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Research Article

Ecology of the Epiphytic Potentially Harmful Dinoflagellate *Ostreopsis* cf. *ovata* (Fukuyo) from Coastal Waters off Alexandria Egypt

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ABSTRACT

Despite macroalgae epiphytes play an important role in algal habitats, available information from Alexandria coastal waters is scarce. To evaluate the role of environmental factors on the inventory of the ephyphytic O*streopsis* c.f *ovata* cells, its abundance on the thalli of 14 different macroalgae collected from two sites at Abou Qir rocky area was done on the basis of sampling carried out for one year. The annual maximum *Ostreopsis* cf. *ovata* cell abundance was observed in May (late-spring), reaching the maximum on the green macroalgae *Ulva linza* in July (mid- summer). Analysis of variance showed significant difference of abundance between seasons and hosts. Water temperature and NO_2, NO_3 and PO_4 seem the major factor affecting on abundance of *Ostreopsis* c.f *ovata*. The present study documented the significant importance of *Ulva* sp. as the suitable host of O. *ovata* in Alexandria waters, in contrasting with others in the Mediterranean Sea that show O. *ovata* most often found on Phaeophyta and Rhodophyta.

Keywords: *Ostreopsis* sp.; Epiphytic; Macroalgae; Harmful algae

INTRODUCTION

Macroalgae provide a suitable substrate for microalgae found as epiphytes, which often provide support for higher trophic levels and might have important implications for food web dynamics [1,2]. Other ecological part played by epiphytes in ecosystem as a protection layer for seagrasses from excessive UV radiance was discussed [3]. However, as epiphyte load increases, it can negatively affect seagrasses in different ways [4].

Species of the dinoflagellate genus *Ostreopsis* Schmidt are unicellular algae preferentially epiphytic/epibenthic and grow in shallow waters of tropical and subtropical areas on abiotic and biotic substrates [5,6]. But cells may detach from the substrate and are found planktonic in the water column [6,7]. However, in the last few decades an increasing number of reports have been documented for a worldwide geographical expansion, and intensive bloom outbreaks of O. *ovata* and O. *siamensis* in warm and cold temperate areas [8,7]. *Ostreopsis* sp. has been expanding geographically in recent decades, especially in temperate regions, with 67% of studies focused on these regions [9]. There are currently eleven described species of *Ostreopsis*, four of which are known to be toxic; O. cf. *ovata*, O. *siamensis*, O. *mascarenensis*, and O. *lenticularis* to which scientists, the aquaculture industry, and government are keenly attentive [10,11]. The toxicity is associated with the presence of palytoxin and its derivatives [12].

Ostreopsis cf. *ovata* (Fukuyo) populations typically proliferate in rocky coasts, forming rusty-brown coloured mucilaginous film [6]. Widespread occurrence of the toxic benthic dinoflagellates genus *Ostreopsis* Schmidt has increased during the last decades [5]. It is recently found in the Mediterranean Sea where the first known bloom recorded in 1998 along the Tuscany coasts [13].

The presence of O. *ovata* in coastal waters may pose a threat to coastal food web and fishery [14]. In the Mediterranean Sea, neurotoxic effects due to toxin accumulation in food web have not yet been reported and *Ostreopsis* species are implicated thus far only in respiratory affections and skin or eyes irritations, causing health problems as dyspnea, fever, conjunctivitis and dermatitis for people near the shore exposed to marine aerosols during bloom events [15]. High levels of ovatoxins were recorded in natural samples; episodes of death of both benthic invertebrates (limpets, sea urchins and mussels) and macroalgae were commonly observed during O. *ovata* blooms.

A review of the existing literatures over the last three decades on harmful red tide blooms in Alexandria coastal waters shows a great attention towards planktonic microalgal bloom forming species while, there is a paucity of information in the literature on the ecology of attached potentially harmful epiphytic dinoflagellates

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in relation to biotic and abiotic regulating factors [7,16]. Ismael and Halim reported epiphytic *Ostreopsis* cf. *ovata* east of Alexandria [17]. Such studies are of increasing importance in the years ahead and that is what this study sets out to address. This *in situ* study investigates the interactions of habitat and ecosystem characteristics in the facilitation/regulation of the abundance of the epiphytic dinoflagellate *O. ovata* living attached to diverse macroalgal species in Alexandria waters, considering multiple environmental stressors assumed to regulate or influence its growth on biotic substrates. The study might provide additional information for better understanding the ecology of the *Ostreopsis* population dynamics in order to depict some considerations on its occurrence trend in this area and provide a basis for effective bloom management and mitigation. We quantified the abundances of *O. ovata* on different macroalgal communities dominated by specific macroalgal species, and measure multiple environment and weather factors to determine their effects on *O. ovata* abundances and endeavored to identify a correlation between *O. ovata* abundance and ambient abiotic factors.

