovata* blooms. High levels of ovatoxins were recorded in natural samples; episodes of death of both benthic invertebrates (limpets, sea urchins and mussels) and macroalgae were commonly observed during *O.* cf. *ovata* blooms.

Adriatic Sea / *Ostreopsis* cf. *ovata* / HABs / Nutrients / Water temperature / Hydrodynamic conditions / Substratum preference

INTRODUCTION

Ostreopsis cf. *ovata* Fukuyo is a toxic benthic dinoflagellate distributed in both tropical and temperate areas (Rhodes, 2011) that, in recent years, occurred also in the Mediterranean region with increasing frequency and bloom intensity (Vila *et al.*, 2001; Aligizaki & Nikolaidis, 2006; Mangialajo *et al.*, 2011).

Ostreopsis cf. *ovata* populations typically proliferate in rocky coasts, forming a rusty-brown coloured mucilaginous film, which covers reefs, pebbles, soft sediments, seaweeds, marine angiosperms and invertebrates (Vila *et al.*, 2001; Aligizaki & Nikolaidis, 2006; Battocchi *et al.*, 2010; Totti *et al.*, 2010; Accoroni

* Correspondence and reprints: s.accoroni@univpm.it

et al., 2011). The presence of *O.* cf. *ovata* in coastal waters may pose a real threat to coastal food web and fishery (Aligizaki *et al.*, 2011). Its toxicity is associated with the presence of palytoxin (PlTX)-like compounds (Ciminiello *et al.*, 2011), causing human health problems, as dyspnoea, fever, conjunctivitis and dermatitis (Tichadou *et al.*, 2010), and mortality of benthic marine organisms (Shears & Ross, 2009). Recently, liquid chromatography-mass spectrometry (LC-MS) disclosed the presence of putative PlTX and five new palytoxin analogues, named ovatoxin-a, -b, -c, -d and -e, in field and in cultured samples of *O.* cf. *ovata* collected along the Italian coasts (Accoroni *et al.*, 2011; Ciminiello *et al.*, 2011).

Ostreopsis cf. *ovata* has been recorded along the rocky coasts of the northwestern Adriatic since 2006 (Monti *et al.*, 2007; Totti *et al.*, 2007). In this study, we illustrate the present knowledge on the ecology of *O.* cf. *ovata* blooms along the Conero Riviera (NW Adriatic Sea), on the basis of samplings carried out in 2006 (Totti *et al.*, 2007), 2007 (Totti *et al.*, 2010), 2009 (Accoroni *et al.*, 2011) and 2010, highlighting the potential role of environmental factors (temperature, hydrodynamism and nutrients, depth and substratum) on the bloom dynamics, in order to depict some general considerations on the *O.* cf *ovata* trend in this area.

MATERIALS AND METHODS

The study area is the Passetto station in the Conero Riviera (Ancona, NW Adriatic Sea) characterized by shallow depth (~1 m) and rocky bottom. Sampling was carried in summer 2006 (Totti *et al.*, 2007), 2007 (Totti *et al.*, 2010), 2009 (Accoroni *et al.*, 2011) and 2010 with a frequency of 15-7 days. Surface temperature (CTD) and meteomarine conditions (Douglas scale) were recorded. Water samples for nutrient analysis (nitrates, nitrites, ammonia, phosphates and silicates) were collected, filtered (0.45 μm), stored in polyethylene bottles at –22°C, analyzed following Strickland & Parsons (1968). Surface seawater samples were collected to analyze the abundance of dinoflagellates in the water column, and preserved with 0.8% neutralized formaldehyde. Undisturbed benthic substrata (macroalgae, pebbles) were collected underwater to avoid the loss of *Ostreopsis* cells. Sampling protocol and laboratory treatments were made following the procedure described in Totti *et al.* (2010). Identification and counting were made using an inverted microscope following Utermöhl (Hasle, 1978).

Differences in the abundances of *Ostreopsis* cells between different substrata, depths and sites at different hydrodynamic conditions were assessed through a one-way analysis of variance (ANOVA) using Statistica (Statsoft) software. When significant differences for the main effect were observed ($p < 0.05$), a Tukey's pairwise comparison test was also performed.

