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Factor driving heterotrophic dinoflagellate in relation to environment conditions in Kerkennah Islands (eastern coast of Tunisa)

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ABSTRACT

Objective: To study the seasonal variability of heterotrophic dinoflagellate in the station of Cercina (southern coast of Tunisia).
Methods: Sampling was done in 2007 in Cercina station located in the western coast of Kerkennah (34°41'27" N; 11°07'45" E) (Southern Tunisia). Three replicates of water samples were taken during 10 days of each month. Environmental variables and nutrients were measured *in situ*.
Results: A significant seasonal difference was observed for temperature and water salinity. The highest values were observed in spring and summer. No significant seasonal difference was, however, detected for nitrite, nitrate, ammonia, silica and phosphate. Sixty-five species of dinoflagellate were identified in the station of Cercina. Abundance of dinoflagellates fluctuated between seasons with values showing a significant seasonal and monthly difference. The highest mean abundance was recorded in spring in April, while the lowest abundance was detected in December in winter. *Protoperidinium granii* was the main species contributing to the dissimilarity between spring and winter with 13.98% followed by *Peridinium* sp. with 12.5% of dissimilarity and by *Polykrikos* sp. with 10.58%.
Conclusions: Heterotrophic dinoflagellates proliferate in spring and summer. This increase was justified by the nutrient availability. *Protoperidinium granii* and *Polykrikos kofoidii* were the main heterotrophic dinoflagellate making difference between seasons and their densities were positively correlated with both temperature and salinity.

1. Introduction

Dinoflagellates are common to abundant in both marine and freshwater environments. They are particularly diverse in the marine plankton where some cause "red tides" and other harmful blooms. Also, dinoflagellates are conventionally categorized into autotrophs and heterotrophs according to the presence or absence of chloroplast pigments. They are biochemically diverse, varying in photosynthetic pigments and toxin production ability[1]. They feed on a broad range of prey species, including phytoplankton, the eggs, early nauplii stages, and adult forms of some metazoans, ciliates, fish bloods and bacteria; at the same time they are important prey for many planktonic consumers, such as some metazoans, ciliates and other dinoflagellates[2]. Heterotrophic dinoflagellates (HDs) categories are prevalent in the marine environments, with an abundance of up to 2×10^5 cells/L under non-bloom conditions. They play an important role in the carbon cycling and energy flow in the marine planktonic community[3].

Heterotrophic and mixotrophic dinoflagellates were the major contributors to total phytoplankton biomass in the gulf of Gabes[4]. Hassen *et al*. proved that the nano- and picophytoplankton were the major contributors to the autotrophic biomass in the gulf[5]. This area had heterotrophic microplankton standing stock feeding on a large variety of prey ranging from picoplankton to diatoms.

This study was aimed at evaluating the importance of HD in the ecosystem of Kerkennah Islands by estimating their biomass and comparing it with the biomass of other plankters. Moreover, this study aimed to find an answer to the possible ways through which the seasonal variation of water-column nutrients and HD dynamics were affected in the Island of Kerkennah, Tunisia.

2. Materials and methods

2.1. Study area

The station of Cercina was located in the northern Gulf of Gabes

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and situated in the western coast of the Island of Kerkennah, with depths ranging from 3 to 5 m (Figure 1). It is influenced by regional water circulation[6] and is directly exposed to the arrival of prevailing cold water from the channels of El Louza (north of Sfax) and warmer water from the channel between Sfax and Kerkennah. The sea bottom morphology of the island is highly complex, characterized by mudholes, marine tide channels, and *Posidonia oceanica* beds of different shapes[7].

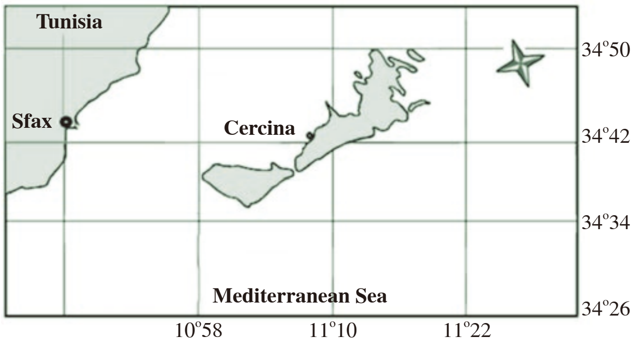

Figure 1. Map of the study area showing the sampling station of Cercina (western coast of Kerkennah Islands).

