

Seasonal and spatial variations of the dinoflagellate *Ostreopsis siamensis* in the Lebanese coastal waters (Eastern Mediterranean)

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Abstract – The dinoflagellate *Ostreopsis siamensis* was studied as part of a broad national monitoring program along the Lebanese coastal waters. The monthly and spatial variations of its density in water samples were carried out at 4 different rocky stations from September 2000 to December 2001 and for a longer period (April 1997-December 2001 and January 2010 to December 2010) in one of the stations.

Results showed that *O. siamensis* is present almost in all rocky shores along the Lebanese coastline from south to north between May and November when the surface seawater temperature ranges from 23 to 30.5 °C, with a maximum in abundance between July and September. In 2001, the population densities reached 10 500 cells/L in July and 5 700 cells/L in September at two stations both located in the southern coast. In 2010, the analysis of larger water volumes revealed the presence of this species in February, March and April; however densities were lower than 1-3 cells/L. Significant positive correlation was observed ($P < 0.01$) between the occurrence of the species and nitrate concentrations, whereas the correlation was negative with orthophosphates. Our results suggest that *O. siamensis* is a thermophilic species. It constitutes a permanent component of the microalgal flora of Lebanese waters and its abundance depends on water enrichment.

Eastern Mediterranean / Lebanon / Dinoflagellate / *Ostreopsis siamensis*

INTRODUCTION

This study was initially reported at the International Conference on *Ostreopsis* Development (Villefranche-sur-Mer, April 2011) which presentations lead to the publication of a special issue in *Cryptogamie Algologie* (Abbate *et al.*, 2012; Accoroni *et al.*, 2012; Asnaghi *et al.*, 2012; Blanfuné *et al.*, 2012; David *et al.*, 2012; Fraga *et al.*, 2012; Guidi-Guilvard *et al.*, 2012; ICOD 2012 a & b; Illoul *et al.*, 2012; Lemée *et al.*, 2012 a & b; Monti & Cecchin, 2012; Moreira *et al.*, 2012; Penna *et al.*, 2012; Rossini & Sala, 2012; Sechet *et al.*, 2012; Vanucci *et al.*, 2012; Vila *et al.*, 2012; Zingone *et al.*, 2012).

In recent years, reports of the presence of toxic algae and associated toxicity in seafood have been studied extensively in many Mediterranean countries (Zingone, 2010). Blooms of benthic dinoflagellates belonging to the tropical genus *Ostreopsis* have been reported as an increasingly common phenomenon in temperate regions worldwide (Shears & Ross, 2009; Aligizaki, 2010).

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The first records of benthic dinoflagellate *Ostreopsis* species in Lebanese coastal waters dates from 1979 (Abboud-Abi Saab, 1989). During the last decade, reports on the occurrence of *Ostreopsis* spp. have increased rapidly along eastern Mediterranean coasts: in Egyptian coast (Ismael & Halim, 2006), and in North Aegean Sea (Aligizaki, 2010). Similar increases have been reported for the western Mediterranean (Ciminiello *et al.*, 2008; Mangialajo *et al.*, 2011). Populations with numerous individuals of *Ostreopsis* species were usually recorded during the warm period, while summer blooms of these species, detected in the Tyrrhenian and South Adriatic Seas in Italian coasts have been associated with human health problems, such as respiratory and skin irritations (Sansoni *et al.*, 2003; Zingone, 2010).

In Lebanon, until now, studies on phytoplankton have shown no *Ostreopsis* species other than *Ostreopsis siamensis*, even though, there are extensive studies from Lebanon on other harmful microalgae such as those causing harmful algal bloom and *Heterosigma akashiwo* that was detected in May 2007 (Abboud-Abi Saab & El-Bakht, 1998; Abboud-Abi Saab *et al.*, 2006; 2008b).

In the framework of a broad national monitoring project, *Ostreopsis siamensis* in addition to other toxic or potentially toxic microalgae, the following parameters were recorded as part of the monitoring program: temperature, salinity, nutrients (nitrites and nitrates, orthophosphates), chlorophyll *a*, fecal coliforms and fecal streptococci. The presence of *O. siamensis* was limited to rocky sites and the neighboring sandy zones and only during warm periods and extending temporally at most of the rocky littoral sites in the warmer months (Abboud-Abi Saab, 2010). The aim of the present study is to look into the temporal and spatial variations of the density of *O. siamensis* in water samples and its possible relationships between abundance and environmental factors at four different rocky sites along the Lebanese coast.

