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RESEARCH PAPER

Temporal and Bathymetric Variation of Epiphytic Microalgae on *Posidonia oceanica* (L.) Delile Leaves in Gökçeada (North Aegean, Turkey)

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Abstract

Epiphytic microalgae attached to *Posidonia oceanica* leaves play a significant role through primary production, nutrient cycling and trophic fluxes in benthic ecosystems. Due to their sensitivity, epiphytic microalgae respond to environmental alterations more quickly than their host. The aim of this study is therefore to evaluate the ecological quality in Gökçeada Underwater Park (North Aegean) using epiphytic microalgal composition and abundance. In this respect, *P. oceanica* shoots are collected at 6 m, 15 m and 28 m in May, July, October 2009 and January 2010 to estimate the microalgal composition and abundance. Also, *P. oceanica* descriptors and physico-chemical parameters in the water column of the meadow lower limit are measured. Leaf surface improvement through greater depths, have provided a convenient substratum for epiphytic colonization. A total of 56 taxa belonging to epiphytic microalgae are composed of Bacillariophyceae (91%), Cyanophyceae (7%) and Dinophyceae (2%). Temporal and bathymetric dynamics of the epiphytic community structure and abundance depend on the environmental parameters; such as leaf surface, nutrients and turbidity. Also, a mucilage phenomenon, resulted in a low diversity due to the dominancy of filamentous cyanobacteria. Necessary data is supplied for a less evaluated region affected by agricultural activities and urbanization in the recent years.

Introduction

Endemic seagrass of the Mediterranean Sea, *Posidonia oceanica* (L.) Delile constitutes a shelter for various epiphytic communities colonizing on the leaf blades and rhizomes (Pasqualini *et al.*, 2006). It is considered as a functional substratum due to its long-life span and wide leaf blades; (Cinelli *et al.*, 1984; Mazzella *et al.*, 1989).

Photosynthetic epiphytes on leaf blades, take part in trophic fluxes in benthic ecosystems as a food source for herbivores and also contribute to the primary production (Mazzella *et al.*, 1992; Belegratis *et al.*, 1999; Giovannetti *et al.*, 2010). Nevertheless, high epiphytic coverage on leaf blades is the most important negative factor declining the photosynthesis rate of the host plant (Round, 1984; Cebrian *et al.*, 1999). Since photosynthetic epiphytes can not have the ability to obtain nutrients directly from the sediment; they colonize on leaf blades, especially close to the apex where they can utilize the sunlight energy (Trautman & Borowitzka, 1999). Moreover, during the colonization processes, they reach to high abundances constituting mono-specific or multi-layered communities on leaf blades therefore, they play an important role in the functioning of the *P. oceanica* ecosystem (Mazzella *et al.*, 1994; De Stefano *et al.*, 2000).

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In the recent years, increasing nutrient levels and organic compounds in the coastal waters, affected *P. oceanica* habitats and also modified the composition of marine flora stimulating the excessive growth of opportunistic species (Ruiz *et al.*, 2001; UNEP/GPA, 2006; Giakoumi *et al.*, 2015). Epiphytic microalgal species respond to environmental abiotic and biotic factors more quickly than their hosts adjusting their composition and abundance (Frankovich & Fourqurean, 1997; Piazzi *et al.*, 2004; Terrados & Pons, 2008; Nesti *et al.*, 2009; Mabrouk *et al.*, 2011). Therefore, photosynthetic epiphytes can be regarded as bio-indicators of environmental alterations (Sand-Jensen & Borum, 1991; Trautman & Borowitzka, 1999; Balata *et al.*, 2008).

Distribution of *P. oceanica* meadows has been the aim of numerous studies (Pergent *et al.*, 1994; Dural & Pergent, 2001; Boyacıoğlu & Dural, 2009; Cirik *et al.*, 2010) in Turkey however, some ecological aspects, such as the relative contribution of microalgae to the epiphytic community has not been investigated. Therefore, this study mainly focused on the evaluation of the ecological quality in Gökçeada Underwater Park (North Aegean) using epiphytic microalgal composition and abundance on *P. oceanica* leaf blades due to their sensitivity to environmental alterations.

Materials and Methods

Study Area

Gökçeada (40°10'10.19"N – 25°50'13.93"E) is the largest island of Turkey (289 km²); located in the North Aegean Sea (Eastern Mediterranean), it sayexhibits distinct hydrological features comparing to the Western Mediterranean Sea due to the Turkish Straits Current System and the river inputs (Uçkaç, 2005). Correspondingly the region is the intersection point of the less intense and brackish Black Sea waters coming from Dardanelles Strait and the intense and saline Levantine waters flowing upwards along the eastern coasts of the Aegean Sea (Pazı, 2008; Siokou-Frangou *et al.*, 2009; Sayın & Beşiktepe, 2010). Study site is the Gökçeada Underwater Park situated in the northern coast of the island harboring hard bottom types with photophilic macroalgal communities and seagrass meadows (Figure 1).

