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Research Article

Variability in the Structure of Planktonic Microalgae Assemblages in Water Column Associated with *Posidonia oceanica* (L.) Bed in Tunisia

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Patterns of phytoplankton in areas with seagrass (*Posidonia oceanica*) and areas without seagrass were quantified in the coast of Chebba (East of Tunisia). Replicate samples were collected at August 2011, from four stations (separated by 500 of metres). The diversity of phytoplankton was high around *P. oceanica* meadows compared to area without seagrass. A possible explanation to this finding is that water motion and hydrodynamics forces cause leaves agitation allowing the passage of epiphytic species in the water column. Our results also show an increase of abundance of potentially toxic dinoflagellates around *Posidonia* bed such as *Alexandrium minitum*, *Amphidinium carterae*, *Karenia selliformis*, *Coolia monatis*, *Karlodinium veneficum*, *Ostreopsis ovata*, *Prorocentrum concavum*, *P. minimum*, *P. rathymum*, and *P. lima*. Installation of fish farms on *Posidonia* beds should be avoided, not only to preserve this vulnerable habitat, but also to avoid fish contamination by toxic species derived from the resuspension of epiphytic community on seagrass substrata to the water column.

1. Introduction

Marine phytoplankton constitutes the base of the pelagic food-web and is responsible for about half of the net annual primary production on Earth [1]. These range from diatoms and dinoflagellates to cyanobacteria. They are a direct as well as indirect food source for many animal species [2]. Leaves of seagrass systems offer substrata suitable for settlement and growth of a number of microcolonists characterized by a high diversity of species [3]. Among the epiphytic community, a large number of potentially toxic species of dinoflagellates (*Alexandrium minutum, Amphidinium carterae, Karenia selliformis, Coolia monatis, Karlodinium veneficum, Ostreopsis siamensis, Prorocentrum concavum, Prorocentrum minimum, Prorocentrum rathymum,* and *Prorocentrum lima*) were found in Tunisia [4, 5] and in the north of the Mediterranean Sea [6]. Some of them are potential toxin producers [7].

Although a good number of surveys have focused on the phytoplankton community in the north basin of the Mediterranean Sea, the south part of the basin has received little attention. Many authors have bemoaned the lack of data in some Mediterranean regions, particularly North Africa [8]. Few studies analyzing changes in phytoplankton assemblages in the north of Tunisia have been published [4, 9] and to our knowledge no studies on plankton assemblages in the east coast have been conducted up to now. This region, with other neighbouring areas, contains 20% of the potential yield of small pelagic fish stocks of the country. Moreover, several fish farms have recently been installed in this area. Knowledge of potentially toxic species of microalgae is necessary to assess the influence of those species on fisheries exploitation and human health.

At Chebba, east of Tunisia, we analyzed the phytoplankton community structure around different habitats. We hypothesized that phytoplankton communities' abundances are higher around *Posidonia* beds.

We thus compared microalgae assemblages around three kinds of *Posidonia oceanica* beds and around unvegetated habitats and examined their variability.

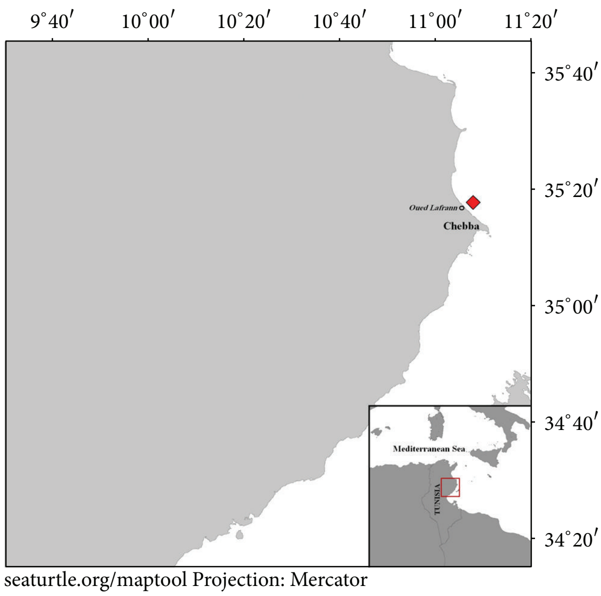

Figure 1: Map of the study area, showing the sampling station.

