



UNIVERSITÀ POLITECNICA DELLE MARCHE  
Repository ISTITUZIONALE

A conceptual model of annual *Ostreopsis cf. ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients

This is the peer reviewed version of the following article:

*Original*

A conceptual model of annual *Ostreopsis cf. ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients / Accoroni, Stefano; Glibert, P. M.; Pichierri, Salvatore; Romagnoli, Tiziana; Marini, M.; Totti, Cecilia Maria. - In: HARMFUL ALGAE. - ISSN 1568-9883. - 45:(2015), pp. 14-25. [10.1016/j.hal.2015.04.002]

*Availability:*

This version is available at: 11566/225994 since: 2022-07-06T11:02:12Z

*Publisher:*

*Published*

DOI:10.1016/j.hal.2015.04.002

*Terms of use:*

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.


(Article begins on next page)

Dear Author,

Please, note that changes made to the HTML content will be added to the article before publication, but are not reflected in this PDF.

Note also that this file should not be used for submitting corrections.

**AUTHOR QUERY FORM**

 <b>ELSEVIER</b>	<b>Journal:</b> HARALG  <b>Article Number:</b> 1110	<b>Please e-mail your responses and any corrections to:</b>  <b>E-mail:</b> <a href="mailto:corrections.eseo@elsevier.thomsondigital.com">corrections.eseo@elsevier.thomsondigital.com</a>
--	---	--

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult <http://www.elsevier.com/artworkinstructions>.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the ‘Q’ link to go to the location in the proof.

Location in article	Query / Remark: <b>click on the Q link to go</b> <b>Please insert your reply or correction at the corresponding line in the proof</b>
<b>Q1</b> <b>Q2</b> <b>Q3</b> <b>Q4</b>  <b>Q5</b>  <b>Q6</b> <b>Q7</b> <b>Q8</b>	<p>Please confirm that given names and surnames have been identified correctly.</p> <p>Please check whether the designated corresponding author is correct, and amend if necessary.</p> <p>Please check the address for the corresponding author that has been added here, and correct if necessary.</p> <p>“Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact <a href="mailto:r.skinner@elsevier.com">r.skinner@elsevier.com</a> immediately prior to returning your corrections.”.</p> <p>The citation “Brissard et al. (2014); Edler and Elbrachter (2010)” has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.</p> <p>One or more sponsor names and the sponsor country identifier may have been edited to a standard format that enables better searching and identification of your article. Please check and correct if necessary.</p> <p>Please provide the grant number for all the grant sponsors, if any.</p> <p>Please supply the name of the city of publication for the reference “Edler and Elbrachter (2010); Pearce et al. (2001)”.</p> <div data-bbox="334 1519 1034 1640"> <p>Please check this box or indicate your approval if you have no corrections to make to the PDF file</p> <input data-bbox="938 1542 1000 1608" type="checkbox"/> </div>

Thank you for your assistance.

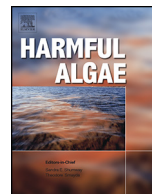


ELSEVIER

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

## Harmful Algae

journal homepage: [www.elsevier.com/locate/hal](http://www.elsevier.com/locate/hal)



### Highlights

#### **A conceptual model of annual *Ostreopsis cf. ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients**

*Harmful Algae xxx (2015) pp. xxx–xxx*

Stefano Accoroni\*, Patricia M. Glibert, Salvatore Pichierri, Tiziana Romagnoli, Mauro Marini, Cecilia Totti

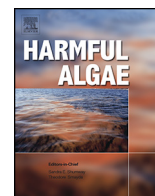
- *O. cf. ovata* bloom is triggered by a combination of optimal temperature and N:P ratio.
- Synergic effects of hydrodynamics, temperature and nutrients affect the bloom onset.
- Water temperature threshold (25 °C) plays a key role on cyst germination.
- N:P ratio around Redfield value is a necessary condition to allow cell proliferation.
- After the onset, bloom development can be sustained at temperature values below 20 °C.



Contents lists available at ScienceDirect

# Harmful Algae

journal homepage: [www.elsevier.com/locate/hal](http://www.elsevier.com/locate/hal)



## A conceptual model of annual *Ostreopsis* cf. *ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients

Stefano Accoroni<sup>a,b,\*</sup>, Patricia M. Glibert<sup>b</sup>, Salvatore Pichierri<sup>a</sup>, Tiziana Romagnoli<sup>a</sup>,  
Mauro Marini<sup>c</sup>, Cecilia Totti<sup>a</sup>

<sup>a</sup> Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

<sup>b</sup> University of Maryland Center for Environmental Science, Horn Point Laboratory, P.O. Box 775, Cambridge MD 21613, USA

<sup>c</sup> Istituto di Scienze Marine (Consiglio Nazionale delle Ricerche), Largo Fiera della Pesca, 60125 Ancona, Italy

### ARTICLE INFO

#### Article history:

Received 7 December 2014

Received in revised form 2 April 2015

Accepted 2 April 2015

#### Keywords:

*Ostreopsis*

N:P ratio

Temperature

Harmful algae

Benthic dinoflagellates

Cysts

### ABSTRACT

The ecology of *Ostreopsis* cf. *ovata* blooms in the Conero Riviera (N Adriatic Sea) was investigated based on sampling carried out from 2007 to 2012, in order to assess the role of environmental factors associated with blooms. Generally, the temporal trend of blooms showed the first cell appearance at the end of July/early August, the maximum abundances in late-summer at end of September/early October reaching up to  $10^6$  cells  $g^{-1}$  fw on macrophyte samples, and the decline of the blooms at end October/early November. Calm conditions appeared to be a prerequisite for blooms. When suitable hydrodynamic conditions exist, *O. cf. ovata* blooms appear to be triggered by a combination of optimal temperature and available nutrients. A water temperature threshold of 25 °C plays a key role on the germination of *O. cf. ovata* cysts and therefore on the bloom onset, and an N:P ratio around Redfield value is a necessary condition to allow cell proliferation. The synergy of higher temperatures and optimal N:P ratios resulted in a higher net growth rate of *O. cf. ovata* cells. After the onset, blooms can be maintained at temperature values even below 20 °C and at N:P ratios that are in excess of the Redfield ratio. Once the bloom is started it may be maintained not necessarily through high growth rates, but likely through other physiological mechanisms. Bloom decline occurred when temperatures dropped below 18 °C. The net effect of the synergy between local hydrodynamic conditions, temperature, and N and P availability may help to explain why blooms in the northern Adriatic Sea occur differently from those in other Mediterranean regions.

© 2015 Published by Elsevier B.V.

### 1. Introduction

Toxic benthic dinoflagellates belonging to the genus *Ostreopsis* are common components of tropical and subtropical marine environments and their presence has increased greatly in temperate waters in recent years (Rhodes, 2011; Parsons et al., 2012). In the last decade, massive *Ostreopsis* blooms, mostly *O. cf. ovata* (Battocchi et al., 2010; Perini et al., 2011), have occurred regularly during summer along Mediterranean Sea coasts (Vila et al., 2001; Turki, 2005; Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2011; Amzil

et al., 2012; Illoul et al., 2012; Ismael and Halim, 2012; Pfannkuchen et al., 2012). In these areas, blooms have been associated with both noxious effects on human health (Gallitelli et al., 2005; Kermarec et al., 2008; Tichadou et al., 2010; Del Favero et al., 2012) and mass mortalities of benthic marine invertebrates and/or macroalgae (Shears and Ross, 2009; Accoroni et al., 2011; Gorbi et al., 2012, 2013; Pagliara and Caroppo, 2012). Mortalities have been attributed to the production of palytoxin-like compounds that include putative palytoxin and ovatoxins-a, b, c, d, e and f (Ciminiello et al., 2011, 2012; Uchida et al., 2013), and mactarenotoxins-a and c (Rossi et al., 2010; Scalco et al., 2012). Furthermore, palytoxin-like compounds produced by *Ostreopsis* spp. have been detected in tissues of both filter-feeding bivalve molluscs and herbivorous echinoderms in some Mediterranean areas (Aligizaki et al., 2008, 2011; Amzil et al., 2012; Brissard et al., 2014) constituting a further possible threat for human health associated with seafood consumption.

\* Corresponding author at: Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy.

Q3 Tel.: +39 071 2204919; fax: +39 071 2204650.

E-mail address: [s.accoroni@univpm.it](mailto:s.accoroni@univpm.it) (S. Accoroni).

Several environmental factors, including hydrodynamics, water temperature, and nutrient concentrations may affect bloom dynamics, growth rate, cell size and toxin production of *Ostreopsis* cf. *ovata*, as previously shown in both field and experimental conditions (Aligizaki and Nikolaidis, 2006; Guerrini et al., 2010; Granéli et al., 2011; Accoroni et al., 2012b; Pezzolesi et al., 2012, 2014; Vanucci et al., 2012; Vidyaratna and Granéli, 2013). Several studies have shown hydrodynamics to be one of the main factors affecting *Ostreopsis* blooms, highlighting that higher abundances are found in sheltered sites compared with exposed ones (Chang et al., 2000; Shears and Ross, 2009; Totti et al., 2010; Mabrouk et al., 2011; Selina et al., 2014). Hydrodynamics may also have an important effect on the bloom temporal variability as stormy events can lead a sudden decrease of cell abundances on the benthic substrata (Totti et al., 2010; Accoroni et al., 2011).

Several authors have proposed that temperature may represent a key factor for bloom development, as *Ostreopsis* spp. need relatively high temperatures to proliferate, and they have suggested that regional warming may have influenced *Ostreopsis* expansion in temperate areas such as the Mediterranean Sea (Hallegraeff, 2010; Granéli et al., 2011). However, it is also recognized that high temperatures may not be a consistent factor for blooms throughout the Mediterranean area (Mangialajo et al., 2011); for example, in the northern Adriatic Sea, the bloom peak occurs in the late summer when temperatures are decreasing rather than when they are at their seasonal maximum (Accoroni et al., 2012a). Nevertheless, a study on the life cycle of *O. cf. ovata* conducted with northern Adriatic strains highlighted that temperature represents a key factor for bloom onset because it affects cyst germination (Accoroni et al., 2014).

