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Potentially harmful *Ostreopsis* spp. in the coastal waters of Alexandria - Egypt

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Abstract

Ostreopsis spp. has been reported for the first time from the Egyptian Mediterranean waters. Macroalgal samples were collected monthly between June 2005 and December 2007, from the rocks at Abu Qir, from less than 1.5 m depth, and their associated microalgae examined. Populations of two *Ostreopsis* morphotypes were found to occur in this location, east of Alexandria, viz., *O. cf. ovata* Fukuyo and *Ostreopsis* morph1.

The *Ostreopsis* spp. was abundant and dominant during the summer. They were more abundant as epiphytes of the brown algae *Padina* sp. and *Sargassum* sp., less abundant on the red algae *Corallina* sp., *Jania* sp., *Laurencia* sp. and even less so on the green algae *Ulva* spp. *Ostreopsis cf. ovata* was also identified during the summer months on the same macroalgal species, although in a much lower abundance. *Ostreopsis* spp. alternated in dominance with the benthic cyanobacteria *Oscillatoria* spp. and the diatom *Licmophora* sp. Other benthic dinoflagellates recorded at low abundance included *Amphidinium carterae*, *Gymnodinium* sp. and *Prorocentrum lima*.

Keywords: *Ostreopsis*, harmful, benthic microalgae, Alexandria, Egypt.

Introduction

Since the description of the species type *Ostreopsis siamensis* by Schmidt in 1902, the genus *Ostreopsis* remained monospecific until 1981, except for the contentious incorporation of *Coolia monotis* Meunier by Lindemann in 1928. In 1981, Fukuyo described *O. lenticularis* and *O. ovata* from French Polynesia and the Ryukyu Islands. Six more *Ostreopsis* spp. were described over the following years: *O. heptagona* Norris *et al.* (1985) from the Florida Keys, *O. mascarenensis* Quod (1994) from the coral reefs on the islands of Mascareignes in the Indian Ocean, *O. labens* Faust and Morton (1995) from Belize and the Japanese Islands Oshigaki and Iriomote, *O. marinus* Faust (1999), *O. belizeanus* Faust (1999), *O. caribbeanus* Faust (1999) from Belize, Puerto Rico and the British Virgin Islands. The first report of *O. siamensis* in the Mediterranean was submitted in 1972 in Villefranche-sur-Mer (Taylor, 1979). From among all the *Ostreopsis* spp. known to date, only *O. cf. ovata* and *O. cf. siamensis* were reported from the Mediterranean, the former appearing to be more widespread than the latter. Since Tognetto *et al.* (1995) recorded *O. ovata* from the Mid Tyrrhenian Sea, Italy, the species was reported from the Spanish coast from the Balearic Islands (Penna, *et al.*, 2005), Tuscany coasts (Sansoni *et al.*, 2003), Ganzirri, Gioia Tauro (Tyrrhenian Sea), Sicily and the Ligurian

coast (Penna, *et al.*, 2005), Campania coast of Italy (Zingone *et al.*, 2006), Northern Adriatic (Monti *et al.*, 2007; Totti *et al.*, 2010) and Taranto Gulf-Ionian Sea (Penna, *et al.*, 2005). When the bloom occurred along the Genoa coast (Italy), they were accompanied by symptoms of skin irritation, fever and respiratory problems (Mangialajo *et al.*, 2008). Invertebrate mass mortalities linked to *Ostreopsis* blooms have been observed by several authors in the Mediterranean Sea (Sansoni *et al.*, 2003; Simoni *et al.*, 2003; Vila *et al.*, 2008; Totti *et al.*, 2010). In the western Mediterranean basin, Penna *et al.* (2005) reported *O. cf. siamensis* and *O. cf. ovata* containing palytoxin. In the eastern Mediterranean basin, the first observation of *Ostreopsis siamensis* was recorded from the Lebanese waters in 1979 (Abi Saab, 1989). It appears now that *Ostreopsis* spp. occurs along the coasts of Lebanon, Greece and Tunisia. Aligizaki and Nikolaidis (2006) reported on the ecology and distribution of *O. siamensis*, *O. ovata* and *Coolia monotis* from 50 sites along the North Aegean coast. In the Tunisian waters *O. siamensis* was reported by Turki *et al.* (2006).

The present work deals with a thriving *Ostreopsis* spp. population associated with macroalgae in the Alexandrian coastal waters in Egypt, confirming the circum-Mediterranean distribution of the genus. The population included two different morphs, *Ostreopsis cf. ovata* and *Ostreopsis* morph1.

