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Comparative growth of three strains of *Ostreopsis ovata* at different light intensities with focus on inter-specific allelopathic interactions

Marina MONTI[*] *& Eleonora CECCHIN*

Istituto Nazionale di Oceanografia e Geofisica Sperimentale, OGS, Department of Biological Oceanography, Via A. Piccard 54, I-34151, Trieste, Italy

Abstract – Three strains of *Ostreopsis ovata* were isolated from sea water and algae collected either along the Tyrrhenian or the Adriatic Sea. Evaluation of growth profile of the three *O. ovata* strains in batch cultures were analyzed at four light intensities (10, 100, 400, 650 µmol m^{-2} s^{-1}). *Ostreopsis ovata* cell densities increased at all light intensities except at 10 µmol m^{-2} s^{-1}. The three strains showed growth rates with values from 0.24 to 0.56 day^{-1}. The allelopathic effect of the filtrate of *O. ovata* culture on growth of *Coscinodiscus granii*, *Prorocentrum minimum* and *Coolia monotis* was studied. Filtrate from culture in exponential and senescent growth phase was considered. The results revealed a weak allelopathic effect on the growth of the three microalgae with an inhibition on the growth of *C. monotis* and *P. minimum*.

Allelopathy / light intensity / culture / microalgae

INTRODUCTION

Ostreopsis ovata is an epiphytic toxic dinoflagellate (Ciminiello *et al.*, 2006; 2008; Guerrini, 2010). It has a world-wide distribution, normally associated with other epiphytic or benthic dinoflagellates. In tropical seas, *O. ovata* is often associated with the genera *Gambierdiscus*, *Coolia* and *Prorocentrum* and therefore was suspected to be involved in ciguatera fish poisoning (Bomber & Aikman, 1989; Tindall & Morton, 1998). *Ostreopsis ovata*, along the Italian coastlines, has been documented since the end of the '90 (Sansoni *et al.*, 2003; Totti *et al.*, 2007; Mangialajo *et al.*, 2008). In the last years, *O. ovata* blooms, in the Tyrrhenian and southern Adriatic Sea, have been related to human health problems, such as breathing and skin irritation (Sansoni *et al.*, 2003; Ciminiello *et al.*, 2006).

The process that select a particular algal species to reach a bloom are influenced by physical factors, such as temperature, salinity, light and by the amount of inorganic nutrients available (Granéli & Flynn, 2006). In aquatic environment the light penetrating the water column is highly variable in both irradiance and spectral quality (Kirk, 1994). White light of different irradiances can induce changes in algal growth and respiration (Brown & Richardson, 1968). Dinoflagellates are capable of adapting to very low irradiances but extreme values of the light intensity can influence their growth rate (Prezelin, 1981).

* Corresponding author: mmonti@ogs.trieste.it

Allelopathy refers to any process involving secondary metabolites (allelochemicals) released by the microalgae that affect competing organisms. Allelochemical products are very important because they can influence algal growth, succession events, competitive strategy and algal blooms (Wolfe, 2000; Fistarol *et al.*, 2004). Many dinoflagellates produce allelochemicals in order to compete with other co-occurring algae under unfavorable environmental conditions for growth (Granéli & Hansen, 2006). *Ostreopsis* species are able to produce and release in the sea water hemolytic compounds (Vila *et al.*, 2001; Lenoir *et al.*, 2004; Granéli *et al.*, 2011), however, no laboratory studies have yet been conducted on allelopathic effects on monocultures of microalgae.

In this study three microalgae, *Coolia monotis* Meunier, *Prorocentrum minimum* (Pavillard) Schiller and *Coscinodiscus granii* Gough, isolated from the Gulf of Trieste, were considered as target species. These species were selected because of their different characteristics. *C. monotis* and *Coscinodiscus* sp. are respectively a dinoflagellate and a diatom living on macroalgae in assemblages with *O. ovata* (Aligizaki & Nikolaidis, 2006; Monti *et al.*, 2007; Vila *et al.*, 2001), while *P. minimum* is a potentially toxic dinoflagellate (Grzebyk *et al.*, 1997) present regularly in the Adriatic Sea (Virgilio, 2008).