MATERIALS AND METHODS

Description of the study sites

The study site is located at the western head of Abu Qir Bay, east of Alexandria City. It is a relatively sheltered rocky shore, characterized by chains of natural exposed rocks extends for about 100 m seaward from the coast. Its massive rocky outcrops provide a suitable substratum for a rich algal flora [18]. This site was selected due to the presence of abundant and highly productive macroalgae community [7]. A transect stretched for about 25 m long was positioned perpendicular to the coast, among which two sites at 0.5 m and 1m depth were chosen. Sampling was carried out in May, July, and October of 2017 and February of 2018 (Figure 1).

Sampling methods

A total of 26 samples of macroalgae including 14 macroalgal species of the different groups Rhodophyta (7 species), Phaeophyta (2 species), and Chlorophyta (5 species) were collected seasonally at a rocky substrate. Depending on their growth cycle and the environmental disturbance, some macroalgae species were missing, particularly during rough sea periods in winter, and therefore could not be collected. Thus, the collected macroalgae here represents the whole algal species present during the different months. The handpicked macroalgae of ca. 20-100 g wet weight collected carefully was placed into individual plastic bags with surrounding seawater, and transported on ice cooler back to the laboratory. In laboratory, each macroalgae was put in a plastic container with 250 mL of fresh filtered seawater, vigorously shaken to dislodge epiphytic cells, and the material passed through several meshed filter (500, 100 and 20 µm mesh) to separate macroalgae and water containing microalgae [19]. The material retained by the filter was suspended in sterile filtered seawater (25 mL) and fixed with the addition of Lugol's acid solution at 1%. For qualitative and quantitative analyses, 2 mL of preserved sample from determined wet weight of each macroalgal sample was counted triplicate using counting chamber. Cell abundances of epiphytic cells expressed here as cells g^{-1} wet weight of macroalgae, following the methodology described by Okolodkov [20]. Identification and nomenclature of microorganisms followed appropriate keys while macroalgae following standard taxonomic keys as described by Aleem et al. [21-24].

Figure 1: Location of the studied site.

Measurement of environmental factors

Water temperature (oC), salinity, dissolved oxygen (mg l-1 and saturation %) and pH values determined using the (hydro lab. HANA, Model HI 9828). Samples intended for nutrient analysis were kept in the dark at 4oC. Concentrations of nitrite, nitrate, ammonia, phosphate and silicate were measured according to the methodology of Strickland and Parsons [25].

Statistical analyses

The Pearson's correlation coefficient "r" and multiregression analyses (STATISTICA 7.0, Stat Soft, Inc.) were applied on the whole data set in order to highlight eventual relationships between *Ostreopsis* abundance and environmental parameters, as well as between the parameters themselves across the two sites.

RESULTS

Environmental characteristics of sites

The sampled sites were natural rocky areas and were highly exposed to hydrodynamic conditions. Each measured environmental parameter showed approximately the same range of values at sampled sites. The physical and chemical parameters of water from locations collecting samples are given in Table 1.

The total temperature range encountered varied within 10.74°C between the lowest in February and the highest in July, reflecting a clear seasonality. Salinity range varied within 1.77, with no particular seasonal variation. The hydrogen ion concentration values lie in the alkaline side and with a very narrow range of fluctuation (0.54). Dissolved oxygen in May and July increased by 3.5-3.9 times greater compared with the minimum in October. Concerning nutrients, nitrite followed typical summer minimum, and it was highest in May. Nitrate concentrations were always high, and expressed 63.21-78.91% of the total nitrogen. Ammonia concentrations were often <3 µM, and reached minimum in October. Silicate values were the lowest in May, and were at a maximum of 5.64 µM in July. Phosphate trend followed that of silicate.

Macroalgae species

The encountered macroalgae species in the present study (14 species) are given in Table 2 Clear seasonality is evident, and *Ulva compressa* represents a perennial form.

Epiphytic species

Generally, the phytoplankton epiphytic species other than O. cf. *ovata* found during the study comprised mainly diatom species of the genera *Achnanthes* (Agardh), *Bacillaria* (Gemlin), *Climacosphenia* (Ehrenberg), *Cocconies* (Ehrenberg), *Cyclotella* (Chang), *Licmophora* (Agardh), *Melosira* (Pritchard), *Navicula* (Cleve & Grunow), *Odentella* (Agardh), *Pleurosigma* (Smith), *Pseudonitzschia* (Cleve), and *Synedra* (Nitzsch), and few rather dinoflagellates mostly belong to the genera *Prorocentrum* (Ehrenberg), *Protoperidinum* (Balech), *Pyrophacus* (Stein), and *Scrippsiella* (Loeblich III). The abundance of epiphytic cells is given in Table 3.

Parameter	oC	Salinity	pH	DO mgL-1	DO%	NO_2 µM	NO_3 µM	NH_4 µM	TN µM	SiO_4 µM	PO_4 µM
May	25.1	37.2	8.37	10.4	61.5	3	11.02	2.5	16.52	0.12	0.66
July	28.37	35.43	8.59	9.35	39.2	1.2	8.34	2.8	12.34	5.64	5.44
October	22.34	36.6	8.11	2.65	139.2	1.5	10.1	1.2	12.8	0.62	1
February	17.63	35.8	8.65	3.58	163.1	2.34	7.8	2.2	12.34	0.45	0.39
Mean	23.36	36.26	8.43	6.50	100.75	2.01	9.32	2.18	13.50	1.71	1.87
S. D	4.54	0.80	0.24	3.94	59.71	0.82	1.50	0.69	2.02	2.63	2.39

Table 1: Temporal distribution of physical and chemical parameters.