Material and Methods

A total of 61 samples were collected from the west Abu Qir area. This site includes a relatively sheltered rocky shore (Fig. 1). Samples of macroalgae (about 100 g fresh weight) were collected monthly from June 2005 to December 2007. All samples were collected from depths between 0.5 and 1.5 m and whenever possible three samples of three different macroalgal species were collected. The macroalgal samples were carefully picked and placed in plastic bags filled to approximately 100 ml with local seawater. Water samples were also collected immediately above each macroalgal stand to determine the salinity using Salintest HANNA model Hi 98203. Temperature values were recorded *in situ* using a digital thermometer HANA model Hi 96127. Macroalgal samples were then vigorously shaken (to dislodge any epiphytic microalgae inadvertently present), removed from the plastic bags and weighed to determine the wet weight. The macroalgae were then identified to species level using the appropriate keys and catalogued (Feldmann, 1937; Aleem, 1993; Abbott & Hollenberg, 1976; Abbott and Huisman, 2004). The water subsamples were examined and the cells counted at X200 and/or X400 under the Zeiss inverted and Olympus CH40 microscopes (Utermöhl, 1958). The data were expressed as cells g⁻¹ fresh weight (fw) algae. For SEM analysis, the cells were fixed with 4% formaldehyde, washed with distilled water and dehydrated in an ethanol series, filtered through 0.45 µm membrane filters, critical point dried, coated with gold and examined under the JEOL JSM-5300 scanning electron microscope.

Ostreopsis spp. abundance data were tested for macroalgal host preference using one-way ANOVA with Tukey pairwise comparisons. Correlation analysis between *Ostreopsis* spp., temperature and salinity was conducted. All statistical tests were performed using STATISTICA 7.

Results

The sea surface temperature and salinity ranged from 16.9 to 30°C and from 37.2 to 39.01, respectively, during

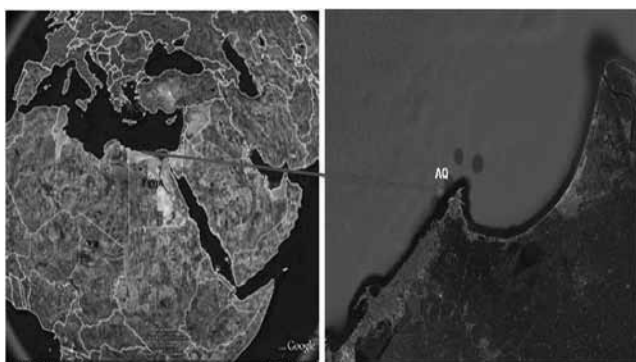


Fig. 1: Abu Qir shore line.

the study period (Fig. 2). *Ostreopsis* morphs were recorded during June to November, at temperatures ranging between 24.2 and 30°C. During the three month study period, the maximum abundance of both species (maximum value 9053 cells g⁻¹ fw in July 2005) was always found to be recorded in July, when temperature rose to above 28°C (Fig.3).

The correlation between the standing crop of the two *Ostreopsis* morphs was significant with temperature ($p < 0.00004$, $r = 0.69596486$) although not so with salinity.

During the study period, 54 epiphytic microalgae were recorded. *Ostreopsis* morphs alternated in abundance with the *Oscillatoria* spp. (cyanobacteria) and *Licmophora* (diatoms) together with the potentially toxic benthic dinoflagellates *Coolia monotis* and *Prorocentrum lima*. The trend of the abundance of the epiphytic microalgae in Abu Qir showed five peaks throughout the 26-month study period. *Ostreopsis* morph 1 and *O. cf. ovata* were responsible for the two peaks recorded during the summer (July 2005 and August 2007), while the diatom *Licmophora* sp. was responsible for the two peaks in February 2006 and 2007. Also, the harmful cyanobacterium *Oscillatoria nigroviridis* was responsible for the peak recorded in November 2006 (Fig. 4).

One-way ANOVA showed significant differences in the abundance of *Ostreopsis* morphs on the macroal-

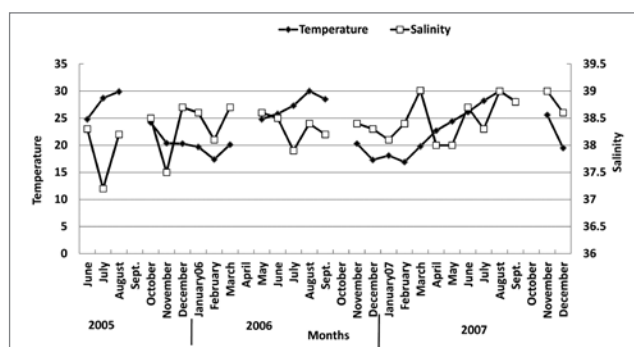


Fig. 2: Distribution of salinity and temperature (°C) during the present study.