MATERIAL AND METHODS

Four rocky sites were selected as part of the National Monitoring Program covering a large part of the Lebanese coast extending from Tripoli in the north to Naqoura in the south. The description and position of the four investigated stations are presented in Table 1 and Fig. 1. Surface water samples were collected on a monthly basis in the morning from September 2000 to December 2001 at all stations. Supplementary samples were collected at Bat-14 station from April 1997 to December 2001 and from January 2010 to December 2010. Sea surface temperature (SST) and salinity (SSS) were measured simultaneously. Fresh samples for nutrient analyses were stored at -20°C until analysis. Orthophosphate (P-PO₄), nitrite (N-NO₂) and nitrate (N-NO₃) concentrations were analyzed according to Strickland and Parsons (1968). Surface water samples were immediately fixed with lugol's solution in order to estimate the abundance of dinoflagellates in the water column using Utermöhl's method (1958). An adequate volume of 50 mL or 100 mL was then left to settle for 48h. During the sampling performed in 2010, for a more detailed investigation, larger volumes up to 3 L, were screened through a 20 μm collector and samples were preserved in 4% borate-buffered formaldehyde.

Spearman non-parametric correlation matrix was applied in order to investigate possible relationships between *O. siamensis* density and five environmental parameters and N/P ratio on 105 samples from four stations using SPSS software.

Table 1. Coordinates and characteristics of the 4 rocky stations monitored on the Lebanese coastal waters

<i>Station & code</i>	<i>Locality in Lebanon</i>	<i>Coordinates</i>	<i>Depth (Z) and distance from the coast (D)</i>	<i>Study period</i>
Tri-20	North	E 35° 44.160' N34° 22.054'	Z = 1m D = 6m	September 2000-December 2001
Bat-14	North-Central	E 35° 39.413' N34° 15.090'	Z = 0.5m D = 4m	April 1997-December 2001 January 2010-December 2010
Sur-8	South	E 35° 18.171' N33° 28.032'	Z = 0.5m D = 10m	September 2000-December 2001
Nak-10	South	E 35° 07.254' N33° 06.977'	Z = 1m D = 10m	September 2000-December 2001

RESULTS

Descriptive statistics (mean, standard deviation, minimum and maximum values) of the parameters measured at the four stations studied between September 2000 and December 2001 are given in Table 2.

Temporal and spatial variations of environmental parameters

During the study period, in all sampling stations, the average values (\pm SD) of the sea surface temperature varied from 24.03°C (\pm 3.93) at Bat-14 to 22.79 °C (\pm 4.65) at Sur-8 and the range from a minimum of 16.2 °C in January (Sur-8) to a maximum 30.5°C in August (Bat-14). The average values (\pm SD) of salinity varied between 39.0 (\pm 0.95) at Tri-20 and 39.34 (\pm 0.21) at Naq-10. A maximum of 39.6 was recorded in November 01 at Bat-14 and a minimum of 35.96 in February at Tri-20 (Table 2). The mean values (\pm SD) of nitrite varied from 0.04 (\pm 0.02) μ M/L at Tri-20 to 0.18 (\pm 0.12) μ M/L at Sur-8. The values ranged between 0.006 (Tri-20, Naq-10) and 0.47 μ M/L (Sur-8). For nitrate, the mean values (\pm SD) vary from 0.55 (\pm 0.04) μ M/L at Naq-10 to 4.45 (\pm 3.23) at Sur-8. A maximum of 11.64 μ M/L was recorded at Sur-8 and a minimum of 0.02 μ M/L at Nak-10 in December 01. The average values (\pm SD) of orthophosphates varied between 0.08 (\pm 0.05) μ M/L at Sur-8 and 0.38 μ M/L (\pm 0.64) at Bat-14. The values ranged between 0.005 and 2.73 μ M/L (January 2001) respectively at Naq-10 and Bat-14 (Table 2).