Group	May	July	October	February
Chlorophyta				
	Ulva fasciata	*Ulva fasciata*	*Ulva compressa*	*Ulva fasciata*
	Ulva compressa	*Ulva compressa*	*Ulva lactuca*	*Ulva compressa*
	Ulva linza	*Cladophora pelluceda*		*Ulva linza*
Rhodophyta				
	Corallina officianalis	*Jania rubens*	*corallina mediterranean*	*Corallina officianalis*
	Laurencia papillosa	*Jania smooth*	*corallina elongata*	*Corallina elongata*
	Pterocladia capillacea	*Pterocladia capillacea*	*corallina officianalis*	*Pterocladia capillacea*
			Jania rubens	
Phaeophyta				
	Cystoseira spinosa	*Padina pavonia*	not recorded	*Cystoseira spinosa*
	Padina pavonia			*Padina pavonia*

Table 2: Monthly occurrence of the host macroalgae.

Macroalgae	Cells g_{-1} w.w. X 10^3		Epiphytic cells g_{-1} w.w. X 10^3		% to total epiphytic cells	
	St. I	**St. II**	**St. I**	**St. II**	**St. I**	**St. II**
Chlorophyta						
May	62.38	51.72	85.09	66.26	73.32	78.06
July	65.48	49.67	75.07	58.69	87.22	84.63
October	56.00	40.00	68.63	44.73	81.59	89.42
February	0.09	0.07	1.36	1.28	6.85	5.74
Rhodophyta						
May	12.24	9.22	12.84	9.96	95.33	92.57
July	19.17	15.94	22.96	23.03	83.49	69.22
October	15.18	11.44	18.98	15.32	79.97	74.70
February	0.27	0.16	1.97	2.75	13.81	5.83
Phaeophyta						
May	6.12	3.76	6.75	5.4	90.67	69.63
July	15.47	15.32	18.11	18.02	85.42	85.01
October	**Absent**					
February	0.22	0.18	2.28	1.72	3.15	3.46

Table 3: Abundance of the *Ostreopsis* cf. *ovata* (average cells g^{-1} wet weight x 10^3) on the different macroalgae groups.

May	Cells g_{-1} w.w. X 10_3		Epiphytic cells g_{-1} w.w. X 10_3		% to total epiphytic cells	
Species	Range		Range		Range	
Chlorophyta	St. I	St. II	St. I	St. II	St. I	St. II
U. compressa	49.8-62.66	39.6-50.5	112.6-130.5	67.8-80.9	42.53-51.03	58.41-64.35
Mean	55.99	45.2	121.97	73.13		
SD	6.44	5.46	8.98	6.88		
U. linza	68.4-75.5	56.8-63.5	78.8-91.5	60.5-75.5	74.75-91.52	79.74-93.88
Mean	71.40	60.17	84.27	68.93		
SD	3.68	3.35	6.53	7.67		
U. fasciata	58.2-60.8	43.1-53.5	65.4-74.3	53.1-63.6	81.83-88.99	80.71-99.41
Mean	59.77	49.80	70.03	56.70		
SD	1.38	5.81	4.46	5.90		

May	Cell g_{-1} w.w x 10_3		Epiphytic cell g_{-1} w.wx103		% to total epiphytic cells	
Species	Range		Range		Range	
Rhodophyta	St. I	St. II	St. I	St. II	St. I	St. II
C. officianalis	27.1-29.9	19.8-24.6	27.9-30.5	20.7-26	96.4-98.92	94.62-96.36
Mean	28.20	21.87	28.83	22.90		
SD	1.49	2.47	1.45	2.76		
P. capillacea	2.1-2.67	1.1-1.5	2.4-3.1	1.5-1.9	79.29-87.50	68.42-83.3
Mean	6.99	3.90	8.30	5.20		
SD	2.33	1.30	2.77	1.73		
L. papillosa	4.4-8.6	3.5-5.8	4.8-9.4	4.5-6.3	84.85-91.67	77.78-92.06
Mean	6.20	4.50	6.90	5.23		
SD	2.16	1.18	2.32	0.95		

May	Cells g_{-1} w.w x 10_3		Epiphytic cell g_{-1} w.w x 10_3		% to total epiphytic cells	
Species	Range		Range		Range	
Phaeophyta	St. I	St. II	St. I	St. II	St. I	St. II
C. spinosa	1.1-2.1	0.7-1.2	1.4-2.5	0.9-1.5	78.57-85.71	56.67-85.71
Mean	1.67	0.92	2.00	1.70		
SD	0.51	0.26	0.56	6.85		
P. pavonia	1.8-2.8	1.7-2.5	2.3-2.9	2.3-2.8	78.26-96.55	73.91-89.29
Mean	2.23	2.02	2.50	2.50		
SD	0.51	0.43	0.35	0.26		

Table 4: Abundance (cells g^{-1} wet weight x10^{3} (of *Ostreopsis* cf. *ovata* cells on macroalgae species during May.