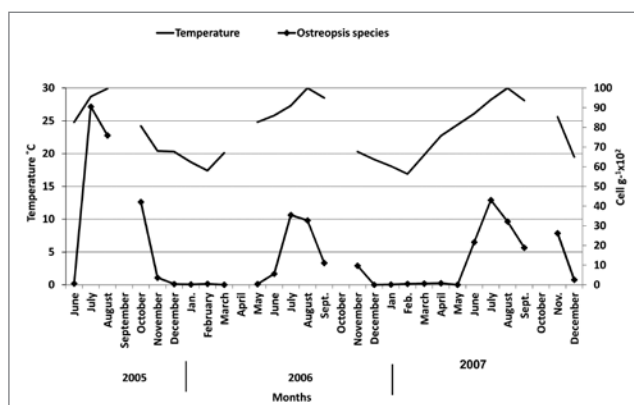


Fig. 3: Distribution of *Ostreopsis* species and temperature during the period of study

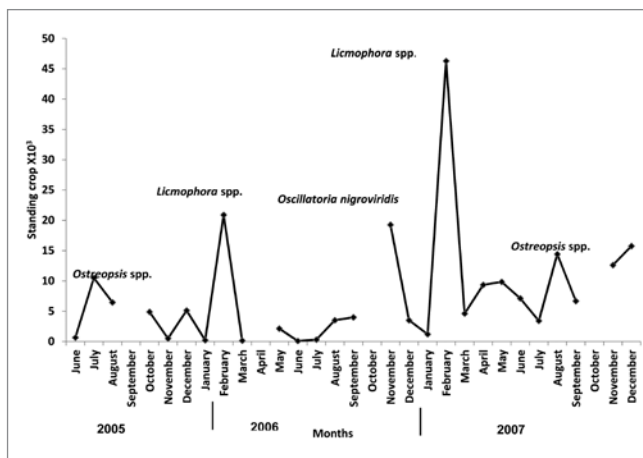


Fig. 4: Total standing crop of epiphytic microalgae recorded during the present study.

gae ($df=12$, $p<0.05$). *Ostreopsis* cf. *ovata* and *Ostreopsis* morph 1 showed a preference for a particular macroalgal species. Both were associated with the brown and red algae, *Hypnea* ($F=8.292$, $p=0.002$), *Laurencia* ($F=2.41$, $p=0.01$), *Jania*, *Corallina* ($F=3.015$, $p=0.002$) and *Sargassum* ($F=2.598$, $p=0.009$). However, generally, preferences for macroalgae were not constant among the microalgal species, indicating specific epiphyte requirements. The *Ostreopsis* spp. was absent or insignificant where *Ulva* was found dominant (Fig. 5).

Morphological characters

Ostreopsis cf. *ovata*

The specimens conform to the species description of Fukuyo (1981), with the exception of having a smaller Po.

Cells are oval (Fig. 6a) and pointed ventrally in the apical view, with the typical plate pattern of the species,

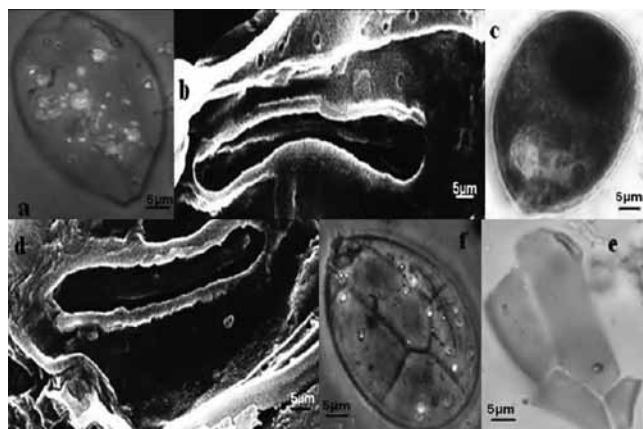


Fig. 6: LM of *Ostreopsis* cf. *ovata* (a), SEM of thecal surface showing thecal pores (b), LM of *Ostreopsis* cf. *ovata* with red-pigmented vacuoles (c), SEM of Po (d), LM of plates 1', 2' and 3' with Po (e), LM of hypothecal view (f).