Spatial and monthly variations of the density of *Ostreopsis siamensis*

Between September 2000 and December 2001, the average values (\pm SD) of the density of *O. siamensis* varied between 212 \pm 534 cells/L at Bat-14 and 648 \pm 1 435 cells/L at Sur-8. The density ranged between zero at all stations and 10 560 cells/L at Sur-8. An annual cycle was detected between May and November with maximum in July, August or September depending on the station or the year, when the surface sea water temperature ranged between 23 and 30.5°C; it was absent between January and May at all stations except Sur-8 where it was detected in low densities in February and March (Fig. 2).

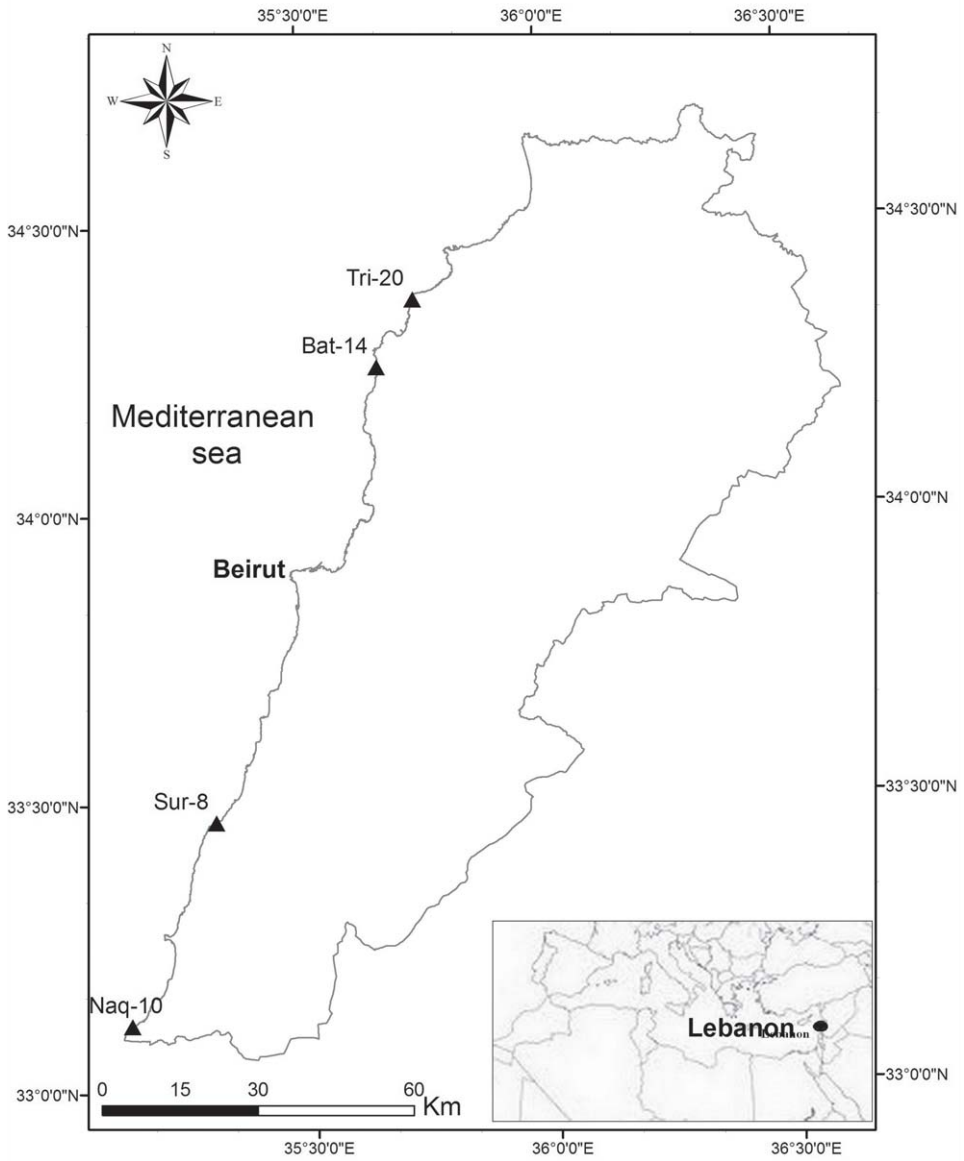


Fig. 1. Sampling sites of *Ostreopsis siamensis* in the Lebanese coastal waters.

