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Interannual variability in *Ostreopsis ovata* bloom dynamic along Genoa coast (North-western Mediterranean): a preliminary modeling approach

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Abstract – The tropical genus *Ostreopsis* has been recorded along Italian coasts of the Mediterranean Sea since the '90s, but large bloom events have been reported only in recent years. In order to describe *O. ovata* bloom dynamics and provide a better understanding of environmental variables involved in triggering blooms, we collected a time series of data in Genoa (North Western Mediterranean) from 2006 to 2010. Cell abundances in the water column and epiphyte on the macroalgae were assessed during the summer months. Water and meteorological variables were concurrently collected.

We elaborated a meaningful explanatory model, performing multiple correlations between bloom magnitude (maximum cell concentration) and length (extent of the bloom event) and water/meteorological features. Such a model highlights a significant role of water temperature, barometric pressure and wind speed in affecting bloom dynamics. It represents a good base for managers in the attempt of forecasting *O. ovata* blooms and, specifically, toxic events, in an ecological, economic and sanitary perspective.

***Ostreopsis ovata* / HABs / temporal variability / bloom modeling**

INTRODUCTION

Blooms of benthic dinoflagellates belonging to the tropical genus *Ostreopsis* have been reported in many temperate regions, such as the Mediterranean area (Aligizaki & Nikolaidis, 2006; Ciminiello *et al.*, 2006;

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Mangialajo *et al.*, 2011; Vila *et al.*, 2001), Japan (Taniyama *et al.*, 2003), New Zealand (Chang *et al.*, 2000; Rhodes *et al.*, 2000) and Australia (Pearse *et al.*, 2001).

As far as the Italian coasts of the Mediterranean Sea the genus *Ostreopsis* has been recorded since '90s (Tognetto *et al.*, 1995), but only in recent years large bloom events have been reported (see Mangialajo *et al.*, 2011). At present, it can be considered a widespread phenomenon, as bloom events in the last years have been reported both for the Tyrrhenian (Congestri *et al.*, 2006; Mangialajo *et al.*, 2008; Simoni *et al.*, 2003) and the Adriatic side (Monti *et al.*, 2007; Totti *et al.*, 2007).

In Italy, since the "Genoa event" of 2005 (Brescianini *et al.*, 2006; Ciminiello *et al.*, 2006), the monitoring of *O. ovata* presence suspended in seawater and epiphytic on the macroalgae has started in some regions and has also been included in the national monitoring program financed by the Ministry of the Environment (2008-2009). In the meanwhile (2006), the Italian central Health Institute (ISS) was put in charge from the Ministry of Health of devising a manual on the management of the risk associated to *Ostreopsis* blooms along the Italian coasts. An expert group, formed by ISS, University and Regional Agencies produced guidelines, published in early 2007, that became then part of the new Italian law about bathing water control (D. Lgs. 116/08). A three-phase monitoring plan was recommended: routine phase, alert phase and emergency phase. A threshold of 10000 cell/l was identified for shifting from the alert to the emergency phase.

In this framework, the development of a forecasting tool for predicting *Ostreopsis* blooms from a suite of environmental variables becomes of great interest in an ecological but also in an economic and sanitary perspective. Operational biological forecasts in the ocean are uncommon, and, more generally, ecological forecasts are difficult because uncertainty and inherent stochasticity in the data, system and models lead to low information content in the forecast (Clark *et al.*, 2001). Meaningful bloom predictive models require long term data series (Bates & Trainer, 2006; Trainer *et al.*, 2000) and some examples are reported in Lane *et al.* (2009). Additional challenges in producing a predictive model are: i) the definition of the actual cell number characterizing a bloom, that cannot be strictly defined, varying intrinsically among species (Smayda, 1997; Spatharis *et al.*, 2009); ii) in the case of benthic species, such as *O. ovata*, the choice of the environmental matrix to consider for bloom accounting (water or macroalgae or rocks; see comments on Mangialajo *et al.*, 2011); iii) the use of explanatory variables that need to be at their turn predicted (*e.g.* meteorological models).