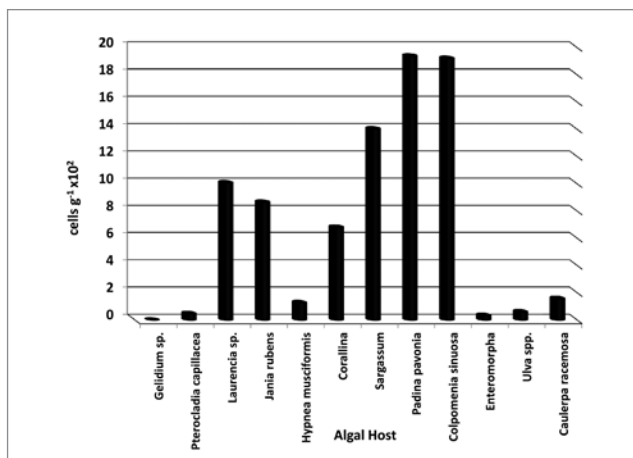


Fig. 5: Cell concentration of *Ostreopsis* spp. on the different macroalgal hosts during the period of study.

Po, 3', 7'', 5''', 2''''', 1P. The thecal plates are thin and delicate. The thecal surface is covered by minute pores 0.2 μm in diameter (Fig. 6b). The cells contain many yellow-brown chromatophores, some cells having one or two large red-pigmented vacuoles on the dorsal side (Fig. 6c).

The species is small, the DV diameter ranges from 45 to 50 μm (average 29.5 μm) and the transdiameter from 25 to 30 μm (average 27.5 μm). The apical pore Po is short and slightly curved (Fig. 6d), located on the left dorsal side of the epitheca, associated with plates 1', 2' and 3' (Fig. 6e). The first apical plate 1' is elongated and hexagonal, in contact with plates 2', 3', 1'', 2'', 6'' and 7'' (Fig. 6e). Plate 3' is pentagonal and situated on the left dorsal side of the epitheca. In the hypotheca, the posterior intercalary plate 1P is long and narrow with a dorsoventral diameter of 26 μm and a transdiameter of 8 μm (Fig. 6f). Plates 1'', 4'' and 5'' are the smallest, while plate 6'' is the largest. This species differs from the morphotype described below by its smaller size, very delicate thecal plates and shorter Po and general cell shape.

Ostreopsis morph 1

Cells of *Ostreopsis* morph 1 are oval (Fig. 7a), with a DV diameter of 60-70 μm (average 65 μm), and a transdiameter of 35-48 μm (average 41.5 μm). The AP diameter ranged from 19 to 31 μm (average 25 μm) and the DV/AP ratio value was 2.25 - 3.15 (average 2.7). *Ostreopsis* morph 1 differs from *O. ovata* in several features. The cells are distinctly larger than *O. ovata*, although the L/W ratio is comparable. The thecal surface is smooth and covered with one type of pores, 0.9 μm in diameter, visible under the light microscope (Fig. 7b). The pores often contain ejected trichocysts (Fig. 7c).

The thecal tabulation of the species is P0, 3', 7'', 5''', P and 2'''' just like other species belonging to the genus. The apical pore plate (Po) is narrow, slightly curved, and

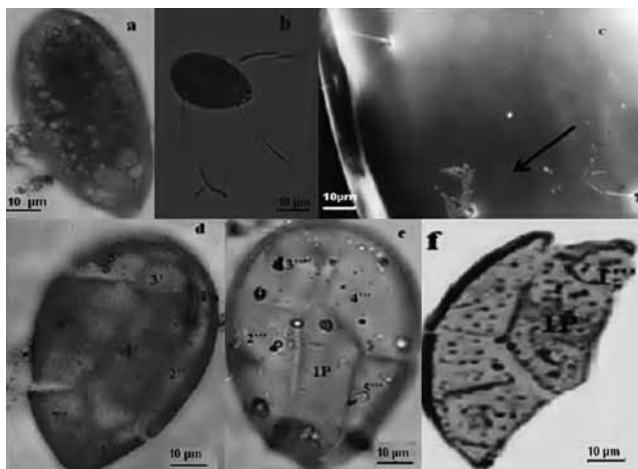


Fig. 7: LM of *Ostreopsis* morph 1 with trypan blue staining (a), LM of thecal surface with pores (b), SEM of thecal plates surface with ejected trichocysts (c), LM of epithecal view of *Ostreopsis* morph 1 (d), hypothecal plates (e), the posterior intercalary plate (f).

of 12.5 μm average length. It is situated in plate 2'. The apical pore plate Po extends to the half of plate 3'. Plate 2' is narrow and long. It is adjacent to plate 1', 3' and connected with plate 2'' through only one point (Fig. 7d).