With regard to the role of nutrients, recent studies have provided increasing evidence of a link between the nutrient enrichment of coastal waters (anthropogenic eutrophication) and harmful algal events (Anderson et al., 2002; Glibert and Burkholder, 2006; Heisler et al., 2008; Glibert et al., 2010). Furthermore, high input of N and P from the land and the atmosphere not only increase their concentrations in the receiving water bodies but may also result in an alteration of nutrient ratios in relation to the optimum Redfield ratio (N:P = 16:1) for algal growth (Redfield, 1934, 1958; Glibert et al., 2013, 2014). There are some conflicting results regarding the relationships of nutrients with *Ostreopsis* bloom trends in field observations. Several authors did not observe any clear relationship between the abundance of *Ostreopsis* cf. *ovata* during blooms and nutrient concentrations in the water column (Vila et al., 2001; Shears and Ross, 2009; Accoroni et al., 2011; Cohu et al., 2011). Others have found positive but variable nutrient relationships. For example, Parsons and Preskitt (2007) found that *Ostreopsis* sp.1 abundance was positively correlated with nutrient availability (nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), and silicate ( $\text{Si(OH)}_4$ ) concentrations in the waters surrounding Hawaii, while Cohu et al. (2013) reported that  $\text{PO}_4^{3-}$  concentrations, rather than those of nitrogen (N) or  $\text{Si(OH)}_4$ , were positively associated with *O. cf. ovata* abundances in northwestern Mediterranean Sea. Studies carried out in experimental conditions have shown that the N:P ratio in the growth medium affects growth rate, cell size, yield, and toxin production of *O. cf. ovata*, suggesting that the Redfield proportion is optimal for its growth (Vanucci et al., 2012; Vidyaratna and Granéli, 2013). It has also been shown that diluted growth media (L/4, f/4 and f/10) seem to be most suitable for an optimal growth of *Ostreopsis* cells (e.g. Guerrini et al., 2010; Mabrouk et al., 2011; Nascimento et al., 2012; Accoroni et al., 2014) because the use of f/2 or GSe causes a decrease of growth rate and the appearance of aberrant cell shape in *Ostreopsis* cultures (Rhodes et al., 2000; Pearce et al., 2001; Nascimento et al., 2012).

Thus, despite the number of studies about the influence of environmental parameters on *Ostreopsis* blooms, the mechanism for bloom development of this dinoflagellate is complex and far from understood. In this study, we analyzed data from blooms in the Conero Riviera, northern Adriatic Sea, from 2007 to 2012, in order to compare the environmental factors associated with bloom onset, maintenance and decline. These observations show how environmental factors, including hydrodynamics, temperature and nutrient availability, and their synergistic effects, may affect bloom dynamics, explaining also the interannual variability of *Ostreopsis* cf. *ovata* blooms in this area. These data permitted the development of a conceptual model of these factors on bloom dynamics in the northern Adriatic Sea.

## 2. Materials and methods

### 2.1. Sampling and sample treatment

Sampling was carried out from spring to fall of 2007–2012 at the station of Passetto in the Conero Riviera, Ancona, N Adriatic Sea (Fig. 1). This station is a sheltered site due to the presence of a natural reef and is characterized by a rocky bottom, shallow depth, and it is moderately affected by human impact during the summer season. This site is also characterized by small caves derived from human boring of the natural cliffs, with some wastewater discharge facilities. The shore is a popular area for summer holidays, when it is subjected to trampling by swimmers.

Sampling was conducted each year from April–June to November, fortnightly until the first appearance of *Ostreopsis* cells, and then about once a week until the bloom declined.

Surface temperature and salinity were measured with a CTD (Model 30 handheld salinity, conductivity and temperature system, YSI, Yellow Spring, OH USA) and wave height was recorded according to the Douglas scale. Water samples for nutrient analysis were collected in all years, except for 2007. Water samples were collected in polyethylene bottles (50 ml) near the benthic substrata (before the collection of biota or substratum, in order to avoid resuspension), filtered through GF/F Whatman filters as soon as possible thereafter (diameter 25 mm, nominal pore size 0.7  $\mu\text{m}$ ) and

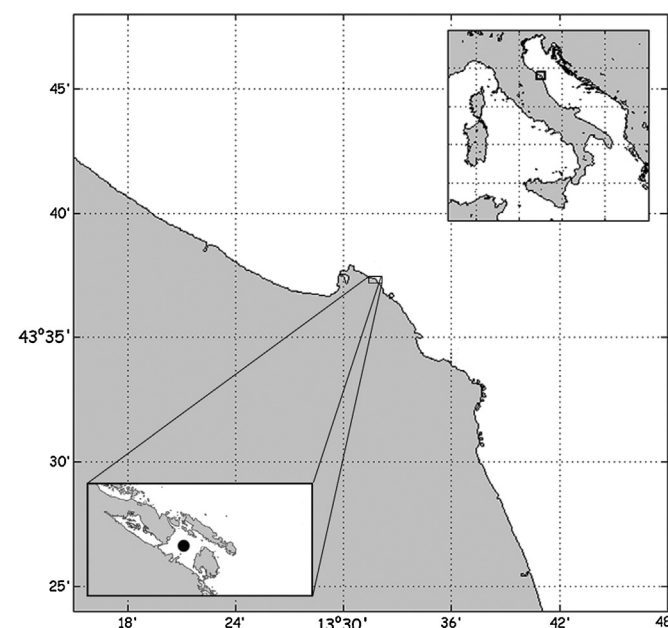


Fig. 1. Map of the study area, showing the location of the sampling station along the Conero Riviera (N Adriatic Sea).



stored in triplicate in 4 ml polyethylene bottles at  $-22^{\circ}\text{C}$  until subsequent analysis.

Undisturbed benthic samples (macroalgae and pebbles) were collected in triplicate at a depth of 0.5 m, following the protocol of Totti et al. (2010). Several seaweed species were collected among those always occurring in the study area during sampling period in all investigated years: *Ulva rigida* (Ulvophyceae), *Dictyota dichotoma* and *Dictyopteris polypodioides* (Phaeophyceae) and *Hypnea musciformis* (Rhodophyceae). Surface seawater (one replicate) was also sampled to analyze the abundance of benthic dinoflagellates in the water column and preserved by adding 0.8% neutralized formaldehyde (Thrandsen, 1978).

## 2.2. Sample treatment

Macroalgae and pebbles were treated following the method described in Totti et al. (2010). Seaweed samples with their storage water were vigorously shaken into plastic jars to allow the dislodgement of epiphytic cells, then thalli were rinsed with filtered sea water (FSW) until the cells of *Ostreopsis* cf. *ovata* were completely dislodged from their substratum. Macrophyte thalli were weighed to determine fresh and dry weight and their area were calculated (Accoroni et al., 2011). In order to obtain a conversion factor useful for estimating the thallus area only by measuring the wet or dry weight, the ratio of 'fresh weight'/'dry weight'/'surface' was determined on numerous samples.

Hard substrata, including pebbles, were accurately scraped with a blade and rinsed with FSW, collecting the scraped material with storage water. The area of the scraped surface was then measured using a tape measure. Finally, the water sample obtained, containing detached cells, rinsing and storage water, was fixed with 0.8% neutralized formaldehyde (Thrandsen, 1978) and stored at  $4^{\circ}\text{C}$  in the dark until analysis.

## 2.3. Microscope analysis

Abundances of *Ostreopsis* cf. *ovata* were estimated using an inverted microscope (Zeiss Axiovert 135) equipped with phase contrast, at  $200\times$  magnification. Subsamples (1–25 ml) were settled in counting chambers after homogenization, according to the Utermöhl's sedimentation method (Edler and Elbrachter, 2010). The identification of *O. cf. ovata* was carried by epifluorescence microscopy, after staining with Calcofluor-White M2R. Counting was performed on 10–30 random fields, 1–2 transects, or the whole sedimentation chamber, in order to count a representative cell number. Identifications were previously confirmed by molecular analysis performed on the same samples (Accoroni et al., 2011; Perini et al., 2011).

## 2.4. Nutrient analysis

Analyses of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , and  $\text{PO}_4^{3-}$  were conducted following the colorimetric method by Strickland and Parsons (1968), adapted for an Autoanalyzer QuAatro Axflow. Limits of detection were  $0.02\ \mu\text{mol l}^{-1}$  for  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , and  $0.03\ \mu\text{mol l}^{-1}$  for  $\text{PO}_4^{3-}$ .

## 2.5. Calculations and statistical analyses

Cell count data for *Ostreopsis* cf. *ovata* were expressed as cells  $\text{g}^{-1}$  fw/dw and cells  $\text{cm}^{-2}$  for macrophytes, cells  $\text{cm}^{-2}$  for pebbles and cells  $\text{l}^{-1}$  for water column. Each annual study period was divided into three phases, identifying a pre-bloom condition (no *O. cf. ovata* cells detected), a bloom onset (the first appearance of *O. cf. ovata* cells), and a bloom maintenance

period (the remaining period during which cells were detected until their decline).

Estimates of the net growth rates ( $\mu\ \text{day}^{-1}$ ) throughout the bloom were calculated according to:

$$\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1) \quad (1)$$

where  $N_2$  and  $N_1$  are *Ostreopsis* cf. *ovata* abundances on each benthic substrata at respective sampling day,  $t_1$  or  $t_2$ .

