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Toxic effects of *Ostreopsis ovata* on larvae and juveniles of *Paracentrotus lividus*

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ABSTRACT

Recently, in the Mediterranean Sea and in other temperate regions, mass mortalities of marine organisms and human diseases have been caused by blooms of the dinoflagellate genus *Ostreopsis*, palytoxin (PLTX) producing species. Such blooms are cause of health, economical and environmental concern, particularly when affecting edible species of commercial relevance that, additionally, play key ecological roles, with cascading effects through the whole ecosystem.

The present research aims at the evaluation of the effects produced by *Ostreopsis ovata* blooms on the early stages of *Paracentrotus lividus* (Lamarck, 1816). This sea urchin species is considered one of the key controllers of the structure and dynamics of Mediterranean algal communities. Further, *P. lividus* is an edible species and its gonads (roe) are considered a culinary delicacy: in many areas the harvesting of this species has lead to overexploitation.

Through three eco-toxicological assays we tested mortality effects produced by four *O. ovata* concentrations (4, 40, 400 and 4000 cell ml^{-1}) on *P. lividus* competent larvae, using *O. ovata* cultured at 20 °C and 25 °C, and on juveniles, using *O. ovata* cultured at 20 °C. Both *O. ovata* culture and growth medium devoid of algal cells were tested.

Juveniles turned out to be more sensitive than larvae to *O. ovata* presence, the latter showing significant mortality only at extremely high *O. ovata* concentrations. Furthermore, temperature at which the algal cultures were grown played an additional role.

The results of the present research are particularly relevant given the commercial and ecological relevance of *P. lividus*.

1. Introduction

In the last 15 years, blooms of the toxic dinoflagellates *Ostreopsis* spp. were observed in temperate and tropical coastal waters in both the northern and southern hemispheres (Rhodes, 2011). In particular, in the Mediterranean region, blooms of *Ostreopsis ovata* (Penna et al., 2005) have been observed with increasing frequency, intensity and distribution (Tognetto et al., 1995; Vila et al., 2001; Monti et al., 2007; Totti et al., 2007; Mangialajo et al., 2008, 2011). Mass mortalities of benthic organisms (Sansoni et al., 2003; Vila et al., 2008; Shears and Ross, 2009, 2010; Totti et al., 2010; Simonini et al., 2011) and human health problems (Brescianini et al., 2006; Ciminiello et al., 2006; Barroso Garcia et al., 2008; Mangialajo et al., 2008; Vila et al., 2008) have been reported during *O. ovata* blooms and were attributed to palytoxin-like compounds (such as ovatoxins) (Ciminiello et al., 2006, 2008, 2010; Guerrini et al., 2010; Rossi et al., 2010; Ciminiello et al., 2011).

PLTX is a toxic marine compound, considered one of the most toxic natural molecules (*e.g.* Steidinger, 1983; Steidinger and Baden, 1984; Faimali et al., 2011). Its main biological target is the Na^+/K^+-ATPase, a plasmatic membrane pump involved in the maintenance of trans-membrane ionic gradients of animal cells, essential for cellular function (Habermann, 1989; Wu, 2009; Rossini and Bigiani, 2011; Tubaro et al., 2011).

Human intoxications reported during the most intense *O. ovata* blooms along Italian and Spanish coasts have been caused by direct contact (dermatitis) and by inhalation of seawater droplets containing *Ostreopsis* cells and/or aerosolized toxins (Mangialajo et al., 2011). In fact, although the genus *Ostreopsis* is usually described as epiphytic on macroalgae and seagrasses, or attached to coral rubble or on sand (Faust et al., 1996; Totti

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et al., 2010), during its blooms large numbers of cells of this dinoflagellate are present in the water column and are likely to affect humans by inhalation through marine aerosol (Gallitelli et al., 2005; Durando et al., 2007).

From an ecological point of view, beyond the acute response to HABs represented by mass mortalities of sensitive organisms, the threaten of natural marine toxins is represented by bioaccumulation along the food chains (Ramos and Vasconcelos, 2010). Such consequences are particularly significant when affecting single ecologically relevant species, with cascading effects through the whole ecosystem (Faimali et al., 2011).

It is well known that sea urchins play a key role in controlling the dynamic, structure and composition of shallow macroalgal assemblages through their grazing activity (Paine and Vadas, 1969; Andrew, 1993; Sala et al., 1998; Hereu et al., 2004). In the Mediterranean, one of the most abundant and pivotal sea urchins is *Paracentrotus lividus*. Whenever present at very high densities, most probably because of the lack of top-down control (*e.g.* overfishing of its natural predators; Sala and Zabala, 1996; Scheibling, 1996; Guidetti, 2004), this species may cause the total depletion of algal assemblages, causing the formation of barren areas that may have detrimental effects on the whole food chain (*e.g.* Sala et al., 1998; Gianguzza et al., 2006; Privitera et al., 2008). Similarly, even at lower densities, its grazing activity can maintain barren areas, whenever the algal cover has been removed by human activities (*e.g.* date mussel harvesting; Guidetti et al., 2003; Privitera et al., 2008; Parravicini et al., 2010).

In addition, this species is largely harvested in many Mediterranean regions (Gianguzza et al., 2006; Pais et al., 2007) where its gonads (roe) are considered a culinary delicacy thus causing, in recent years, a large stock reduction due to overfishing (Pais et al., 2011). Because of this, an increasing interest for *P. lividus* aquaculture in many European regions has emerged both for re-population and direct commercialization (*e.g.* Chiantore et al., 2010; Carboni et al., 2011).