The hypotheca is composed of eight plates (Fig. 7e). The posterior intercalary plate (1P) is pentagonal and symmetric (Fig. 7f). It is 31.25 μm long and 10.9 μm wide (L/W ratio of 2.86).

Discussion

This study represents the first report of the genus in the Egyptian Mediterranean coastal waters. Blooms of *Ostreopsis* spp. have recently been associated with human health problems by the inhalation of the marine aerosols as well as with invertebrate mass mortality along the beaches in the Tyrrhenian, northern and Southern Adriatic Sea (Sansoni *et al.*, 2003; Mangialajo *et al.*, 2008; Vila *et al.*, 2008; Totti *et al.*, 2010). Thus far, the small blooms of *Ostreopsis* morphs in Abu Qir, Alexandria, beaches remain harmless. They, however, represent a potential threat to the local tourism and require monitoring on a continuous basis.

Water temperature plays an important role in the appearance of the genus *Ostreopsis* in the Alexandrian waters. *Ostreopsis* sp. and *O. cf. ovata* occurred at temperatures between 24.2 and 30°C, respectively, with the highest abundance at 28.7°C. The affinity of the genus for higher temperatures could be an indication of a tropical origin. The proliferation of *Ostreopsis* spp. under relatively high temperatures was also documented by Sansoni *et al.* (2003); Aligizaki & Nikolaidis (2006); Monti *et al.* (2007) and Mangialajo *et al.* (2008) and Shears & Ross (2009). Carlson and Tindall (1985) and Vila *et al.* (2001), however, found that temperature did not seem to affect the genus distribution, while, Mangialajo *et al.*

(2011) observed that temperature did not play the same role in all the Mediterranean areas.

The peak concentration of *Ostreopsis* morph 1 on macroalgae in this study (9.05×10^3 cells g^{-1} fw) was comparatively lower than the other records of *Ostreopsis* worldwide, such as 1.41×10^6 cells g^{-1} fw in New Zealand (Shears & Ross, 2009), 5.96×10^5 cells g^{-1} fw in Costa Brava, Spain (Vila *et al.*, 2001), 2.54×10^6 cells g^{-1} fw in Genoa, Italy (Mangialajo *et al.*, 2008).

During this study, *Ostreopsis* morphs exhibited a preference for certain macroalgal hosts. They were significantly found more abundantly on *Hypnea* sp., *Laurencia* sp., *Jania* sp., *Corallina* sp. and *Sargassum* sp. They were absent or insignificant on the *Ulva* spp. This is compatible with the observations of Vila *et al.* (2001) and Aligizaki & Nikolaidis (2006), where the highest density of *Ostreopsis* corresponded mainly to Rhodophyta and Phaeophyta. On the contrary, Parsons and Preskitt (2007) showed that *Ostreopsis* spp. exhibited a preference for macroblades, specifically *Ulva fasciata*. As mentioned earlier, the *Ostreopsis* population occurring in the Abu Qir bay waters included two morphs; *Ostreopsis cf. ovata* and *Ostreopsis* morph 1, the latter being more abundant.

Based on morphological features recorded during this study, *O. cf. ovata* conforms to the species description of Fukuyo (1981), both in size and general morphology. However, the morphological features of the dominant morph, *Ostreopsis* morph 1, do not match any of the *Ostreopsis* species described (Fukuyo, 1981; Norris *et al.*, 1985; Faust & Morton 1995; Faust *et al.*, 1996 and Faust, 1999). The species recorded during this study bears some similarity to *O. ovata* and *O. siamensis*, but four characters distinguish it from the other two: a) the size of the apical pore plate (Po), 12 μm in length; b) the shape of the suture between plate 1' and plate 3'; c) the thecal pore size of 0.9 μm , and d) the ratio DV/AP. The latter (2.25-3.15) is higher than that recorded for *O. cf. ovata* (1.25-2.8) by Monti *et al.* (2007). Based on these results, there could be a new *Ostreopsis* sp. in the Egyptian Mediterranean waters. Further research including the molecular characterization of the genus in these waters is essential to assess whether the differences observed in the most abundant morphs warrant the establishment of a new species.

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