Sampling Design

Field study was performed in May, July, October 2009 and January 2010 by SCUBA diving in a continuous *Posidonia oceanica* seagrass bed (40°14'13.35" N – 25°54'14.05" E). Ecological status of the meadow bed is evaluated by measuring meadow density (shoot m^{-2}) and meadow cover (%) in three replicated 60 cm × 60 cm quadrats placed randomly in the upper (6 m), intermediate (15 ± 1 m) and lower limit (28 m) depths of the meadows (Aktan *et al.*, 2011). Moreover, 10 shoots of *P. oceanica* are collected randomly from each quadrat in order to measure the phenological parameters and the epiphytic microalgal abundance.

In the laboratory, leaves are sorted according to the protocol of Giraud (1979) to measure the phenological parameters: leaf number (leaf $shoot^{-1}$), leaf surface (cm^2 $shoot^{-1}$) and epiphytic microalgal abundance (cells 50 g^{-1} fresh weight of *Posidonia*). After the detachment from their sheet, leaves are weighted to 50 g with a precision balance to record their wet mass (Turki, 2005; Aligizaki & Nikolaidis, 2006). Epiphytic cover is removed from the leaves by scraping gently with a scalpel (Kendrick & Lavery, 2001). The removed material is washed through a 500-µm sieve in order to separate larger particles. The final epiphytic material is collected in 50 ml distilled water and quantitatively subsampled for cell counting to estimate the epiphytic microalgal abundance. Cleaning in H_2SO_4 acid solution, permanent slides are prepared for the identification of microalgae. The subsamples are also fixed with 4% formaldehyde solution for further taxonomic identification process. Cell counts are performed under the microscope at ×40 magnification examining the whole surface. The total number of epiphytic microalgal cells are expressed as the number of individuals per 50 g of fresh weight of *Posidonia* (fw). In terms of Cyanophyceae, the cells are estimated present in the trichomes (Findenegg, 1974). During the identification process; the terminology of Hustedt (1930; 1959; 1961-1966; 1985); Patrick and Reimer (1966-1975); Krammer and Lange-Bertalot (1986); Poulin (1986); Round *et al.* (1990); Tomas (1995); Anagnostidis and Komárek (1988) and Komárek and Anagnostidis (1986) are followed.

Physico-chemical water quality parameters (temperature, salinity, dissolved oxygen, pH) in the water column (0 m, 15 m, 28 m) are measured in situ with YSI Model Professional Plus multiparameter and the water transparency is measured three times at each site using a Secchi disc. In addition, water samples for determining nutrient concentrations are collected in 100 ml polyethylene bottles and kept in deep freeze (20 $^{\circ}$C) until their analysis in the laboratory. Dissolved inorganic nitrogen (NO_2^{-1}+NO_3^{-1}+NH_4) concentrations are analyzed spectrophotometrically (APHA, 1999). Total phosphorus (TP), Silicate (SiO_4) analyses are carried out by the methods described by Parsons *et al.* (1984).

Data Analysis

In order to reveal temporal and bathymetric variations of each epiphytic microalgal group abundances and *Posidonia oceanica* descriptors, multivariate analysis of variance (MANOVA) is used with the model consisted of three quadrats per combination of the factor levels: depth and month. Before the analysis, Kolmogorov–Smirnov test and Levene's test are applied on the data in order to investigate the normality of distribution and the homogeneity of

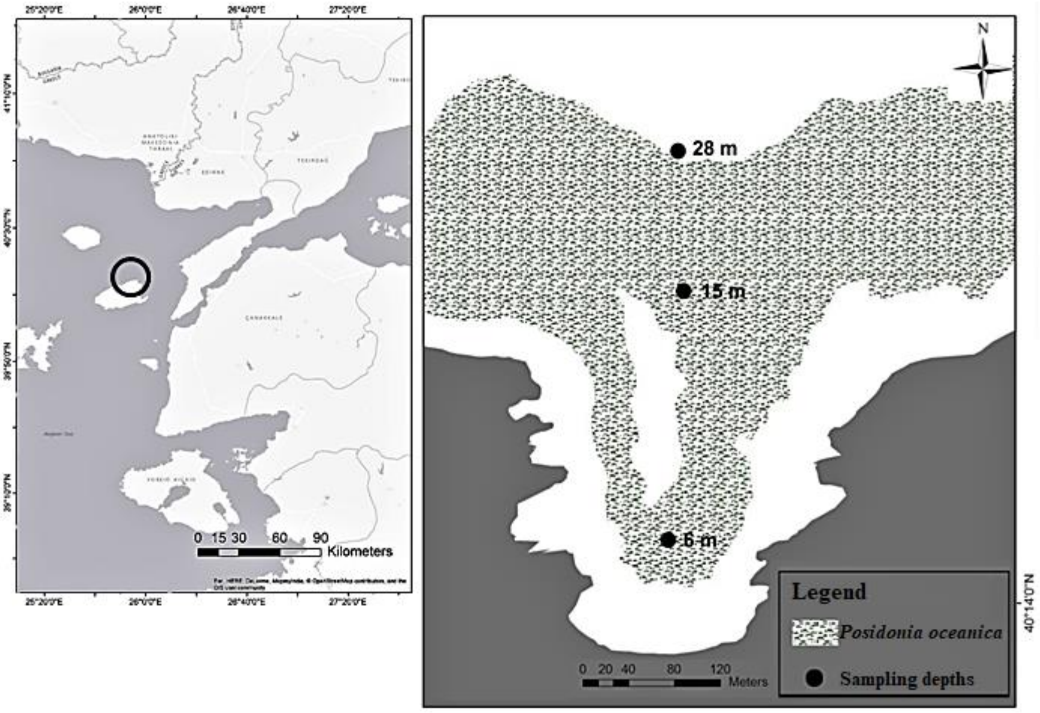

Figure 1. Study site.