2. Materials and Methods

This study was conducted in the locality of Oued Lafrann (35°15′18′′N, 11°07′28′′E) in the region of Chebba (east of Tunisia). The climate is semiarid(average precipitation: 350 mm year^{-1}) and sunny with strong northerly winds. This locality is characterized by a good seagrass status with barrier and fringing reefs in addition to an extensive plain meadow that has the highest cover and shoot densities in the east of Tunisia [10]. In this region, we found a tiger meadow [10] with tiger-shaped cords of seagrass from 10 to 30 m long and spaced about 1–1.5 m with dead matte [11]. This area is under consideration as a marine protected area (submitted in Italo-Tunisian project iTunES) because of its biocenotic richness and its distinctive ecosystems.

Prospecting and sampling were conducted during August 2012 in four stations (i) a fringing reef meadow, (ii) tiger meadow, (iii) plain meadow, and (iv) unvegetated habitat (Figure 1). At each station, twelve replicate water samples (about 10 m apart) were selected. Water column samples, using 1-litre glass bottle, were conducted by SCUBA diving above canopy for vegetated stations or on the bottom for unvegetated station. All samplings were collected about noon.

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. Settling long glass tubes used for sedimentation procedure were 2 cm wide by 21 cm long and have a base plate that contains a coverslip onto which the algae settle. To mix the sample, the bottle was gently tilted back and forth 10 times before pouring. A 50 mL subsample was poured into the settling chamber and left to settle for 24 h. Subsamples were examined in an inverted microscope at medium (×200) magnification by scanning the entire surface of the settling chamber to enumerate epiphytic microalgae [12]. The total number of microalgae individuals (N) contained in 1 litre [expressed as number of individual per litre] is obtained by the following conversion: $N = (n \times 1000)/v$; with n = number of individuals counted and v = volume of the sedimentation chamber (50 mL). The identified taxa were divided into groups (diatoms, dinoflagellates, cyanobacteria).

Table 1: Results of analysis of similarity ANOSIM of phytoplankton abundances in water column according to habitat. Transformation $\log(X + 1)$.

Sources	R global	P	Number of permuted statistics greater than or equal to global R
Habitats	0.790	0.01	0

2.1. Data Analysis. Data were tested for normality using the Kolmogorov-Smirnov test [13] and for heteroscedasticity using Cochran's C test, and transformed if necessary [14].

Analyses of similarity (ANOSIM) randomization tests (with $\text{Log}(x + 1)$-transformed data) were used to test for differences in species abundance between stations [15]. Differences found using ANOSIM were followed up using the *SIMPER* analysis to identify which species primarily accounted for the observed differences between stations. SIMPER generates a ranking of the species responsible for the significant differences. These analyses used a matrix composed of Bray-Curtis similarity coefficient generated with $\text{Log}(x + 1)$-transformed species abundance data.

A one-way *Analysis of variance (ANOVA)* was used to test the hypothesis that the abundance of each of the most abundant taxa differed between stations. The Tukey's HSD (Honestly Significant Difference) test was applied for multiple comparisons of means.

3. Results

The highest mean value of dinoflagellates abundance was recorded in the fringing reef meadow, whereas the lowest mean value has been detected in the tiger meadow. The lowest mean value of diatoms abundance was recorded in the unvegetated station, whereas the highest mean value was detected in fringing reef meadow. Cyanobacteria abundance was very low in all stations (Figures 2(a), 2(b), 2(c), and 2(d)).

