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REVIEW

The toxic benthic dinoflagellates of the genus *Ostreopsis* in temperate areas: a review

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ABSTRACT

The genus *Ostreopsis* includes species largely distributed from tropical to temperate marine areas worldwide. Among the nine species of the genus, *O. siamensis*, *O. mascarenensis*, *O. lenticularis* and *O.* cf. *ovata* can produce toxins of the palytoxin group. In the last decade *Ostreopsis* cf. *ovata* and *O.* cf. *siamensis* originated intense blooms in all the rocky Mediterranean Sea coastal areas, typically during summer-late summer. The correct identification of *Ostreopsis* species in field samples is often problematic as *Ostreopsis* species are morphologically plastic and hardly discriminable under light microscopy and, therefore, molecular analyses are required. *Ostreopsis* blooms are often associated with noxious effects on health of both humans and benthic marine organisms mainly carried by aerosol and direct contact with seawater. Environmental factors have been shown to affect toxin content of *Ostreopsis* which generally produces more toxins per cell when growing under suboptimal conditions. *O.* cf. *ovata* is able to produce both temporary and resting cysts. In particular, the resting cysts are able to germinate in laboratory conditions for as long as 5 months after their formation at 25°C, but not at 21°C; the presence of a temperature threshold affecting cyst germination in the laboratory suggests that temperature represents a key factor for *Ostreopsis* cf. *ovata* bloom onset in natural environments as well. Several studies conducted to assess the role of abiotic factors (mainly hydrodynamics, water temperature and nutrients) on the bloom dynamics, revealed that the synergic effects of hydrodynamics, temperature and N:P ratios would lead the *Ostreopsis* blooms in temperate areas. *Ostreopsis* abundances showed a significant decrease with depth, likely related to light availability, although there are conflicting data about the relationship between light intensity and *Ostreopsis* growth in experimental conditions. The relationship between *Ostreopsis* blooms and salinity is not completely clear, complicated by the influence of high nutrient levels often associated to low salinity waters. Finally, *Ostreopsis* colonize a variety of substrata, although living substrata seems to allow lower concentration of epibionts than any other substrate, probably due to the production of some allelopathic compounds.

Key words: Ostreopsis; palytoxin; harmful algae; benthic dinoflagellates; Mediterranean Sea.

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INTRODUCTION

The genus *Ostreopsis* belongs to the family of Ostreopsidaceae (Gonyaulacales, Dinophyceae, Dinophyta). It was first described by Schmidt (1901) after the finding of *O. siamensis* Schmidt in plankton samples collected in the Gulf of Siam in 1900. Since then, this species has rarely been encountered in the phytoplankton, due to its predominantly benthic habit.

Ostreopsis species were reported since a long time in tropical ciguatera endemic areas, associated with the benthic toxic dinoflagellate *Gambierdiscus toxicus* (Ballantine *et al*., 1985; Carlson and Tindall, 1985; Bomber and Aikman, 1989) and, therefore, improperly considered in association with ciguatera syndrome (Tosteson, 1995). Indeed, some *Ostreopsis* species are toxic, but their toxins (mostly belonging to the palytoxin group) are not those implicated in ciguatera.

In the last decade, *Ostreopsis* blooms have become common in temperate areas as well, and regularly occur in the Mediterranean Sea during summer-autumn (Vila *et al*., 2001; Turki, 2005; Aligizaki and Nikolaidis, 2006; Mangialajo *et al*., 2008; Totti *et al*., 2010; Illoul *et al*., 2012; Ismael and Halim, 2012; Pfannkuchen *et al*., 2012), and in other temperate areas of the world (Chang *et al*., 2000; Rhodes *et al*., 2000; Pearce *et al*., 2001; Taniyama *et al*., 2003; Shears and Ross, 2009; Selina *et al*., 2014). In these areas, *Ostreopsis* is well-known since its blooms are often associated with noxious effects on health of both humans (Gallitelli *et al*., 2005; Kermarec *et al*., 2008; Tichadou *et al*., 2010; Del Favero *et al*., 2012) and benthic marine organisms (Pagliara and Caroppo, 2012; Gorbi *et al*., 2013; Carella *et al*., 2015). Additionally, *Ostreopsis* often appeared in association with other toxic or potentially toxic benthic dinoflagellates such as *Prorocentrum* spp., *Amphidinium* spp. and *Coolia monotis* in both several Mediterranean (Tognetto *et al*., 1995; Vila *et al*., 2001; Aligizaki and Nikolaidis, 2006; Monti *et al*., 2007; Mabrouk *et al*., 2011) and world areas (Okolodkov *et al*., 2007; Parsons and Preskitt, 2007; Kim *et al*., 2011; Selina and Levchenko, 2011).

Given their negative implications on both marine ecosystem functioning and human health and activities, *Ostreopsis* blooms attracted the attention of researchers in the last decade. This paper is meant to be a narrative review of the existing information about taxonomy, geographical distribution, toxin production, life cycle and ecology of *Ostreopsis* in temperate areas.

TAXONOMY

There is a considerable confusion regarding the descriptions of the *Ostreopsis* species, since its first description carried out by Schmidt (1901): in the original drawings of *Ostreopsis siamensis*, both the epitheca and the hypotheca in anteroposterior view were presented but with marked different shapes (one appeared rounded and the other appeared elongated) insomuch hardly they can belong to the same cell. Much later, Fukuyo (1981) re-described *O. siamensis* and described two new species *O. lenticularis* and *O. ovata*. The rounded shape reported by Schmidt for *O. siamensis* was one of the main differences between *O. lenticularis* and *O. siamensis*, so probably in the original description Schmidt confused these two species. *O. ovata* was distinguished from the other two species by having a more ovoid shape and a smaller size. In the following years, several other species have been described by other authors: *O. heptagona* (Norris *et al*., 1985), *O. mascarenensis* (Quod, 1994), *O. labens* (Faust and Morton, 1995), *O. marinus, O. belizeanus* and *O. caribbeanus* (Faust, 1999) (recently, the latter three have been renamed as *O. marina, O. belizeana, O. caribbeana,* Hoppenrath *et al*., 2014). So, at present, nine species of *Ostreopsis* have been described.