Temporal trend of *Ostreopsis*

The surface distribution of O. *ovata* reflects its nature an epiphytic form that attached on the thalli of the whole tested macroalgal groups with different frequencies, and during the different seasons. Based on average values (Table 3), and neglecting multiple factors as the seasonal variation of both macroalgal and microalgal species diversity and community structure, the magnitude of the abundance is arranged as Chlorophyta>Rhodophyta> Phaeophyta. The results highlighted the highest abundance observed between spring and autumn culminated the peak on chlorophycean species in July, while, it was sharply declined in winter when the abundance was at minimum in February.

The diatom *Navicula* sp. during February was the major contributor to total epiphytic diatom density (35% of the total diatom abundance). Meanwhile, no epiphytic O. *ovata* were found on Phaeophyta sp. in October. This species contributes significantly to the total epiphytic counts at the two sampling sites with higher abundances at the relatively far station comparing with each other's, following the same seasonal trend.

Abundance on macroalgae species

The field work involved the manipulation of naturally occurring macroalgal community omplexities in shallow rocky shore. The results show noticeable differences in the abundance of O. *ovata* depending on macroalgal host species, without statistically highlighting a clear substrate preference. The Chlorophytes *U. linza*, *U. compressa* and *U. fasciata* were particularly able to support very high abundances of *Ostreopsis* during May and July, and it was relatively less on *U. lactuca* in October. The maximum abundance on *U. linza* during May attained almost 1900 times the values estimated during February on the same species. The results declared Chlorophytes might be the optimal macroalgae for epiphytic *Ostreopsis*. As for the Rhodophyta, the maximum abundance was found on *C. officianalis* in July and October. The occurrence of the Phaeophyta species was restricted to May and July, and hence, abundance on the species was very low, among them, *P. pavonia* sustained the highest abundances.

July	Cells g^1 fwx10^3		Epiphytic cells g^1 fwx10^3		% to total epiphytic cells	
Species	Range		Range		Range	
Chlorophyta	St. I	St. II	St. I	St. II	St. I	St. II
C. pelluceda	49.60-67.89	30.10-36.50	63.20-70.90	38.30-40.20	75.73-95.75	78.59-90.80
Mean	56.60	33.00	66.53	39.07		
SD	9.87	3.24	3.95	1.00		
U. fasciata	63.90-71.20	47.70-56.30	69.50-79.20	52.20-68.90	89.46-89.90	78.81-91.38
Mean	66.53	51.40	73.60	61.60		
SD	4.05	4.42	5.02	8.55		
U. compressa	67.40-80.20	60.60-67.70	78.80-89.90	70.10-80.60	77.92-91.75	80.26-96.58
Mean	73.30	64.60	85.07	75.40		
SD	6.46	3.63	5.69	5.25		

July	Cells g^1 w.w x 10^3		Epiphytic cells g^1 w.w x 10^3		% to total epiphytic cells	
Species	Range		Range		Range	
Rhodophyta	St. I	St. II	St. I	St. II	St. I	St. II
J. rubens	28.60-36.60	27.60-31.80	36.70-40.20	35.40-38.40	77.93-91.04	77.86-85.03
Mean	34.87	29.77	36.27	37.07		
SD	5.66	2.10	4.17	1.53		
J. smooth	12.60-21.20	10.40-16.70	19.90-26.10	20.20-36.80	70.11-83.14	45.38-78.22
Mean	17.37	14.30	23.83	25.80		
SD	4.38	3.41	3.42	9.53		
P. capillacea	3.40-6.70	3.10-4.80	7.90-9.80	5.40-7.40	43.04-68.37	57.41-64.86
Mean	5.27	3.77	8.77	6.23		

July	Cells g^1 w.w x 10^3		Epiphytic cells g^1 w.w x 10^3		% to total epiphytic cells	
Species	Range		Range		Range	
Phaeophyta	St. I	St. II	St. I	St. II	St. I	St. II
P. pavonia	40.40-51.10	36.50-53.30	48.50-60.10	43.30-63.50	83.30-85.29	83.94-89.17
Mean	45.97	46.40	54.33	54.07		
SD	5.36	8.79	5.80	10.17		

Table 5: Abundance (cells g^{-1} wet weight x10^3 (of *Ostreopsis* cf. *ovata* cells on macroalgae species during July.

In May (Table 4), the highest abundance of *Ostreopsis* appeared attached mainly to the Chlorophyta species in order of magnitude *U. linza*>*U. fasciata*>*U. compressa*, representing 42.53-99.44% of the total epiphytes. Much lower densities found on Rhodophyta species, but, mainly were restricted to the thalli of *C. officianalis*, contributing >94% of the total. A few thousand cells were gathered on the surface of *L. papillosa*, higher than on *P. capillacea*. The lowest abundance appeared on the Phaeophyta *C. spinosa*.

