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Occurrences of the toxic dinoflagellate *Ostreopsis ovata* in relation with environmental factors in Kerkennah Island (Southern coast of Tunisia)

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ABSTRACT

Objective: To study the seasonal and monthly variability of the toxic dinoflagellate *Ostreopsis ovata* (*O. ovata*) in relation to environmental parameters in Kerkennah Island.
Methods: Three water samples replicate of one-litter were taken daily for ten consecutive days on 12 months. All sampling water was kept in the dark at ambient temperature until their microscopic observation. Environmental variables such as salinity and temperature were measured in the field concomitantly as phytoplankton sampling. Nutrients (ammonium, nitrite, nitrate, phosphate and silicate) were analysed in laboratory with Auto-analyser Luebbe type. Cell identification and enumeration in water samples were performed with an inverted microscope after the sedimentation.
Results: The highest abundance of *O. ovata* was recorded in summer. Analysis of variance showed significant difference of abundance between seasons, whereas no significant difference for month was detected. Factorial analysis ordination showed a positive correlation of *Ostreopsis* mainly with temperature and low correlation with nitrite and nitrate whereas the second axis (with 26.30% of variance) showed that *Ostreopsis* was correlated with temperature and salinity.
Conclusions: The maximum abundance of *Ostreopsis* was reached in summer when temperature was high and a low relationship between *O. ovata* and nutrient was detected.

1. Introduction

Events of harmful algal blooms have increased all around the world over the past several decades, with an increase in the diversity of the harmful species and the number of areas affected[1]. For instance, worldwide occurrence of the toxic benthic dinoflagellates genus *Ostreopsis* Schmidt has increased during the last 15 years[2].

Species of the genus *Ostreopsis* are preferentially epiphytic/epibenthic and grow in shallow waters, on macrophytes or directly on the abiotic substrates. But with rapid proliferation, cells may detach from the substrate and are found in the water column[3]. Cells in the water column can form dense aggregates occurring as floating clusters[4]. *Ostreopsis* is known to produce palytoxin and analogues; Palytoxin is one of the most potent phycotoxins in tropical seafood intoxications[5-7]. 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, in events in Italy and Spain[8]. These syndromes may be caused by simple contact and/or inhalation of cells (or toxins), and can affect people near the shore exposed to marine aerosols during *Ostreopsis* bloom events[8,9].

The phenomenon of bloom occurs frequently in the Gulf of Gabes. In 1935, Fremy and Feldman[10] described a particular phenomenon of algal phytoplankton proliferation in Kerkennah island caused by cyanobacterium *Trichodesmium* and named "Muffa". In July 1988, a similar phenomenon was described in

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different areas of the Gulf of Gabes. The phytoplankton species responsible for this bloom was the Cyanobacterium *Oscillatoria* called early-*Lyngbya*[11] and currently notified *Trichodesmium erytreum*. Until 1994, the same phenomenon is repeated annually during the summer in the Gulf of Gabes[12].

Abdennadher *et al.*[13] reported that the phytoplankton community sampled from 1997 to 2006 consisted of dinoflagellates, diatoms, Cyanobacteriae and Euglenophyceae with the first two groups dominating. Among the dinoflagellates, the Gymnodiniaceae were the largest family, with *Karenia selliformis* as the most abundant taxa (81% of total dinoflagellate abundance). The diatom *Navicula* sp. was the major contributor to total diatom density (35% of the total diatom abundance). Cyanobacteriae and Euglenophyceae contributed only slightly to phytoplankton abundance with 9% and 3%, respectively. Drira *et al.*[14] identified 18 toxic species, belonging mainly to the groups of dinoflagellates and cyanobacteria and which represent respectively 5% and 11% of the total phytoplankton and dinoflagellates constitute 36% of total abundance of toxic phytoplankton.

Turki *et al.*[15] revealed that toxic microalgae in the gulf of Gabès were dominated by *Prorocentrum lima* and *Ostreopsis* sp. and these species are known to be benthic or epiphytic.

Relatively few studies on our areas have examined in situ abundances of *Ostreopsis* species variability with the environmental factors. Consequently this present work was carried in Kerkennah Island to provide a better insight on the structure and the seasonal dynamics of the harmful dinoflagellate *Ostreopsis ovata* (*O. ovata*) and their relationships with abiotic environmental parameters.