The aim of this study was to analyse if different parameters, such as light and competition with other microalgae, can influence the success of *O. ovata*. The primary objectives were: (1) to investigate on the light intensity ranges able to influence the growth of three algal strains of *O. ovata*; (2) to provide a preliminary investigation on the possible *O. ovata* allelopathic activity on three microalgae.

MATERIALS AND METHODS

Growth curves

Three strains of *O. ovata* were isolated from sea water and macroalgae collected either along the Tyrrhenian (Campania region: D483 strain, isolated in September 2008) or the Adriatic (Marche region: CBA-T strain isolated in 2008 and Friuli Venezia Giulia region: OS2T strain, isolated in September 2006). The cells were cultured at 25°C under 15:9 h L:D cycle, intensity light of 50 µmol m^{-2} s^{-1} cool white lamp, at salinity 36 in K/2 medium. The cultures were not axenic.

Evaluation of growth profile of the three *O. ovata* strains in batch cultures were analyzed at four light intensities (10, 100, 400, 650 µmol m^{-2} s^{-1}). Experiments were conducted in triplicates using 100 ml flasks. The initial cell inoculum was of about 200 cell ml^{-1}. Every second-third day 1 ml subsamples were analyzed at a Leitz Labovert inverted microscope (200×). Evaluation of growth profile was complicated by the presence of mucous aggregates in which *O. ovata* cells were included. In order to overcome this problem the samples were treated with Na2EDTA (final concentration 0.01 M) and shaken for 30 sec.

At each light intensity, the specific growth rate was calculated using the formula: $K = (\ln C_2 - \ln C_1)/(t_2 - t_1)$ with C_1 the algal concentration at time t_1 and C_2 at time t_2.

Differences in growth rates (3 strains considered together) at different light intensities were established using a 1-way ANOVA.

Allelopathy

The allelopathic effect of the filtrate from *O. ovata* OS2T culture on growth of the diatom *C. granii*, and the dinoflagellates *P. minimum* and *C. monotis* was studied.

Filtrate from *O. ovata* culture in exponential (EXP) and senescent (SEN) growth phase was considered. The filtrate from the exponential phase was collected at day 3, the one from the senescent phase at day 13.

Ostreopsis ovata culture, maintained at the standard conditions, was previously passed through 10 µm net and then by gentle filtration (a pressure lower than – 1.5kPa) through 0.22 µm Millipore filter. Freshly prepared filtrates (25 ml) were added to culture flasks (100 ml) containing 25 ml of the target species maintained at their original culture conditions (15°C, 50 µmol $m^{-2}s^{-1}$). *Ostreopsis* filtrates and the target species cells were combined to give the final concentration corresponding at different ratio (Tab. 1). The different *O. ovata*: target species final ratios were due to the different *O. ovata* concentration at the two growth phases as the target species were always considered at their stationary growth phase.

Every experiment was conducted in triplicate and a control was considered. The controls were made by adding 25 ml of target species filtrate (0.22 µm Millipore filter) to 25 ml of target species culture to reach the same amount as in the experiment flasks.

The allelopathic effect on the target cells was measured by comparing the cell numbers in the filtrate treatments with the controls after 6, 24 and 48 h by directly counting the cells on a Leitz Labovert inverted microscope.

The difference between the cell numbers in the controls (Ctn) and in the filtrate treatments (Ftn) for the same sampling occasion, normalized by the cell numbers in the control, and expressed as percentage is called allelopathic effect (AE) and was calculated following the formula: $AE= [(Ctn-Ftn)/Ctn] \times 100$, with cell concentration in the control = Ctn and in the filtrate = Ftn. AE represents the percentage of decrease or increase of cells in the filtrate relative to the control.