For microalgae, 61 species were counted including 60 species around *P. oceanica* bed stations and 34 at the unvegetated station (see the appendix). Analysis of similarity (ANOSIM) of epiphytic microalgae species abundances showed significant differences between stations (Table 1).

Abundances of microalgae and cyanobacteria were included in the univariate analyses of variances. Dinoflagellates differed significantly between stations with high abundance in fringing reef meadow (Tukey test). Significant differences were detected for diatoms with high abundance in the plain meadow (Tukey test). When the abundance of toxic dinoflagellates and cyanobacteria are grouped, significant differences were detected between stations with high abundance in vegetated habitats (ANOVA, Table 2).

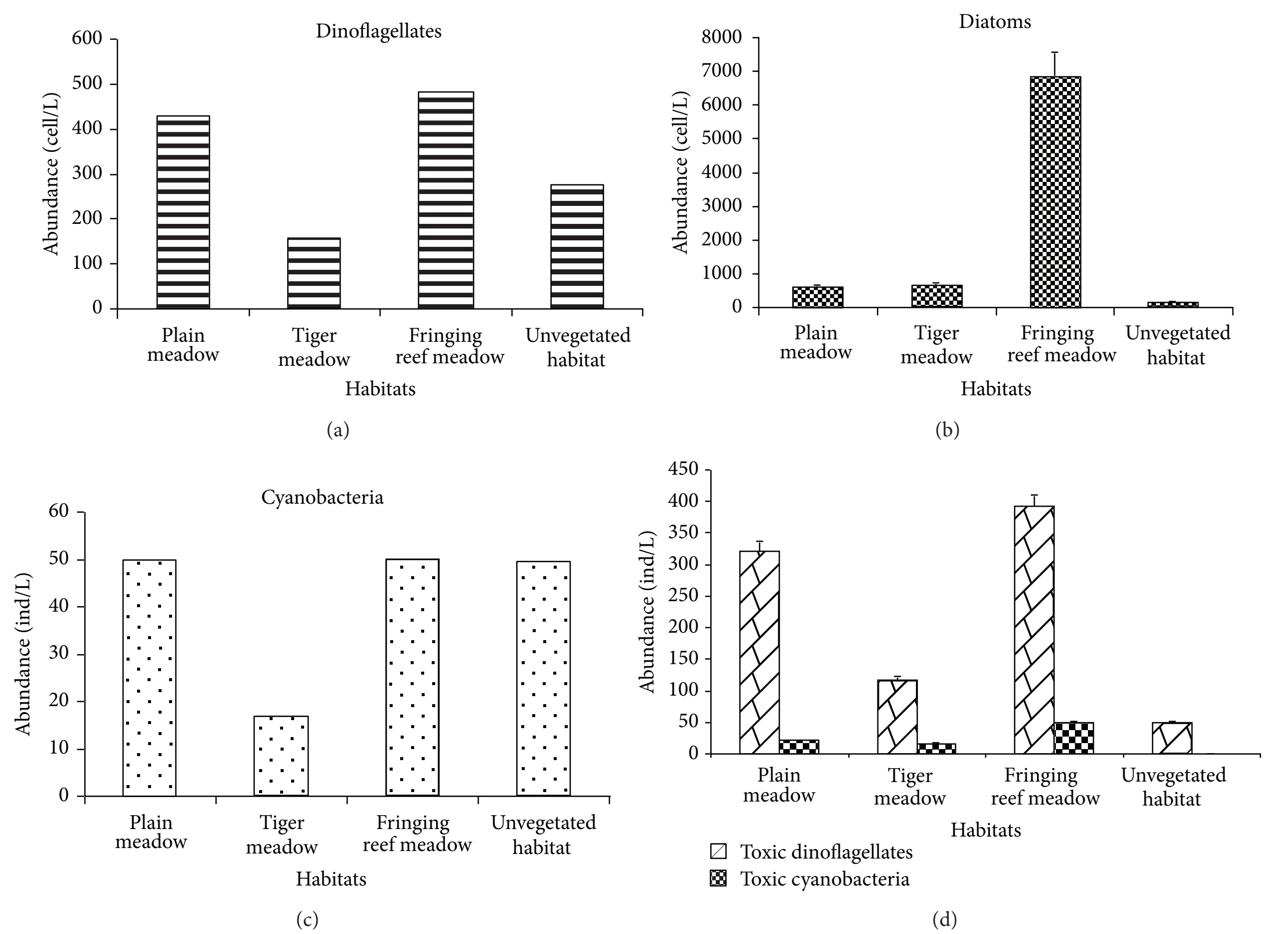

Figure 2: Average abundance of phytoplankton in water column in prospected stations.

Analyses of similarity percentage (SIMPER) showed that the average dissimilarity between vegetated and unvegetated stations is high (89.15%). This procedure also allowed determining the species that contribute to this dissimilarity, they are *Pleurosigma* sp. *Nitzschia fontifugua, Guinardia* sp., *Ostreopsis ovata, Licmophora* sp., *Coolia monotis, Polykrikos* sp., *Prorocentrum micans,* and *Coscinodiscus* sp. that are absent at unvegetated stations and the cyanobacteria *Spirulina subsalsa* that was absent around *Posidonia* beds. Species *Navicula* sp., *Peridinium* sp., and *Prorocentrum lima* have unequal abundance between the groups of stations (Table 3).

4. Discussion

Our study shows two key results: (1) a high abundance of phytoplankton in the water column around *P. oceanica* beds and (2) high number and abundance of potentially toxic dinoflagellates species around vegetated habitats.

The diversity of phytoplankton was high around *P. oceanica* meadows. A possible explanation to this finding is that water motion in our study site causes blade agitation (and other substratum) allowing the passage of epiphytic (and benthic) species into the water column as found by other previous studies [4, 6, 16]. For example, positive and significant correlations between epiphytic and planktonic *Ostreopsis* cell abundances have been found previously [6, 16] which supports the hypothesis of cell resuspension from the macrophyte surfaces to the water column.