variances, respectively. Significancy of levels are tested at P<0.05. In addition, the relationships between epiphytic microalgal group abundances, *P. oceanica* descriptors and water quality parameters are evaluated by using the Pearson correlation coefficient.

Hierarchical clustering of Bray – Curtis is also used to detect the similarities between epiphytic microalgal abundances among sampling months and depths. Bray – Curtis similarity coefficient (produced from log (x+1)-transformed data) in order to balance the contributions of very abundant species and rare species) created a matrix for the Analysis of Similarity (ANOSIM) used to identify the differences in the abundances and for the Analysis of Similarity of Percentages (SIMPER) used to detect the species responsible for the dissimilarities among sampling dates (Clarke, 1993; Clarke & Warwick, 1994; 2001). Considering the algal composition; the Shannon-Weaver (H´) diversity index is calculated. Biostatistical analyses are conducted using PRIMER 5 software.

Results

Ecological Status of *Posidonia oceanica*

Meadow density decreased significantly (MANOVA *f* = 4.178, P<0.05) along depths; that the highest (mean 207 ± 12 shoot m^{-2}) at 6 m whereas; the lowest (mean 75 ± 16.4 shoot m^{-2}) at 28 m. Meadow cover showed significant decline parallelly to the meadow density (MANOVA *f* = 5.031, P<0.05) with the highest value (mean 89 ± 8%) at 6 m and the lowest value (mean 37 ± 7%) at 28 m. On the contrary, leaf surface increased significantly along the depth gradient (MANOVA *f* = 3.945, P<0.05); with the highest value (mean 220 cm^2 shoot^{-1}) at 28 m and the lowest value (mean 140.6 cm^2 shoot^{-1}) at 6 m. In spite of these results, total leaf number is stable at all depths (MANOVA *f* = 0.746, P>0.05); changed temporally from July to January with the mean values of 7.0 ± 0.8 leaf shoot^{-1} and 5.0 ± 0.5 leaf shoot^{-1}, respectively.

Water Quality Parameters

The results of the water quality parameters in the water column of the meadow lower limit are shown in the Figure 2. It can be seen that temperature dynamics are typically temporal; the lowest temperature (13.2 °C) is measured in January; while the highest (26.4 °C) is measured in July at surface water. Water transparency varied between 10 m and 18 m with an average of 12.8 ± 3.5. The lowest transparency (10.3 m) is measured in May however contrary to expectation, January has also low values (10.6 m) due to the massive mucilage formation.

Salinity exhibited a variation among both sampling months and depths; lowest value (27.0%) is recorded at surface water in October, while highest value (39.0%) is recorded in July at deeper. The mean value of pH is recorded as 7.8 ± 0.2 and is similar both in the annual cycle and in the vertical profile. The lowest value (6.0) of pH is noted in July at deeper waters. Nutrient levels are recorded at their maximum at 28 m in January; 10.3 µg l^{-1} for dissolved inorganic nitrogen; 0.20 µg l^{-1} for total phosphorus and 0.04 µg l^{-1} for silicate. Considering the mean annual values for dissolved inorganic nitrogen, total phosphorus and silicate values ranged from 10.3 µg l^{-1} to 1.2 µg l^{-1}, 0.3 µg l^{-1} to 0.1 µg l^{-1} and 0.09 µg l^{-1} to 0.01 µg l^{-1}, respectively during the study period.