2. Material and methods

2.1. Study area and sampling station

This study was conducted in the station of Cercina (34°41'27" N, 11°07'45" E) located in western coast of Kerkennah Island. Kerkennah is an emerged part of a vast submarine shelf whose shoals extend from 9 to 50 km around the islands. The surrounding waters range from 2 to 30 m in depth, averaging < 2 m[16]. Cercina station was directly exposed to the arrival of prevailing cold water from the channels of Louza (north of Sfax) and warmer water from the channel between Sfax and Kerkennah.

Three water samples replicate of one-litter were taken daily by Kuttner bottle, for ten consecutive days for 12 months. Samples were fixed with Lugol's solution and finally preserved in 5% formalin. All sampling water was kept in the dark at ambient temperature until their microscopic observation. Environmental variables such as salinity and temperature were measured in the field concomitantly as phytoplankton sampling. Additionally, nutrients (ammonium, nitrite, nitrate, phosphate and silicate) were analysed in a laboratory with Auto-analyser Luebbe type. Cell identification and enumeration in water samples were performed with an inverted microscope after the sedimentation of subsamples (10 mL) in chambers following Uthermöl's method[17]. Abundances of microalgae were expressed in number of organisms per liter of sample.

2.2. Statistical analysis

One-way nested ANOVA was used to test the hypothesis that the abundance *O. ovata* differed between seasons and months. Data were ln(x + 1) transformed if necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's C-tests).

Principal component analysis was employed to assess the relationships between total abundance of *O. ovata*, temperature, salinity and nutrient (nitrite, nitrate, phosphate and silicate) variables.

3. Result

The highest mean value of toxic *Ostreopsis* (86.67 ± 40.20 cell/L) abundance was recorded in summer on July Figure 1). ANOVA of variance showed significant difference of abundance between seasons, whereas no significant difference for month was detected (Table 1). Student-Newman-Keuls test revealed that the highest abundance was registered in summer, while no significant difference was detected between autumn, winter and spring.

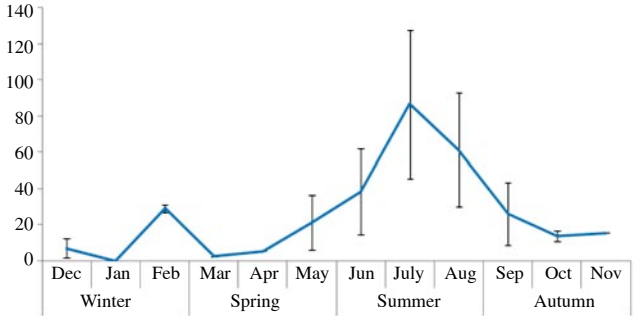

Figure 1. Seasonal and monthly abundance (cell/L ± SD) of the toxic dinoflagellate *Ostreopsis ovata* in Kerkennah Island.

Table 1

Nested ANOVA results for *O. ovata* during all seasons and months.

Source of variation	*df*	Mean square	F	Probability
Season	3	41.41	13.57	0.000
Month (season)	8	42.85	1.88	0.062
Residual	348	3.05		
Cochran's C-test	C = 0.466 ns			
Transformation	Ln(x+1)			
SNK test	Winter = Autumn = Spring < Summer			

F: F of Fischer; SNK: Student-Newman-Keuls.

The first axis (with 48.53% of variance) of the principal component analysis ordination shown in Figure 2, revealed a positive correlation of *Ostreopsis* with temperature, salinity and nitrate while the second axis (with 34.72% of variance) show a positive correlation of *Ostreopsis* with temperature, nitrate, nitrite and phosphate.

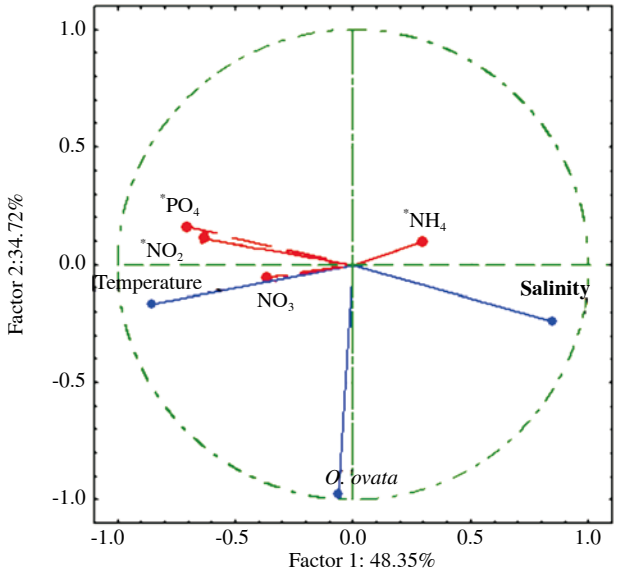

Figure 2. Principal component analysis ordination showing *Ostreopsis ovata* with respect to the environmental variables.