Vegetated ecosystems are ideal habitats for benthic diatoms and other epiphytes [17] since seagrass leaves and algal thalli may represent an order of magnitude greater surface area for the colonization and growth of diatoms [18]. These high abundances in the presence of macrophytes may be due to an increase in (i) the amount of physical structure (usable as living space), (ii) the number of microhabitats, (iii) sediment deposition and stabilization, and (iv) food resources and (v) protection from predators, and also to (vi) a reduction of hydrodynamic forces [19]. Vila et al. [20] suggest that benthic dinoflagellates are particularly affected by hydrodynamics, as the mat is only loosely attached, and they are easily resuspended in the water column. Brahim et al. [21] have found that abundances of micro- and macroorganisms were higher in the *P. oceanica* leaf habitat than in the water column. This may be explained by the very high predatory pressure exerted by young and planktivorous (larvae, juveniles, and adults) fish [22] on animals remaining in the water column.

Table 2: Analysis of variance ANOVA of the abundances of major groups of microalgae and cyanobacteria in prospected stations.

	df	MS	*F*	*P*	Tukey test
Total abundance					
Habitat	3	132843498	852.876	<0.0001	F > P = T > U
Residuals	44	155759.422			
Total	**47**				
Dinoflagellates					
Habitat	3	262690.972	7.221	<0.0001	F = P > T = U
Residuals	44	36377.841			
Total	**47**				
Diatoms					
Habitat	3	124622282	1675.147	<0.0001	F > T = P > U
Residuals	44	74394.839			
Total	**47**				
Cyanobacteria					
Habitat	3	3333.333	2.047	0.121	n.s.
Residuals	44	1628.788			
Total	**47**				
Toxic dinoflagellates					
Habitat	3	316857.639	11.126	<0.0001	F = P > T = U
Residuals	44	28480.114			
Total	**47**				
Toxic cyanobacteria					
Habitat	3	5190.972	9.533	<0.0001	F > P = T = U
Residuals	44	544.508			
Total	**47**				

F: fringing reef meadow, P: plain meadow, U: unvegetated station, and T: tiger meadow, ns.: not significant.