Table 1. Different ratio *Ostreopsis ovata*: target species

Exponential growth phase	
Ostreopsis ovata: *Coscinodiscus granii*	1:4
Ostreopsis ovata: *Prorocentrum minimum*	1:20
Ostreopsis ovata: *Coolia monotis*	1:2
Senescent growth phase	
Ostreopsis ovata: *Coscinodiscus granii*	1:1
Ostreopsis ovata: *Prorocentrum minimum*	1:50
Ostreopsis ovata: *Coolia monotis*	1:10

RESULTS

Growth curves

Ostreopsis ovata cell densities increased at all light intensities except at 10 µmol m^{-2} s^{-1}. Maximum cell densities were obtained for all strains on days 5-10 followed by a rapid decrease. No strains showed the stationary growth phase but they presented a sharp decline immediately after the maximum value.

Table 2. Growth rate k (day^{-1}) of three *O. ovata* strains at different light intensities

Light $\mu mol\ m^{-2}\ s^{-1}$	*OS2T* $k\ (day^{-1})$	*CBA-T* $k\ (day^{-1})$	*D483* $k\ (day^{-1})$
10	–	–	–
100	0.56	0.36	0.41
400	0.42	0.33	0.29
650	0.49	0.24	0.28

The strain D483 showed the highest cell densities with $16.25 \pm 4 \times 10^3$ (SD) cell ml^{-1} at 100 µmol $m^{-2}\ s^{-1}$. CBAT reached the highest values at 400 µmol $m^{-2}\ s^{-1}$ with $7.1 \pm 0.3 \times 10^3$ (SD) cell ml^{-1} and OST2T at 650 µmol $m^{-2}\ s^{-1}$ with $51.0 \pm 0.7 \times 10^3$ (SD) cell ml^{-1}.

The growth rate (K) varied from 0.24 to 0.56 day^{-1} (Tab. 2). K values at the different light intensities showed no significant differences (ANOVA, $F = 0.94$; $p > 0.4$) and at 10 µmol $m^{-2}s^{-1}$ no growth was present in all the strains.

Allelopathy

The results revealed a weak allelopathic effect on the growth of the three microalgae. Only the growth of *C. monotis* (ratio 1:2) and *P. minimum* (ratio 1:20) seems inhibited by the filtrate of the exponential *O. ovata* growth (Fig. 1). AE varied from about 10% for *P. minimum* to more than 20% for *C. monotis*.

The filtrate from senescent *O. ovata* growth did not show any negative effect on the target species.

The strain used in the experiment, independent of the growth phase considered, had no negative allelopathic effect on *C. granii*, even if the senescent filtrate seemed to have a weak positive effect on the microalga.

Analyses at the microscope had not shown any cells of the target organisms lyzed or damaged after the exposure to *O. ovata* exponential and senescent filtrate.

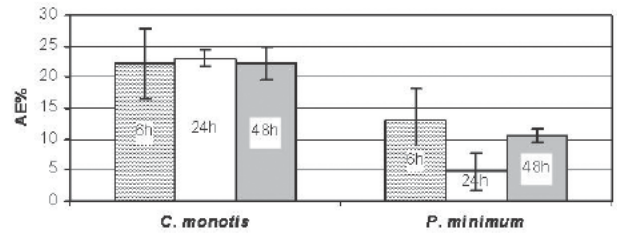

Fig. 1. Allelopathic effect of *Ostreopsis ovata* strains OS2T on *Coolia monotis* and *Prorocentrum minimum* after 6, 24 and 48 h of exposure to cell-free filtrate in exponential growth phase (n = 3, mean ± SD).

DISCUSSION AND CONCLUSIONS

The success of a microalga depends on physicochemical (light, temperature, salinity, nutrients) and biological (competition, grazers) factors (Smayda 1980). However some studies (Keating, 1977; Vardi *et al.*, 2002) clearly demonstrated the influence of compounds produced by phytoplankton organisms on succession events, and allelopathy is becoming more commonly used to explain phytoplankton population dynamics (Vardi *et al.*, 2002).