As a first step to develop a true predictive model, we analyzed through a multiple correlative approach a relatively long data set, in which the response variables were represented by the magnitude and the temporal extent of bloom events. *O. ovata* is a benthic species and its occurrence in the water is caused by detachment from the substrate and resuspension that occur because of water turbulence and/or because of exceedingly high abundances (Totti *et al.*, 2010; Vila *et al.*, 2001). Consequently, from an ecological perspective, the most appropriate response variable would be the abundance of cells on the substrate (see comments on Mangialajo *et al.*, 2011). Yet, in the present monitoring, local agencies collect mostly water samples, according to the Ministry of Health guidelines. Given that the aim of this work is to provide a tool to the agencies, we decided to consider blooms in the water column. The choice of planktonic algae as the environmental matrix to model, was based also on a sanitary perspective, as cells in the water column are the ones more directly affecting humans by contact or inhalation.

MATERIALS AND METHODS

In order to assess the relative importance of a range of possible predictor variables on *O. ovata* blooms, we used a data set from Genoa – Quarto dei Mille (Ligurian Sea, North-western Mediterranean; Fig. 1), very close to the Genoa city center. Samples were collected during the summer months, from July 2006 to August 2010, with increasing frequency from June to July (from fortnightly to daily).

For each sampling date, water and macroalgae, the brown alga *Halopteris scoparia* (Linnaeus) Sauvageau, samples were collected in order to evaluate cell abundance. Concurrently, additional water samples were collected to estimate nutrient (NH_3, NO_3+NO_2, PO_4) concentrations. Water samples (2 Liters) were collected at nearly 50 cm depth, at a distance of approximately 20 cm from vegetated natural rocks. The collection of macroalgae (two replicates of *Halopteris scoparia*) in bottles (250 mL) was performed at the same depth with care to minimize the loss of cells. Both water and macrophyte samples for *O. ovata* counting were fixed in 4% formalin solution.

Water features (seawater temperature, salinity) were measured *in situ* using a multiparametric probe (Hanna 9328). Meteorological variables (air temperature, barometric pressure, wind speed and direction, sea condition) were gathered from the meteorological service of ARPAL (http://meteo.meteoliguria.it/).

Cell identification and enumeration in water samples were performed with an inverted microscope after the sedimentation of subsamples (100 mL) in chambers following Uthermöl's method (1958). For quantification of cell abundance on macroalgae, samples were vigorously shaken. Macroalgae were isolated from the water and weighed (fresh weight) and cells were counted in 2 mL chambers, following the method described above.

Fig. 1. Location of the study site, Quarto dei Mille (Ligurian Sea, North-western Mediterranean).

Blooms were defined as consecutive days in which planktonic cell concentrations (PC) exceeded 4000 cell/L (corresponding to epiphytic cell abundances – EA – exceeding 80000 cell/gFW of macroalga, according to the positive strict correlation between epiphytic and planktonic cells reported in Mangialajo *et al.*, 2008; 2011). This threshold, chosen on the basis of previous investigations and authors' personal observations (data not presented), cautionary encompasses the alarm value set by the Health Ministry and provides a large amount of replicability of bloom events in order to increase statistical inference.

Response variables taken into account were bloom magnitude, the largest abundance of cells in any bloom event, and bloom length, the number of days that each bloom event lasted, calculated according to the planktonic cell concentrations.

Predictors of the model were a set of physical-chemical features of the water and some local meteorological variables. Among these, those purportedly playing a major role were seawater temperature (although its role is still debated, see Mangialajo *et al.*, 2011 and Faimali *et al.*, 2012) and the marine weather conditions (Mangialajo *et al.*, 2008; Pistocchi *et al.*, 2011; Shears & Ross, 2009), according to literature data and authors' experience.

Multiple linear regressions were performed between each response variable and a set of predictors. Predictors for the bloom length were average values measured during the bloom for the following variables: *O. ovata* PC and EA concentrations (respectively Log cell/L and Log cell/gFW), seawater temperature (°C), salinity (PSU), NH_3 (µM), NO_3+NO_2 (µM), PO_4 (µM), air temperature at noon (°C), barometric pressure (mbar), wind speed (m/sec) and direction (degrees).

Predictors for bloom magnitude were highest *O. ovata* EA during the bloom event and values of the same set of environmental variables as above, measured on the day of largest cell occurrence.