In the Mediterranean Sea, only two species have been recorded until now, *O.* cf. *ovata* and *O.* cf. *siamensis* (Vila *et al*., 2001; Penna *et al*., 2005, 2010, 2012; Battocchi *et al*., 2010; Totti *et al*., 2010; Mangialajo *et al*., 2011; Perini *et al*., 2011; Mabrouk *et al*., 2012). The correct identification of these *Ostreopsis* species in field samples is often highly problematic. The most suitable taxonomical character used to discriminate *O.* cf. *ovata* and *O.* cf. *siamensis*, is the dorsoventral/anteroposterior diameter ratio (DV/AP) which is <2 and >4, respectively (Penna *et al*., 2005; Aligizaki and Nikolaidis, 2006; Selina and Orlova, 2010). However, *O.* cf. *ovata* cells in the Adriatic Sea (Mediterranean Sea) have a DV/AP ratio slightly higher than 2 (*i.e.*, 2.3-2.4) (Monti *et al*., 2007; Guerrini *et al*., 2010; Accoroni *et al*., 2012b). Since the molecular analyses clearly confirmed the presence of only *O. ovata* species in the N Adriatic samples (Perini *et al*., 2011), it can be hypothesized that the Adriatic population has a different cell morphology, with cells more flattened than in other areas. These morphological problems and the lack of genetic data for the holotype specimens from which the original species descriptions of both *O. siamensis* and *O. ovata* were made, lead to the conclusion that *O. siamensis* and *O. ovata* examined nowadays should be referred to *O.* cf. *siamensis* and *O.* cf. *ovata* respectively, until more accurate morphological data and genetic sequences will be gathered to clearly define each species (Penna *et al*., 2005, 2010).

Given these ambiguities in defining morphological characteristics, many researchers have been induced to revise the description of *Ostreopsis* species by sequencing the ITS and 5.8S rDNA regions, using these data in combination with morphometric ones. Leaw *et al*. (2001) isolated several *Ostreopsis* strains from Malaysian coastal waters and showed that *O.* cf. *ovata* isolates were separated into two genetically distinct geographic groups, a Malacca Strait group and a South China Sea group, while there were minor morphological differences among the strains. The 5.8S and ITS sequences of these Malaysian strains differed from Mediterranean strains ones (Penna *et al*., 2005), suggesting a genetic variability in relation to the geographic distribution within the species. Nowadays, the ITS-5.8S and LSU rDNA allowed to distinguish various clades among the *Ostreopsis* species (Penna *et al*., 2014): the species complex *Ostreopsis* cf. *ovata* includes the Atlantic/Mediterranean/Pacific clade (*i.e.*, isolates from Japan Sea, and Mediterranean Sea), the Atlantic/Indian/Pacific clade (*i.e.*, isolates from Belize) and the Pacific clade (*i.e.*, isolates from Vietnam); *Ostreopsis* cf. *siamensis* forms an Atlantic/Mediterranean clade. The *Ostreopsis* cf. *lenticularis*/*O.* cf. *labens* contains isolates from Hawaii and Pacific Asia.

DISTRIBUTION IN TEMPERATE AREAS

The first record of *Ostreopsis* in the Mediterranean Sea dates back to 1972 in Villefranche-sur-Mer (France) by Taylor (1979). Later, *O.* cf. *ovata* was detected in 1994 along both the Italian coasts of the Tyrrhenian Sea (Tognetto *et al*., 1995) and the Catalan coast in Spain in 1997-1998 (Vila *et al*., 2001). In the last decade, *Ostreopsis* spp. blooms have been more intense, frequent, and widely distributed in many Mediterranean areas, including Spain, France, Greece, Italy, Algeria, Tunisia, Turkey (Turki, 2005; Aligizaki and Nikolaidis, 2006; Ciminiello *et al*., 2006, 2008; Riobó *et al*., 2006; Turki *et al*., 2006; Monti *et al*., 2007; Riobó *et al*., 2008; Guerrini *et al*., 2010; Mabrouk *et al*., 2011, 2012; Mangialajo *et al*., 2011; Illoul *et al*., 2012). As reported above, genetic analyses indicate that two genotypes corresponding to the morphotypes *O.* cf. *ovata* and *O.* cf. *siamensis* are present in the Mediterranean Sea (Penna *et al*., 2010, 2012). Along the Mediterranean rocky coasts, the genotype *ovata* is the most abundant and widely distributed (Battocchi *et al*., 2010; Perini *et al*., 2011). The genotype *siamensis* was detected along the Catalan coast, in the eastern Atlantic coast of Morocco, Portugal, northern Spain and southern Italy (Vila *et al*., 2001; Amorim *et al*., 2010; Bennouna *et al*., 2010; Laza-Martinez *et al*., 2011; Ciminiello *et al*., 2013) and its morphotype has also been reported along the northern African coast (Turki, 2005; Turki *et al*., 2006; Mabrouk *et al*., 2011, 2012). Moreover, Penna *et al*. (2012) found a new genotype, probably corresponding to a new species of *Ostreopsis*, in both the Atlantic coast (Canary Islands) and Mediterranean Sea (Greece and Cyprus).

Ostreopsis spp. have been recorded in other temperate areas as well. In Japan, toxic strains of both *O. siamensis* and *O. ovata* have been recorded not only in sub-tropical southern Okinawan waters in late 1970s (Fukuyo, 1981; Nakajima *et al*., 1981; Yasumoto *et al*., 1987) but also in the more temperate northern waters of western Kyushu, eastern Miyazaki, Kochi and Shikoku (Taniyama *et al*., 2003; Adachi *et al*., 2008; Sagara, 2008). *Ostreopsis* cf. *siamensis* cells were found in temperate New South Wales and Tasmanian waters (Murray, 2010) and, in the latter, in the gut contents of wild mussels (Pearce *et al*., 2001). Moreover, *O.* cf. *siamensis* has become a major bloom former in New Zealand, causing extensive mats covering seaweeds in the eastern Northland waters, and has been reported as far south as temperate Wellington waters (Rhodes *et al*., 2000, 2010; Shears and Ross, 2009); in the northern New Zealand waters also *O. lenticularis* and *O.* cf. *ovata* have been recorded (Chang *et al*., 2000). Finally, *Ostreopsis* cf. *ovata* and *O*. cf. *siamensis* were a constant component of the epiphytic communities during the summer–fall period in Peter the Great Bay, Sea of Japan since 2006 (Selina and Orlova, 2010; Selina *et al*., 2014).