2.2. Sampling and laboratory procedures

Samples were collected monthly during 2007. The sampling campaigns were performed at the station of Cercina for 10 successive days. The tidal amplitude of Kerkennah is ≈ 1.60 m[8]. Environmental variables, such as salinity and temperature, were measured in the field concomitantly with phytoplankton sampling. Moreover, nutrient (ammonium, nitrite, nitrate, phosphate, silicate) analysis was performed at a laboratory by using an Auto-analyzer (Luebbe, Germany). Three replicate 1 L water samples were collected by Kuttner bottles and fixed with formaldehyde (5%). Microalgae enumeration was performed with an inverted microscope after fixation with a Lugol solution (final concentration 1% v/v) and settled for 48 h in accordance with the method of Uthermöhl[9]. Abundances were expressed in the number of organisms per liter of sample.

2.3. Statistical analysis

The data recorded for the dinoflagellate were submitted to ANOVA for analysis of difference in terms of abundance rates between seasons. Data were transformed where it is necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's C-test). Student-Newman-Keuls (SNK) test was employed for a posteriori multiple comparisons of means. A similarity percentage analysis was used to identify the contribution of individual dinoflagellates species with the pattern of similarity and dissimilarity between each season. A One-way analysis of similarity was used to test significant differences in community composition between seasons.

3. Results

A total of 65 species of dinoflagellate were identified in the station of Cercina (Table 1). Among them, 36.92% species had mode of nutrition as heterotrophic, 1.53% species were autotrophic and 1.53% was endosymbionts.

Table 1

List of dinoflagellate species found in Cercina station with their mode of nutrition.

Dinoflagellate species	Mode of nutrition
Akashiwo sanguinea (K.Hirasaka) G.Hansen & Ø.Moestrup	Mixo-heterotrophic
Alexandrium minutum Halim	Mixo-heterotrophic
Alexandrium pseudogonyaulax (Biecheler)	Mixo-heterotrophic
Alexandrium sp.	Mixo-heterotrophic
Amphidinium carterae Hulburt	Mixo-heterotrophic
Amphidinium operculatum Herdman	-
Amphidinium sp.	-
Amylax triacantha (Jörgensen) Sournia	-
Ceratium fusus (Ehrenberg) Dujardin	Mixo-heterotrophic
Ceratium lineatum var. *robustum* Cleve	Mixo-heterotrophic
Ceratium macroceros (Ehrenberg) Vanhöffen	Mixo-heterotrophic
Ceratium furca (Ehrenberg) Claparède & Lachmann	Mixo-heterotrophic
Coolia monotis Meunier	Mixo-heterotrophic
Ebria sp.	Heterotrophic
Dinophysis sp.	Endosymbionts
Goniodoma sphaericum Murray & Whitting	-
Gonyaulax polyedra F.Stein	-
Gonyaulax digitale (Pouchet) Kofoid	-
Gonyaulax sp.	Mixo-heterotrophic
Gonyaulax spinifera (Claparède & Lachmann) Diesing	Mixo-heterotrophic
Gymnodinium sp.	-
Gymnodinium catenatum H.W.Graham	-
Gyrodinium sp.	-
Gyrodinium fusiforme Kofoid & Swezy	Mixo-heterotrophic
Hermesinum sp.	-
Heterocapsa sp.	-
Karenia selliformis A.J.Haywood, K.A.Steidinger	-
Karlodinium veneficum (D.Ballantine) J.Larsen	-
Kryptoperidinium foliaceum (F.Stein) Lindemann	-
Ostreopsis ovata Fukuyo	-
Ostreopsis sp.	-
Oxyrrhis marina Dujardin	Mixo-heterotrophic
Peridinium sp.	Autotrophic
Podolampas sp.	Heterotrophic
P. kofoidii Chatton	Heterotrophic
Prorocentrum compressum (Bailey) Abé ex Dodge	-
Prorocentrum concavum Fukuyo	-
Prorocentrum gracile Schütt	-
Prorocentrum lima (Ehrenberg) F.Stein	-
Prorocentrum micans Ehrenberg	Mixo-heterotrophic
Prorocentrum minimum (Pavillard) J.Schiller	Mixo-heterotrophic
Prorocentrum rathymum Loeblich, Shirley & Schmidt	-
Prorocentrum triestinum J.Schiller	Mixo-heterotrophic
Protoperidinium ovum (Schiller) Balech	-
Protoperidinium bipes (Paulsen) Balech	-
Protoperidinium conicum (Gran) Balech	-
Protoperidinium curvipes (Ostenfeld) Balech	Mixo-heterotrophic
Protoperidinium depressum (Bailey) Balech	Mixo-heterotrophic
Protoperidinium diabolus (Cleve) Balech	-
Protoperidinium divergens (Ehrenberg) Balech	Mixo-heterotrophic
Protoperidinium globulus (Stein) Balech	-
P. granii (Ostenfeld) Balech	Mixo-heterotrophic
Protoperidinium leonis (Pavillard) Balech	-
Protoperidinium minutum (Kofoid) Loeblich III	Mixo-heterotrophic
Protoperidinium mite (Pavillard) Balech	-
Protoperidinium pellucidum Bergh	-
Protoperidinium pentagonum (Gran) Balech	-
Protoperidinium pyriforme (Paulsen) Balech	Mixo-heterotrophic
Protoperidinium quinquecorne (Abé) Balech	-
Protoperidinium sp.	-
Protoperidinium steinii (Jørgensen) Balech	Mixo-heterotrophic
Pyrophacus sp.	-
Scrippsiella spinifera G.Honsell & M.Cabrini	-
Scrippsiella subsalsa (Ostenfeld) Steidinger & Balech	-
Scrippsiella trochoidea (Stein) Balech ex Loeblich III	Mixo-heterotrophic