The macroalgal community changed in July compared with May as *C. pelluceda* replaced *U. linza*, Rhodophyta species were well diversified (4 spp.), and species belong to Phaeophyta were not recorded. The diversified algal community might contribute to the abundance of *O. ovata* observed all over the year (Table 5).

The Chlorophytes were still the hosts having the highest abundances of *Ostreopsis* in the order of magnitude *U. compressa*> *U. fasciata*> *C. pelluceda*. Among the Rhodophytes, *J. rubens* seems the preferable host, where *Ostreopsis* contributed 77.86-91.04% of the total epiphytes. Again, the lowest abundance detected on *P. capillacea*.

Chlorophyta in October comprised *U. compressa* and *U. lactuca*, but, with almost similar *Ostreopsis* abundances. Among the four recorded Rhodophytes, the highest abundance, in May was found on *C. officianalis*, followed by *C. elongata* as the second important host, while, the lowest density observed on *C. mediterranean* (Table 6).

Despite the well diversified macroalgal community in February (8 species), the epiphyte density dropped to minimum compared with other months. *Ostreopsis* abundance did not exceed 12, 80 and 35 cell g^{-1} wet weight on the Chlorophyta, Rhodophyta and Phaeophyta species. The highest *Ostreopsis* abundance was restricted to the Rhodophytes *C. elongata* and *P. capillacea*. Moreover, its contribution to the total epiphytes was low, almost nil on *P. capillacea* and *P. pavonia*. The thalli of Phaeophyta *P. pavonia* and *C. spinosa* sustained the lowest abundance.

Interactions of the abundance of *Ostreopsis cf. ovata* with measured physical and chemical environmental parameters

The results of the statistical analyses (Tables 7-9) revealed water temperature the major environmental contributory affecting the occurrence *O. ovata* on examined macroalgae. The nutrients, NO_3 and PO_4 contribute significantly to the abundance of *Ostreopsis* on Chlorophyta spp., while, NO_2, SiO_4 beside PO_4 link significantly with its abundance on Rhodophyta. Salinity variations exhibit a weak, insignificant influence on the abundance attached to Chlorophyta spp., and it is almost of a negligible effect for Rhodophyta spp. A linear insignificant negative correlation was

October	Cells g^{1} wwx10^{3}		Epiphytic cells g^{1} wwx10^{3}		% to total epiphytic cells	
Species	Range		Range		Range	
Chlorophyta	St. I	St. II	St. I	St. II	St. I	St. II
U. compressa	50.60-58.30	39.50-40.10	63.20-76.90	40.20-48.70	74.25-92.25	83.02-98.26
Mean	55.33	40.67	68.53	45.73		
SD	4.14	1.53	7.34	4.80		
U. lactuca	50.60-67.10	32.40-46.10	63.80-76.90	38.70-48.30	77.25-87.26	83.72-95.45
Mean	56.67	39.33	68.73	43.73		

October	Cells g^{1} w.w x 10^{3}		Epiphytic cells g^{1} w.w x 10^{3}		% to total epiphytic cells	
Species	Range		Range		Range	
Rhodophyta	St. I	St. II	St. I	St. II	St. I	St. II
C. mediterranean	5.60-8.40	0.90-5.60	6.50-10.60	2.50-7.40	71.28-86.15	36-75.68
Mean	6.90	4.00	8.83	5.77		
SD	1.41	2.69	2.11	2.83		
C. elongata	14.10-17.30	11.20-18.40	18.60-20.40	16.40-21.40	75.49-89.18	68.29-86.83
Mean	15.60	14.70	19.47	18.17		
SD	1.61	3.60	0.90	2.80		
C. officianalis	17.30-33.40	13.40-21.20	19.40-40.40	18.70-26.40	83.67-89.18	65.69-80.30
Mean	24.93	16.37	29.47	21.83		
SD	8.08	4.22	10.53	4.05		
J. rubens	11.40-14.30	9.50-11.20	16.40-19.40	14.40-16.40	69.51-75.81	60.51-77.78
Mean	13.27	10.70	18.13	15.50		
SD	1.62	1.04	1.55	1.01		

Table 6: Abundance (cells g^{1} wet weight x10^{3} (of *Ostreopsis* cf *ovata* cells on macroalgae species during October.