TEMPORAL TREND OF *OSTREOPSIS* CF. *OVATA* BLOOM

Ostreopsis cf. *ovata* was detected in all sampling sites characterized by rocky bottom, while no cells were observed in stations characterized by soft bottoms. PCR amplifications revealed the presence of the sole genotype *O.* cf. *ovata* (Battocchi *et al.*, 2010; Perini *et al.*, 2011). The temporal trend of *O.* cf. *ovata* blooms on benthic substrata and in the water column was similar in each investigated year, with the first cell appearance at the end of July/early August, then the maximum abundances recorded in late-summer (end of September/early October) and decline of the blooms at end October/early November, often in concomitance with prolonged conditions of stormy sea (Figs 1-3).

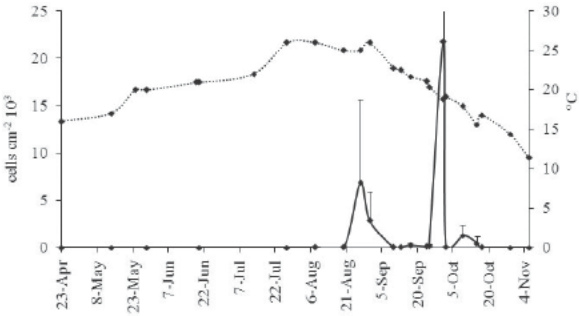

Fig. 1. Temporal variability of *Ostreopsis* cf. *ovata* abundance (cells cm^{-2}) at Passetto station on macroalgae in 2007. Abundances are expressed as mean values (± standard error) calculated for all seaweeds for each sampling date (modified from Totti *et al.*, 2010). Surface seawater temperature values throughout the study period are shown.

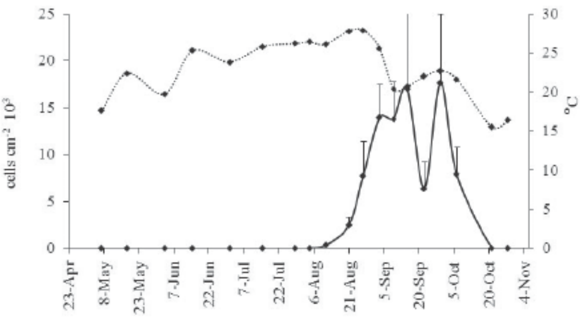

Fig. 2. Temporal variability of *Ostreopsis* cf. *ovata* abundance (cells cm^{-2}) at Passetto station on rocks in 2009 (mean values ± standard deviations, modified from Accoroni *et al.*, 2011). Surface seawater temperature values throughout the study period are shown.

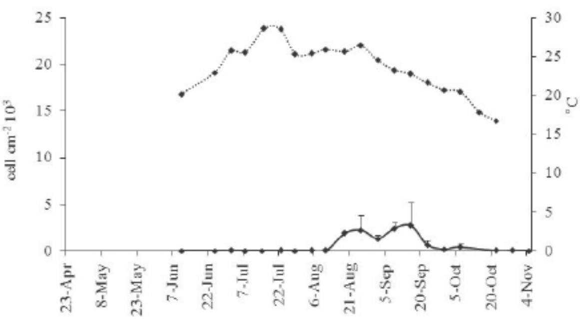

Fig. 3. Temporal variability of *Ostreopsis* cf. *ovata* abundance (cells cm^{-2}) at Passetto station on *Dictyopteris polypodioides* in 2010 (mean values ± standard deviations). Surface seawater temperature values throughout the study period are shown.

Ostreopsis cf. *ovata* blooms at the Conero Riviera seem to be among the most intense ones within the Mediterranean Sea. *O.* cf. *ovata* maximum abundances reached yearly 10^4 cells cm^{-2} (10^6 cells g^{-1} fw, 10^7 cells g^{-1} dw) (Totti *et al.*, 2010; Accoroni *et al.*, 2011). These abundances are comparable with the maximum values reported in the Mediterranean Sea, as in the Ligurian Sea and Catalonia (Mangialajo *et al.*, 2011). In the water column *O.* cf. *ovata* cells appeared a few days after their record on benthic substrata and showed quite variable abundances.