P. kofoidii: *Polykrikos kofoidii*; *P. granii*: *Protoperidinium granii*.

The highest value of temperature (26.74 °C) was recorded in summer while the lowest (14.84 °C) value occurred in autumn (Figure 2). The temperature was also noted to vary significantly from season to season. Furthermore, a significant seasonal difference was observed for water salinity (Table 2). In fact, salinity ranged from 41.70 g/L in summer to 38.27 g/L in spring (Figure 3). Nutrients fluctuated between seasons (Figures 4 and 5) and no significant difference was, however, detected for nitrite, nitrate, ammonia, silica and phosphate (Table 3). Abundance of dinoflagellates fluctuated between seasons (Figure 6) with values showing a significant seasonal and monthly difference (Table 4). The highest mean abundance (5600 ± 742.11) was recorded in spring in April, while the lowest abundance [(495.3 ± 60.27) cell/L] was detected in December in winter. SNK test revealed a significant difference among seasons. The highest abundance was registered in spring, summer, winter and autumn respectively. Principal component analysis ordination showed a clear correlation mainly between dinoflagellate and salinity and temperature (Figure 7). The first axis (with 38.93% of variability) showed a correlation of dinoflagellate with salinity, phosphate and nitrite while the second axis (with 22.76% of variability) showed a correlation of dinoflagellate with salinity, phosphate, temperature and nitrite. The main species contributing to the dissimilarity between spring and winter were shown in Table 5 by similarity percentage analysis. *P. granii* was the main species contributing to the dissimilarity between spring and winter with 13.980% followed by *Peridinium* sp. with 12.500% of dissimilarity and by *Polykrikos* sp. with 10.580%. The One way analyses of similarity revealed significant differences between each pair of seasons. The highest values of similarity coefficient R were registered between spring and winter (0.552) and between summer and winter (0.421), whereas the lowest similarity coefficient R values were detected between autumn and summer (0.226) and between summer and spring (0.214).

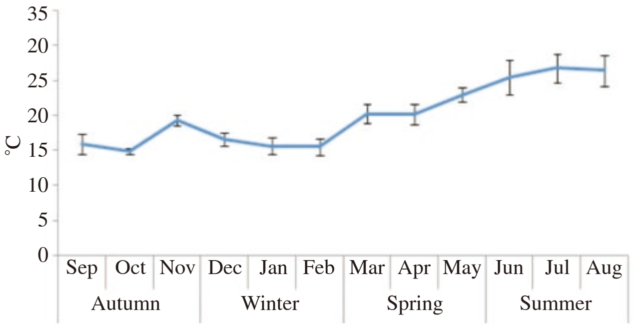

Figure 2. Variation of sea water temperature between seasons and months on the station of Cercina.

Table 2

ANOVA result of salinity and temperature among seasons on the station of Cercina.

Source of variation		Salinity			Temperature		
	df	MS	*F*	*P*	MS	*F*	*P*
Season	3	3.06	6.91	0.013	67.94	31.08	0.000
Residual	8	0.44			2.18		
SNK test		Au = Su > Sp = Wi			Au = Wi < Sp < Su		

MS: Mean square; Au: Autumn; Su: Summer; Sp: Spring; Wi: Winter.