February	Cells g^{1} w.w x 10^{3}		Epiphytic cells g^{1} w.w x 10^{3}		% to total epiphytic cells	
Species						
Chlorophyta	St. I	St. II	St. I	St. II	St. I	St. II
U. linza	0.04	0.05	2.40	2.10	1.67	2.38
U. fasciata	0.12	0.10	1.50	1.60	8.00	6.25
U. compressa	0.12	0.07	0.19	0.13	63.16	53.85
Total	0.28	0.22	4.09	3.83		

February	Cells g^{-1} w.w x 10^{3}		Epiphytic cells g^{-1} w.w x 10^{3}		% to total epiphytic cells	
Species						
Phaeophyta	St. I	St. II	St. I	St. II	St. I	St. II
P. pavonia	0.01-0.22	0.07-0.12	1.1-2.3	1.36-2.1	0.48-20	4.12-8.89
Mean	0.14	0.10	1.83	1.72		
SD	0.12	0.03	0.64	0.38		
C. spinosa	0.25-0.35	0.19-0.32	2.1-3.3	1.05-2.1	7.58-16.67	9.05-30.48
Mean	0.30	0.26	2.73	1.72		
SD	0.05	0.07	0.60	0.58		

Table 7: Abundance (cell g^{1} wet weight x10^{3}(of *Ostreopsis* cf *ovata* cells on macroalgae species during February.

found with the pH variations. The highly significant correlation between salinity and nutrient concentrations indicate discharged fresh water the main source of nutrients supply.

The multiple regression equation for *O. ovata* (Cell g^{1} wet weight) on Chlorophyta is expressed as:

= -147.49 - 4.87* oC - 28.92* NO3 + 20.22*PO4..... R^{2} =0.88, n=27

explain the significant importance of these parameters in combination, on Rhodophyta as:

= -20.44+2.09* oC - 6.47*NO2 - 2.10* SiO4 + 2.2 *PO4... R^{2} =0.36, n=39

explain about 36% of the *O. ovata* variations.

The relation between *Ostreopsis* abundance on the green algae *U. compressa* and measured environmental factors was also done. This macroalgal species was chosen being a perennial form. The results Table 10 support others previously mentioned factors for all Chlorophyta spp., but, salinity negatively correlated with its abundance and PO4 concentration seem more effective.

Variables	St. I	St. II	°C	Salinity	pH	DO	DO%	NO_2	NO_3	NH_4	SiO_4	PO4
St. I	**1**											
St. II	**0.90**	**1**										
oC	**0.80**	**0.73**	**1**									
Salinity	0.14	0.21	-0.28	**1**								
pH	-0.24	-0.19	0.28	**-0.67**	**1**							
DO	**0.52**	**0.58**	**0.74**	0.09	**0.45**	**1**						
DO%	-0.10	0.02	0.25	-0.05	**0.77**	**0.76**	**1**					
NO_2	-0.15	-0.01	-0.32	**0.81**	-0.12	0.33	**0.49**	**1**				
NO_3	**0.38**	**0.42**	-0.05	**0.96**	**-0.71**	0.20	-0.11	**0.69**	**1**			
NH_4	0.20	0.26	**0.64**	-0.33	**0.84**	**0.87**	**0.89**	0.11	-0.28	**1**		
SiO_4	0.33	0.24	**0.73**	**-0.86**	**0.60**	0.31	0.14	**-0.76**	**-0.71**	**0.55**	**1**	
PO_4	**0.39**	0.30	**0.78**	**-0.82**	**0.57**	0.35	0.14	**-0.75**	**-0.66**	**0.56**	**1**	**1**
Values in bold are different from 0 with a significance level alpha=0.05												

Table 8: Pearson's correlation values for *Ostreopsis cf. ovata* on Chlorophyta species.

Variables	St. I	St. II	oC	Salinity	pH	DO	DO%	NO_2	NO_3	NH_4	SiO_4	PO_4
St. I	**1**											
St. II	**0.97**	**1**										
oC	**0.53**	**0.54**	**1**									
Salinity	0.03	-0.02	-0.03	**1**								
pH	-0.26	-0.21	-0.04	**-0.68**	**1**							
DO	0.23	0.25	**0.76**	0.09	**0.36**	**1**						
DO%	-0.25	-0.22	0.05	-0.23	**0.87**	**0.63**	**1**					
NO_2	**-0.34**	**-0.36**	-0.31	**0.61**	0.15	0.31	**0.57**	**1**				
NO_3	0.23	0.19	0.27	**0.94**	**-0.76**	0.23	**-0.34**	**0.38**	**1**			
NH_4	0.00	0.04	**0.47**	**-0.36**	**0.81**	**0.83**	**0.89**	0.25	-0.31	**1**		
SiO_4	**0.35**	**0.39**	**0.71**	**-0.73**	**0.43**	**0.43**	0.16	**-0.67**	**-0.47**	**0.54**	**1.00**	
PO_4	**0.39**	**0.43**	**0.77**	**-0.66**	**0.36**	**0.47**	0.12	**-0.66**	**-0.38**	**0.53**	**0.99**	**1**
Values in bold are different from 0 with a significance level alpha=0.05												

Table 9: Pearson's correlation values for *Ostreopsis* cf. *ovata* on Rhodophyta species.