ROLE OF ENVIRONMENTAL FACTORS

Many authors suggested that *Ostreopsis* spp. need relatively high temperatures to proliferate, suggesting that the global warming may be influence *Ostreopsis* expansion in the Mediterranean Sea (Hallegraeff, 2010; Granéli *et al.*, 2011). However, although *Ostreopsis* proliferations are often reported in the warmest period of the year, it has been proved that the relationship with the seawater temperature is not the same in all the geographic areas (Mangialajo *et al.*, 2008; Selina & Orlova, 2010). In the NW Adriatic Sea, the highest abundances of *O.* cf. *ovata* were always recorded in late summer when temperature was decreasing (Monti *et al.*, 2007; Totti *et al.*, 2010; Accoroni *et al.*, 2011).

Often, and particularly in coastal areas, eutrophication appears to be directly linked to the occurrence of harmful algal blooms (Glibert *et al.*, 2010). However, such a cause-effect relationship between *Ostreopsis* blooms and trophic conditions has not been proved so far. Indeed, worldwide *Ostreopsis* spp. appear to proliferate both in eutrophicated (Accoroni *et al.*, 2011) and oligotrophic areas (Shears & Ross, 2009). At the Conero Riviera, nutrient concentrations showed a marked variability, with values falling within the expected range for a shallow area subjected to a moderate anthropic impact (Marini *et al.*, 2002). In 2009, we observed the peak of abundances in concomitance with a decrease of nutrient concentrations (Fig. 4), while in 2010 the nutrient concentrations showed a very

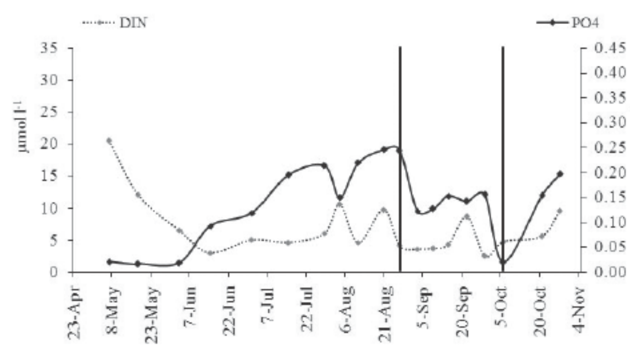

Fig. 4. Temporal variability of nutrient concentration (µmol l^{-1}) at Passetto station in 2009 (modified from Accoroni *et al.*, 2011). Dissolved Inorganic Nitrogen (left y-axis) and phosphate (right y-axis). Vertical bars indicate the period of *Ostreopsis* cf. *ovata* bloom.

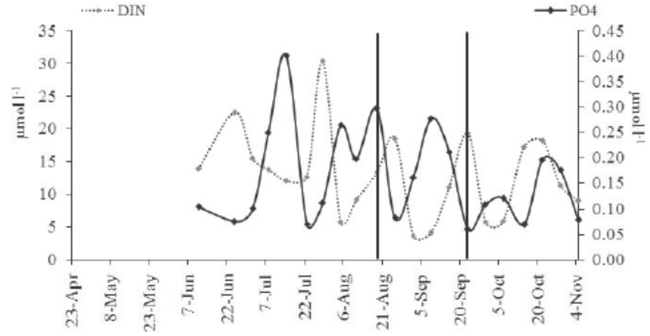

Fig. 5. Temporal variability of nutrient concentration (µmol l^{-1}) at Passetto station in 2010. Dissolved Inorganic Nitrogen (left y-axis) and phosphate (right y-axis). Vertical bars indicate the period of *Ostreopsis* cf. *ovata* bloom.

oscillating trend (Fig. 5). However, we cannot relate the bloom trend to any particular trophic condition. This observation agrees with that reported by other authors (Vila *et al.*, 2001; Parsons & Preskitt, 2007; Shears & Ross, 2009; Cohu *et al.*, 2012) who did not observe any clear relationship between epiphytic *Ostreopsis* abundances and the concentrations of inorganic nutrients. Further studies are needed to clarify the trophic behaviour of *Ostreopsis* spp. Mixotrophy was hypothesized for these species (Barone, 2007; Burkholder *et al.*, 2008) and may play an important role in *Ostreopsis* development, as already observed in other potentially toxic microalgae (Cucchiari *et al.*, 2008; Heisler *et al.*, 2008).