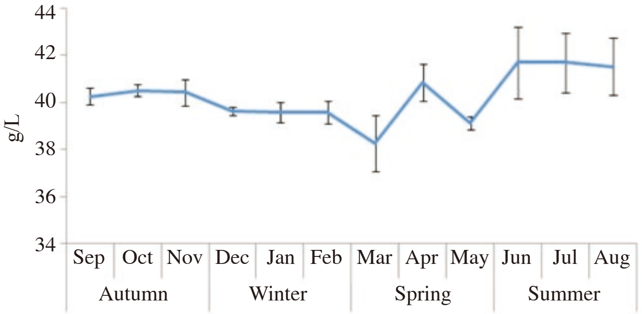

Figure 3. Variation of sea water salinity between seasons and months on the station of Cercina.

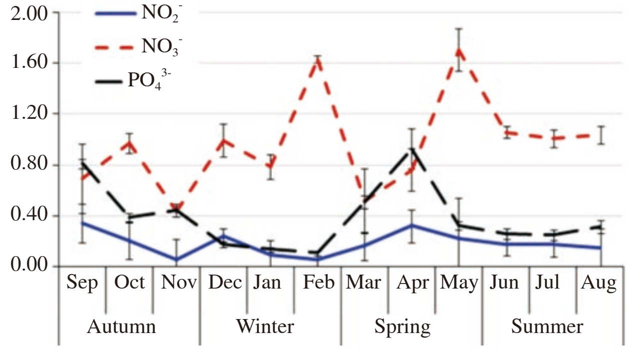

Figure 4. Variation of sea water nutrient between seasons and months on the station of Cercina.

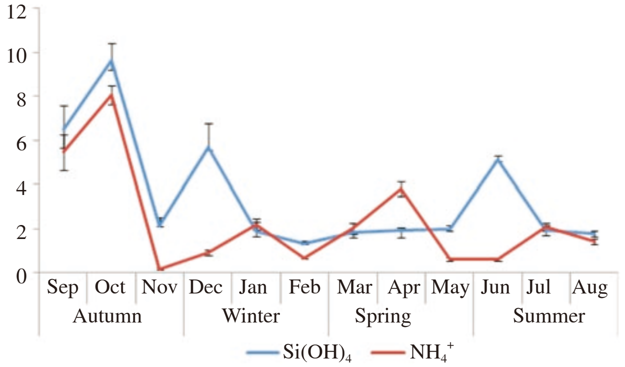

Figure 5. Variation of sea water nutrient between seasons and months on the station of Cercina.

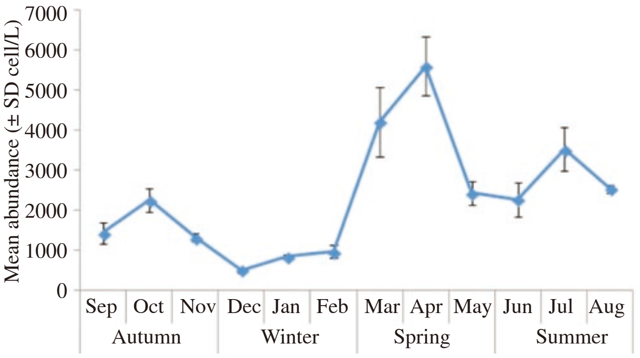

Figure 6. Seasonal mean abundance of dinoflagellate in the station of Cercina.

Table 3

ANOVA results of nutrient variability among seasons on the station of Cercina.

Source of variation		PO_4^{3-}			NH_4^+			$Si(OH)_4$			NO_3^-			NO_2^-		
	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Season	3	0.14	3.70	0.062	6.00	1.24	0.357	9.00	1.38	0.316	0.11	0.64	0.606	0.006	0.638	0.612
Residual	8	0.038			4.83			6.5			0.17			0.009		
SNK test		-			-			-			-			-		

MS: Mean square.

Table 4

Nested ANOVA results for HD variability during all seasons and months.

Source of variation	*df*	MS	*F*	*P*
Season	3	14.90	105.15	0.000
Month	8	1.09	7.15	0.000
Residual	348	0.14		
Cochran's C-test	C = 0.466 ns			
Transformation	Ln(x+1)			
SNK test	Winter < Autumn < Summer < Spring			

MS: Mean square.

Table 5

Similarity percentage analysis showing the main species contributing to the dissimilarity between spring and winter (cut off at 50% of cumulative).