The ecological role of this species is tightly connected to another very common sea urchin, *Arbacia lixula*, and much debate is reported in literature about their interactions and the relative role of the two species in barren areas formation and maintenance (Benedetti-Cecchi et al., 1998; Sala et al., 1998; Bulleri et al., 1999; Micheli et al., 2005; Gianguzza et al., 2006; Guidetti and Dulčić, 2007; Privitera et al., 2008). Additionally, a possible feedback mechanism between barren substrate availability and settlement of urchin juveniles has been recently reported (Privitera et al., 2011).

The latter process focuses attention on the larval/juvenile stages, since for marine benthic invertebrates with a planktonic larva, such as *P. lividus*, recruitment to the adult population is assured by larval dispersal, settlement and juvenile survival (Cameron and Schroeter, 1980; Harrold et al., 1991).

All the above makes *P. lividus* a key species in ecosystem functioning of shallow temperate reefs. This species has already been shown to be sensitive to *Ostreopsis* blooms: mortality events have been reported (Sansoni et al., 2003; Ciminiello et al., 2006), as well as for other temperate reef sea urchins (Shears and Ross, 2010).

The aim of the present research was to evaluate *O. ovata* direct toxicity on larval and juvenile stages of *P. lividus*. The knowledge of the effects produced on these crucial stages of *P. lividus* life cycle, will highlight the potential influence of *O. ovata* on the population dynamics of this species and, consequently, on the whole shallow temperate reef ecosystem functioning. Further, an understanding of the effects produced in particular on juveniles may represent a useful tool for enterprises and municipalities that are involved in *P. lividus* aquaculture and re-population strategies.

2. Materials and methods

2.1. O. ovata cultures

Laboratory cultures of *O. ovata* were obtained from environmental samples collected the previous summer (2010) in Quarto dei Mille (Genoa, NW Mediterranean Sea, Italy). Cells isolation was performed at the laboratory of University of Urbino from the team of Dr. Antonella Penna. Algae from this strain were cultured into several 200 ml sterilized plastic flasks closed with transpiring caps. Each flask, at the start, was filled with 20 ml of *O. ovata* culture from the master, added with 20 ml of filtered (GF/F 0.22 μm) and autoclaved sterilized marine water and Guillard growth medium F/4 (1 ml l^{-1}). All flasks were maintained at 20 °C and 25 ± 0.5 °C in a 16:8 h light:dark (L:D) cycle (light intensity 85–135 μE m^{-2} s^{-1}) inside a thermostatic culture chamber. Every two-three days culture volumes were doubled with the addition, under vertical laminar flow hood, of growth medium till 200 ml volume and checked under microscope. Cell counts were performed in three 1 ml replicates/flask. Before being used for the toxicological tests, algal cultures were diluted in order to obtain the following concentrations of *O. ovata* cells: 4000–400–40–4 cell ml^{-1} (as in Faimali et al., 2011). Such concentrations have been chosen in order to encompass the regular and maximum order of magnitude of cell concentrations recorded during bloom events along Ligurian coasts from summer 2006 to summer 2011 (Mangialajo et al., 2008, 2011).

2.2. P. lividus larval and juvenile production

Wild adult specimens of *P. lividus* (test diameter >3 cm) have been collected in Pontetto, a coastal rocky site in the neighborhood of Genoa city (Ligurian Sea, NW Mediterranean) where high concentration of *O. ovata* are usually recorded during summer. Specimens have been maintained in a 2000 l aquarium (in the IBF-CNR laboratory located in Camogli) in a flow through system. Specimens were fed *ad libitum* every three days with the brown algae *Dictyota dichotoma* and *Stypocaulon scoparium*, collected from the wild for 30 days before spawning induction.

Gamete release has been induced through intracoelomic injection of 0.5 ml acetylcholine chloride (5×10^{-1} M) (Kurihara et al., 2004). Eggs from four female specimens were collected into 100 ml Falcon tubes, counted under stereoscope and introduced into three 1 l autoclaved beakers at a concentration of 40 eggs ml^{-1}. Dry sperm from four male specimens was collected using a Pasteur pipette and placed into a 2 ml Eppendorf tube. Afterwards, 20 μl of dry sperm was added to the beakers and gently mixed with the eggs. After 15 min, eggs were checked for fertilization. Fertilized eggs were maintained in the dark for two days, then the three 1 l beakers were mixed in a 5 l one and two and four armed plutei were randomly split into three 15 l bottles at a concentration of 2 larvae ml^{-1}. From day two after fertilization, larvae were fed every 48 or 72 h with *Cricosphaera elongata*. This microalgal species (a planktonic dinoflagellate, diameter around 10 μm), strain gently provided by Viking Fish Farm (Ardtoe, Scotland), is considered one of the best food items for *P. lividus* larvae (Carboni et al., 2011). Not axenic *C. elongata* cultures were grown in a Guillard F/2 medium at 18 °C. Larvae were fed throughout development until the competent stage (rudiment larger than the stomach, total body length around 1 mm) was reached, with increasing number of algal cells according to the larval stage and density, following the protocol from Falugi and Angelini (2002): 500 *C. elongata* cells/larva for 2- and 4-armed plutei, 1250 *C. elongata* cells/larva for 6-armed plutei, 2500 *C. elongata* cells/larva for 8-armed plutei and following stages. *C. elongata* density in the culture was measured by cell counts in erythro-cytometre under microscope. Each time larvae were fed,