TOXIN PROFILE AND FACTORS AFFECTING TOXIN PRODUCTION

Ostreopsis species produce different toxins, mostly belonging to the palytoxin group. Among the nine species of the genus *Ostreopsis*, toxicity has been demonstrated in *O. siamensis*, *O. mascarenensis*, *O. lenticularis* and *O.* cf. *ovata* (Nakajima *et al*., 1981; Yasumoto *et al*., 1987; Holmes *et al*., 1988; Mercado *et al*., 1994; Meunier *et al*., 1997; Lenoir *et al*., 2004; Ciminiello *et al*., 2006; Scalco *et al*., 2012; Uchida *et al*., 2013; Brissard *et al*., 2015; García-Altares *et al*., 2015). Moreover, *O. heptagona* was determined to be toxic as methanol extracts of culture of this species isolated from Knight Key (Florida) were weakly toxic to mice (Babinchak, according to Norris *et al*., 1985).

Palytoxin (PlTX) has a molecular formula of $C_{129}H_{221}N_3O_{54}$ and a molecular weight of 2680 Da (Moore and Bartolini, 1981). It has been primarily isolated from the marine zoanthid *Palythoa toxica* (Moore and Scheuer, 1971), from which the name comes. PlTX is a very complex molecule with both lipophilic and hydrophilic groups and is slightly less toxic than maitotoxin in total potency. The PlTX analogues produced by *Ostreopsis* species have a similar chemical structure as the parent PlTX, as well as a similar mode and site of action. Ostreocin-D was the first PlTX analogue isolated from cultures of *O. siamensis* (Usami *et al*., 1995; Ukena *et al*., 2001). This compound has the chemical formula $C_{127}H_{220}N_3O_{53}$ and a molecular weight of 2634 Da, a little lower than PlTX. Another PlTX analogue, mascarenotoxin (McTX), was isolated from *O. mascarenensis* (Lenoir *et al*., 2004) and *O.* cf. *ovata* (Rossi *et al*., 2010; Scalco *et al*., 2012). The molecular weight of the three identified mascarenotoxin congeners ranges from 2500 to 2628 Da (Rossi *et al*., 2010). In addition, a third PlTX analogue was isolated from *O.* cf. *ovata*, the ovatoxin (OvTx) (Ciminiello *et al*., 2008, 2010, 2012a; Rossi *et al*., 2010). Mediterranean cultures of *O.* cf. *ovata* were found to produce isobaric palytoxin, ovatoxin-a, b, c, d, e, f, g and h and mascarenotoxin-a and c (Scalco *et al*., 2012; García-Altares *et al*., 2014; Brissard *et al*., 2015). On the contrary, the Mediterranean *O.* cf. *siamensis* strain seems to be devoid of any appreciable toxicity (Ciminiello *et al*., 2013). Finally, ostreotoxins (produced by *O. lenticularis*) do not display the same mode and site of action as PlTX-analogues and the classification of these compounds as PlTX analogues is still unclear (Mercado *et al*., 1994; Meunier *et al*., 1997).

As far as the studies on the action mechanism are concerned, almost all studies refer to the commercial PlTX standard (Tubaro *et al*., 2014). PlTX targets membrane sodium-potassium pumps (Na+/K+-ATPase) responsible for maintaining ionic gradients (Artigas and Gadsby, 2003). Characteristic aspects of PlTX include delayed haemolysis with a loss of potassium, converting Na/K pump into a non-specific ionic channel leading to the disruption of ion homeostasis exerted on excitable tissues (Habermann *et al*., 1981). This results in nausea, vomiting, hyper-salivation, abdominal cramps, diarrhoea, numbness of extremities, severe muscular spasms and respiratory distress (Yasumoto *et al*., 1986; Alcala *et al*., 1988; Kodama *et al*., 1989). On the contrary, the possible effects recorded in non-excitable cells are less clear: in this case, the toxin can affect different sets of proteins and signalling pathways, stressing the complexity of the mode of action of PlTX (Bellocci *et al*., 2011; Rossini and Bigiani, 2011; Wattenberg, 2011). For example, protein kinases involved in the control of cell proliferation can be activated by PlTX (Wattenberg, 2011), providing a possible role of this toxin in the tumour-promoting activity (Fujiki *et al*., 1986; Fujiki and Suganuma, 2009). However, PlTX has been also shown to possess a potent cytotoxic activity (Bellocci *et al*., 2011).

Environmental factors (*e.g.* temperature, salinity, light, nutrients) and the characteristic of the strains (*e.g.*, isolation site, growth phase and the age of the strain) have all been shown to affect toxin content in *Ostreopsis* cultures (Guerrini *et al*., 2010; Ciminiello *et al*., 2012a,b; Pezzolesi *et al*., 2012; Scalco *et al*., 2012; Vanucci *et al*., 2012b). Several phytoplankton species produce more toxins per cell when growing under suboptimal conditions (Johansson and Granéli, 1999a,b; Etheridge and Roesler, 2005). In the same way, some authors observed that *O*. cf. *ovata* isolated from both the Tyrrhenian and Adriatic Seas produces higher toxin contents per cell when growing under suboptimal temperature and salinity conditions (Granéli *et al*., 2011; Pezzolesi *et al*., 2012; Vidyarathna and Granéli, 2013), which differ among strains from different geographical areas (see Paragraph 7). On the contrary, optimal nutrient conditions seem to be required for toxin production and both P- and N-depleted media decreased *O*. cf. *ovata* toxicity (Vanucci *et al*., 2012b). The growth phase affects *Ostreopsis* toxicity as well. Although toxin production rate has been found to increase during the exponential phase (Pezzolesi *et al*., 2014), toxins concentration on a per cell basis increased from the exponential to the senescent phase, independently of the growth conditions (Guerrini *et al*., 2010; Pistocchi *et al*., 2011; Vanucci *et al*., 2012a, 2012b). This behaviour was recently explained (Pinna *et al*., 2015) as due to the strong influence of the internal nutrient status (*i.e.*, carbon to nutrient ratio) on toxin synthesis. On the contrary, the toxin profile of *O*. cf. *ovata* was relatively stable during the growth stages and independent of culture conditions (Pistocchi *et al*., 2011; Scalco *et al*., 2012; Pezzolesi *et al*., 2014).