Taxon	Contribution	Cumulative %	Mean abundance (Winter)	Mean abundance (Spring)
P. granii	13.980	16.06	14.7	813
Peridinium sp.	12.500	30.42	125.0	813
P. kofoidii	10.580	42.58	139.0	530
Protoperidinium sp.	5.729	49.16	69.3	211
Prorocentrum micans	5.429	55.40	63.3	223

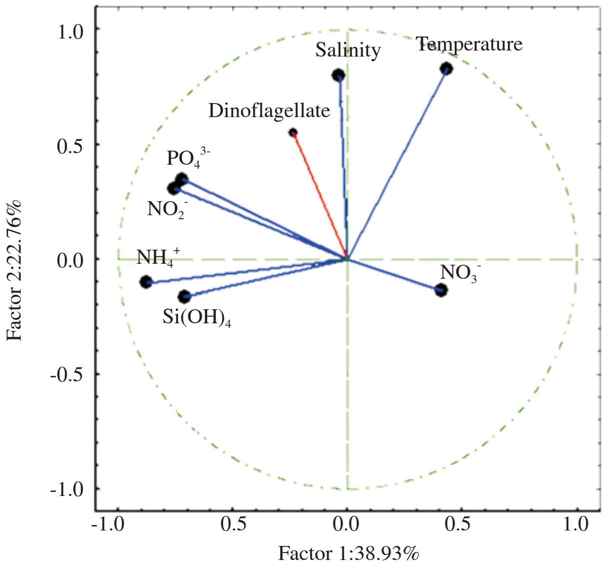

Figure 7. Principal component analysis ordination applied on the abundance of dinoflagellate, abiotic variable (temperature and salinity) and nutrients [PO_4^{3-}, NO_2^-, NO_3^-, $Si(OH)_4$ and NH_4^+] on the station of Cercina.

4. Discussion

The present study provides the first direct measurement of seasonal abundances of HD communities of Cercina in western coast of Kerkennah Islands. The seasonal variability of HD shows an increase during spring and summer compared to winter and fall. This pattern was revealed by Feki-Sahnoun *et al*. in the same area (southern coast of Tunisia) where dinoflagellate illustrated a marked seasonal cycle opposing winter-spring species to summer-fall species and this was related to the increase of dinoflagellate species abundance during summer and fall[10]. This model illustrates the basic characteristics of phytoplankton succession in temperate coastal waters described elsewhere[11] and mainly justified by the nutrient availability along seasons[12]. Similar result was revealed by Ltaief *et al*. in the Gulf of Gabes where obvious proliferation of heterotrophic and mixotrophic dinoflagellates was the distinctive feature of this summer cruise[4]. Also dinoflagellate density was positively correlated to both temperature (Spearman correlation coefficient R = 0.772) and salinity (Spearman correlation coefficient R = 0.765) suggesting a good adaptation of this group to the warm and salty waters in the inshore region. Dominance of dinoflagellates species during spring has been already reported in previous studies in the coastal water and over the continental shelf area of the Gulf of Gabes[13-16]. *P. granii* was the main HD contributing to the dissimilarity between spring and winter. This species was ascribed by Feki-Sahnoun *et al*. in the Gulf of Gabes (southern coast of Tunisia) affecting harbors subjected to intense marine traffic of chemical materials and near the discharge point of industrial zone subjected to crude phosphate treatment and chemical industry waste[10]. Its proliferation in spring was coincided by the increased abundance of diatom[15] and so *P. granii* dynamic was likely to be governed by their feeding preferences, and thus it was likely to be diatom grazers[17-19]. Gribble *et al*. mentioned that heterotrophic *Protoperidinium* had the potential to consume 30%–80% of the dinoflagellate or diatom[20]. In addition to food requirements, physical variables such as temperature, salinity and nutrients are superimposed to the dynamic of *Protoperidinium* population. Temperature may be of secondary importance, however, considering that the majority of *Protoperidinium* species are widespread in spring and summer in our study area. In general, food availability may be the most important factor regulating seasonal dynamics of individual *Protoperidinium* species. The genus *P. kofoidii* was among HD to make seasonal dissimilarity. The high abundance of *P. kofoidii* in spring was coincided with the high abundance of dinoflagellate [(5 600.20 ± 742.11) cell/L] and diatom. Matsuyama *et al*. estimate that the pseudocolonial HD *P. kofoidii* was likely able to prey 2.7 to 16.2 *Gymnodinium catenatum* in a day and this ability probably contributes to the comparatively high estimate of ingestion rates[21]. This physiological aspect appears to have a significant ecological effect of reducing the grazing pressure during the course of bloom formation in harmful dinoflagellates[19,22].

On the other hand, recent research has revealed that harmful dinoflagellate blooms are greatly regulated by the co-occurrence of HD[23]. Our study reveals a low abundance of naked dinoflagellate in the study area and this may likely be attributed to considerable predation by *P. kofoidii* on a natural *Gymnodinium catenatum* bloom in geographically distant areas suggesting that populations of toxic dinoflagellates are often regulated by the proliferation of HD predators worldwide.

Conflict of interest statement

We declare that we have no conflict of interest.

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