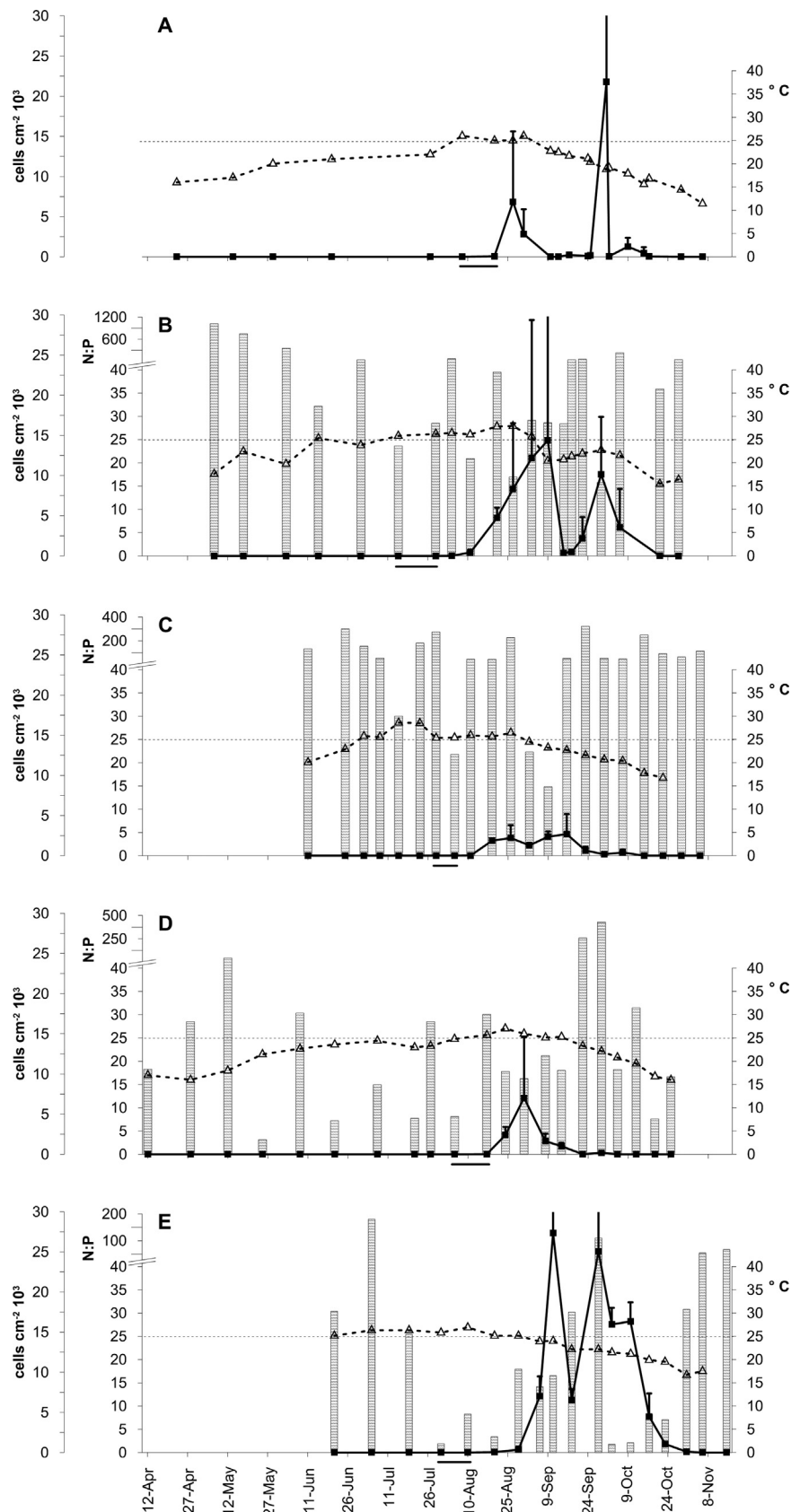
Differences in environmental parameter values, i.e. water temperature, salinity and concentrations of nutrients  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , and  $\text{PO}_4^{3-}$  and total dissolved inorganic N (as sum of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ) and the ratio of inorganic N:P between the three bloom phases (pre-bloom condition, bloom onset and bloom maintenance) were assessed through a one-way analysis of variance (ANOVA). Differences in terms of *Ostreopsis* cf. *ovata* *in situ* growth rates between (i) low ( $<20^{\circ}\text{C}$ ) and high ( $\geq 20^{\circ}\text{C}$ ) water temperature and (ii) high ( $>24.5$ ) and low ( $\leq 24.5$ ) N:P ratio, were also assessed through an ANOVA test, while the effects of water temperature and nutrient condition on *in situ* growth rate were tested using a two-way ANOVA. The abundances of *Ostreopsis* cf. *ovata* were also tested for significant correlations (Pearson) with all recorded environmental parameters and between abundances on benthic substrata and those of water column. When significant differences for the main effect were observed ( $p < 0.05$ ), a Tukey's pairwise comparison test was also performed. The statistical analyses were conducted using Statistica (Statsoft) software.

## 3. Results

### 3.1. Temporal trend

Blooms of *Ostreopsis* cf. *ovata* were detected in all sampling years at the Passetto station. Generally, the first cell appearance on benthic substrata occurred at the end of July/early August, the maximum abundances were recorded in early autumn (end of September/early October), and the decline of the blooms occurred at end October/early November (Fig. 2).

During summer 2007, the first cells appeared on 20<sup>th</sup> August, maximum abundances were recorded on 1<sup>st</sup> October ( $1.7 \times 10^6\ \text{cells g}^{-1}\ \text{fw}$ , corresponding to  $62.0 \times 10^3\ \text{cells cm}^{-2}$ ) and the bloom declined during the last days of October (Fig. 2A). During summer 2009, the first cells appeared on 29<sup>th</sup> July, maximum abundances were recorded on 9<sup>th</sup> September ( $1.3 \times 10^6\ \text{cells g}^{-1}\ \text{fw}$ , corresponding to  $63.8 \times 10^3\ \text{cells cm}^{-2}$ ) and the bloom declined during the last days of October (Fig. 2B). In contrast to these two years, in 2010 the first appearance of *Ostreopsis* cf. *ovata* was recorded early, on 11<sup>th</sup> June, but abundances remained very low and sporadically observed until the end of July (Fig. 2C). The bloom started at the beginning of August reaching the maximum value on 19<sup>th</sup> August ( $1.2 \times 10^6\ \text{cells g}^{-1}\ \text{fw}$  corresponding to  $47.8 \times 10^3\ \text{cells cm}^{-2}$ ), and a second increase of abundances was observed on 16<sup>th</sup> September, with lower values. The bloom decline occurred in the first week of November (Fig. 2C). The pattern of the bloom recorded in summer 2011 was the most different of the years studied: although the first cells were recorded on 5<sup>th</sup> August, the bloom developed very quickly, reaching the maximum abundances on 31<sup>st</sup> August ( $1.6 \times 10^6\ \text{cells g}^{-1}\ \text{fw}$  corresponding to  $25.6 \times 10^3\ \text{cells cm}^{-2}$ ), much earlier than in previous years (Fig. 2D). Abundances rapidly decreased although the end of the bloom occurred only in the last days of October (Fig. 2D). Lastly, in summer 2012, the trend of the *Ostreopsis* bloom was similar to that of the earlier years of the study, with the first cell appearance on 10<sup>th</sup> August, maximum abundances on 11<sup>th</sup> September ( $1.9 \times 10^6\ \text{cells g}^{-1}\ \text{fw}$ , corresponding to  $33.3 \times 10^3\ \text{cells cm}^{-2}$ ),



**Fig. 2.** Trend in *Ostreopsis cf. ovata* abundance on seaweeds expressed in cells cm<sup>-2</sup> (bold lines, 1st left y-axis), N:P ratio (bars, 2nd left y-axis) and water temperature (dotted lines, right y-axis), in years 2007 (A), 2009 (B), 2010 (C), 2011 (D) and 2012 (E). N:P ratio in 2007 is missing. Error bars indicate standard deviation. Bold lines under the x-axis mark dates of bloom onset in each year. The horizontal dotted line represents both (i) the lower temperature threshold permitting cyst germination and therefore the bloom onset and (ii) the upper N:P ratio threshold below which better nutrient conditions are supplied to *O. cf. ovata* growth.



and the bloom decline occurred during the first part of November (Fig. 2E). In this year, the bloom was the most intense in terms of abundances recorded and duration (Fig. 2E).

In all years, *Ostreopsis* cf. *ovata* was also observed in the water column, but with variable abundances (with maximum abundances of  $14.7 \times 10^3$  cells  $l^{-1}$  in 2007,  $92.5 \times 10^3$  cells  $l^{-1}$  in 2009,  $10.2 \times 10^4$  cells  $l^{-1}$  in 2010,  $39.5 \times 10^3$  cells  $l^{-1}$  in 2011 and  $77.9 \times 10^3$  cells  $l^{-1}$  in 2012). The correlation between *O. cf. ovata* abundances on benthic substrata and those in water column was positive and significant ( $n = 98$ ;  $r = 0.44$ ;  $p < 0.001$ ; Fig. 3A). Hydrodynamic events influenced this correlation as, excluding sampling days when sea state was not calm (Douglas scale  $\leq 1$ ), the correlation improved ( $n = 32$ ;  $r = 0.66$ ;  $p < 0.001$ ; Fig. 3B).

Among co-occurring benthic dinoflagellates, three species of potentially toxic microalgae were most often found with *Ostreopsis* cf. *ovata*, although with abundances 1–3 orders of magnitude lower: *Prorocentrum lima* and *Amphidinium* cf. *carterae* were found on all the analyzed substrata ( $6 \pm 1$  and  $16 \pm 10$  cells  $cm^{-2}$ , respectively)

and they did not display any clear seasonal pattern. *Coolia monotis* was found as well ( $104 \pm 20$  cells  $cm^{-2}$ ), but this species has been recently removed from the list of harmful algae ([www.marinespecies.org/hab/index.php](http://www.marinespecies.org/hab/index.php)).

### 3.2. Relationships between *Ostreopsis* cf. *ovata* bloom and environmental parameters

#### 3.2.1. Temperature and salinity

Surface temperature ranged from 14.4 to 28.6 °C in all of these 5 years during the period when *Ostreopsis* cf. *ovata* was detected on benthic substrata. The highest temperature values, between 25 and 28.6 °C, were generally observed from early July and were maintained until middle August and generally corresponded with bloom onset. Bloom onset was always observed on average about 30 days after a temperature of 25 °C was reached, with the only exception observed in summer 2011 (Fig. 4). The bloom peak was generally 70–100 days past this temperature threshold.

The bloom peak was mainly observed when surface temperature was decreasing from the seasonal maximum (18.8–24 °C), with values in the middle of the two previously described extremes. However, in summer 2011 the early bloom peak was recorded close to the seasonal maximum (27 °C) with water temperature of 25.9 °C. Water temperature was significantly lower in bloom maintenance phase ( $22.7 \pm 3.3$  °C) than in the onset ( $26.5 \pm 1.1$  °C, Tukey HSD test,  $p < 0.05$ ). Lowest temperature values were recorded at the end of the bloom, with values ranging from 14.4 to 17.5 °C (Fig. 5).

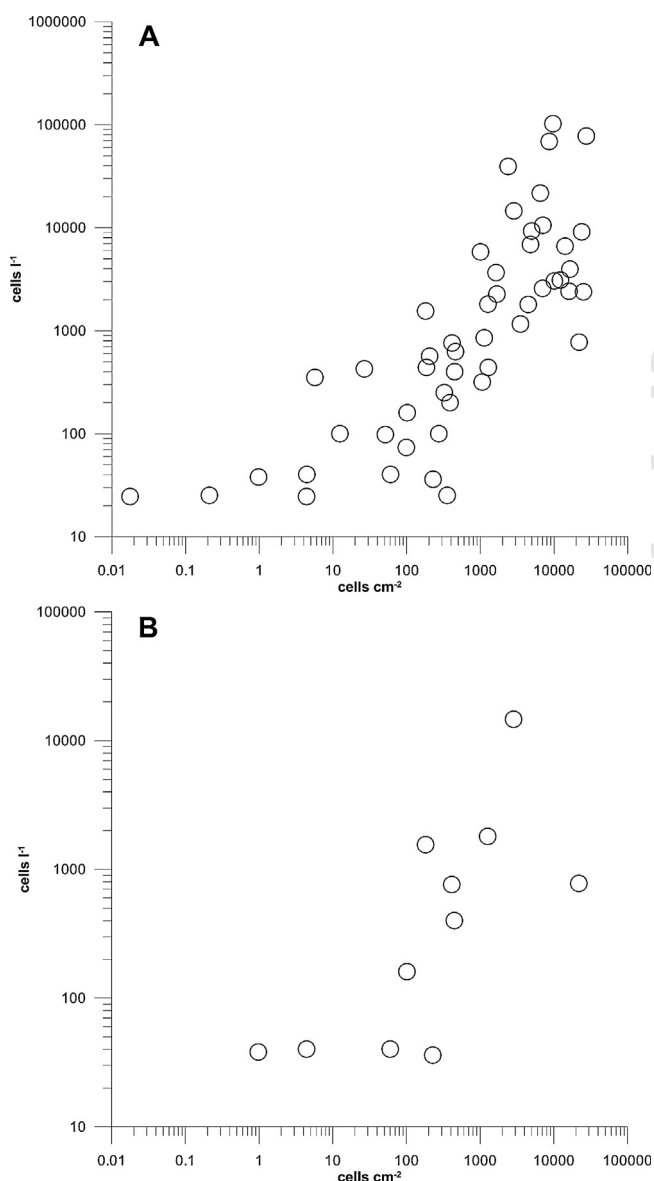
During the period when *Ostreopsis* cf. *ovata* was detected on benthic substrata, the salinity ranged from 31.3 to 39.1 ‰ (not shown).