VECTORS OF EXPOSITION AND EFFECTS ON ORGANISMS

The main vectors for *Ostreopsis* intoxication of humans include marine aerosol (Casabianca *et al*., 2013, 2014; Ciminiello *et al*., 2014), direct contact (Tichadou *et al*., 2010) and the *per os* ingestion (the latter mainly associated to clupeotoxism syndrome, see below). Blooms of *O*. cf. *ovata* caused serious problems on human health, mainly due to inhalation of sea water droplets containing *Ostreopsis* cells or fragments and/or aerosolized toxins (Gallitelli *et al*., 2005; Kermarec *et al*., 2008; Tichadou *et al*., 2010; Honsell *et al*., 2011; Del Favero *et al*., 2012). One of the most intense episode occurred in summer 2005, when about 200 people exposed to marine aerosols along the Ligurian coasts required medical first aid due to similar symptoms of respiratory intoxications, and 20 persons were subjected to extended hospitalization (Brescianini *et al*., 2006; Durando *et al*., 2007). The typical intoxication symptoms of *Ostreopsis* aerosol and direct contact exposure (fever, dyspnoea, broncho-constriction, conjunctivitis and skin irritations) resolve within a few days.

Regarding the oral ingestion, although *Ostreopsis* has not been confirmed as the source of toxin in clupeotoxism yet, it was strongly suspected of that intoxication in several events (*e.g.*, Onuma *et al*., 1999; Randall, 2005). Clupeotoxism is one of human intoxications due to consumption of contaminated sardines and herrings (Clupeidae) or anchovies (Engraulidae). Symptomology of clupeotoxism is similar to that of ciguatera (Yasumoto *et al*., 1986), though the former has a much higher mortality rate (Onuma *et al*., 1999). Several outbreaks were reported in tropical insular areas of the Pacific and the Caribbean during the last 30 years (Yasumoto *et al*., 1986; Fukui *et al*., 1987; Gleibs *et al*., 1995). Recently, clupeotoxism occurred in the southwestern Indian Ocean, mainly in Madagascar where palytoxin analogues were involved in fatalities occurred after consumption of *Sardinella* fish (Yasumoto, 1998; Hansen *et al*., 2001).

Ostreopsis toxins may contaminate seafood: ostreocin-D produced by *O*. cf. *siamensis* were accumulated in wild mussels (*Mytilus edulis planulatus*) from Tasmanian coasts (Pearce *et al*., 2001). Rhodes *et al*. (2002), feeding New Zealand mussels (*Perna canaliculus*), Pacific oysters, and scallops (*Pecten novaezealandiae*) with *O*. cf. *siamensis* cells detected trace amounts of palytoxin-like compounds in some of the fed animals. In the Mediterranean Sea, Aligizaki *et al*. (2008) analysing field samples of shellfish (*Mytilus galloprovincialis*, *Venus verrucosa*, *Modiolus barbatus*) reported that shellfish toxicity coincided with seasonal peaks in *Ostreopsis* abundance, providing the most compelling evidence to date that *Ostreopsis*-borne palytoxin analogues likely accumulate in shellfish. Although it has been shown that the oral toxicity of palytoxin and 45-hydroxy palytoxins is about 1000-fold less than that observed by intraperitoneal injection (Sosa *et al*., 2009; Munday, 2011; Tubaro *et al*., 2011), a regulatory threshold of 30 μg kg^{-1} has been proposed for shellfish flesh (EFSA, 2009). However, the effects of the ingestion of products contaminated by *O*. cf. *ovata* toxin are still unknown.

Ostreopsis blooms are often accompanied by mortality of benthic marine organisms, such as sea urchins, limpets, mussels, crustaceans, holothurians, sponges and even macroalgae (Di Turi *et al*., 2003; Shears and Ross, 2009, 2010; Accoroni *et al*., 2011). In fact, several recent studies have shown that *Ostreopsis* toxicity affects also various marine organisms, both invertebrates and fish (Gorbi *et al*., 2012, 2013; Simonini *et al*., 2011; Faimali *et al*., 2012; Pezzolesi *et al*., 2012; Privitera *et al*., 2012; Carella *et al*., 2015), interfering with embryonic development as well (Pagliara and Caroppo, 2012).

A study carried out on natural banks of *Mytilus galloprovincialis* sampled during various phases of *O*. cf. *ovata* bloom in the north-western Adriatic Sea (Mediterranean Sea) demonstrated a significant accumulation of algal toxins in mussels exposed, which exhibited a marked inhibition of the Na+/K+-ATPase activity and alterations of immunological, lysosomal and neurotoxic responses (Gorbi *et al*., 2012).

LIFE CYCLE AND CYST FORMATION

As the largest part of marine dinoflagellates, *Ostreopsis* has a haplontic life cycle with a dominant motile haploid biflagellate stage (Pfiester and Anderson, 1987; Litaker *et al*., 2002).

The asexual and sexual reproduction of *Ostreopsis* has been studied mainly in *Ostreopsis* cf. *ovata*, both in the field and in cultures isolated from the Mediterranean Sea (Bravo *et al*., 2012; Accoroni *et al*., 2014). The asexual reproduction occurs with the division of haploid vegetative cells that takes place in the motile stage: cells divide by desmoschisis in the sagittal plane, with each daughter cell inheriting part of the parental thecal plates (Bravo *et al*., 2012).