Best reduced models were obtained through the AIC procedure (Akaike Information Criteria; Akaike, 1974), as a measure of the relative goodness of fit of the statistical model, through backward and forward selection of variables, using the software Brodgar 2.6.6 from Highland Statistics Ltd.

RESULTS

Ostreopsis ovata cell abundances during the summer months showed large inter-annual variability along the study period, from 2006 to 2010 (Fig. 2), although higher cell abundances were always recorded at the end of July. Among these annual maxima, higher values were recorded in those years when at that time of the year seawater temperature showed higher values. Conversely, in colder summers, as occurred in 2009, in which seawater temperature increased much slower and top higher temperature values were recorded at the end of August, *O. ovata* was still more abundant at the end of July, although with lower values, and no late summer (late August) blooms were observed.

A total of 19 bloom events were recorded during the study period, ranging in magnitude from 4310 to 137103 cell/L and in length from 1 to 30 days. Longer and more intense blooms were recorded in 2006, 2007 and 2008, while in later years blooms were shorter and showed a lower magnitude.

A summary of the results from the multiple regressions is reported in Table 1. For each response variable R-squared and p values are reported for the

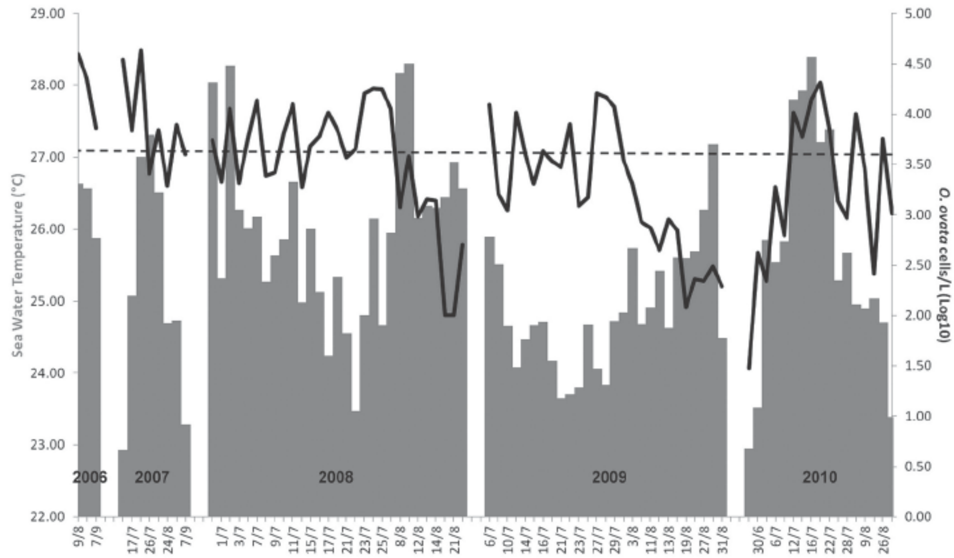

Fig. 2. Log transformed *Ostreopsis ovata* planktonic cells concentrations (PC; black line) and sea water temperature (grey bars) in summer months from 2006 to 2010. The dashed line represents the 4000 cell/L threshold (corresponding to 3.6 on a log base): each bloom event is represented by the portions of the cell concentration line that is above the threshold.

Table 1. Summary of modeling results on *Ostreopsis ovata* bloom length and magnitude. PC: planktonic cell concentrations; EA: epiphytic cell abundances. Significance codes: ***: 0.001; **: 0.01; *: 0.05

Bloom length	Full Model	Response: number of days of each bloom event	Predictors: full set of 12 variables	Multiple R-squared: 0.9583 P value: 0.2254
	Reduced model	Response: number of days of each bloom event	Predictors: Log PC (+)*** Log EA (+)* Barometric pressure (+)* Wind speed (+)*** NH_3 (-)***	Multiple R-squared: 0.9072 P value: 2.774e-06 AIC: 41.36
Bloom magnitude	Full Model	Response: highest PC concentration of each bloom event	Predictors: full set of 11 variables	Multiple R-squared: 0.9416 P value: 0.1246
	Reduced model	Response: highest PC concentration of each bloom event	Predictors: Log EA (+)*** PO_4 (+) Water temperature (+)** NO_3+NO_2(-) Wind speed (-) Salinity (-)	Multiple R-squared: 0.7968 P value: 0.001344 AIC: 264.25

full model (accounting for all the predictor variables) and the reduced model (accounting for the predictors selected through the AIC procedure). Reduced models produced for both bloom length and magnitude are largely significant and explain a quite large proportion of the variability, around 80% for bloom magnitude and 90% for bloom length.