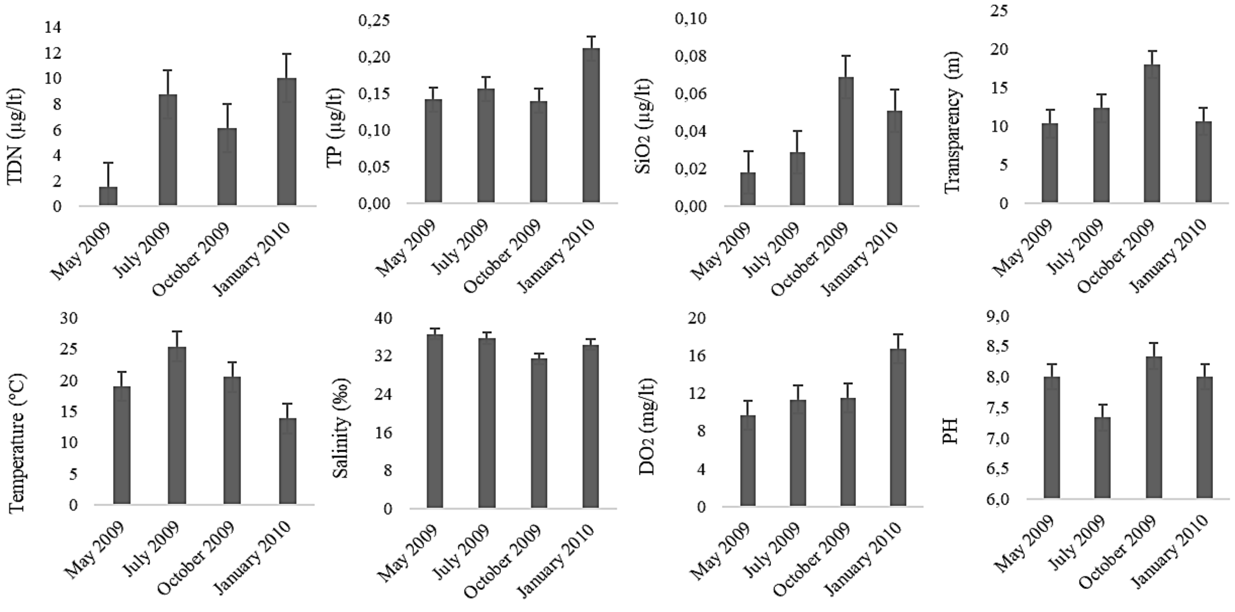

Figure 2. Mean values and standard deviations of the temporal variations of the water quality parameters.

Epiphytic Microalgal Groups

Throughout the study period; a total of 56 taxa belonging to the epiphytic microalgal groups; Bacillariophyceae (91%), Cyanophyceae (7%) and Dinophyceae (2%) are identified on *Posidonia oceanica* leaf blades (Table 1).

Bacillariophyceae revealed significant variations temporally (MANOVA *f* = 3.491, P<0.05); highest abundances are recorded in May (864 cells 50 g^{-1} fw *Posidonia*) and July (570 cells 50 g^{-1} fw *Posidonia*) whereas; lowest abundances (317 cells 50 g^{-1} fw *Posidonia*) are recorded in October. Followingly, in January, epiphytic community has substituted with Cyanophyceae (66517 cells 50 g^{-1} fw *Posidonia*) and Dinophyceae (262 cells 50 g^{-1} fw *Posidonia*) on the leaf blades. Cyanophyceae and Dinophyceae didn't show significant variations among sampling months (MANOVA *f* = 1.262, P>0.05); however, reached to their highest abundances in the winter period due to the massive mucilage formation on the sea bottom. Total epiphytic abundance in October (ave. sim. 50.40) and January (ave. sim. 13.19) showed an obvious separation from May (ave. sim. 30.77) and July (ave. sim. 59.47), according to the Bray–Curtis clustering (Figure 3). SIMPER results also showed that group dissimilarities are obviously high (ave. dis. 79.33%) between May and January due to the contribution of *Lyngbya* sp. (40.29%) that showed an extreme growth in the winter period. Furthermore, *Lyngbya* sp. (35.13%) and *Cocconeis scutellum* (6.89%) are the responsible species for the high group dissimilarities (ave. dis. 72.22%) between May and October (Table 2).

In terms of the mean epiphytic microalgal species diversity (H′) differed between sampling months; the lowest values are recorded as 0.69 ± 0.97 in October and as 1.29 ± 0.17 in January whereas; the highest value is recorded as 2.30 ± 0.11 in May (Figure 4).

Considering the bathymetric profile, epiphytic microalgal abundances differed between 6 m (ave. sim. 34.18) and 28 m (ave. sim. 28.23) depths; *Lyngbya* sp. (42.84%) and *Calothrix* sp. (7.1%) are the responsible species for the high dissimilarities (ave. dis. 69.80%) (Table 3). On the group basis, Bacillariophyceae, also showed significant variations bathymetrically (MANOVA, P<0.05); reaching high abundances through the vertical profile; the lowest (494 cells 50 g^{-1} fw *Posidonia*) and the highest (982 cells 50 g^{-1} fw *Posidonia*) abundances are detected at 6 m and 28 m, respectively.

As for the relationship between the epiphytic microalgal abundance and leaf surface area (r = 0.787, P<0.05) and leaf number (r = 0.647, P<0.05) is verified with the Pearson coefficient both showing the same increasing trend towards to 28 m depth. Also, there is a correlation between epiphytic microalgal abundance and total phosphorus (r = 0.873, P<0.05); dissolved inorganic nitrogen (r = 0.550, P<0.05) values.

Discussion

Epiphytic microalgal composition and abundance on *Posidonia oceanica* leaf blades estimated within this study, have provided relevant information on the ecological quality in Gökçeada Underwater Park (North Aegean). Due to their sensitivity to environmental alterations, community structure and abundance of the epiphytic microalgal groups on leaf blades, showed temporal and bathymetric variation during the sampling period.