In general, sexual reproduction of dinoflagellates usually begins with the production of gametes that can fuse with each other forming a diploid zygote. The actual details of gamete formation vary, but in any case, two haploid cells (both typically motile) fuse to yield a diploid cell. The sexual reproduction in *O*. cf. *ovata* has been observed in both natural and cultured populations (Bravo *et al*., 2012). Gamete pairs are observed in either intercrosses or intracrosses of different strains (Bravo *et al*., 2012) and nutrient limitation seems to stimulate sexual reproduction, which however occurs also in normal culture conditions (Accoroni *et al*., 2014). In *O*. cf. *ovata*, two types of mating gametes were identified: i) gametes joined by epitheca, with the point of attachment positioned almost centrally (Bravo *et al*., 2012), and ii) gametes joined laterally with the two cingula perpendicular to each other, with melting of the two thecae (Accoroni *et al*., 2014), as observed in *Coolia monotis* by Faust (1992). In dinoflagellates, the newly formed motile diploid cell produced when karyogamy and plasmogamy are complete, is known as a planozygote (Pfiester, 1989). The planozygote of some species undergoes meiosis in the plankton, while that of most other dinoflagellates swims for a variable amount of time (hours to weeks), sheds its flagella, rounds up, and settles to form a non-motile hypnozygote. Hypnozygotes often differentiate into long-term resting stages (hypnocysts) that accumulate in sediments and may remain dormant for years before germinating (Wall, 1975; Anderson *et al*., 1987). Different types of cysts were identified for *O*. cf. *ovata*; some of them are non-dormant as germinate within 3 days (temporary cysts), while some others are resting cysts able to germinate for as long as 5 months after their formation. A study on the life cycle of *O*. cf. *ovata* conducted with northern Adriatic strains highlighted that resting cysts germinated in laboratory conditions at 25°C, but not at 21°C (Accoroni *et al*., 2014). The presence of a temperature threshold affecting cyst germination in laboratory conditions would highlight that temperature could represent a key factor for *Ostreopsis* cf. *ovata* bloom onset also in natural environments.

ROLE OF ENVIRONMENTAL PARAMETERS ON BLOOMS

Hydrodynamic conditions

Several studies considered hydrodynamic condition as the main factor affecting *Ostreopsis* bloom trends, highlighting that higher abundances are observed in sheltered sites compared with exposed ones (Barone, 2007; Shears and Ross, 2009; Totti *et al*., 2010; Mabrouk *et al*., 2011). The abundances of benthic dinoflagellates are highly affected by wave action, since they are only loosely attached to the substrata and can be easily removed and re-suspended in the water column, although in literature we often found controversial data. Chang *et al*. (2000) suggested that *O*. cf. *siamensis* was more abundant on the northern New Zealand's eastern coast because it is a less energetic, more stable environment than the western one. This suggestion has been confirmed later by Shears and Ross (2009) that observed higher *Ostreopsis siamensis* abundances at sheltered *vs* exposed locations. Vila *et al*. (2001), on the other hand, concluded that *Ostreopsis* sp. prefers 'moderately shaken' waters in the NW Mediterranean and Parsons and Preskitt (2007) observed higher abundance of *O*. cf. *ovata* on the windward coast of the island of Hawaii, whereas *Ostreopsis* sp.1 was more prevalent on the leeward coast.

Observations in the northern Adriatic Sea highlighted that 1) significantly higher abundances were observed in the sheltered sites compared with the exposed ones; 2) hydrodynamics may have an important effect on the temporal variability of bloom, because stormy events can result in a sudden decrease of cell abundances on the benthic substrata, with cell proliferation being re-established at high densities after some days of calm sea conditions (Totti *et al*., 2010; Accoroni *et al*., 2012a). Moreover, it has been highlighted that turbulence can affect *O*. cf. *ovata* growth rate and consequently its cell size (Accoroni *et al*., 2012b).

The effect of hydrodynamics has been separately tested for each bloom phase, *i.e.* initial (no more than 10^2 cells cm^{-2} recorded over all substrata), proliferation (period of intense cell division, when *O*. cf. *ovata* rapidly increased abundances until reaching maximum peak) and decline phase (decrease in cell abundances and bloom decline), and it has been shown that cell abundances in sheltered sites were significantly higher than those in the exposed ones during the proliferation phase (Accoroni *et al*., 2012b). This result suggested that hydrodynamics affect *O*. cf. *ovata* abundances mainly during phases when the highest abundances are reached. In fact, a well-developed benthic mat (*i.e.*, brownish pellicle loosely attached to benthic substrata) is produced only during the most intense proliferation period (Totti *et al*., 2010) and this structure is easily removed by effect of the hydrodynamic conditions. This result may explain why such effects linked to hydrodynamic conditions were not observed in those areas where high abundances were not reached and a mat did not develop, as observed in the Tyrrhenian Sea (Zingone, *personal communication*), and in Johnston Atoll (Pacific Ocean) (Richlen and Lobel, 2011).

Water temperature

Many authors suggested that *Ostreopsis* spp. need relatively high temperatures to proliferate, proposing that the global warming might have influenced *Ostreopsis* expansion in temperate areas such as the Mediterranean Sea (Hallegraeff, 2010; Granéli *et al*., 2011), but a more careful analysis of literature data shows that temperature role is not the same in all coastal areas around the world (Tab. 1). *Ostreopsis* blooms are summer events in temperate areas, although comparing the bloom trend in several Mediterranean areas it can be observed that peaks can occur from spring to autumn, with a certain inter-annual variability. In the northern Adriatic Sea, the peaks of the blooms occur generally in September-October (Monti *et al*., 2007; Totti *et al*., 2010; Accoroni *et al*., 2015a). On the contrary, in the Ligurian Sea highest cell abundances were mostly recorded in mid-summer (end of July) (Mangialajo *et al*., 2008). Vila *et al*. (2001) observed abundance peaks for *Ostreopsis* sp. in the north-western Mediterranean even early, in springtime. Also in the Aegean Sea, Spatharis *et al*. (2009) found *O*. cf. *ovata* being most abundant in May, contrarily to what observed in the same area by Aligizaki and Nikolaidis (2006) that reported peak abundances from midsummer to late fall.

