Harmful Algae 49 (2015) 147–155

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Allelopathic interactions between the HAB dinoflagellate *Ostreopsis* cf. *ovata* and macroalgae

Stefano Accoroni [a,*], Isabella Percopo [a,1], Federica Cerino [a,2], Tiziana Romagnoli [a], Salvatore Pichierri [a], Cesira Perrone [b,3], Cecilia Totti [a]

[a] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, Ancona 60131, Italy
[b] Dipartimento di Biologia, Università di Bari, via Orabona, 4, Bari 70124, Italy

ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 31 August 2015
Accepted 31 August 2015
Available online 8 October 2015

Keywords:
Ostreopsis
Macroalgae
Allelopathy
Harmful algae
Growth inhibition
Cysts

ABSTRACT

Intense blooms of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata* have been a recurrent phenomenon along several Mediterranean coasts during summer in the last few years. These blooms are often associated with noxious effects on humans and deaths of benthic invertebrates. Previous studies carried out on the Conero Riviera (northern Adriatic Sea) highlighted that *Ostreopsis* abundances recorded on rocks were significantly higher than on the surface of seaweeds, suggesting that some allelopathic interactions might occur between *Ostreopsis* and macroalgal substrates. In this study we investigated under experimental conditions the interactions between *O.* cf. *ovata* and three of the most common macroalgae in this area: *Dictyota dichotoma* (brown alga), *Rhodymenia pseudopalmata* (red alga) and *Ulva rigida* (green alga). Three different experiments were set up: *O.* cf. *ovata* was grown (i) together with fresh macroalgal tissues, (ii) in media in which macroalgae were previously cultured, and (iii) in media with the addition of dry macroalgal powder at different concentrations. The results indicated that all the investigated seaweeds exerted negative effects toward the benthic dinoflagellate *O.* cf. *ovata*. *D. dichotoma* inhibited the growth of *O.* cf. *ovata* in all tested experimental conditions; *U. rigida* had inhibitory effect both in form of fresh thalli and dry powder but not as growth medium filtrate, suggesting that either *Ulva* does not release any allelopathic compound in the medium in absence of *O.* cf. *ovata* or the alleged released allelochemicals are rapidly degradable. Neither the fresh thalli of *R. pseudopalmata* or the filtrate of its culture medium showed any inhibitory effects, while a negative effect was only observed at high concentrations of dry thallus powder. With the exception of *D. dichotoma* co-culture experiment, a complete algicidal effect was never observed partly because *O.* cf. *ovata* produced a large amount of resting stages, which permitted its survival.

1. Introduction

In the last decade, blooms of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata* Fukuyo have been a recurrent phenomenon along several Mediterranean coastal areas during summer (Vila et al., 2001; Turki, 2005; Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2008, 2011; Totti et al., 2010; Amzil et al., 2012; Illoul et al., 2012; Ismael and Halim, 2012; Pfannkuchen et al., 2012; Casabianca et al., 2014). In these areas, *O.* cf. *ovata* is well-known for its toxin production, including both palytoxin-like compounds (isobaric palytoxin and ovatoxin-a, b, c, d, e, f and g) and mascarenotoxin-a and c (Rossi et al., 2010; Ciminiello et al., 2011, 2012; Scalco et al., 2012; Uchida et al., 2013; García-Altares et al., 2015) that cause both mortality of benthic marine organisms (Shears and Ross, 2009; Accoroni et al., 2011; Gorbi et al., 2012, 2013; Pagliara and Caroppo, 2012) and noxious effects on human health (Gallitelli et al., 2005; Kermarec et al., 2008; Tichadou et al., 2010; Del Favero et al., 2012).