#### 3.2.2. Nutrients

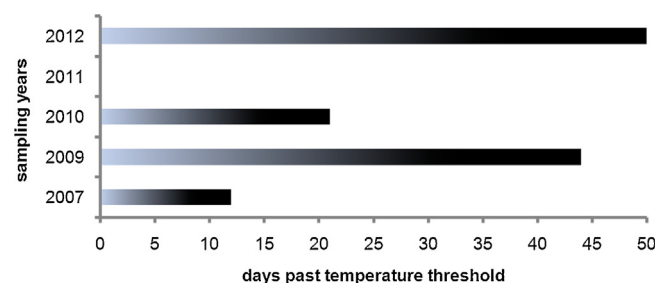
Concentrations of DIN were highly variable between and within the *Ostreopsis* cf. *ovata* blooms, ranging from 0.55 to  $19.40 \mu\text{mol } l^{-1}$  (Fig. 6). In 2009, 2010 and 2011,  $\text{NH}_4^+$  was the major contributor to DIN from June to early-October (Fig. 6A–C), while in 2012  $\text{NO}_3^-$  represented the dominant N source for the entire sampling period (Fig. 6D).

Concentrations of  $\text{PO}_4^{3-}$  ranged from 0.02 to  $0.49 \mu\text{mol } l^{-1}$  during the blooms (Fig. 6).  $\text{PO}_4^{3-}$  concentrations were significantly higher in the bloom onset periods ( $0.35 \pm 0.27 \mu\text{mol } l^{-1}$ ) than in pre-bloom ( $0.10 \pm 0.09 \mu\text{mol } l^{-1}$ , Tukey HSD test,  $p < 0.001$ ) or bloom maintenance periods ( $0.18 \pm 0.10 \mu\text{mol } l^{-1}$ , Tukey HSD test,  $p < 0.01$ ). A recognizable  $\text{PO}_4^{3-}$  seasonal trend was observed in 2009, when an increase in concentrations from the end of May to mid-August was followed by a decrease until early October and a new increase at the end of October when the bloom declined (Fig. 6A), while during the other studied years, a clear seasonal trend was not observed (Fig. 6B, C and D).

Considering the ratios of inorganic N:P, they were generally far above the Redfield ratio with values  $> 1000$ . Ratios of N:P far below



**Fig. 3.** Correlation between *Ostreopsis* cf. *ovata* abundances on benthic substrata (x-axis, cells  $cm^{-2}$ ) and in water column (y-axis, cells  $l^{-1}$ ) for all the study years, on a logarithmic scale, considering all the hydrodynamic conditions (A) and excluding sampling days when sea state was not calm (Douglas scale values  $\leq 1$ ) (B).



**Fig. 4.** Timing of the bloom onset of *Ostreopsis* cf. *ovata* blooms in terms of days past temperature threshold of 25 °C, in each sampling year.

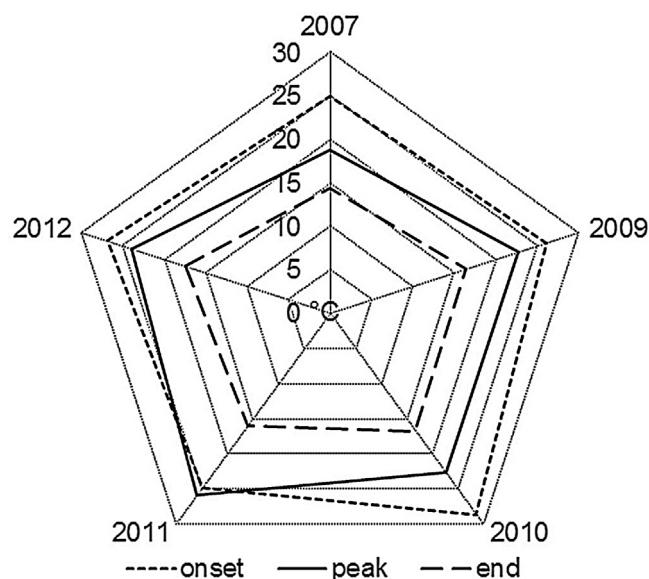


Fig. 5. Water temperature recorded each year at bloom onset, peak and end. Each radius represents the temperature values axis (°C) of year indicated at the corner.

*O. cf. ovata* show a significantly higher growth rate in optimal nutrient conditions and temperature  $\geq 20^\circ\text{C}$  ( $0.09 \pm 0.04 \text{ day}^{-1}$ ) than at higher N:P ratios and temperatures  $< 20^\circ\text{C}$  ( $-0.31 \pm 0.10 \text{ day}^{-1}$ , Tukey HSD test,  $p < 0.05$ ).

No significant correlations were found between salinity values and both benthic *Ostreopsis cf. ovata* abundances and *in situ* growth rate.

#### 4. Discussion

The Passetto station, Conero Riviera, northern Adriatic Sea, has been defined a hot-spot area for *Ostreopsis cf. ovata* blooms by the Italian Regional Agencies for the Protection of the Environments (ISPRA, 2012). In this area, *O. cf. ovata* blooms are among the most intense of the entire Mediterranean coasts (Mangialajo et al., 2011), with maximum abundances reaching the magnitude of  $10^6 \text{ cells g}^{-1} \text{ fw}$  (corresponding to  $10^7 \text{ cells g}^{-1} \text{ dw}$  and  $10^4 \text{ cells cm}^{-2}$ ; Accoroni et al., 2012a). Given the human health and ecological health problems associated with these blooms (Shears and Ross, 2009; Del Favero et al., 2012), there is a great need to understand the factors contributing to their proliferation. Although several studies have highlighted the influence of environmental factors on bloom dynamics of *O. cf. ovata* in field conditions (Aligizaki and Nikolaidis, 2006; Totti et al., 2010; Cohu et al., 2011, 2013), the complexity of conditions leading to blooms of this dinoflagellate are still far from understood.

A clear and important role of the hydrodynamic condition on the trend of *Ostreopsis* blooms has been previously recognized and investigated in the same area (Totti et al., 2010; Accoroni et al., 2011, 2012a). Although blooms occur even in exposed sites, higher abundances are observed under sheltered conditions (Chang et al., 2000; Shears and Ross, 2009; Totti et al., 2010; Mabrouk et al., 2011; Selina et al., 2014). Moreover, the removing of the artificial breakwater reefs from a documented *Ostreopsis cf. ovata* bloom hot-spot area in Conero Riviera (i.e. Numana, see Totti et al., 2010), led to a marked decrease of *Ostreopsis* abundances in the following years (unpublished data). The data herein further highlight how hydrodynamic events may influence the correlation between the abundances of *O. cf. ovata* on benthic substrata and those in water column. In fact, a relatively good correlation between epiphytic and planktonic cell abundances was found only if the hydrodynamic effect was excluded. The *O. cf. ovata* mat is only loosely attached to the substrata and is easily resuspended in the water column; thus, hydrodynamic events can either increase planktonic cell abundance by resuspension or decrease them by dilution if a hydrodynamic event is very strong.

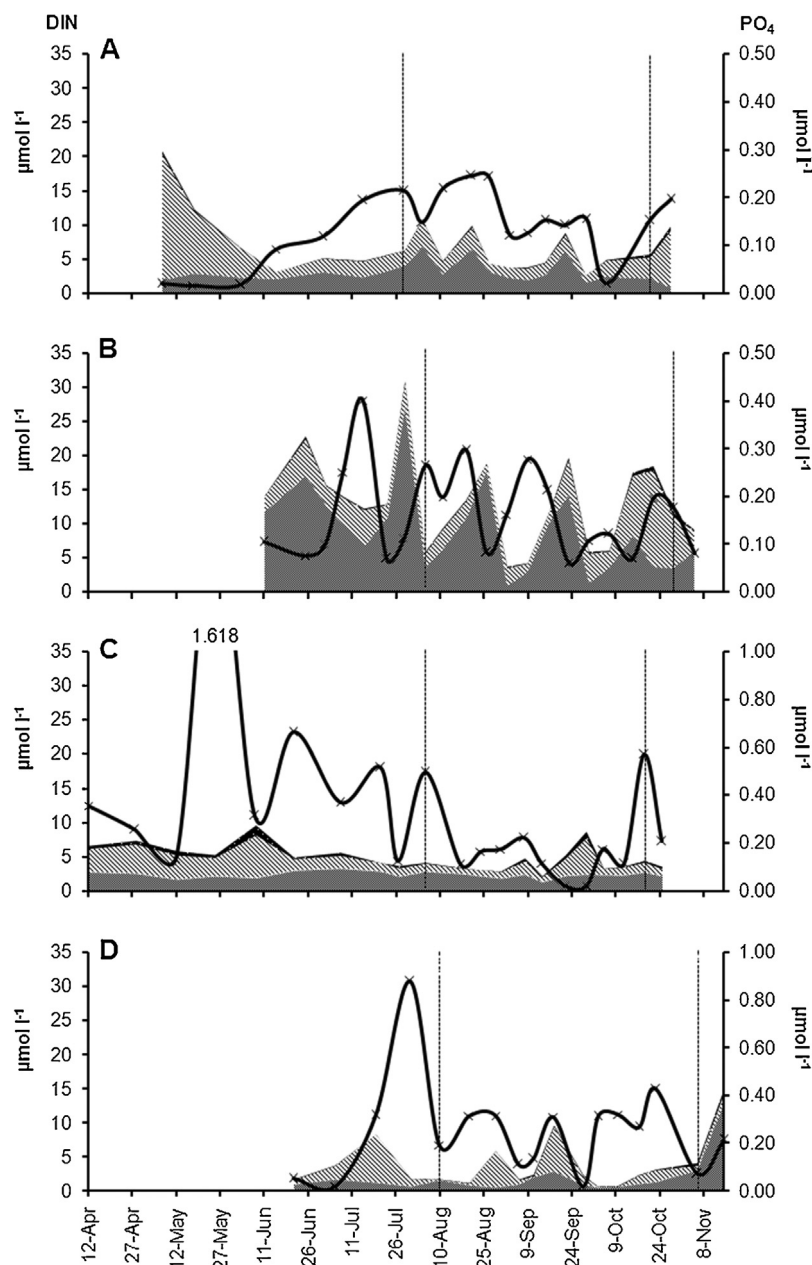
On the contrary, there are some conflicting results regarding the relationships between *Ostreopsis* bloom dynamics and both water temperature and nutrient concentrations. *Ostreopsis* blooms are summer events in temperate areas and maximum abundances have been associated with high temperatures (Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2008), except for the northern Adriatic Sea (Monti et al., 2007; Accoroni et al., 2012a) and the Sea of Japan (Selina et al., 2014). Our data from the Conero Riviera show that the bloom onset was always observed at high temperature ( $25\text{--}28.6^\circ\text{C}$ ). This seems to suggest that *Ostreopsis* needs to reach a temperature threshold to start its bloom, probably linked to cyst germination that generally occurs around values of  $25^\circ\text{C}$  (Accoroni et al., 2014). Once *Ostreopsis* cysts are germinated, its vegetative forms seem to actively proliferate even if temperature values decrease. In this area, the peak in bloom abundance was found to occur in the late summer when temperatures were decreasing from the seasonal maximum ( $18.8\text{--}24^\circ\text{C}$ ) and water temperatures were significantly lower in the bloom maintenance phase ( $22.7 \pm 3.3^\circ\text{C}$ ) than in the bloom onset period ( $26.5 \pm 1.1^\circ\text{C}$ ). In this area, *Ostreopsis cf. ovata* blooms can persist on benthic substrata