Table 2. Descriptive statistics (Mean, Standard Deviation, Minimum and Maximum values) of the parameters measured at the 4 stations studied between September 2000 and December 2001 in the Lebanese coastal waters. Highest and lowest values across the four stations are in bold

Stations Parameters	Tri-20	Bat-14	Sur-8	Nak-10
	Mean \pm SD Min-Max	Mean \pm SD Min-Max	Mean \pm SD Min-Max	Mean \pm SD Min-Max
SST (°C)	23.71 \pm 3.95 18.1 – 29.5	24.03 \pm 3.93 19 – 30.5	22.79 \pm 4.65 16.2 – 29.3	23.7 \pm 4.11 18 – 30
SSS	39.00 \pm 0.95 35.96 – 39.51	39.13 \pm 0.27 38.73 – 39.6	39.04 \pm 0.26 38.59 – 39.47	39.34 \pm 0.21 38.99 – 39.57
N-NO ₂ (µM/L)	0.04 \pm 0.02 0.01 – 0.07	0.085 \pm 0.05 0.006 – 0.183	0.18 \pm 0.12 0.05 – 0.47	0.06 \pm 0.07 0.006 – 0.28
N-NO ₃ (µM/L)	1.43 \pm 2.67 0.1 – 11.02	1.61 \pm 0.44 0.35 – 1.98	4.45 \pm 3.23 0.85 – 11.64	0.55 \pm 0.4 0.02 – 1.33
P-PO ₄ (µM/L)	0.3 \pm 0.25 0.08 – 0.9	0.38 \pm 0.64 0.014 – 2.73	0.08 \pm 0.05 0.006 – 0.20	0.13 \pm 0.13 0.005 – 0.53
N/P ratio	7 \pm 12 0.37 – 49	8 \pm 14 0.6 – 59	59 \pm 39 13 – 158	10 \pm 11 0.3 – 38
<i>O. siamensis</i> density(cells/L)	501 \pm 1 150 0 – 3 465	212 \pm 534 0 – 2 112	1 500 \pm 3 010 0 – 10 560	648 \pm 1 435 0 – 5 742

The study was performed for a longer period at Bat-14: density was generally very low in 1997 (in September), 1998 (in May and July) and in 2000 (in June and September); in 2001, *Ostreopsis* was found only between June and October with maximum numbers in August (2 112 cells/L) while in 2010 its presence was extended to almost the whole year (Fig. 2 and 3).

Most cells were opaque after being fixed in lugol's solution. In November-December, more empty cells and free epitheca and hypotheca were found, which were useful to observe the tabulation. No resting stage has been observed in the water samples.

It is interesting to note that *O. siamensis* was not the only epiphytic dinoflagellate found in the samples; association with the genera *Prorocentrum*, (*P. lima*, *P. mexicanum*, *P. gracile*, *P. arcuateum*) and *Amphidinium* (mainly *A. carterae*) were observed. Moreover, benthic diatoms of the genera: *Cocconeis*, *Coscinodiscus*, *Licmophora*, *Cylindrotheca*, *Striatella* and *Nitzschia* were also found along with the epiphytic dinoflagellates.

Statistic results

The Spearman correlation analysis (n = 105) (Table 3) showed that *Ostreopsis* density shows a high negative correlation ($p < 0.01$) with P-PO₄ ($r = -0.75$). Lower positive correlations are found with nitrates ($r = 0.57$), nitrites ($r = 0.31$) and N/P ratio ($r = 0.34$).