	St. I	St. II	oC	Salinity	pH	DO	DO%	NO_2	NO_3	NH_4	SiO_4	PO_4
St. I	1											
St. II	0.96	1										
oC	0.88	0.95	1									
Salinity	-0.12	-0.24	-0.30	1								
pH	-0.09	0.10	0.34	-0.64	1							
DO	0.45	0.56	0.75	0.03	0.56	1						
DO%	-0.10	0.04	-0.32	-0.10	0.82	0.81	1					
NO_2	-0.38	-0.40	-0.27	0.77	-0.02	0.38	0.52	1				
NO_3	0.14	0.01	-0.08	0.97	-0.69	0.12	-0.16	0.64	1.00			
NH_4	0.24	0.40	0.65	-0.35	0.87	0.89	0.91	0.19	-0.31	1		
SiO_4	0.56	0.68	0.74	-0.86	0.61	0.36	0.21	-0.70	-0.72	0.56	1	
PO_4	0.62	0.73	0.78	-0.82	0.58	0.39	0.21	-0.69	-0.66	0.57	1.00	1

Table 10: Pearson's correlation values for *Ostreopsis* cf. *ovata* on Ulva compressa.

The Pearson linear correlation between the epiphytic O. *ovata* concentrations was significant ($p<0.01$, N = 55, rs = 0.71), with an explained percentage of variation of R2 = 0.50.

DISCUSSION

The present study represents the first specific contribution on the epiphytic O. *ovata* in the Egyptian Mediterranean waters. Previously, this species was recorded planktonic form the investigated area, with a maximum abundance over 1980 cell l^{-1} [7]. The study declared O. *cf. ovata* is not an obligate epiphytic species; since it is able to colonize the macroalgae thalli of 14 species of different groups include 5 Chlorophyta, 7 Rhodophyta and 2 Phaeophyta. A reasonable body of ecological studies has been conducted on the potential role of environmental on the dynamic occurrence of *Ostreopsis*. However, in many cases, contrasting results are found for the same taxon from different areas and the role of ecological habitats in the facilitation of attachment has rarely been considered [7,11]. Most of the research focused on the effects of seawater temperature, nutrients, and salinity on *Ostreopsis* sp. populations.

The statistical analyses illustrate how environmental factors in the sampling sites might influence epiphytic *Ostreopsis* abundance, particularly water temperature and the dissolved nitrate and phosphate concentrations; temperature was the major factor. However, the impact of temperature alone during the summer period, which was represented here by July, seems less evident may be due to strong interactions with other unmeasured physical parameters as light intensity and photoperiod wave activity and some biotic factors that could also be involved such as the availability of some seasonal macroalgal substrates and the competition with other microphytes [20,26]. Also allelopathy has been often suggested among the biotic factors crucial to *Ostreopsis* [27]. A concise summary of the effects of surface seawater temperature, salinity and nutrient concentrations on *Ostreopsis* sp. populations in temperate regions can be found in Accoroni and Totti's review [11].

The maximum abundance of *Ostreopsis* in July took place at the highest water temperature (28.37°C), and a relative decrease of abundances observed in May and October at temperature between 22.34°C and 25.1°C, reaching its minimum in February associated with the lowest temperature all over the year (17.63°C). A bloom event reported with temperature not exceeding 22.5°C while, the results of Cohu et al. highlighted substantial seasonal variations in the abundance of *Ostreopsis* within an optimum growth temperature ranging from 23°C to 27.5°C. Moreover, temperatures from 22 to 30°C seem to favor *Ostreopsis* sp. blooms in temperate zones areas the Mediterranean Sea and the New Zealand coasts but the link between temperature and *Ostreopsis* growth is still not so clear and the relationship with the seawater temperature is not the same in all the geographic areas [28-30]. Also, *Ostreopsis* strains sampled from the tropics have exhibited growth optima in the range 25-30°C [26]. Until now, O. *ovata* and O. *siamensis* have been detected at the minimal temperatures of 13°C-14.5°C in the Mediterranean Sea, yet were also recorded when water temperature dropped below 8°C [31,32]. Such results reveal a high tolerance to extreme climate changes [33]. Therefore, Yamaguchi et al. based on field and experimental studies demonstrated a wide tolerance ability of O. *ovata* to a wide temperature ranges, offering some reasons to explain its recent geographical expansion and occurrence in temperate areas [34]. These conflicting results may likely reflect geographical differences [8]. In the Mediterranean Sea, the seasonal trend of *Ostreopsis* sp. clearly follows temperature seasonality [31]. The observed marked heterogeneity in the abundance of O. *ovata* among the examined macroalgae of different groups, beside other substrates could partially explain its spatial variability [35].

Other ecological parameters of weak contribution to the variation of O. *ovata* were salinity and pH, in particular on Chlorophyta. The maximum abundance of this species occurred at 35.43 in July, while, at the same salinity level (35.8) in February, the abundance was at minimum. The results thus explain the insignificant correlation of salinity with abundance of *Ostreopsis*, and its negligible effect on *Rhodophyta* sp. The intermediate salinity levels, a sign of incoming discharged waters might have positive impact on the epiphytic *Ostreopsis* to proliferate. According to Delgado et al. freshwater source should have a negative impact on O. *ovata* growth via a decrease of salinity, as observed on O. *lenticularis* and others dinoflagellates [36]. Morton et al. reported that O. *siamensis and* O. *heptagona* displayed maximal growth at a salinity value of 33 [26]. In contrast, O. *ovata* isolated from Hawaii were found to be negatively correlated with salinity in agreement with Carlson and Tindall [37,38].