Since seagrass leaf blades are ideal habitats for benthic algal colonization (Mabrouk *et al.*, 2014), the presence of *P. oceanica* meadows around Gökçeada

Table 1. List of epiphytic microalgal species on *Posidonia oceanica* leaf blades

	May - 6 m	May - 15 m	May - 28 m	Jul - 6 m	Jul - 15 m	Jul- 28 m	Oct- 6 m	Oct - 15 m	Oct - 28 m	Jan - 6 m	Jan- 15 m	Jan - 28 m
Bacillariophyceae												
Calothrix sp.	+	-	-	+	+	+	-	-	+	-	-	+
Lyngbya sp.	+	-	-	+	+	+	+	+	+	-	+	+
Phormidium sp.	-	-	-	-	-	-	-	-	+	-	-	+
Spirulina sp.	-	-	-	-	-	+	-	-	-	-	-	+
Achnanthes sp.	+	-	+	+	+	+	-	-	+	-	-	+
Amphiprora sp.	+	+	-	-	-	-	-	+	-	-	-	-
Amphora sp.	-	+	-	-	-	+	-	-	-	-	-	-
Amphora costata W. Smith	-	-	-	-	+	-	-	+	-	-	-	-
Amphora delicatissima Krasske	-	-	-	-	+	+	-	+	+	-	-	-
Amphora hyalina Kützing	-	-	+	-	-	-	-	-	-	-	-	-
Biddulphia alternans (Bailey) Van Heurck	-	-	-	-	-	+	-	-	-	-	-	-
Campylodiscus decorus Brébisson	-	-	+	-	-	-	-	-	-	-	-	-
Cocconeis sp.	-	-	-	-	-	-	-	-	+	+	+	-
Cocconeis pseudomarginata Gregory	-	-	-	-	-	-	-	+	+	-	-	-
Cocconeis placentula Ehrenberg	-	+	+	+	-	+	-	-	-	-	-	-
Cocconeis scutellum Ehrenberg	+	+	+	+	+	+	-	+	+	+	+	+
Diploneis sp.	+	-	+	+	+	+	-	+	-	-	-	-
Diploneis bombus (Ehrenberg) Cleve	-	-	-	-	-	-	-	-	-	-	-	-
Diploneis constricta (Grunow) Cleve	-	-	-	-	-	+	-	-	+	-	-	-
Diploneis crabro Ehrenberg	-	-	-	-	+	-	-	-	-	-	-	-
Diploneis didyma (Ehrenberg) Cleve	-	-	-	-	-	-	-	-	+	-	-	-
Diploneis incurvata (Gregory) Cleve	-	-	-	-	-	-	+	-	+	-	-	+
Diploneis smithii (Brébisson) Cleve	-	-	+	-	-	-	-	-	-	-	-	-
Grammatophora marina (Lyngbye) Kützing	+	+	-	+	-	+	-	-	+	-	-	+
Grammatophora angulosa Ehrenberg	-	-	-	-	-	-	-	-	-	-	+	-
Gyrosigma acuminatum (Kützing) Rabenhorst	+	-	+	-	-	+	-	+	+	-	-	-
Hemiaulus hauckii Grunow ex Van Heurck	+	-	-	-	-	-	+	-	-	-	-	-
Licmophora sp.	+	-	-	-	-	-	-	-	-	-	-	-
Licmophora ehrenbergii (Kützing) Grunow	-	+	+	-	+	+	-	+	+	-	-	-
Licmophora gracilis (Ehrenberg) Grunow	-	-	+	-	-	+	-	+	+	-	-	-
Lichmophora grandis (Kützing) Grunow	-	-	-	-	+	+	-	-	-	-	-	-
Lichmophora quadriplacata Mereschkowsky	-	-	-	-	-	-	-	+	-	-	-	-
Mastogloia acutiuscula Grunow	-	+	-	-	-	-	-	-	-	-	+	-
Navicula sp.	+	-	+	+	+	+	-	+	+	+	-	+
Navicula placenta Ehrenberg	-	-	-	-	-	-	-	-	+	-	-	-
Navicula rostellata Kützing	-	-	-	-	-	-	-	-	+	-	-	-
Nitzschia sp.	+	+	-	+	-	+	-	+	+	-	+	-
Nitzschia closterium (Ehrenberg) Smith	-	-	-	-	+	-	-	+	+	-	-	-
Nitzschia constricta (Kützing) Ralfs	+	-	-	-	-	-	-	-	-	-	-	-
Nitzschia frustulum (Kützing) Grunow	-	-	-	-	-	-	-	+	-	-	-	-
Nitzschia linearis (Agardh) Smith	-	-	-	-	-	+	-	-	-	-	-	-
Odontella sp.	-	-	-	-	-	+	-	-	-	-	-	-
Pinnularia sp.	+	+	+	-	-	+	-	-	-	+	+	+
Pinnularia gibba var. *linearis* Hustedt	-	-	-	-	-	-	-	+	-	-	-	-
Pinnularia viridis (Nitzsch) Ehrenberg	-	-	-	-	-	+	-	-	-	-	-	-
Plagiogramma interruptum (Gregory) Ralfs	+	-	-	-	-	-	-	-	-	-	-	-
Pleurosigma sp.	-	-	-	-	-	-	-	-	-	-	-	-
Podocystis adriatica (Kützing) Ralfs	-	-	-	-	+	-	-	-	-	-	-	-
Stauroneis sp.	+	-	-	+	-	-	-	-	+	-	-	-
Surirella ovalis Brébisson	-	-	-	+	-	-	-	-	-	-	-	-
Surirella didyma Kützing	+	+	-	+	-	+	-	-	+	-	-	-
Synedra sp.	-	-	-	-	-	-	+	-	+	-	+	+
Synedra tabulata (Agardh) Kützing	-	-	+	-	+	+	-	+	+	-	+	-
Synedra ulna (Nitzsch) Ehrenberg	-	-	-	-	-	+	-	-	-	-	-	-
Tabellaria fenestrata (Lyngbye) Kützing	+	-	-	+	-	-	-	-	-	-	-	-
Cyanophyceae												
Calothrix sp.	+	-	-	+	+	+	-	-	+	-	-	+
Lyngbya sp.	+	-	-	+	+	+	+	+	+	-	+	+
Phormidium sp.	-	-	-	-	-	-	-	-	+	-	-	+
Spirulina sp.	-	-	-	-	-	+	-	-	-	-	-	+
Dinophyceae												
Prorocentrum lima (Ehrenberg) Dodge	+	+	-	-	+	-	-	+	+	+	-	+