Bloom length is fundamentally driven by *O. ovata* abundances both in the water column and epiphyte on macroalgae; length increases with increasing barometric pressure and wind speed, while depletion of NH_3 is observed whenever blooms last longer.

Bloom magnitude is again mostly driven by epiphytic cell abundances. Other variables play a role in the model, but water temperature is the only one that is strongly significant: the higher the temperature, the larger the bloom.

DISCUSSION

Toxic algal blooms are becoming a serious threat also in temperate waters for humans and for coastal communities (Shears & Ross, 2009), and a strong need for understanding and predicting the environmental conditions which are likely to trigger bloom development has emerged.

O. ovata blooms in the Northern sector of the Mediterranean have been reviewed recently by Mangialajo *et al.* (2011), pointing out how the timing of the bloom occurrence changes with the Mediterranean sector (*e.g.* Ligurian Sea *vs* Adriatic Sea). Yet, within each sector, larger blooms consistently occur at the same time of the year and such periodicity has been observed also in the present study, showing how in the Ligurian Sea, the end of July-early August period is the most threatened by *O. ovata* proliferations.

The tight coupling between EA and PC is proven by the strong positive correlation between the magnitude of the bloom (measured in the water column) and the abundance of *O. ovata* cells on the macroalgae: the larger is the source stock the larger is the availability of cells in the water. Concurrently, water temperature plays a positive role in enhancing the magnitude of the bloom, most probably directly affecting epiphytic cell proliferation.

With regard to the length of the bloom, that is the number of days that the bloom lasts, the variable exerting the strongest positive effect is the abundance of cells in the water (PC) and, secondly, on the macroalgae (EA), although their relative role is of course impaired by the fact that bloom length is assessed on PC. True predictors, in sense of environmental drivers, affecting bloom length are barometric pressure and wind speed. The role of these two variables could appear controversial, as the first one suggests that calm days (high barometric pressure) have a positive effect, while the second suggests that windy days (high wind speed) have a positive effect. Yet, as we are trying to understand which are the variables that make cell concentrations in the water stay high for a long period of time, the role of the two variables is less controversial. In fact, for bloom length we fed the model with the average values of the predictor variables along each bloom event and the results show that in order to have a long lasting bloom (not necessarily an intense bloom, but a prolonged period of relatively high concentration of cells in the water), an interplay between windy days (summer breeze that resuspends cells) and stable barometric conditions is required. In contrast, strong barometric changes cause too strong windy and stormy days that wash out cells and abruptly interrupt the proliferation.

The role of nutrients on *Ostreopsis* spp. growth and toxicity is almost unknown (Pistocchi *et al.*, 2011) and also in our study both bloom length and magnitude do not show any relevant role of nutrients as a limiting or triggering factor for the bloom development, except for PO_4 showing a small positive effect on bloom length, while NH_3 and NO_3+NO_2 are depleted, the former as a consequence of intense blooms and the latter as a consequence of long blooms.

CONCLUSIONS

Present study has provided a range of significant correlations between *O. ovata* bloom magnitude and length in the Ligurian Sea and a set of local environmental variables, among which seawater temperature and hydrodynamics, as for other toxic benthic dinoflagellates' blooms (Ballantine *et al*., 1988; Hallegraeff *et al*., 1995; Morton *et al*., 1992; Tindall & Morton, 1998; Pistocchi *et al*., 2011), while nutrient limitation seems unlikely. The percentage of variation explained by a relatively small set of variables is remarkably large, highlighting the likelihood that, in the future, a small set of key seawater and meteorological variables could be used as good predictors for managers in forecasting toxic events.

All the above paves the way to building true predictive models based on a small set of variables (mostly meteorological) that could be used by managers for risk forecasting in ecological, sanitary and economic perspectives.

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