Redfield ratio were a more rare event, observed only in some sampling days in 2011 and 2012 (Fig. 2). Noteworthy, the summer of 2011 was characterized by frequent and lasting periods of low N:P, around Redfield ratio during both pre-bloom conditions and bloom development. For all years of study for which nutrient data are available, the N:P ratio was significantly lower in the bloom onset ( $24.5 \pm 7.6$ ) than in pre-bloom conditions ( $139.2 \pm 40.8$ , Tukey HSD test,  $p < 0.05$ ). Moreover, the N:P ratio during the bloom maintenance phase ( $68.2 \pm 13.7$ ) was higher than on the bloom onset and lower than in pre-bloom conditions, although not significantly in both cases.

#### 3.2.3. Relationship between environmental parameters and the *in situ* growth rate

The net *in situ* growth rate of *Ostreopsis cf. ovata* ranged from  $-5.77$  to  $1.36 \text{ day}^{-1}$ . The maximum growth rates for each year ( $1.36$ ,  $0.62$ ,  $0.80$ ,  $1.16$  and  $0.80 \text{ day}^{-1}$ ) were recorded on 13th September 2007, 4th August 2009, 5th August 2010, 17th August 2011, and 29th August 2012, respectively. For those years for which nutrient data were available, these values corresponded with inorganic N:P ratio values ranging from  $16.06$  to  $45.85$  (Fig. 7) as well as temperature values ranging from  $24.5$  to  $26.3^\circ\text{C}$ . In 2009 and 2012, all the positive values of growth rate were detected at inorganic N:P values below 100, while in 2010 and 2011 a small percentage of them (12% and 7%, respectively) were recorded also at inorganic N:P ratios  $> 100$  (Fig. 7). On the contrary, the lowest values, in fact negative net growth rates, were recorded on 29th September 2009, 15th October 2010, 29th September 2011, and 17th October 2012, when inorganic N:P ratios generally exceeded 50 (Fig. 7) and when temperatures were  $< 25^\circ\text{C}$ .

Water temperatures affected the *Ostreopsis cf. ovata in situ* growth rates: at temperatures  $\leq 20^\circ\text{C}$ , *O. cf. ovata* average net growth rates were significantly lower ( $-0.23 \pm 0.05 \text{ day}^{-1}$ ) than at temperatures  $\geq 20^\circ\text{C}$  ( $0.03 \pm 0.03 \text{ day}^{-1}$ , Tukey HSD test,  $p < 0.01$ ). Assuming the average N:P ratio recorded during bloom onset ( $24.5$ ) as the threshold below which better nutrient conditions are supplied to *O. cf. ovata* growth, *O. cf. ovata* showed growth rate higher in optimal nutrient conditions ( $0.06 \pm 0.04 \text{ day}^{-1}$ ) than in higher N:P ratio values ( $-0.02 \pm 0.03 \text{ day}^{-1}$ ), although not significantly. However, analyzing these data considering also the effect of the temperature,

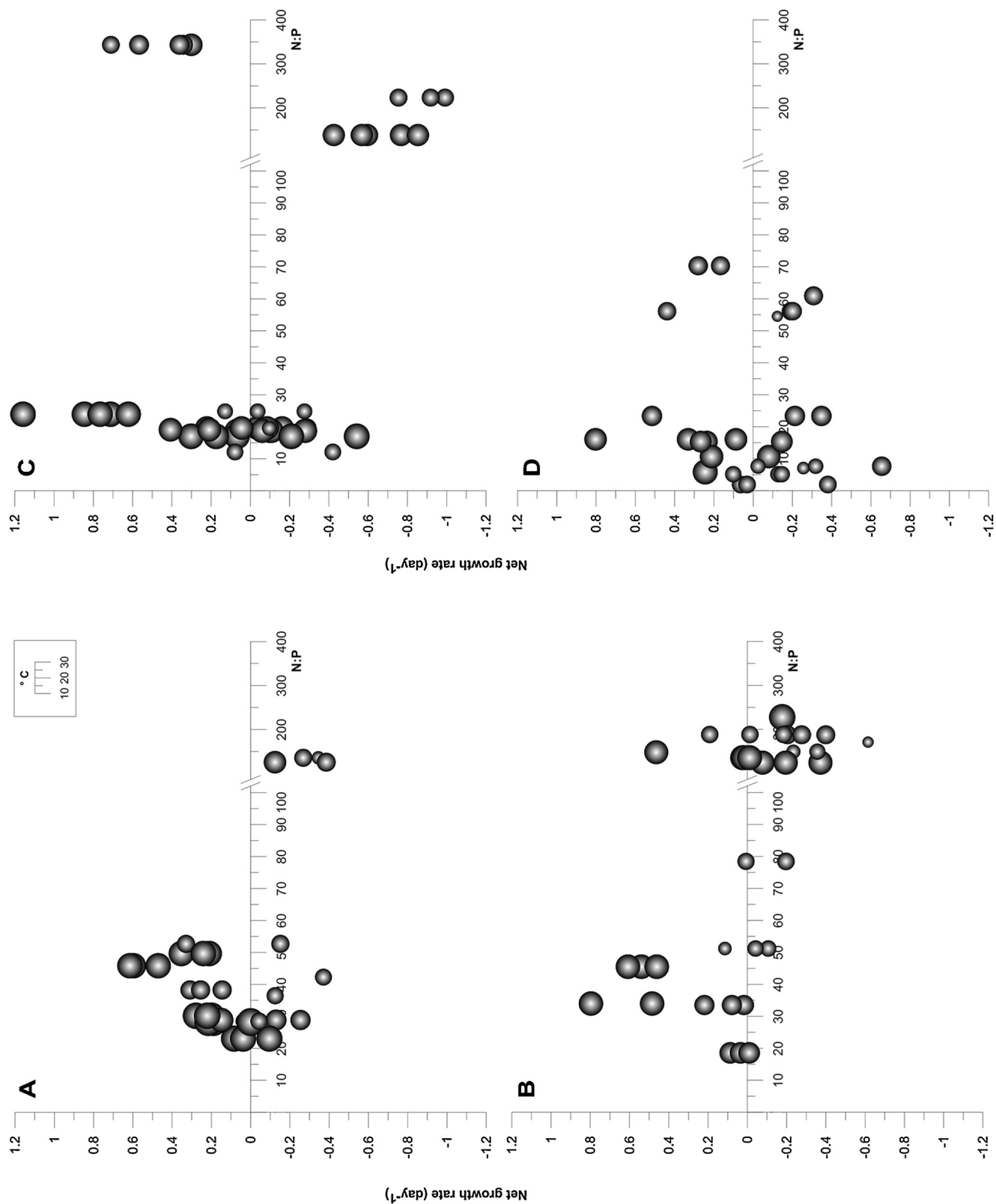


**Fig. 6.** Temporal variability of nutrient concentration ( $\mu\text{mol l}^{-1}$ ) in years 2009 (A), 2010 (B), 2011 (C) and 2012 (D): dissolved inorganic nitrogen (DIN) with the detail of  $\text{NO}_3^-$  (crossed),  $\text{NO}_2^-$  (black) and  $\text{NH}_4^+$  (gray) contribution (left y-axis) and  $\text{PO}_4^{3-}$  (bold line, right y-axis). Vertical bars indicate the period of *Ostreopsis cf. ovata* blooms. (A): modified from Accoroni et al., 2011; (B) modified from Accoroni et al. (2012a).

until temperature values decrease to values ranging from 14.4 to 17.5 °C. These observations completely agree with what observed in experimental conditions about the ecophysiology of *O. cf. ovata* from the northern Adriatic Sea: although *Ostreopsis* strains can growth at 20, 25, and 30 °C, the highest growth rates were recorded at 20 °C (Pezzolesi et al., 2012) and *O. cf. ovata* resting cysts were able to germinate after a 5-month-dormancy period only at 25 °C and not at 21 °C (Accoroni et al., 2014). Binder and Anderson (1987) also found that in experimental conditions the optimal temperature range for the germination of the dinoflagellate *Scrippsiella trochoidea* does not coincide exactly with the optimal range for growth.

In all the studied years except 2011, the bloom onset was observed on average about 30 days after reaching a temperature threshold of 25 °C, suggesting that other environmental factors beside temperature may affect the development of *Ostreopsis cf. ovata* blooms. Recent studies have provided increasing evidence of

a link between the nutrient enrichment of coastal waters (anthropogenic eutrophication) and harmful algal events (Glibert and Burkholder, 2006; Heisler et al., 2008; Glibert et al., 2010). However, there is very limited information on relationships between nutrient concentrations and trends in development of benthic dinoflagellate blooms. Vila et al. (2001) and Cohu et al. (2011) in the NW Mediterranean Sea and Shears and Ross (2009) in NE New Zealand, did not find any relation between epiphytic *O. cf. ovata* and nutrients, while Parsons and Preskitt (2007) found that *Ostreopsis* sp.1 abundance was positively correlated with all inorganic nutrients concentrations in the waters surrounding Hawaii. Cohu et al. (2013) reported that  $\text{PO}_4^{3-}$  concentration, rather than N or  $\text{Si(OH)}_4$ , was also positively associated with *O. cf. ovata* abundances and that N:P ratio was significantly and negatively related to *Ostreopsis* abundances in the northwestern Mediterranean Sea. In fact, if the bloom cells are consuming nutrients, one



**Fig. 7.** In situ net growth rates of *Ostreopsis cf. ovata* expressed in  $\text{day}^{-1}$  (y-axis), in relation to N:P ratio (x-axis), in 2009 (A), 2010 (B), 2011 (C) and 2012 (D).



would not necessarily expect a positive and direct relationship between the abundance of cells and the nutrient availability.