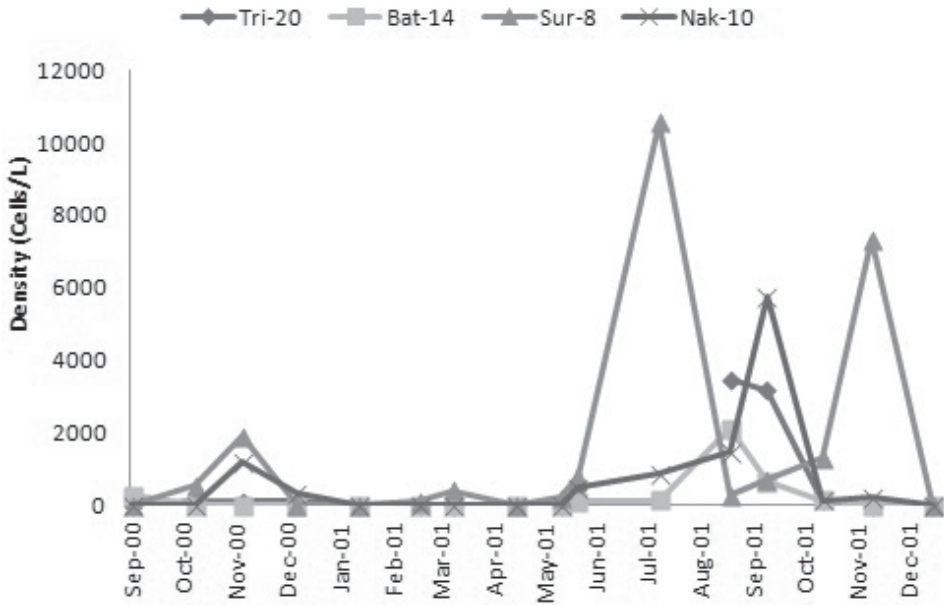


Fig. 2. Monthly variations of the abundance of *Ostreopsis siamensis* at 4 sites from September 2000 to December 2001 in the Lebanese coastal waters.

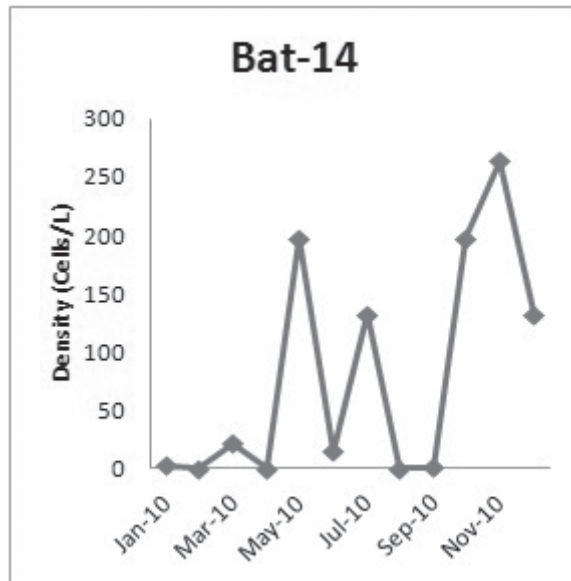


Fig. 3. Monthly variations of the abundance of *Ostreopsis siamensis* from January to December 2010 at one site in the Lebanese coastal waters.

Table 3. Correlation coefficient matrix (Spearman correlation) between parameters at 4 stations in Lebanese coastal waters

	SST	SSS	P-PO4	N-NO2	N-NO3	N/P	<i>Ostreopsis</i>
SST	1	0.53**	-0.12	0.03	-0.15	0.48**	0.05
SSS		1	-0.03	0.08	-0.25**	0.33*	-0.11
P-PO4			1	-0.10	-0.04	-0.30**	-0.75**
N-NO2				1	0.44**	0.19**	0.31**
N-NO3					1	0.11	0.57**
N/P						1	0.34**
<i>Ostreopsis</i>							1

K = n-2 = 103 Correlation at the level: *P < 0.05 **P < 0.01.

DISCUSSION

According to previous results, the values of SST and SSS match with the majority of the results reported from Lebanese coastal waters (Abboud-Abi Saab *et al.*, 2008a). The broad inter-station variability of nutrients reflects the impact of the terrestrial input in such littoral stations despite the fact that the studied stations are a long way from direct continental input (stations Tri-20 and Sur-8) (Table 2). The latter stations displayed also a wide range of standard deviation of nitrate while Tri-20 and Bat-14 of orthophosphates due to high monthly variations.

These nutrients are mostly low in other sampling sites but generally with higher values in coastal stations than in offshore ones (Abboud-Abi Saab *et al.*, 2008a). The large amount of nitrate reaching the seawater near the river (Sur-8) and the great inflow of phosphate discharges into the seawater at the Selaata factory (station Bat-14) and at Tri-20 in the direction of the current, often lead to a dystrophic situation where N/P ratio is frequently unbalanced. The autotrophic species *O. siamensis* can benefit from these discharges and thus a significant correlation was observed between its abundance and nutrients.