There are several studies that consider hydrodynamism as a major factor affecting the *Ostreopsis* abundance trend (Shears & Ross, 2009; Totti *et al.*, 2010; Accoroni *et al.*, 2011). The abundances of these benthic dinoflagellates are particularly affected by wave action, since they only loosely attach to the substrata and can be easily removed and re-suspended in the water column. Observations by Totti *et al.* (2010) highlighted that (1) significantly higher abundances were observed in the sheltered sites compared with the exposed ones; (2) hydrodynamics may have an important effect on the temporal variability of bloom, because stormy events can result in a sudden decrease of cell abundances on the benthic substrata, with cell proliferation being re-established high densities after some days of calm sea conditions.

The role of depth was assessed by Totti *et al.* (2010) at target sites where samples were collected at different quotes between 0.5 and 9.4 m. *O.* cf. *ovata* abundances showed a significant decrease with depth, in agreement with what observed by Richlen & Lobel (2011), suggesting that this trend is related to the decrease in light intensity. This may explain why *Ostreopsis* blooms mainly develop in shallow waters. However, such effect cannot be observed in shallow sites affected by high hydrodynamism, such as on the fringing reefs of the higher infralittoral plane, where *O.* cf. *ovata* abundances were lower than those recorded immediately deeper, due to the hydrodynamic effect of wave actions (Totti *et al.*, 2010). Similarly, Mabrouk *et al.* (2011) did not find a marked decrease of *O.* cf. *ovata* abundances with depth due the high hydrodynamics in their shallow stations.

It is well known that *O.* cf. *ovata* is not an obligate epiphytic species, since it is able to colonize a variety of substrata living as epiphytic, epilithic and epizoic; it was shown that, comparing the mean abundances of *O.* cf. *ovata* settled on seaweed thalli with those growing on hard substrata, significantly higher values were observed in the latter (Totti *et al.*, 2010), suggesting that living substrata support lower concentration of epibionts probably due to the production of some hypothetical allelopathic compounds (Jin & Dong, 2003), while the non-living ones do not exert any contrasting mechanism. Previous studies (Grzebyk *et al.*, 1994) have demonstrated that some macroalgae exude organic substances that stimulate *Gambierdiscus* growth (e.g., *Portieria hornemanii*), while others produce inhibitory compounds (e.g., *Halymenia floresia*). A number of studies underline the importance of host thallus architecture (Lobel *et al.*, 1988; Bomber *et al.*, 1989). Vila *et al.* (2001) observed that three-dimensional flexible thalli are more suitable for the growth of *Ostreopsis* spp. However, the extent of epiphytic colonization is affected by the interaction of several factors; the higher abundances found by Totti *et al.* (2010) in branched than in flattened thalli, might be explained by a different response of such morphotypes to the wave action.

Toxin analysis on natural samples revealed a high toxin content (up to 72 pg cell^{-1}) with ovatoxin-a being the major component (Accoroni *et al.*, 2011). During *O.* cf *ovata* blooms, deaths of both benthic invertebrates (limpets, sea urchins and mussels) and several macroalgae commonly found in the area were observed. In particular, macroalgal thalli showed a bleaching of the distal part of the thallus or completely disappeared from the sampling area (Accoroni *et al.*, 2011). These mass mortalities have been also observed in other world areas affected by *Ostreopsis* blooms (e.g., (Shears & Ross, 2009) and also in experimental conditions (Gorbi *et al.*, 2010; Simonini *et al.*, 2011; Faimali *et al.*, in press;). 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.

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