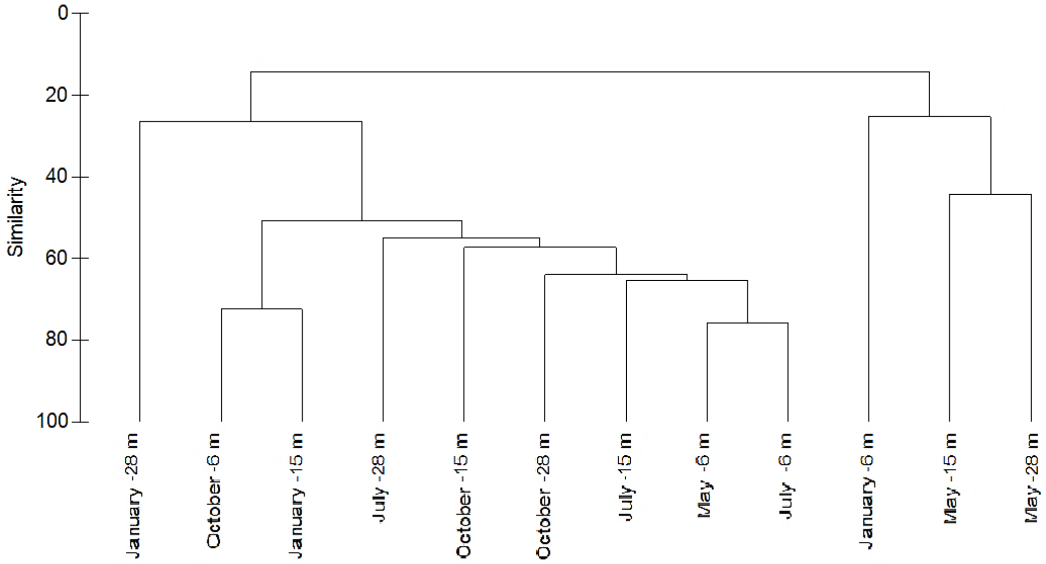

Figure 3. Bray-Curtis similarity % dendogram of epiphytic microalgal abundance according to the sampling months and depths.

Table 2. SIMPER results among sampling months

	Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Groups May & July	*Lyngbya* sp.	11.89	50.31	27.30	1.55	42.68	42.68
Average dissimilarity= 63.95	*Calothrix* sp.	3.83	9.10	5.52	1.48	8.63	51.31
	Achnanthes sp.	2.76	5.14	2.79	1.33	4.36	55.67
Groups May & October	*Lyngbya* sp.	11.89	37.48	25.37	1.37	35.13	35.13
Average dissimilarity = 72.22	*Cocconeis scutellum*	7.62	4.14	4.98	1.19	6.89	42.02
	Calothrix sp.	3.83	2.87	3.15	0.90	4.36	46.38
Groups July & October	*Lyngbya* sp.	50.31	37.48	8.90	0.96	20.05	20.05
Average dissimilarity = 44.40	*Calothrix* sp.	9.10	2.87	4.24	1.38	9.54	29.59
	Cocconeis scutellum	6.14	4.14	2.88	1.42	6.50	36.08
Groups May & January	*Lyngbya* sp.	11.89	95.17	31.96	1.25	40.29	40.29
Average dissimilarity = 79.33	*Cocconeis scutellum*	7.62	2.88	6.43	0.99	8.10	48.39
	Calothrix sp.	3.83	14.38	5.09	1.01	6.41	54.80
Groups July & January	*Lyngbya* sp.	50.31	95.17	31.63	1.60	45.78	45.78
Average dissimilarity = 69.10	*Calothrix* sp.	9.10	14.38	7.40	2.78	10.71	56.49
	Achnanthes sp.	5.14	1.49	2.99	1.08	4.32	60.81
Groups October & January	*Lyngbya* sp.	37.48	95.17	32.29	1.38	46.69	46.69
Average dissimilarity = 69.16	*Calothrix* sp.	2.87	14.38	4.38	1.00	6.34	53.03
	Phormidium sp.	4.53	8.97	3.92	0.89	5.66	58.70