Table 3: Results of SIMPER procedure showing distribution of discriminating species between stations around *Posidonia* bed and unvegetated habitat.

Species	Stations around *Posidonia* bed	Unvegetated habitat				
	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib%	Cum.%
Navicula sp.	2108.33	58.33	33.89	1.17	38.02	38.02
Peridinium sp.	29.17	225	12.92	1.18	14.49	52.51
Pleurosigma sp.	87.5	0	6.62	0.96	7.43	59.94
Nitzschia fontifugua	227.78	0	4.7	1.09	5.28	65.21
Guinardia sp.	44.44	0	4.08	0.73	4.58	69.79
Ostreopsis ovata	79.17	0	3.52	0.64	3.95	73.74
Spirulina subsalsa	0	50	3.22	0.97	3.61	77.35
Licmophora sp.	121.25	0	2.89	0.96	3.24	80.6
Prorocentrum lima	38.89	50	2.81	0.82	3.15	83.75
Coolia monotis	56.94	0	2.41	0.94	2.71	86.45
Polykrikos sp.	38.89	0	1.38	0.64	1.55	88.01
Prorocentrum micans	37.5	0	1.08	0.55	1.22	89.22
Coscinodiscus sp.	33.33	0	1.07	0.56	1.2	90.42

The blades of *P. oceanica* furnish these animals with refuge and protection from predatory fish [19]. A similar observation was made by Mabrouk et al. [5] in the eastern coast of Tunisia (Mahdia), where a significant correlation between the leaf area index and the abundance of epiphytic species indicates that the phenological parameters of the host plant influence the abundance of epiphytic microorganisms.

It seems that benthic dinoflagellates have no preference for specific macrophytes, but they prefer three- dimensional, flexible, and high surface area as proposed by Vila et al. [20]. On the contrary, Turki [9] had found that epiphytic dinoflagellates displayed host substratum preference on *Cymodocea nodosa* where the cell abundance was higher than on *P. oceanica*. An important variability in the abundance of *Ostreopsis*

Table 4: Species list of microalgae and cyanobacteria identified on water column around *P. oceanica* bed and in unvegetated station.