Several studies have been conducted to assess the role of abiotic factors (mainly hydrodynamics, water temperature and nutrients) on the bloom dynamics (Chang et al., 2000; Vila et al., 2001; Monti et al., 2007; Shears and Ross, 2009; Totti et al., 2010; Accoroni et al.,

* Corresponding author. Tel.: +39 071 2204919; fax: +39 071 2204650.
E-mail address: s.accoroni@univpm.it (S. Accoroni).
[1] Present address: Servizio di Tassonomia e Identificazione del Fitoplancton Marino, Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli 80121, Italy.
[2] Present address: Oceanography Section, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS), via A. Piccard 54, Trieste 34151, Italy.
[3] Now retired.

http://dx.doi.org/10.1016/j.hal.2015.08.007
1568-9883/

2012a,b, 2015; Mabrouk et al., 2011; Mangialajo et al., 2011; Selina et al., 2014), while biotic factors such as allelopathic interactions between *Ostreopsis* and other organisms have only been partially addressed. The allelopathic interactions have been well documented among toxic dinoflagellates and co-occurring microalgae under unfavorable environmental conditions (Fistarol et al., 2003, 2004; Granéli and Johansson, 2003; Granéli and Hansen, 2006; Prince et al., 2008), and Monti and Cecchin (2012) showed that *O.* cf. *ovata* had a weak allelopathic activity toward other benthic dinoflagellates as well. However, to the best of our knowledge, allelopathic interactions between *Ostreopsis* and macroalgae have never been considered. *O.* cf. *ovata* commonly grows over all benthic substrata (rocks, pebbles, seaweed thalli, mollusc shells, etc.), and during periods of intense proliferation produces a conspicuous brownish mat which is only loosely attached to the substrata. Previous studies on the Conero Riviera, where *O.* cf. *ovata* blooms reach abundances among the highest of the entire Mediterranean coasts (Mangialajo et al., 2011), highlighted that *Ostreopsis* abundances on rocks were significantly higher than those recorded on seaweeds suggesting that some allelopathic interactions might occur between *Ostreopsis* and its macroalgal hosts (Totti et al., 2010).

Algal-bloom control is an important issue for the protection of the water environment due to the negative impacts on human economy and health, especially when the involved bloom-forming species are toxic. Moreover, the development of environment-friendly and cost-effective strategies for controlling algal blooms, such as using the allelopathy of aquatic macrophytes has gained great interest and has been suggested by several authors (Jeong et al., 2000; Nan et al., 2004; Jin et al., 2005; Wang et al., 2007a,b; Hu and Hong, 2008; Tang and Gobler, 2011). The interactions between microalgae and macroalgae have been investigated between phytoplankton species that form blooms and a number of both freshwater (Gross, 2003; Hu and Hong, 2008) and seawater macrophytes (Gross, 2003; Jin and Dong, 2003; Nan et al., 2004, 2008; Jin et al., 2005; Wang et al., 2007a,b; Ye and Zhang, 2013) but no information is available about macroalgae and benthic dinoflagellate interactions.

In this study, we investigated the interactions between *Ostreopsis* cf. *ovata* and three macroalgal species under laboratory conditions. The macroalgae were chosen among the most common species on the Conero Riviera during the bloom period of *Ostreopsis*: *Dictyota dichotoma* (Hudson) J.V. Lamouroux (brown alga), *Rhodymenia pseudopalmata* (J.V. Lamouroux) P.C. Silva (red alga) and *Ulva rigida* C. Agardh (green alga). Three different experiments were carried out: *O.* cf. *ovata* was grown (i) together with fresh macroalgal tissues, (ii) in filtered culture media in which macroalgae were previously grown, and (iii) in media with addition of dry macroalgal powder at different concentrations.