Although no clear relationship was found between nutrient concentrations and *Ostreopsis* cf. *ovata* abundances in this study, it was found that in the bloom onset period  $\text{PO}_4^{3-}$  concentrations were significantly higher than in either the bloom maintenance phase or the pre-bloom conditions, resulting in an N:P ratio that was significantly lower in the bloom onset period ( $24.5 \pm 7.6$ ) than in pre-bloom conditions ( $139.2 \pm 40.8$ ). Both Vanucci et al. (2012) and Pezzolesi et al. (2014) found, in experimental studies of different Adriatic *O. cf. ovata* strains, that the depletion of P was proportionately more rapid than that of N, highlighting the strong P demand of this dinoflagellate. A decrease in N:P ratio has previously been associated with the onset of a number of planktonic dinoflagellate blooms; comparatively fewer reports are available of this relationships for benthic dinoflagellates. For example, planktonic *Prorocentrum* species generally achieve maximum growth rates at N:P ratios just below Redfield proportions (Zhang and Hu, 2011; Glibert et al., 2012); furthermore, Hodgkiss and Ho (1997), in their study of dinoflagellate blooms in Hong Kong water found that annual frequency of blooms increased with declines in the N:P ratio well below Redfield proportions. Bloom onset is the result of both cyst germination and survival/proliferation of germinating cells. Although water temperature has been identified as the most important factor in cyst germination of dinoflagellates (Anderson and Morel, 1979; Anderson, 1980), nutrient availability may modulate the cyst germination, strongly influencing the timing and the rate of excystment or the growth of cells following excystment (Anderson and Wall, 1978; Anderson and Morel, 1979; Dale, 1983; Anderson and Keafer, 1987; Binder and Anderson, 1987; An et al., 1992; Hallegraeff et al., 1998; Rengefors and Anderson, 1998; Figueroa et al., 2005). To date the only information about the nutritional demand of *Ostreopsis* cysts during germination is based on studies in which an N:P ratio of 24 was used to perform germination experiments on resting cysts (Accoroni et al., 2014). Assuming the N:P ratio value recorded during bloom onset (24.5) as the threshold below which better nutrient conditions are supplied to *O. cf. ovata* growth, the growth rate is higher when proportionately more  $\text{PO}_4^{3-}$  is available and when the N:P is  $\leq 24.5$ . The synergy of higher temperatures and lower N:P ratio resulted in a higher net growth rate.

Interestingly, blooms were maintained in a condition of elevated N:P ratios, well above Redfield proportions, a condition normally taken to be indicative of P limitation. However,  $\text{PO}_4^{3-}$  was never depleted from the water column, and thus true P limitation likely never developed physiologically. However, net growth rates were slower than during the initial phase of blooms development. Such a progression of bloom growth in relation to N:P ratios would

be consistent with the progression of *Prorocentrum* blooms previously described by Glibert et al. (2012). They suggested that such blooms may be initiated at low N:P levels (even less than Redfield ratio, but not in severely N limiting conditions), often stimulated by a “flush” of nutrients or organic materials that may allow a latent resident population (in our case, the newly germinating cells) to increase growth rate, as observed in blooms in Tolo Harbor, Chesapeake Bay, and Neuse River Estuary. After that growth rate increase, bloom biomass is able to increase often accompanied by N:P values greater than Redfield. At this point, these blooms are able to sustain high biomass levels, not necessarily by high growth rates, but also through other mechanisms, including (i) metabolic dissipatory strategies that allow the maintenance of cellular nutrient and energy balance in an environment where energy flow and nutrients are provided at unbalanced proportions and (ii) allelopathic and mixotrophic interactions with other species. Thus, as suggested by our observations on *Ostreopsis* blooms as well, while high growth rates (stimulated by an injection of P-rich water) may allow blooms to initiate, adaptive physiology would enable the maintenance of blooms at less than maximal growth rates and at non-optimal N:P ratios.

Therefore, a synergic action of optimal water temperature and optimal nutrient conditions may explain why in the N Adriatic Sea there is a delay between the maximum temperature values and the peak *Ostreopsis* cf. *ovata* bloom. Generally, these simultaneous conditions happen between the second part of July and early August. An exception to this trend was observed in 2011, as after water temperature reached the threshold of 25 °C, optimal nutrient conditions occurred, allowing the immediate bloom development. Noteworthy, in 2010 nutrient conditions remained unfavorable for rapid bloom development, and only in three sampling days N:P ratios  $\leq 24.5$  were recorded, leading to a proportionately smaller bloom and very low abundances compared to what observed during the other years.

However, other factors both abiotic and biotic, such as ability to use organic forms of nutrients and interactions with other organisms, should be investigated to further clarify the *Ostreopsis* cf. *ovata* natural bloom mechanism. Actually, many HAB genera can use organic (dissolved or particulate) forms of nutrients for their nutritional demands (Cucchiari et al., 2008; Heisler et al., 2008) and mixotrophy has been hypothesized in *O. cf. ovata* that showed a particular defensive strategy where small organisms remain trapped in its mucilaginous web (Barone, 2007). Moreover, allelopathic interactions between *Ostreopsis* and other organisms should be considered as well: although a weak inhibitory effect of *O. cf. ovata* was observed towards other

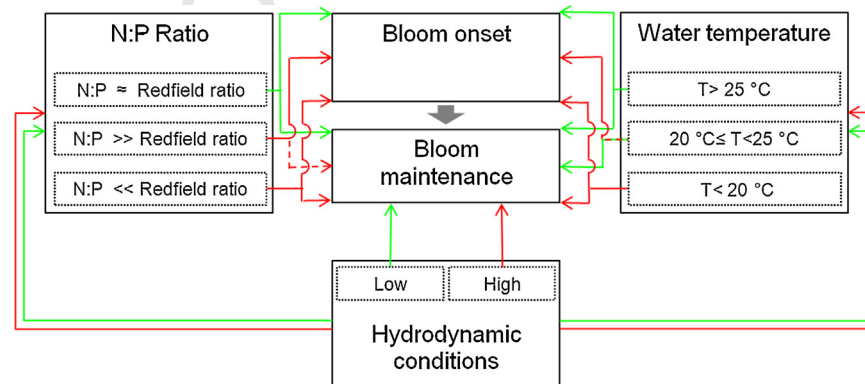
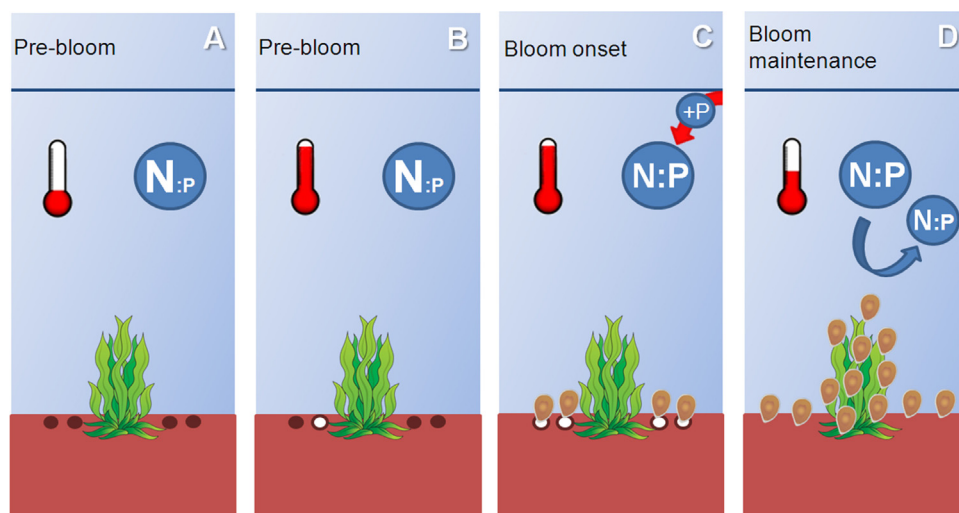


Fig. 8. Conceptual model of the effect of N:P ratio, water temperature and hydrodynamic conditions on both bloom onset and maintenance of *Ostreopsis* cf. *ovata*. Green and red arrows symbolize positive and negative effect, respectively. Dotted line represents a slight effect relative to solid line.





**Fig. 9.** Conceptual diagram of the effect of N:P ratio and water temperature on *Ostreopsis cf. ovata* bloom. Panel A represents the typical pre-bloom condition, showing low temperature and very high N:P ratio. Panel B represents the pre-bloom condition just before the bloom onset: although the temperature ( $\sim 25^\circ\text{C}$ ) is optimal for cyst germination, the high N:P ratio does not allow the start of the bloom. Bloom onset (panel C) occurs at  $T > 25^\circ\text{C}$  and at N:P ratio (around 24) balanced by an injection of P-rich water, allowing both cyst germination and cell proliferation. Panel D shows the optimal environmental conditions for the bloom maintenance: temperature is not limiting, as bloom peak occurs at  $T$  around  $20^\circ\text{C}$ , and the slight increase of N:P ratio accompanied by a general decrease of N and P could be attributable to *O. cf. ovata* growth.

benthic dinoflagellates (Monti and Cecchin, 2012), studies about the interactions between *O. cf. ovata* and the co-occurring microphytobenthic community have not been developed yet, and they may further clarify the *O. cf. ovata* natural bloom mechanism.

In conclusion, our data suggest that in the northern Adriatic Sea *Ostreopsis cf. ovata* blooms are triggered by a combination of calm hydrodynamic conditions, optimal temperature and favorable nutrients (Figs. 8 and 9). Calm conditions are a prerequisite for blooms, and only when this condition exists do temperature and nutrient start to have a decisive effect. The water temperature threshold of  $25^\circ\text{C}$  plays a key role in the germination of *O. cf. ovata* cysts and therefore in bloom onset, and an N:P ratio around Redfield value is a necessary condition to allow cell proliferation. After the bloom onset, the decrease of water temperature to values around  $20^\circ\text{C}$  and slight increase of N:P ratio (likely attributable to *O. cf. ovata* uptake) allow the bloom maintenance, as indicated by the values of *in situ* growth rate. Hydrodynamic conditions enhance these effects both directly acting on the benthic dinoflagellate abundances and indirectly influencing the values of both temperature and nutrient concentration in sea water. This synergic effect on the bloom might explain both (i) the interannual variability of *O. cf. ovata* blooms along Conero Riviera and (ii) why in the northern Adriatic Sea the *O. cf. ovata* blooms occur in the late summer when temperature values are decreasing, differently from other Mediterranean areas.