Experimental studies about the response of *Ostreopsis* growth to temperature provide a possible explanation for the above different temporal trends observed *in situ*: Guerrini *et al*. (2010) and Pezzolesi *et al*. (2012) highlighted that *O*. cf. *ovata* strains from different Italian coasts (in the Tyrrhenian and Adriatic Seas) displayed different growth temperature optima that parallel with the *in situ* temperature values typical of the blooming period of the single strain. Scalco *et al*. (2012) observed that Mediterranean *O*. cf. *ovata* strains grew within a window of 18-30°C with the best performances recorded between 22 and 26°C, suggesting that *O*. cf. *ovata* is adapted to intermediate temperatures and day length conditions, such are those recorded in the natural environment at the beginning of summer and/or of autumn (Mangialajo *et al*., 2011). In

Tab. 1. Summary of environmental conditions recorded during *Ostreopsis* species blooms in temperate areas. Values refer to periods of *Ostreopsis* maximum proliferation; data not explicated in the main text of the references, wherever possible, have been extrapolated from tables or figures.

Area	Species	Sampled substrata	Maximum proliferation period	Temperature (°C)	Salinity	Nutrients (µM)	Reference
Mediterranean Sea, N Adriatic Sea	*Ostreopsis* cf. *ovata*	Macroalgae, invertebrates, rocks, water column	September-October	16.8-27.9	31.3-39.3	0.55-19.4 (DIN) 0.01-0.49 (PO_4)	Monti *et al*., 2007; Totti *et al*., 2010; Accoroni *et al*., 2011, 2012a, 2015a; Mangialajo *et al*., 2011
Mediterranean Sea, S Adriatic Sea	*Ostreopsis* cf. *ovata*	Water column	August	26.4-27.1	36.8-38.4	10.3-37.10 (TN) 0.11-0.15 (PO_4)	Ungaro *et al*., 2005
Mediterranean Sea, Tyrrhenian Sea	*Ostreopsis* cf. *ovata*	Water column	August-early October	24.5-28			Tognetto *et al*., 1995
N Mediterranean Sea, Ligurian Sea	*Ostreopsis* cf. *ovata*	Macroalgae, water column	July-August	22.6-30	38.0-38.2		Ciminiello *et al*., 2006; Mangialajo *et al*., 2008, 2011; Cohu *et al*., 2011
NW Mediterranean Sea, Gulf of Lion	*Ostreopsis* cf. *ovata*	Macroalgae, water column	July-August	19.2-21.5			Mangialajo *et al*., 2011
NW Mediterranean Sea, Catalan Sea	*Ostreopsis* cf. *ovata* *Ostreopsis* cf. *siamensis*	Macroalgae, soft sediments, water column	End March- October	18-28.3	30.3-38.1	0.76-7.74 (DIN) 0.11-0.86 (PO_4)	Vila *et al*., 2001; Mangialajo *et al*., 2011; Carnicer *et al*., 2015
Mediterranean Sea, Aegean Sea	*Ostreopsis* cf. *ovata* *Ostreopsis* cf. *siamensis*	Macroalgae, angiosperms, soft sediments, water column	May-early November	13.9-29.7		0-85 (DIN) 0.5-6.5 (DIP)	Aligizaki and Nikolaidis, 2006; Spatharis *et al*., 2009
Mediterranean Sea, Gulf of Tunis	*Ostreopsis* cf. *siamensis*	Angiosperms	August -October	20-27	36.6-37		Turki, 2005
New Zealand	*Ostreopsis ovata* *Ostreopsis siamensis* *Ostreopsis lenticularis*	Macroalgae, water column	End February-April	17.8-22.1		1.6-3.8 (DIN) 0.33-1.10 (PO_4)	Chang *et al*., 2000; Shears and Ross, 2009
Sea of Japan	*Ostreopsis* cf. *ovata* *Ostreopsis* cf. *siamensis*	Macroalgae	August-October	9-25	30-34		Selina and Orlova, 2010; Selina *et al*., 2014

the same way, Tawong *et al*. (2015) observed that optimal and tolerable temperature conditions differ among *Ostreopsis* cf. *ovata* subclades: strains of *O*. cf. *ovata* Thailand subclade and *O*. cf. *ovata* South China Sea subclade showed the semi-optimal temperature ranges of 22.7-27.4°C and 27.9-30.8°C, with optimal temperature of 25°C and 30°C, respectively.

Although the reaching of the highest abundances of *Ostreopsis* is not in concomitance with the highest water temperature values in all areas, a temperature threshold would seem to be important to let the bloom start: a study conducted along the Conero Riviera (northern Adriatic Sea) showed that, although the bloom peak occurred in late summer (when temperatures ranged between 18.8 and 24°C, decreasing from the seasonal maximum), the bloom onset was always observed at higher temperature (25-28.6°C), suggesting that *Ostreopsis* needs to reach a fairly well fixed temperature threshold to start its bloom, probably in relation to the cyst germination that generally occurs at around 25°C (Accoroni *et al*., 2014). As in this area the bloom can persist until temperature values are much lower (14.4-17.5°C) than that threshold, one would guess that once *Ostreopsis* cysts are germinated, its vegetative forms seem to actively proliferate even if temperature values decrease. This discrepancy between optimal temperature range for the cysts germination and the algal growth has been reported for the dinoflagellate *Scrippsiella trochoidea* as well by Binder and Anderson (1987) in experimental conditions. However, in the northern Adriatic Sea the bloom onset is often observed about 30 days after the reaching of the 25 °C-temperature threshold, suggesting that other environmental factors, besides temperature, may affect the development of *O*. cf. *ovata* blooms. In this regard, *O*. cf. *ovata* blooms appear to be triggered by a combination of optimal temperature and available nutrients, where the temperature threshold plays a key role on the germination of *O*. cf. *ovata* cysts and an N:P ratio around the Redfield value is a necessary condition to allow cell proliferation.