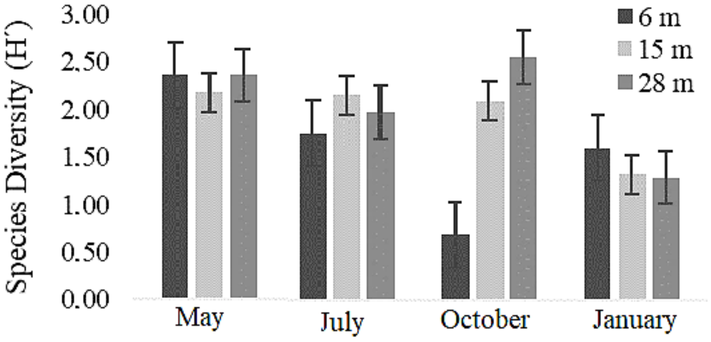

Figure 4. Shannon–Weaver species diversity (H´) among sampling months and depths.

Table 3. SIMPER results among depths

	Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Groups 6 m & 15 m	*Lyngbya* sp.	25.83	28.62	17.12	0.86	28.42	28.42
Average dissimilarity = 60.22	*Cocconeis scutellum*	3.24	7.62	5.36	1.09	8.89	37.32
	Calothrix sp.	4.64	3.17	4.13	0.97	6.86	44.18
Groups 6 m & 28 m	*Lyngbya* sp.	25.83	91.69	29.90	1.40	42.84	42.84
Average dissimilarity = 69.80	*Calothrix* sp.	4.64	14.82	4.95	1.28	7.10	49.93
	Phormidium sp.	0.00	10.13	3.79	0.91	5.43	55.37
Groups 15 m & 28 m	*Lyngbya* sp.	28.62	91.69	28.01	1.41	42.61	42.61
Average dissimilarity = 65.74	*Calothrix* sp.	3.17	14.82	4.91	1.32	7.47	50.08

Island (North Aegean) is vital for the coastal ecological status of the region contributing to the ecosystem services. However, water turbidity affects the meadow density and meadow cover through greater depths, where the light diffusing to the leaves diminishes. Also, same results from the Mediterranean Basin are supported by Pergent *et al.* (1995); Tsirika *et al.* (2007) and Mabrouk *et al.* (2014).

On the other hand, extreme leaf movement at the sea bottom, affect the settlement of epiphytes on seagrass leaves and macroalgal thalli (Borowitzka & Lethbridge, 1989; Borowitzka *et al.*, 2006). Therefore, leaf blades have lost their functional substratum characteristics for epiphytic communities since they are exposed to strong hydrodynamism (eg. waves and currents) at shallower depths where leaf movement is greater (Aktan *et al.*, 2011). *P. oceanica* leaf surface improvement through greater depths, have provided a convenient substratum for epiphytic colonization. Relation between epiphytic microalgal abundance and leaf surface area ($r = 0.787$, $P<0.05$), highlighted that leaf surface improvement support the epiphytic succession to form a diverse benthic community.

In this study, Bacillariophyceae is the principal group among epiphytic microalgal groups in terms of species composition, dominated by the pennate diatom taxa; *Navicula*, *Pinnularia*, *Achnanthes*, *Cocconeis*, *Nitzschia*, *Licmophora* and *Synedra*. They demonstrate widespread micro-epiphytism reaching to high abundances on benthic substrates (Sullivan, 1979; Mazzella *et al.*, 1994; Mabrouk *et al.*, 2014). As stated by Patrick (1977), diatoms are the dominant group of macrophyte-associated microalgae leading other groups to proceed the colonization on leaf blades.

Totti, Poulin, Romagnoli, Perrone and Pennesi (2009) stated that diatom taxa having high adhesive strength characteristics (*Cocconeis* and *Achnanthes* species), tolerate strong hydrodynamic conditions. Due to their adaptation capability to hydrodynamic conditions, *Cocconeis* and *Achnanthes* are abundant at shallower depths where strong hydrodynamism prevailing; while erect growth forms attached to the substratum by mucilaginous stalks (*Nitzschia*, *Lichmophora*, *Grammatophora* and *Synedra*) are abundant at greater depths (15 m and 28 m) where hydrodynamism is lower. In particular, the genus *Cocconeis* Ehrenberg, family Cocconeidaceae, order Achnanthales is frequent in all seasons firmly attached to the leaf blades through their raphe-sternum valves. The genus *Cocconeis* protect themselves from grazers and hydrodynamism by forming monospecific biofilms during their colonization process, however generally subjected to the cell exudation of the host plant (Round, 1984; Mazzella *et al.*, 1994).