The role of depth was assessed at two target sites where samples were collected at 0.5 m and 1 m depth. Despite the very narrow distance between the two sites, the abundance of *Ostreopsis* was always higher at the relatively far site; more strong hydrodynamic action at St. II may be involved affecting abundance [6]. The abundance at St. II compared with St. I contributed 71.43-82.91% for Chlorophyta, 58.78-83.19% for Rhodophyta and 61.44-99.03% for Phaeophyta. While several authors found a significant negative relation of *Ostreopsis* with depth, other denied such conclusion [39,40]. However, one study contradicts these conclusions indicating that *Ostreopsis* sp. is better adapted to 'slightly shaken' environments [41]. The oxygen saturation decrease in summer was related to the increase in seawater temperature. It is might also be linked to the higher consumption of oxygen related to organic matter decomposition during O. *ovata* main occurrence; the negative correlation between these two factors might explain such observation.

The results showed the measured nutrient concentrations values falling within the expected range for a shallow area subjected to a moderate/temporally anthropic impact [7]. The high NO_3 concentrations with the main *Ostreopsis* occurrence periods, and that of PO_4 in July as well certainly indicate arrival of nutrients supply through eutrophication processes. Since the study area is a summer resort that starts annually from May, the increased population density might represent a crucial anthropogenic reason to promote the occurrence of *Ostreopsis* in such urbanized area, similar to other data [42]. The statistical analyses declared highly significant correlation between salinity and nutrient concentration indicates same source of nutrient supply. The analyses showed the positive significant correlation between NO_3, PO_4 and abundance of O. *ovata* on Chlorophytes, and the same for NO_2 and PO_4 for Rhodophytes, and also with a significant positive correlation with SiO_4, in agreement with Parsons and Preskitt [37]. Such high nutrient concentrations provide an indication of eutrophication that might play a significant part to control the epiphytic *Ostreopsis* abundance. Subsequently, there are some conflicting results regarding the relationships of nutrients with *Ostreopsis* proliferation trends in field observations of tropical and temperate areas [39]. However, often, and particularly in coastal areas, eutrophication appears to be directly linked to the occurrence of harmful algal blooms and worldwide *Ostreopsis* sp. appears to proliferate both in eutrophicated and oligotrophic areas [43-45]. Meanwhile, anthropogenic nutrient enrichment might have an indirect impact on *Ostreopsis* sp. abundance by inducing a shift in macroalgae coverage frequency and/or composition, which lead to an additional difficulty in ecological studies [46]. Silicate seems to match with the abundance of O. *ovata* on *Rhodophyta* species; it is unexpected for dinoflagellates which do not specifically use silicate for their growth, in contrast to diatoms.

Another environmental factor seems to affect the abundance of O. *ovata* was the N:P ratios. The N:P ratios calculated based on the Redfield model [47]. Working on this principle, high N:P ratios during May and February may be taken as indicative of low P availability, whereas low values (<16) during July and October might indicate N- limitation [48]. The N:P ratios matched in strong negatively correlation with the abundance of *Ostreopsis* on *Chlorophyta* sp. (r=0.38 and 0.25 at St. I and II, respectively, n=27, p at 0.5), and corresponded in high significance with its abundance on Rhodophytes (r=0.54 and 0.55). The indirect effect of N:P ratio through changed macroalgal composition was discussed [49,50].

The results declared O. *ovata* found on all the examined macroalgae, exhibits the different host preferences; Ulva species were the optimal macroalgae for the epiphytic O. *ovata*. However, factors as algae structure and surface area, regional occurrence and coverage percentage, and presence of allelopathic compounds may determine the host preference of the species [7]. According to the field observations, *Ulva* sp. predominated all the investigated period, with different frequency degrees. As far as macroalgae are concerned, Cohu et al. [42] were unsuccessful in establishing significant preferences, while, Accoroni et al. [11] noted *Ostreopsis* sp. to display algal host preference, and certain macoalgae species have the ability of support very high abundance of O. *ovata* offering evidences that algal host preferences is species-specific [8]. The present study documented the significant importance of *Ulva* sp. as the suitable host of O. *ovata* in Alexandria waters, in contrasting with others in the Mediterranean Sea that show O. *ovata* most often found on Phaeophyta and *Rhodophyta* sp. [31].

CONCLUSION

The potential toxic O. *ovata* represents another additional threat to economy and public health in Alexandria. To our knowledge, this is its first study as epiphytic form in SE Mediterranean Sea taking into account diverse ecological and biological factors. Temperature, PO_4 and NO_3 seem major contributors influencing its abundance. *Ostreopsis ovata* had the ability to attach to a diversified macroalgae species, with preference to Chlorophyta. Since O. *cf. ovate* cells are loosely attach to the substrata and can be easily removed and re-suspended in the water column, future study of wave action in Alexandria waters presents untapped field of research with great potential.

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