Role of salinity and nutrients

Contrasting results are reported in the literature about the effect of salinity on the development of *Ostreopsis* blooms. Rhodes *et al*. (2000) observed that *O*. cf. *siamensis* had a preferred salinity range of 28-34 in cultures isolated from Northland (New Zealand). Salinity measured in the north-western Mediterranean Sea during the *Ostreopsis* spp. blooms showed values around 37-38 (Vila *et al*., 2001; Mangialajo *et al*., 2008) and similar salinity levels were measured in the Gulf of Trieste (northern Adriatic Sea) by Monti *et al*. (2007) and in the southern Adriatic Sea by Ungaro *et al*. (2005); in the Conero Riviera (northern Adriatic Sea) during the *Ostreopsis* blooms occurred from 2007 to 2012, salinity ranged in a much wider range, from 31.3 to 39.3 (Accoroni *et al*., 2015a).

Several authors suggested that benthic dinoflagellates proliferation is favoured by low salinity waters. In the Virgin Islands, abundance maxima of *Ostreopsis* were correlated with the period of maximal rainfall (Carlson and Tindall, 1985), and the same negative correlation with salinity has been found for *O. ovata* along the Hawaiian coasts (Parsons and Preskitt, 2007).

On the contrary, in the north-western coast of Cuba, in Catalonian and in French Mediterranean coasts, Delgado *et al*. (2006), Blanfuné *et al*., (2015) and Carnicer *et al*., (2015) suggested that low salinity values possibly hinder *Ostreopsis* spp. as they found conspicuously lower *Ostreopsis* abundances in sites more affected by river plumes (*i.e*., Jaimanitas River, Rhone River and Ebro Delta) than in the rest of the studied areas. No significant correlation between cell abundances and salinity values were found in the Gulf of Mexico for *O. heptagona* (Okolodkov *et al*., 2007) and in the Conero Riviera for *O*. cf. *ovata* (Accoroni *et al*., 2015a)

When the effect of salinity has been investigated in experimental conditions, it has been shown that such effect may be strain-specific: Tawong *et al*. (2015) showed that optimal and tolerable salinity conditions differed among *Ostreopsis* cf. *ovata* subclades as the optimal salinities for the *O*. cf. *ovata* Thailand and South China Sea subclades were 30 and 25, respectively. Pezzolesi *et al*. (2012) demonstrated that an Adriatic *O*. cf. *ovata* strain cultured at salinity ranging from 26 to 40 showed that although the lowest growth rates were observed at the lowest salinity, growth rates did not significantly differ in different salinity conditions.

Indeed, the relationships between algal blooms in the field and salinity are more complicated, and other factors, such as nutrient levels (which are typically associated to low salinity waters) have to be considered. 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; Glibert *et al*., 2010). However, there is very limited information on the relationships between nutrient concentrations and the occurrence of *Ostreopsis* blooms (Tab. 1). Vila *et al*. (2001) and Cohu *et al*. (2011) in north-western Mediterranean Sea and Shears and Ross (2009) in north-eastern New Zealand did not find any relation between epiphytic *O*. cf. *ovata* abundance and nutrients, while Parsons and Preskitt (2007) found that *Ostreopsis* sp.1 abundance was positively correlated with nutrient concentrations in the waters surrounding Hawaii. In the Conero Riviera (northern Adriatic Sea), although no clear relationship was found between nutrient concentrations and *O*. cf. *ovata* abundances, it was observed that in the bloom onset period, PO_4 concentrations were significantly higher than in the rest of the study period. Interestingly, the following bloom development is maintained in a condition of elevated N:P ratios, suggesting that such blooms may be initiated at low N:P levels (possibly stimulated by a 'flush' of nutrients or organic materials) that may allow the newly germinating cells to increase growth rate while other adaptive mechanisms (*e.g.*, metabolic dissipatory strategies, allelopathic and mixotrophic interactions), would enable the maintenance of blooms at less than maximal growth rates and at not-optimal N:P ratios (Accoroni *et al*., 2015a). A decrease in N:P ratio values has previously been associated with the onset of a number of planktonic dinoflagellate blooms as well (Hodgkiss and Ho, 1997; Zhang and Hu, 2011; Glibert *et al*., 2012). In this regard, experimental studies conducted on different Adriatic *O*. cf. *ovata* strains showed that the depletion of P was proportionately more rapid than that of N, highlighting the strong P demand of this dinoflagellate (Vanucci *et al*., 2012b; Pezzolesi *et al*., 2014).

Moreover, further studies are needed to clarify the trophic behaviour of *Ostreopsis* spp., considering that for these species also mixotrophy was hypothesized (Barone, 2007; Burkholder *et al*., 2008), which may play an important role in *Ostreopsis* development, as already observed in other potentially toxic microalgae (Cucchiari *et al*., 2008; Heisler *et al*., 2008).

Depth

The role of depth on *Ostreopsis* abundances along the Conero Riviera (northern Adriatic Sea) was investigated in 2007 (Totti *et al*., 2010) in target sites where samples were collected at depths comprised between 0.5 and 10 m. *O*. cf. *ovata* abundances showed a significant decrease with depth, in agreement with what observed by Richlen and Lobel (2011) and Cohu and Lemée (2012), suggesting a potential effect of light intensity. This may explain why *Ostreopsis* blooms mainly develop in shallow waters. However, such effect has not been observed in shallow sites affected by high hydrodynamics, such as on the fringing reefs of the higher infralittoral shelf, where *O*. cf. *ovata* abundances were lower than those recorded immediately deeper, due to the hydrodynamic effect of wave actions (Totti *et al*., 2010).