Ostreopsis ovata generally blooms during summer, in shallow and sheltered waters (Vila *et al.*, 2001). In the northern Adriatic, Totti *et al.* (2010) suggested a relation between the decrease of *O. ovata* abundance and light intensity.

Guerrini *et al.* (2010) conducted a study on two *O. ovata* strains collected along the Adriatic and Tyrrhenian coasts and grown in culture. During the exponential phase growth rates were 0.37 and 0.32 day^{-1} respectively. In the stationary phase the Adriatic strain reached the maximum density of about 10.000 cells ml^{-1} and the Tyrrhenian one 8.000 cells ml^{-1}. The maxima were reached after 13-14 days at 90 µmol $m^{-2}s^{-1}$. Recently Nascimento *et al.* (2012) showed lower growth rates (0.15 and 0.10 day^{-1}) in two strains isolated along the Brazilian coast. The strains were maintained at 24°C, 12 h:12 h light/dark cycle and irradiance of 60 µmol $m^{-2}s^{-1}$.

In comparison to these previous researches, in our study the growth rates reached higher values and the maxima were reached at 5-10 days. The three *O. ovata* strains utilized in our study did not show any growth at 10 µmol $m^{-2}s^{-1}$. This result indicates a low capability to grow in shadow areas. Otherwise the higher light intensities tested did not show a clear preference and they all produced a rapid growth, followed by a sharp decrease, passing directly from the exponential to the senescent phase without any stationary growth phase.

Normally the allelopathic effect is caused by cells that are growing exponentially, the effects decrease in the stationary phase, and the senescent cells do not have allelopathic properties (Schmidt & Hansen, 2001; Suikkanen *et al.*, 2004). Several authors have observed that *Ostreopsis* species are able to produce and release hemolytic compounds and polysaccharides in the sea water (Vila *et al.*, 2001; Lenoir *et al.*, 2004). Furthermore, *O. ovata* produces palytoxin-like compounds (Lenoir *et al.*, 2004; Penna *et al.*, 2005; Ciminiello *et al.*, 2008; Guerrini *et al.*, 2010) and the highest production of toxin is observed at the end of the stationary phase (Guerrini *et al.*, 2010).

In this study *O. ovata* showed only a weak allelopathic activity, contrary to many studies that showed how toxic dinoflagellates produce allelochemical in order to compete with other co-occurring algae under unfavorable environmental conditions for growth (Tillmann & John, 2002; Fistarol *et al.*, 2003; 2004; Granéli & Johansson, 2003; Suikkanen *et al.*, 2004; Granéli & Hansen, 2006).

Since allelopathy is mediated by chemical release into the medium, its effect depends also on the cell concentration of the allelopathic organism (Tillmann & John, 2002). We are aware that the differences in *O. ovata* cell densities, present in this study for the exponential and senescent growth phase filtrates, could have determined the absence of allelopathic effect in the senescent *Ostreopsis* filtrate experiments, but the use of constant abundance of the target species was privileged. This choice was dictated by the need to use target species abundance similar to those present in the Gulf of Trieste (Cabrini *et al.*, 2010), to avoid unrealistic scenario, and to maintain them at their stationary growth phase,

to avoid further physiological variables. Furthermore our result is supported from other researches where authors underlined that the intensity of the allelopathic effect depends on the growth phase of the species tested (Schmidt & Hansen, 2001), demonstrating that the allelopathic effect is caused by cells that are growing exponentially (Suikkanen *et al.*, 2004).

Our results suggest that *O. ovata* can express allelopathic, although weak, effects on other dinoflagellates, often co-occurring in the natural environment, but not influence negatively the diatom selected for the experiments.

Based on the results of these preliminary experiments it can be suggested that both light intensity and allelopathy may play a role in the success of *O. ovata* controlling the geographical expansion and biomass increase.

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