## Acknowledgments

The authors wish to thank Federica Colombo, Giacomo Ciampi and Angela Pastore for field and laboratory assistance. This research was partly funded by MURST (PRIN 2007), ISPRA-Italian Ministry of the Environment and ENPI M3-HABs project. This contribution number XXX from the University of Maryland Center for Environmental Science.[SS]

## References

- Accoroni, S., Romagnoli, T., Colombo, F., Pennesi, C., Di Camillo, C.G., Marini, M., Battocchi, C., Ciminiello, P., Dell'Aversano, C., Dello Iacovo, E., Fattorusso, E., Tartaglione, L., Penna, A., Totti, C., 2011. *Ostreopsis cf. ovata* bloom in the

- northern Adriatic Sea during summer 2009: ecology, molecular characterization and toxin profile. *Mar. Pollut. Bull.* 62 (11), 2512–2519.
- Accoroni, S., Colombo, F., Pichierri, S., Romagnoli, T., Marini, M., Battocchi, C., Penna, A., Totti, C., 2012a. Ecology of *Ostreopsis cf. ovata* blooms in the northwestern Adriatic Sea. *Cryptogam. Algol.* 33 (2), 191–198.
- Accoroni, S., Romagnoli, T., Pichierri, S., Colombo, F., Totti, C., 2012b. Morphometric analysis of *Ostreopsis cf. ovata* cells in relation to environmental conditions and bloom phases. *Harmful Algae* 19, 15–22.
- Accoroni, S., Romagnoli, T., Pichierri, S., Totti, C., 2014. New insights on the life cycle stages of the toxic benthic dinoflagellate *Ostreopsis cf. ovata*. *Harmful Algae* 34, 7–16.
- Aligizaki, K., Nikolaidis, G., 2006. The presence of the potentially toxic genera *Ostreopsis* and *Coolia* (Dinophyceae) in the north Aegean sea, Greece. *Harmful Algae* 5 (6), 717–730.
- Aligizaki, K., Katikou, P., Nikolaidis, G., Panou, A., 2008. First episode of shellfish contamination by palytoxin-like compounds from *Ostreopsis* species (Aegean Sea, Greece). *Toxicol.* 51 (3), 418–427.
- Aligizaki, K., Katikou, P., Milandri, A., Diogène, J., 2011. Occurrence of palytoxin-group toxins in seafood and future strategies to complement the present state of the art. *Toxicol.* 57 (3), 390–399.
- Amzil, Z., Sibat, M., Chomerat, N., Gossel, H., Marco-Miralles, F., Lemee, R., Nezan, E., Sechet, V., 2012. Ovatoxin-a and Palytoxin accumulation in seafood in relation to *Ostreopsis cf. ovata* blooms on the French Mediterranean coast. *Mar. Drugs* 10 (2), 477–496.
- An, K.H., Lassus, P., Maggi, P., Bardouil, M., Truquet, P., 1992. Dinoflagellate cyst changes and winter environmental-conditions in Vilaine Bay, Southern Brittany (France). *Bot. Mar.* 35 (1), 61–67.
- Anderson, D.M., 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarens* (dinophyceae) hypnozygotes. *J. Phycol.* 16 (2), 166–172.
- Anderson, D.M., Keafer, B.A., 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarens*. *Nature* 325 (6105), 616–617.
- Anderson, D.M., Morel, F.M.M., 1979. The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarens* hypnocyts. *Est. Coast. Mar. Sci.* 8 (3), 279–293.
- Anderson, D.M., Wall, D., 1978. Potential importance of benthic cysts of *Gonyaulax tamarens* and *Gonyaulax excavata* in initiating toxic dinoflagellate blooms. *J. Phycol.* 14 (2), 224–234.
- Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25, 562–584.
- Barone, R., 2007. Behavioural trait of *Ostreopsis ovata* (Dinophyceae) in Mediterranean rock pools: the spider's strategy. *Harmful Algae News* 33, 1–3.
- Battocchi, C., Totti, C., Vila, M., Masó, M., Capellacci, S., Accoroni, S., Reñé, A., Scardi, M., Penna, A., 2010. Monitoring toxic microalgae *Ostreopsis* (dinoflagellate) species in coastal waters of the Mediterranean sea using molecular PCR-based assay combined with light microscopy. *Mar. Pollut. Bull.* 60 (7), 1074–1084.
- Binder, B.J., Anderson, D.M., 1987. Physiological and environmental control of germination in *Scrippsiella trochoidea* (dinophyceae) resting cysts. *J. Phycol.* 23 (1), 99–107.
- Brissard, C., Herrenknecht, C., Séchet, V., Hervé, F., Pisapia, F., Harcouet, J., Lémée, R., Chomérat, N., Hess, P., Amzil, Z., 2014. Complex toxin profile of french