During the sampling period, we noticed that temporal and bathymetric dynamics of the epiphytic microalgal composition and abundance depend on nutrient loadings in the water column. Bacillariophyceae are influenced by silicate (SiO_2), restrictive element for the growth of diatoms (Round *et al.*, 1990) whereas; dissolved inorganic nitrogen (DIN) and total phosphorus (TP) levels have played an important role in the development of Cyanophyceae and Dinophyceae groups. Dissolved inorganic nitrogen (DIN) and total phosphorus (TP) concentrations have reached to maximum values at 28 m depth; 10.3 µg l^{-1} and 0.20 µg l^{-1}, respectively in January when extensive mucilaginous aggregations accumulated both on the sea bottom and on the *P. oceanica* meadows. As termed by Precali *et al.* (2005), observed aggregate type was ‘blanket’ forming homogenous layers of combined aggregates on the benthic habitats. This mucilage phenomenon is attributed to the excessive growth of phytoplanktonic species (Aktan & Gümüşoğlu, 2010), at the same time, Bacillariophyceae have substituted with Cyanophyceae and Dinophyceae in terms of group abundances.

Coastal zones of Gökçeada (North Aegean), have oligotrophic characteristics, however, the study site near Kaleköy Port with recreational activites, is characterized as mesotrophic or eutrophic due to nutrient inputs. In general, low nutrient levels in the spring period, are linked to algal productivity, whereas high levels of nutrients in the winter period, are attributed to algal growth limitation due to low water temperature and light intensity at deeper waters of the region (Aktan & Gümüşoğlu, 2010). In this study, high levels of nutrients and turbidity (Secchi depth; 11 m) are typical in the water column throughout the mucilage precipitation, also promoted the growth of toxin-producer epiphytic genera; *Lyngbya* and *Prorocentrum*. As known to be potential endotoxin (lyngbyatoxin-a) producers (Hamisi *et al.*, 2004); *Lyngbya* colonies dominated the surface of the substratum as filamentous mats in order to compete with other photosynthetic

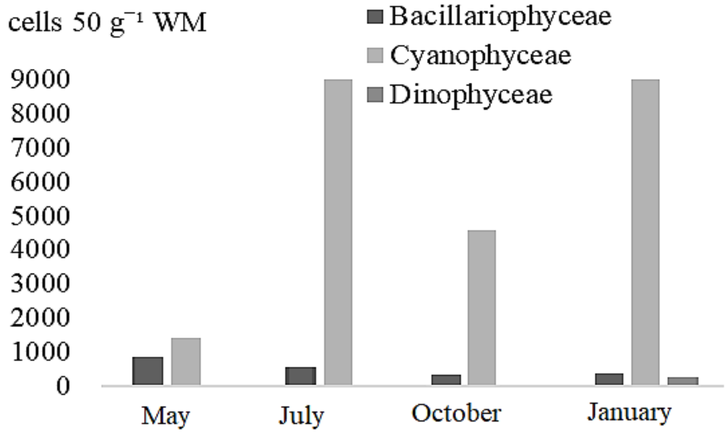

Figure 5. Epiphytic microalgal abundance throughout the study period.

epiphytes for light energy. Meanwhile; okadaic acid and dinophysistoxin producer (Nakajima *et al.*, 1981; Marr *et al.*, 1992; Barbier *et al.*, 1999); *Prorocentrum* occurred solitarily on leaf blades. Responsible from the diarrhetic shellfish poisoning (DSP) (Prokic *et al.*, 1998); *Prorocentrum lima* reached to maximum abundance in this period when environmental conditions have been destroyed as also confirmed by other studies (Belegratis *et al.*, 1999; Bouchouicha *et al.*, 2013; Mabrouk *et al.*, 2014). At the end of the winter period, resistance of the mucilageous precipitation has influenced negatively when warmer waters diffuse to the aggregates, as also stated by Alldredge & Crocker (1995).

Throughout the study period; low diversity of the epiphytic microalgal species, is related to the dominancy of filamentous cyanobacteria due to their tolerance to high nutrient levels and temperatures (Paerl & Huisman, 2009). According to the previous studies (Turley *et al.*, 2000; Ignatiades *et al.*, 2002; Balkıs, 2009), the North Aegean Sea can be regarded as an oligotrophic system due to low nutrient levels. However, coastal waters of Gökçeada Island (North Aegean) are affected by increasing levels of nutrients and organic compounds as a consequence of agricultural activities and urban discharges in the recent years. Moreover, considering these outputs in terms of an early warning of changing climatic conditions; higher temperatures (>14 °C) in winter periods, might be promoted the excessive growth of cyanobacteria in such a temperate region (Figure 5).

In case of the occurrence of mucilage phenomena along the Aegean coasts and intensive colonizations of opportunistic species; biodiversity of the benthic community within the meadow habitats will be affected by the deterioration in the water quality. Taking all these facts into consideration, epiphytic microalgal assemblages responding quickly to environmental alterations, should be used as bio-indicators in the monitoring programs to distinguish and minimize the pressures on coastal ecosystems. However; the deficiency of monitoring studies through epiphytes; is linked to their disadvantageous feature; a time-consuming process including a detailed laboratory work. Therefore, this study can be used as a necessary data for the future management plans for critical ecosystems, especially in less evaluated regions of the Eastern Mediterranean Sea.

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