4. Discussion

The importance of temperature for the development of benthic harmful dinoflagellate is reflected since the highest abundance of *O. ovata* was detected in summer. In the fact these events occur mainly in tropical waters or in temperate areas during summer[18]. *O. ovata* is considered a tropical or subtropical species, but records in colder waters are increasing. In the Mediterranean Sea, *Ostreopsis* blooms in summer[19] when nutrients are relatively low and light and temperature are high.

As reported by Totti *et al.*[3] who revealed that *Ostreopsis* proliferate when the water is warm although in some cases the bloom may occur after the temperature maximum. High water temperatures, high irradiance and high remineralisation are factors that converge to create an environment favouring benthic harmful algal blooms. These studies were concurred with our results. In fact the maximum abundance of *Ostreopsis* was reached in summer when temperature was high. The same result was revealed by Mabrouk *et al.*[20] in Mahdia (eastern coast of Tunisia) where abundance of *Ostreopsis* depends on the sampling date and the highest values were recorded in July. Our result indicates that *Ostreopsis* recorded in concomitance with relatively high salinity. Pezzolesi *et al.*[21] revealed that the highest abundance of *Ostreopsis* was reached at high salinity ranged between 36‰ and 40‰. Our result was also in concordance with Tanimoto *et al.*[22] who found that optimal temperature of growth for *Ostreopsis* ranged between 24 ℃ and 30 ℃. However, the highest abundance of *Ostreopsis* in our study was founded in summer where the temperature was high. There are some conflicting results regarding the relationships of nutrients with *Ostreopsis* proliferation trends in field observations. Physical and chemical parameters were similar in Kerkennah Island (water temperature was about 20 ℃ to 24 ℃ and water salinity varying between 37.4‰ to 39.7‰ in the present study. Our study shows a low relationship between *O. ovata* and nutrient as demonstrated in Figure 2. This finding has been reported by Cohu *et al.*[23] who mentioned no clear pattern between nutrients and *Ostreopsis* development. Phosphates, nitrogen (both nitrates and nitrites) and silicates seemed to have no impact. Other studies have already mentioned that inorganic nitrogen, phosphorus and silicate concentrations appeared unassociated with benthic dinoflagellate distributions in tropical and Mediterranean areas. Accoroni *et al.*[19] assumed that *Ostreopsis ovata* blooms are triggered by a combination of calm hydrodynamic conditions, optimal temperature and favorable nutrients. It has also been shown by Parsons and Preskitt[24] which found that *Ostreopsis* sp. abundance was positively correlated with nutrient availability (Nitrate, nitrite, phosphate, and silicate) concentrations in the waters surrounding Hawaii. Our results are in agreement with this finding since the abundances founded were low. This situation may be explained by the fact that the study area was located on a continental shelf exposed to high tide and current tide regime and so the calm conditions were not offered to the *O. ovata* bloom. As reported by Blanfuné *et al.*[25] *Ostreopsis* spp. are frequently associated with two other Dinophyceae, *Prorocentrum lima* and *Coolia* spp., and no obvious competition or facilitation were found between them. Moreover abundance of *Ostreopsis* spp. was function of variety of substrates, seagrasses and macroalgae associated.

The maximum abundance of *Ostreopsis* was reached in summer when temperature was high. A low relationship between *O. ovata* and nutrient was detected. The distribution of harmful dinoflagellate *O. ovata* in Kerkennah Island have integrated all the biotopes (pelagic, benthic and epiphytic community) to document the knowledge concerning harmful algae blooms occurring in marine waters, a pre-requisite for designing responsible strategies for better management of marine resources.

Further studies are needed to confirm the effects and the interaction between environmental parameters, including investigation of *Ostreopsis* mixotrophic nutrition, parameter potentially important in population dynamics.

Conflict of interest statement

We declare that we have no conflict of interest.

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