Groups	Species	Stations around Posidonia bed	Unvegetated station
Diatoms	*Achnanthes* sp.	+	−
	Amphiprora constricta (Ehrenberg)	+	−
	Amphora sp.	+	+
	Bacillaria sp.	+	−
	Biddulphia sp.	+	+
	Chamaesiphon sp.	+	−
	Chaetoceros sp.	+	+
	Climacosphenia moniligera (Ehrenberg)	+	−
	Coscinodiscus sp.	+	−
	Guinardia sp.	+	−
	Grammatophora sp.	+	+
	Gyrosigma sp.	+	+
	Leptocylindrus sp.	+	−
	Licmophora sp.	+	−
	Melosira sp.	+	−
	Navicula sp.	+	+
	Nitzschia fontifuga (Cholnoky)	+	−
	Nitzschia sp.	+	+
	Pinnularia sp.	+	+
	Plagiotropis sp.	+	+
	Pleurosigma sp.	+	−
	Pseudonitzschia sp.	+	−
	Rhizosolenia sp.	+	+
	Skeletonema sp.	+	+
	Striatella unipunctata (Lyngbye) C. Agardh	+	−
	Synedra sp.	+	−
	Thalassionema nitzschioides (Grunow) Mereschkowsky	+	−
Dinoflagellates	*Alexandrium* sp.	+	−
	Amphidinium carteri (Hulburt)	+	+
	Ceratium furca. (Ehrenberg) Claparède and Lachmann,	+	+
	Ceratium tripos (Müller)	+	+
	Coolia monotis (Meunier)	+	−
	Gymnodinium sp.	+	+
	Cyst of *Karenia selliformis*	+	+
	Cyst of *Polykrikos*	+	+
	Ostreopsis ovata (Schmidt)	+	−
	Peridinium sp.	+	+
	Polykrikos sp.	+	−
	Prorocentrum concavum (Fukuyo)	+	+
	Prorocentrum gracile (Schütt)	+	−
	Prorocentrum lima (Ehrenberg) Dodge	+	+
	Prorocentrum micans Ehrenberg	+	−
	Prorocentrum minimum (Pavillard) Schiller	+	+
	Prorocentrum rathymum (Loeblich) Shirley and Schmidt	+	+
	*Prorocentrum triestinum (*Schiller)	+	+
	Protoperidinium curtipes (Jorgensen) Balech	+	+
	Protoperidinium depressum (Bailey) Balech	+	+
	Protoperidinium divergens (Ehrenberg) Balech	+	−
	Protoperidinium mite (Pavillard) Balech	+	+
	Protoperidinium ovum (Schiller) Balech	+	+
	Protoperidinium steinii (Jørgensen) Balech	+	−
	Protoperidinium sp.	+	+
	Scrippsiella sp.	+	−

Table 4: Continued.

Groups	Species	Stations around Posidonia bed	Unvegetated station
Cyanobacteria	*Anabeana* sp.	+	−
	Chroccus sp.	+	−
	Lyngbya sp.	+	−
	Merismopedia sp.	+	+
	Mesodinium rubrum Leegaard	+	−
	Oscillatoria sp.	+	+
	Pseudanabaena sp.	+	+
	Spirulina subsalsa Oersted	−	+

depends on the macroalgal host species, Dictyota species seem particularly able to support very high abundances of *Ostreopsis* during blooms.

Seagrass *Posidonia* beds concentrate a wide variety of organism that feed on their epiphytes [2]. Grazing has a negative effect on the abundance of phytoplankton in the water column around the seagrass beds [23]. Despite this effect, the abundance of phytoplankton in vegetated areas remains higher than in the unvegetated station.

The effect of other environmental parameters should be mentioned, such as wave activity [24], nutrient availability, salinity, and light intensity [25]. Nutrient concentration is higher around seagrass [26] and as a consequence the plankton population is more diversified around *Posidonia* bed.

Our results also show an increase of abundance of potentially toxic dinoflagellates around *Posidonia* beds such as *Alexandrium minutum*, *Amphidinium carterae*, *Karenia selliformis*, *Coolia monatis*, *Karlodinium veneficum*, *Ostreopsis ovata*, *Prorocentrum concavum*, *P. minimum*, *P. rathymum,* and *P. lima*. *Prorocentrum* species are mainly associated with okadaic acid and the production of analogues [27]. Moreover, Marr et al. [28] concluded that the underestimation of toxic dinoflagellates associated with a toxic event might be due, in part, to the lack of sampling of the benthic and epiphytic communities.

No evidence of pollution at our studied area since we did not detect nutrient enrichment and the phenological parameters of *P. oceanica* are good [29]. Moreover, this station is quite far from any anthropogenic disturbance.

5. Conclusion

The results of this survey highlight the increases of toxic dinoflagellates around vegetated area. We, thus, believe that our data may be, on a North African scale, not only worthwhile but also useful in initiating other investigations. Finally, a study by FAO [30] recommends that aquaculture installation on *Posidonia* beds should be avoided whenever possible and our data support this recommendation, not only to preserve this vulnerable habitat [31], but also to avoid fish contamination by toxic species derived from the passage of epiphytic community on seagrass substrata to the water column.

Appendix

See Table 4.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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