2. Materials and methods

2.1. *Ostreopsis* cf. *ovata* cultures

A strain of *Ostreopsis* cf. *ovata* was isolated by the capillary pipette method (Hoshaw and Rosowski, 1973) from seawater samples collected from the bloom that occurred on the Conero Riviera (N Adriatic Sea) in summer 2007 (strain OoAPn0807/E). After initial growth in microplates, cells were cultured at 21 ± 0.1 °C under a 12:12 h L:D cycle and an irradiance of 90–100 μmol m^{-2} s^{-1}, in modified f/4 medium prepared by adding macronutrients at a f/4 medium concentration without silica (Guillard and Ryther, 1962) and selenium to filtered and autoclaved natural seawater (salinity 35). Trace metals, iron, vitamins (H, B1 and B12) and HEPES pH 7.1 were added at levels corresponding to f/2 medium. The same physico-chemical conditions were used in all experiments described below, with the addition of germanium dioxide (6 mg l^{-1}), when specified, to prevent the growth of diatoms.

Microalgae were cultured to the exponential phase before inoculation in the following experiments. All the experiments were carried out in three replicates.

2.2. Macroalgae sampling and pre-treatment

Thalli of three macroalgal species, i.e., *Dictyota dichotoma*, *Rhodymenia pseudopalmata* and *Ulva rigida*, were collected on the Conero Riviera in summer 2009 and treated to remove the epiphytes from their surface as follows: they were washed carefully in filtered sea water (FSW) containing 1% of surfactant, rinsed in FSW and then observed at a stereo-microscope in order to mechanically remove the epiphytes with scalpels and tweezers; afterwards, a 3-min dip in tap water followed by a washing with chloramphenicol (50 ppm in FSW) led to the complete removal of residual epiphytic cells and bacteria respectively. After rinsing in FSW, macroalgal thalli were cut into fragments of approximately 9 cm^2 and acclimated for one week in FSW (containing 6 mg l^{-1} germanium dioxide).

2.3. Co-cultures of Ostreopsis cf. ovata and fresh macroalgal thalli

The co-cultures were set up with 1 g of thallus segments of each tested seaweed in 500 ml of medium containing germanium dioxide. For each species, 3 flasks were inoculated with 100 cells ml^{-1} of *O.* cf. *ovata*, and 3 flasks containing 100 cells ml^{-1} of *Ostreopsis* cf. *ovata* without macroalgal thalli were used as control. The flasks were incubated for 20 days in a culture chamber at the conditions previously described. Every 2 days, 3 aliquots (2 ml) were taken from each flask (after gentle shaking of each thallus with a pair of tweezers and a homogenization of the medium) and preserved with Lugol's solution in the dark to assess the cell densities, and 1 aliquot (4 ml) was filtered (GF/F Whatman, diameter 25 mm, nominal pore size 0.7 μm) and stored in polyethylene bottles at −22 °C for nutrient analysis. Every 7 days, pH was checked and adjusted to maintain it to value of 8 until the end of the experiment.

2.4. Cultures of Ostreopsis cf. ovata in macroalgal culture medium filtrate

For each macroalgal species, 24 g of thallus segments pre-treated as above were maintained in 1 l FSW for one week. Afterwards, macroalgal thalli were removed and the culture medium was filtered (0.22 μm pore size) and used to prepare the medium for the experiment: the pH was adjusted to 8 and the nutrients were added to obtain a modified f/4 medium as described previously. Culture flasks containing 250 ml of medium were inoculated with *Ostreopsis* cf. *ovata* cells to obtain a final concentration of 500 cells ml^{-1}. As a control, 500 cells ml^{-1} of *O.* cf. *ovata* were inoculated in fresh modified f/4 medium. Subsamples (2 ml) were sampled every 2–3 days for 23 days and fixed with Lugol's solution.

2.5. Cultures with dry powder of macroalgae

Fresh thalli of each macroalga were dried at room temperature and pulverized using a mortar and a pestle. Different amounts of dry powder (0.4, 0.8, 1.6 g l^{-1}) were added to flasks containing *Ostreopsis* cultures (500 cells ml^{-1}) in 300 ml modified f/4 medium. Microalgal cultures without addition of dry macroalgal powder were used as controls. Cultures were maintained for 18 days during which subsamples (2 ml) were taken every 2 days and fixed with Lugol's solution.