It is interesting to note that the site Bat-14 where *Ostreopsis* abundance was lowest, is located in a station with a higher mean of P-PO4 levels, whereas Sur-8 which shows the highest abundance levels of *O. siamensis* (10 560 cells/) have highest mean values of nitrate. In a previous study (Abboud-Abi Saab *et al.*, 2008a), Naq-10 and Bat-14 were reported as having the lowest annual mean value of chl-*a* (0.09 and 0.11 µg/L respectively), whereas station Tri-20 showed a slightly higher annual mean value (0.22 µg/L) and was thus considered as slightly enriched either by orthophosphates or nitrate while Sur-8 which is affected indirectly by fresh water input showed 0.56 µg/L. It seems plausible that in the case of this station, nitrate favor the development of phytoplankton and the epiphytic populations of *O. siamensis*.

In this study, *O. siamensis* was detected in the water column only between May and November and the highest densities were recorded simultaneously with the warm months in the first 3 stations while its occurrence at Sur-8 was extended from February to November presumably because this

station is under the influence of continental discharges in its vicinity that are loaded with fertilizers and other domestic waste. It is possible that *O. siamensis* was previously missed in this station or not noted due to Utermöhl' method which is based on a relatively small water volume; it becomes obvious during the period of low abundance levels which corresponds to the relative cold temperature months (December-April); in recent data (2010) at Bat-14, some cells were detectable in February and March in larger volume (3 L of screened samples through a 20 µm collector). It is therefore likely that this species is present all year around, as initially postulated by Penna *et al.* (2005).

Our data on the study of *Ostreopsis* are “relatively” old (pre-2000) and are rare as very few had studied this species before it became a nuisance (Zingone 2010). Comparing with the result of these old data, density was limited to warmer months (Figs 2 & 3) while in latest data the presence of *Ostreopsis* was extended to almost the whole year.

The pattern of seasonal distribution is congruent with the results found in North Aegean Sea and along Italian coasts (Aligizaki & Nikolaidis, 2006; Pistocchi *et al.*, 2011): high densities during warm months, characterized by high temperature, salinity, and water column stability. These results in Bat-14 are in agreement with the observed trends in North Western Mediterranean Sea by Mangialajo *et al.* (2011), who also reported higher cell abundances mostly in mid-summer (end of July), and a second bloom in November. In this respect water temperature acts as an additional stimulus to initiate the development as also observed by Pistocchi *et al.* (2011), who consider water temperature and nutrient availability controlling and promoting the occurrence and intensity of blooms.

However, nutrients and water temperature are not the only factors affecting the growth and distribution of epiphytic dinoflagellates. The presence of this species sometimes outside of the range cited above or far in water or in sandy places could be due to the position of the site or the hydrodynamic conditions; in rough seas, more cells may detach and disperse. Also, the geomorphology of coastal environment is an important factor in the accumulation or the dispersion of cells. In Lebanon, there are no closed bays and water is being continuously mixed preventing water stagnation which helps the dispersion of nutrients, pollutants and cells; this likely explain why some cells of *Ostreopsis* were found in stations hundred of meters away from the rocky stations. Further studies should consider other factors such as duration and intensity of light, morphology of the coastline and substrate availability that appear also to influence growth and distribution.

Concerning human health and despite the extensive presence of rocky shores and substrate availability in Lebanon, so far no remarkable or undesirable events have been recorded. Effects on the health of sea urchins in relation to the density of *O. siamensis* suggest strong negative impact on this herbivore: the sea urchin densities declined by 56-60% at bloom sites in a study reported from northern New Zealand in 2004 (Shears & Ross, 2009). The development of this epiphytic species may partly explain the scarcity of the sea urchin *Paracentrotus lividus* from the Lebanese coast. Relatively little is known about these blooms or their impact on other local organisms.

This preliminary study presents a baseline for future studies. Further studies should also consider benthic sampling, since an increase in the development of monospecific green algae has been noticed in many Lebanese rocky zones (personal observations) especially in the threatened vermetid facies, referred to also as platforms or terraces which are an emblematic feature of the Lebanese and Levantine coasts.

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