As previously suggested, the role of depth may be related to light availability, although there are conflicting data about the relationship between light intensity and *Ostreopsis* growth in experimental conditions. Morton *et al*. (1992) reported that *O*. cf. *siamensis* and *O*. *heptagona* isolated from the Florida Keys displayed maximal growth at approximately 200 μmol photons m^{-2} s^{-1} and did not grow rapidly at over 240 μmol photons m^{-2} s^{-1} (*i.e.*, >10% full sun light). Scalco *et al*. (2012), analysing the growth performance of some Italian *O*. cf *ovata* strains, observed that this species grew better at relatively low photon flux density (50 instead of 200 μmol photons m^{-2} s^{-1}). Yamaguchi *et al*. (2014) observed that *Ostreopsis* sp. from Japan grew proportionally when light intensity was increased from 49.5 to 199 μmol photons m^{-2} s^{-1}, but its growth appeared to be inhibited slightly at >263 μmol photons m^{-2} s^{-1}. Heil *et al*. (1993) observed that *O*. cf. *siamensis* cultured in spinner flasks would stay planktonic when light intensities are maintained low (25 μmol photons m^{-2} s^{-1}), but would produce mucus and settle at the bottom at higher light intensities (75 μmol photons m^{-2} s^{-1}). This has been interpreted as a protective measure to shade the cells. Therefore, in experimental conditions *Ostreopsis* sp. seems to suffer too high light intensities, therefore being exposed to potentially detrimental consequences of photodamaging.

In field conditions, there are only few data about the relationship between *Ostreopsis* abundances and light intensity, which, moreover, seem to disagree with the experimental evidences described above. A study carried out along several Italian coastal areas affected by *Ostreopsis* blooms showed that during the blooms, the values of light intensity at depth of *Ostreopsis* sampling were quite high, up to 1800 μmol photons m^{-2} s^{-1} (ISPRA, 2012). However, it is known that light availability amidst macroalgal vegetation is generally low (Raniello *et al*., 2004), and Ballantine *et al*. (1988) suggested that *Ostreopsis* cells can migrate to shaded areas of the algal host thallus to escape high light levels.

Anyway, further studies are required to clarify the real role of light intensity in the bloom dynamics of *Ostreopsis*.

Substratum

Ostreopsis has often been indicated to be preferentially epiphytic on macroalgae (Bomber *et al*., 1989; Vila *et al*., 2001), although it has been recorded on a variety of other substrata (Table 1), including marine angiosperms (Turki, 2005; Aligizaki and Nikolaidis, 2006; Turki *et al*., 2006; Battocchi *et al*., 2010; Mabrouk *et al*., 2012), rocks (Bottalico *et al*., 2002; Totti *et al*., 2010; Accoroni *et al*., 2011), coral rubble (Norris *et al*., 1985), soft sediments (Vila *et al*., 2001; Aligizaki and Nikolaidis, 2006), and invertebrates (Bianco *et al*., 2007; Totti *et al*., 2010). They also can be found as free-living in the plankton (Faust and Morton, 1995; Tognetto *et al*., 1995; Chang *et al*., 2000; Totti *et al*., 2010; Accoroni *et al*., 2011).

The possibility of *Ostreopsis* to colonize a variety of substrata, living either as epiphytic, epilithic, or epizoic, indicates that this species is not an obligate epiphyte.

A number of studies underlined the importance of host thallus architecture (Lobel *et al*., 1988; Bomber *et al*., 1989). Vila *et al*. (2001) observed that three-dimensional flexible thalli are more suitable for the growth of *Ostreopsis* spp. It has been suggested that the higher abundances found in branched than in flattened thalli, might be explained by a different response of such thallus morphotypes to the wave action (Totti *et al*., 2010).

Indeed, the relationships between *Ostreopsis* and macrophytes are more complicated. In studies carried out on natural populations of *O.* cf. *ovata*, significantly higher abundances were reported on pebbles than on macroalgae (Totti *et al*., 2010; Accoroni *et al*., 2011), suggesting that living substrata allow lower concentration of epibionts than any other substrate, probably due to the production of some hypothetical allelopathic compounds (Jin and Dong, 2003). In this regard, a study conducted to assess any possible allelopathic interactions between *Ostreopsis* cf. *ovata* and macroalgae showed that all the investigated seaweeds [*Dictyota dichotoma* (brown alga), *Rhodymenia pseudopalmata* (red alga) and *Ulva rigida* (green alga)] exerted negative effects toward the benthic dinoflagellate, with the highest inhibitory effect observed in *D. dichotoma* and the lowest in *R. pseudopalmata* (Accoroni *et al*., 2015b).

UNRESOLVED ISSUES

Despite the number of studies on *Ostreopsis* biology, ecology and toxin production and actions, several aspects about the environmental concerns associated with this genus remain still unclear. Regarding the action mechanism of the implicated toxins, almost all studies refer to the commercial PlTX standard, while a more accurate analysis should be addressed on both effects and action mechanism of all toxins produced by *Ostreopsis* species. Moreover, among the vectors of *Ostreopsis* intoxication *i.e.*, marine aerosol, direct contact and *per os* ingestion, the latter needs certainly further studies given its possible implications on human health almost unknown in temperate areas nowadays.

Numerous studies have highlighted the influence of environmental factors on bloom dynamics of *Ostreopsis* and the complexity of conditions leading to blooms of this dinoflagellate is becoming clearer but not totally understood. Although the mechanisms for bloom onset seems clarified, those driving both bloom development and decline are still far from being understood, and other both abiotic and biotic factors, such as the interactions with other organisms and the ability to use organic forms of nutrients, should be investigated. Actually, several HAB genera have been shown to use organic forms of nutrients for their nutritional demands (Cucchiari *et al*., 2008; Heisler *et al*., 2008) and a mixotrophic behavior has been hypothesized in *Ostreopsis* (Barone, 2007; Burkholder *et al*., 2008; Pinna *et al*., 2015). Effects of biotic interactions on *Ostreopsis* should be considered as well and only few studies have been carried out on bacteria (Vanucci *et al*., 2012a), diatoms (Pichierri *et al* 2015) and macroalgae (Accoroni *et al*., 2015b). In this regard, the role of viruses, bacteria and parasites in both cysts formation and bloom termination have been recognized in several microalgae (Nagasaki *et al*., 1994; Nagasaki *et al*., 2000; Tarutani *et al*., 2001; Mizumoto *et al*., 2008; Garcés *et al*., 2013) and should be studied in *Ostreopsis* bloom dynamics as well.

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