- Mediterranean *Ostreopsis* cf. *ovata* strains, seafood accumulation and ovatoxins pre-purification. Mar. Drugs 12, 2851–2876.
- Chang, F.H., Shimizu, Y., Hay, B., Stewart, R., Mackay, G., Tasker, R., 2000. Three recently recorded *Ostreopsis* spp. (Dinophyceae) in New Zealand: temporal and regional distribution in the upper North Island from 1995 to 1997. N. Z. J. Mar. Freshw. Res. 34 (1), 29–39.
- Ciminiello, P., Dell'Aversano, C., Dello Iacovo, E., Fattorusso, E., Forino, M., Tartaglione, L., 2011. LC-MS of palytoxin and its analogues: state of the art and future perspectives. Toxicon 57 (3), 376–389.
- Ciminiello, P., Dell'Aversano, C., Dello Iacovo, E., Fattorusso, E., Forino, M., Tartaglione, L., Battocchi, C., Crinelli, R., Carloni, E., Magnani, M., Penna, A., 2012. Unique toxin profile of a Mediterranean *Ostreopsis* cf. *ovata* strain: HR LC-MS<sup>n</sup> characterization of ovatoxin-f, a new palytoxin congener. Chem. Res. Toxicol. 25, 1243–1252.
- Cohu, S., Thibaut, T., Mangialajo, L., Labat, J.-P., Passafiume, O., Blanfuné, A., Simon, N., Cottalorda, J.-M., Lemée, R., 2011. Occurrence of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation with environmental factors in Monaco (NW Mediterranean). Mar. Pollut. Bull. 62 (12), 2681–2691.
- Cohu, S., Mangialajo, L., Thibaut, T., Blanfuné, A., Marro, S., Lemée, R., 2013. Proliferation of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation to depth, biotic substrate and environmental factors in the North West Mediterranean Sea. Harmful Algae 24 (0), 32–44.
- Cucchiari, E., Guerrini, F., Penna, A., Totti, C., Pistocchi, R., 2008. Effect of salinity, temperature, organic and inorganic nutrients on growth of cultured *Fibrocapsa japonica* (Raphidophyceae) from the northern Adriatic Sea. Harmful Algae 7 (4), 405–414.
- Dale, B., 1983. Dinoflagellate resting cysts: benthic plankton. In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge University Press, Cambridge, UK, pp. 69–136.
- Del Favero, G., Sosa, S., Pelin, M., D'Orlando, E., Florio, C., Lorenzon, P., Poli, M., Tubaro, A., 2012. Sanitary problems related to the presence of *Ostreopsis* spp. in the Mediterranean Sea: a multidisciplinary scientific approach. Annali dell'Istituto Superiore di Sanità 48 (4), 407–414.
- Edler, L., Elbrachter, M., 2010. The Utermöhl method for quantitative phytoplankton analysis. In: Karlson, B., Cusack, C., Bresnan, E. (Eds.), Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis, 55. IOC UNESCO, Manuals and Guides, pp. 13–20.
- Figuerola, R.I., Bravo, I., Garcés, E., 2005. Effects of nutritional factors and different parental crosses on the encystment and excystment of *Alexandrium catenella* (Dinophyceae) in culture. Phycologia 44 (6), 658–670.
- Gallitelli, M., Ungaro, N., Addante, L.M., Silver, N.G., Sabba, C., 2005. Respiratory illness as a reaction to tropical algal blooms occurring in a temperate climate. JAMA—J. Am. Med. Assoc. 293 (21), 2599–2600.
- Glibert, P.M., Burkholder, J.M., 2006. The complex relationships between increases in fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer, Berlin Heidelberg, pp. 341–354.
- Glibert, P.M., Allen, J.L., Bouwman, A.F., Brown, C.W., Flynn, K.J., Lewitus, A.J., Madden, C.J., 2010. Modeling of HABs and eutrophication Status, advances, challenges. J. Mar. Syst. 83 (3–4), 262–275.
- Glibert, P.M., Burkholder, J.M., Kana, T.M., 2012. Recent insights about relationships between nutrient availability, forms, and stoichiometry, and the distribution, ecophysiology, and food web effects of pelagic and benthic *Prorocentrum* species. Harmful Algae 14, 231–259.
- Glibert, P.M., Kana, T.M., Brown, K., 2013. From limitation to excess: the consequences of substrate excess and stoichiometry for phytoplankton physiology, trophodynamics and biogeochemistry, and the implications for modeling. J. Mar. Syst. 125, 14–28.
- Glibert, P.M., Maranger, R., Sobota, D.J., Bouwman, L., 2014. The Haber Bosch—harmful algal bloom (HB–HAB) link. Environ. Res. Lett. 9, 105001, <http://dx.doi.org/10.1088/1748-9326/9/10/105001>.
- Gorbi, S., Bocchetti, R., Binelli, A., Bacchiocchi, S., Orletti, R., Nanetti, L., Raffaelli, F., Vignini, A., Accoroni, S., Totti, C., Regoli, F., 2012. Biological effects of palytoxin-like compounds from *Ostreopsis* cf. *ovata*: a multibiomarkers approach with mussels *Mytilus galloprovincialis*. Chemosphere 89 (5), 623–632.
- Gorbi, S., Avio, G.C., Benedetti, M., Totti, C., Accoroni, S., Pichiari, S., Bacchiocchi, S., Orletti, R., Graziosi, T., Regoli, F., 2013. Effects of harmful dinoflagellate *Ostreopsis* cf. *ovata* exposure on immunological, histological and oxidative responses of mussels *Mytilus galloprovincialis*. Fish Shellfish Immunol. 35 (3), 941–950.
- Granéli, E., Vidyarthna, N.K., Funari, E., Cumararatunga, P.R.T., Scenati, R., 2011. Can increases in temperature stimulate blooms of the toxic benthic dinoflagellate *Ostreopsis ovata*? Harmful Algae 10 (2), 165–172.
- Guerrini, F., Pezzolesi, L., Feller, A., Riccardi, M., Ciminiello, P., Dell'Aversano, C., Tartaglione, L., Dello Iacovo, E., Fattorusso, E., Forino, M., Pistocchi, R., 2010. Comparative growth and toxin profile of cultured *Ostreopsis ovata* from the Tyrrhenian and Adriatic Seas. Toxicon 55 (2–3), 211–220.
- Hallegraeff, G.M., 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J. Phycol. 46 (2), 220–235.
- Hallegraeff, G.M., Marshall, J.A., Valentine, J., Hardiman, S., 1998. Short cyst-dormancy period of an Australian isolate of the toxic dinoflagellate *Alexandrium catenella*. Mar. Freshw. Res. 49 (5), 415–420.
- Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoerck, D.K., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8 (1), 3–13.
- Hodgkiss, I.J., Ho, K.C., 1997. Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352, 141–147.
- Illoul, H., Hernandez, F.R., Vila, M., Adjias, N., Younes, A.A., Bournissa, M., Koroghli, A., Marouf, N., Rabia, S., Ameer, F.L.K., 2012. The genus *Ostreopsis* along the Algerian coastal waters (SW Mediterranean Sea) associated with a human respiratory intoxication episode. Cryptogamie, Algologie 33 (2), 209–216.
- Ismael, A., Halim, Y., 2012. Potentially harmful *Ostreopsis* spp. in the coastal waters of Alexandria-Egypt. Mediterr. Mar. Sci. 13 (2), 208–212.
- ISPRA, 2012. *Ostreopsis* cf. *ovata* lungo le coste italiane: monitoraggio 2011. Rapporti 173/2012. ([http://www.isprambiente.gov.it/en/publications/reports/ostreopsis-ovata-along-the-italian-coast-monitoring-2011?set\\_language=en](http://www.isprambiente.gov.it/en/publications/reports/ostreopsis-ovata-along-the-italian-coast-monitoring-2011?set_language=en))
- Kermarec, F., Dor, F., Armengaud, A., Charlet, F., Kantin, R., Sauzade, D., De Haro, L., 2008. Health risks related to *Ostreopsis ovata* in recreational waters. Les risques sanitaires liés à la présence d'*Ostreopsis ovata* dans les eaux de baignade ou d'activités nautiques. Environnement, Risques Santé 7 (5), 357–363.
- Mabrouk, L., Hamza, A., Ibrahim, M.B., Bradai, M.N., 2011. Temporal and depth distribution of microepiphytes on *Posidonia oceanica* (L.) Delile leaves in a meadow off Tunisia. Mar. Ecol. 32 (2), 148–161.
- Mangialajo, L., Bertolotto, R., Cattaneo-Vietti, R., Chiantore, M., Grillo, C., Lemee, R., Melchiorre, N., Moretto, P., Povero, P., Ruggieri, N., 2008. The toxic benthic dinoflagellate *Ostreopsis ovata*: quantification of proliferation along the coastline of Genoa, Italy. Mar. Pollut. Bull. 56, 1209–1214.
- Mangialajo, L., Ganzin, N., Accoroni, S., Asnaghi, V., Blanfuné, A., Cabrini, M., Cattaneo-Vietti, R., Chavanon, F., Chiantore, M., Cohu, S., Costa, E., Fornasaro, D., Grossel, H., Marco-Miralles, F., Masó, M., René, A., Rossi, A.M., Sala, M.M., Thibaut, T., Totti, C., Vila, M., Lemée, R., 2011. Trends in *Ostreopsis* proliferation along the Northern Mediterranean coasts. Toxicon 57 (3), 408–420.
- Monti, M., Cecchin, E., 2012. Comparative growth of three strains of *Ostreopsis ovata* at different light intensities with focus on inter-specific allelopathic interactions. Cryptogam. Algol. 33 (2), 113–119.
- Monti, M., Minocci, M., Beran, A.L.I., 2007. First record of *Ostreopsis* cf. *ovata* on macroalgae in the Northern Adriatic Sea. Mar. Pollut. Bull. 54 (5), 598–601.
- Nascimento, S.M., Corrêa, E.V., Menezes, M., Varela, D., Paredes, J., Morris, S., 2012. Growth and toxin profile of *Ostreopsis* cf. *ovata* (Dinophyta) from Rio de Janeiro, Brazil. Harmful Algae 13 (0), 1–9.
- Pagliara, P., Caroppo, C., 2012. Toxicity assessment of *Amphidinium carterae*, *Coolia* cf. *monotis* and *Ostreopsis* cf. *ovata* (Dinophyta) isolated from the northern Ionian Sea (Mediterranean Sea). Toxicon 60 (6), 1203–1214.
- Parsons, M.L., Preskitt, L.B., 2007. A survey of epiphytic dinoflagellates from the coastal waters of the island of Hawai'i. Harmful Algae 6 (5), 658–669.
- Parsons, M.L., Aligizaki, K., Bottein, M.-Y.D., Fraga, S., Morton, S.L., Penna, A., Rhodes, L., 2012. Gambierdiscus and *Ostreopsis*: reassessment of the state of knowledge of their taxonomy, geography, ecophysiology, and toxicology. Harmful Algae 14 (0), 107–129.
- Pearce, I., Marshall, J.A., Hallegraeff, G., 2001. Toxic epiphytic dinoflagellates from east coast Tasmania, Australia. In: Hallegraeff, G., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.), Harmful Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO, pp. 54–57.
- Perini, F., Casabianca, A., Battocchi, C., Accoroni, S., Totti, C., Penna, A., 2011. New approach using the real-time PCR method for estimation of the toxic marine dinoflagellate *Ostreopsis* cf. *ovata* in marine environment. PLoS One 6 (3), e17699.
- Pezzolesi, L., Guerrini, F., Ciminiello, P., Dell'Aversano, C., Iacovo, E.D., Fattorusso, E., Forino, M., Tartaglione, L., Pistocchi, R., 2012. Influence of temperature and salinity on *Ostreopsis* cf. *ovata* growth and evaluation of toxin content through HR LC-MS and biological assays. Water Res. 46 (1), 82–92.
- Pezzolesi, L., Pistocchi, R., Fratageli, F., Dell'Aversano, C., Dello Iacovo, E., Tartaglione, L., 2014. Growth dynamics in relation to the production of the main cellular components in the toxic dinoflagellate *Ostreopsis* cf. *ovata*. Harmful Algae 36 (0), 1–10.
- Pfannkuchen, M., Godrijan, J., Maric Pfannkuchen, D., Ivesa, L., Kruzic, P., Ciminiello, P., Dell'Aversano, C., Dello Iacovo, E., Fattorusso, E., Forino, M., Tartaglione, L., Godrijan, M., 2012. Toxin-producing *Ostreopsis* cf. *ovata* are likely to bloom undetected along coastal areas. Environ. Sci. Technol. 46 (10), 5574–5582.
- Redfield, A.C., 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnstone Memorial Volume. University of Liverpool Press, Liverpool, pp. 176–192.
- Redfield, A.C., 1958. The biological control of chemical factors in the environment. Am. Sci. 46 (3), 205–221.
- Rengefors, K., Anderson, D.M., 1998. Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. J. Phycol. 34 (4), 568–577.
- Rhodes, L., 2011. World-wide occurrence of the toxic dinoflagellate genus *Ostreopsis* Schmidt. Toxicon 57 (3), 400–407.
- Rhodes, L., Adamson, J., Suzuki, T., Briggs, L., Garthwaite, I., 2000. Toxic marine epiphytic dinoflagellates, *Ostreopsis siamensis* and *Coolia monotis* (Dinophyceae), in New Zealand. N. Z. J. Mar. Freshw. Res. 34 (2), 371–383.
- Rossi, R., Castellano, V., Scalco, E., Serpe, L., Zingone, A., Soprano, V., 2010. New palytoxin-like molecules in Mediterranean *Ostreopsis* cf. *ovata* (dinoflagellates) and in *Palythoa tuberculosa* detected by liquid chromatography-electrospray ionization time-of-flight mass spectrometry. Toxicon 56 (8), 1381–1387.
- Scalco, E., Brunet, C., Marino, F., Rossi, R., Soprano, V., Zingone, A., Montresor, M., 2012. Growth and toxicity responses of Mediterranean *Ostreopsis* cf. *ovata* to seasonal irradiance and temperature conditions. Harmful Algae 17 (0), 25–34.

- Selina, M.S., Morozova, T.V., Vyshkvartsev, D.I., Orlova, T.Y., 2014. Seasonal dynamics and spatial distribution of epiphytic dinoflagellates in Peter the Great Bay (Sea of Japan) with special emphasis on *Ostreopsis* species. *Harmful Algae* 32 (0), 1–10.
- Shears, N.T., Ross, P.M., 2009. Blooms of benthic dinoflagellates of the genus *Ostreopsis*; an increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. *Harmful Algae* 8 (6), 916–925.
- Strickland, J.D.H., Parsons, T.R., 1968. A practical handbook of seawater analysis. J. Fish. Res. Board Can. 167, 310.
- Thronsdon, J., 1978. Preservation and storage. In: A. S. (Ed.), *Phytoplankton Manual*. UNESCO, Paris, pp. 69–74.
- Tichadou, L., Glaizal, M., Armengaud, A., Grossel, H., Lemee, R., Kantin, R., Lasalle, J.L., Drouet, G., Rambaud, L., Malfait, P., de Haro, L., 2010. Health impact of unicellular algae of the *Ostreopsis* genus blooms in the Mediterranean Sea: experience of the French Mediterranean coast surveillance network from 2006 to 2009. *Clin. Toxicol.* 48 (8), 839–844.
- Totti, C., Accoroni, S., Cerino, F., Cucchiari, E., Romagnoli, T., 2010. *Ostreopsis ovata* bloom along the Conero Riviera (northern Adriatic Sea): relationships with environmental conditions and substrata. *Harmful Algae* 9 (2), 233–239.
- Turki, S., 2005. Distribution of toxic dinoflagellates along the leaves of seagrass *Posidonia oceanica* and *Cymodocea nodosa* from the Gulf of Tunis. *Cah. Biol. Mar.* 46 (1), 29–34.
- Uchida, H., Taira, Y., Yasumoto, T., 2013. Structural elucidation of palytoxin analogs produced by the dinoflagellate *Ostreopsis ovata* IK2 strain by complementary use of positive and negative ion liquid chromatography/quadrupole time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 27 (17), 1999–2008.
- Vanucci, S., Pezzolesi, L., Pistocchi, R., Ciminiello, P., Dell'Aversano, C., Iacovo, E.D., Fattorusso, E., Tartaglione, L., Guerrini, F., 2012. Nitrogen and phosphorus limitation effects on cell growth, biovolume, and toxin production in *Ostreopsis cf. ovata*. *Harmful Algae* 15 (0), 78–90.
- Vidyarathna, N.K., Granéli, E., 2013. Physiological responses of *Ostreopsis ovata* to changes in N and P availability and temperature increase. *Harmful Algae* 21–22, 54–63.
- Vila, M., Garcés, E., Masó, M., 2001. Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean. *Aquat. Microb. Ecol.* 26 (1), 51–60.
- Zhang, Q., Hu, G., 2011. Effect of nitrogen to phosphorus ratios on cell proliferation in marine micro algae. *Chin. J. Oceanol. Limnol.* 29, 739–745.

832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850