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Epiphytic bryozoan community of *Posidonia oceanica* (L.) Delile leaves in two different meadows at disturbed and control locations

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Abstract

The impact of fish farming activities on *Posidonia oceanica* meadows in the Aegean Sea have been detected using bryozoan epiphytes as indicators of disturbance. Samples were collected by SCUBA diving in September 2004 in order to compare assemblages between disturbed (I1:0-5 m; I2:5-10 m and I3:10-15 m) and control meadows (C1:0-5 m; C2:5-10 m and C3:10-15 m) located at different depths. Regarding the mean percentage coverage of bryozoans, significant differences were found between stations, leaf sides, depths and their interactions ($p<0.05$). Bryozoan species densely colonized the back side of adult leaves. *Bantariella verticillata*, *Alcyonidium* sp., *Aetea truncata*, *Chorizopora brongniartii*, *Fenestrulina joannae* were frequently observed on *P. oceanica* leaves. *B. verticillata* showed statistically significant side and station differences, while other species showed only significant side differences. Moreover, the mean coverage of *Aetea truncata* revealed no station or side variations. *B. verticillata* could be evaluated as an appropriate key species studying any environmental changes. In the impacted meadow, higher mean coverage values of the species were particularly determined on front side of the leaves. These results suggest that locality, leaf side and leaf part can influence the coverage value of epiphytic bryozoan species.

Keywords: Epiphyte, Bryozoan, Fish farming, *Posidonia oceanica*, Aegean Sea, Spatial variability.

Introduction

In the seagrass ecosystem, leaves and rhizomes provide an alternative substrate for various groups of organisms growing on the plant such as algae, hydrozoa, bryozoa, molluscs, crustaceans, which are called epiphytes. Seagrass epiphytic algae are important primary producers and contribute significantly to the food web. They can account for more than 50% of standing stock production in the sea grass meadow. Seagrass also shows high levels of N_2 fixation via the epiphytic cyanobacteria living on it (Borowitzka *et al*., 2006).

Seagrasses and their epiphytes are sensitive to enhanced nutrient concentrations in the water column, which contributes to epiphyte coverage (Frankovich & Fourqurean, 1997; Borg *et al*., 2006; Terrados & Medina-Pons, 2008). Previous studies have focused on changes in abundance and composition of epiphyte assemblages derived from nutrient enrichment (Balata *et al*., 2008; 2010; Ben Brahim *et al*., 2010). Among abiotic factors, exposure to waves and currents are considered to have negative effects on the accumulation of epiphyte biomass on seagrass leaves (Frankovich & Fourqurean, 1997; Cornelisen & Thomas, 2004). Nevertheless, changes in epiphyte biomass have been investigated in various studies; knowledge about epiphyte species sensitivity to anthropogenic disturbances is limited (Ben Brahim *et al*., 2010). Epiphytes of *P. oceanica* showed an increase in biomass related to fish farm nutrient loading (Delgado *et al*., 1999; Ruiz *et al*., 2001; Cancemi *et al*., 2003). Differences between meadows appeared to be linked to local differences in environmental factors influenced by human disturbance (Balata *et al*., 2007).

Bryozoans are one of the most common sessile organisms in the epiphytic community of *P. oceanica* meadows (Pardi *et al*., 2006). The growth pattern of bryozoan epiphytes is affected by the flexibility and duration of the substratum. Moreover, they remain in small colonies and begin reproduction activity at a small size in order to adapt to life on *P. oceanica* leaves (Hayward, 1974).

In this study, species composition and coverage of epiphytic bryozoan assemblages on *Posidonia oceanica* leaves were investigated near fish farm suspended cages and a reference site selected at the same depth range. The response of epiphytic bryozoan species to fish farming activities was investigated as appropriate indicators of disturbance among bryozoan epibiont species.

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Material and Methods

Sampling area

The study area, located at Engeceli Bay (Aegean Sea) has sandy-muddy sediments. Northern winds were dominant in the area and the current speed changed between 4.06-5.49 cm.sn^{-1} (Gier *et al*., 2007). In the Bay, farming activities began in 1990 above a *P. oceanica* bed. The facility has 36 cages of 12 m in diameter and another 20 cages with dimensions of 5x5x5 m, which cover a surface of approximately 3500 m^2. During 2004, the annual production was 300 t. At the facility, 360 g of faeces were produced per kg of fish production (Gier *et al*., 2007). Dead *P. oceanica* rhizomes beneath the cages indicate that the meadow totally disappeared. The impacted location was chosen in the facility area, approximately 300 m from the net cages. At this station, sea grass beds were less dense (452 ± 21 shoot per m^2) than those selected as control (501 ± 12 shoot per m^2) (Kocak *et al*., 2011). The impacted stations (I) were located at three different depth ranges (I1:0-5 m; I2:5-10 m and I3:10-15 m). The other selected site included control locations (C) at a distance of 1200-1500 m from the fish cages and at the same depth ranges (C1, C2 and C3) as the impacted ones (Fig 1.). Three replicates were collected about 100 m apart from each other, at each of the six stations, using a 20x20 cm quadrate. Five shoots randomly selected from each quadrate were uprooted and preserved in 4% formalin-seawater solution. The samples of *P. oceanica* and sediments (0-5 cm) from the stations were collected by scuba diving in September 2004 when epiphyte assemblages are known to reach their maximum development (Balata *et al*., 2007).

Laboratory and data analyses

The bryozoan epiphytes colonising adult *Posidonia* leaves were studied at species level. Adult leaves were determined according to Giraud, 1977. Both the internal (Back side) and external faces (Front side) of the leaves of each shoot were examined under stereo and dissecting microscope. *Posidonia* leaves were divided into 5 cm pieces from the leaf apex, the oldest part of leaves (Pardi *et al*., 2006; Balata *et al*., 2008; Balata *et al*., 2010). Percentage coverage of the frequently observed five bryozoan species on these parts was calculated to investigate possible preferences. Species coverage was determined by using areas of 1 mm^2. The number of areas covered by bryozoan species on all blade fragments was counted. The mean percentage cover of each species found on adult leaves was calculated as the ratio between squares in which the bryozoan species were present versus the total of the squares on the leaf side for each station.

In order to determine nutrient concentrations, water samples were taken from just above the meadows using a Nansen bottle. The concentrations of ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), orthophosphate phosphorus (oPO_4-P) and total phosphate phosphorus (TPO_4-P) were measured using an auto analyzer and the colorimetric methods. Water samples were filtered through GF/F filters for chlorophyll-a analysis and the concentrations were measured by Sequoia-Turner Fluorometer (Strickland & Parsons, 1972). Dissolved oxygen (DO) values were measured according to the Winkler method. The amount of organic matter (%) in sediment samples was determined spectrophoto-

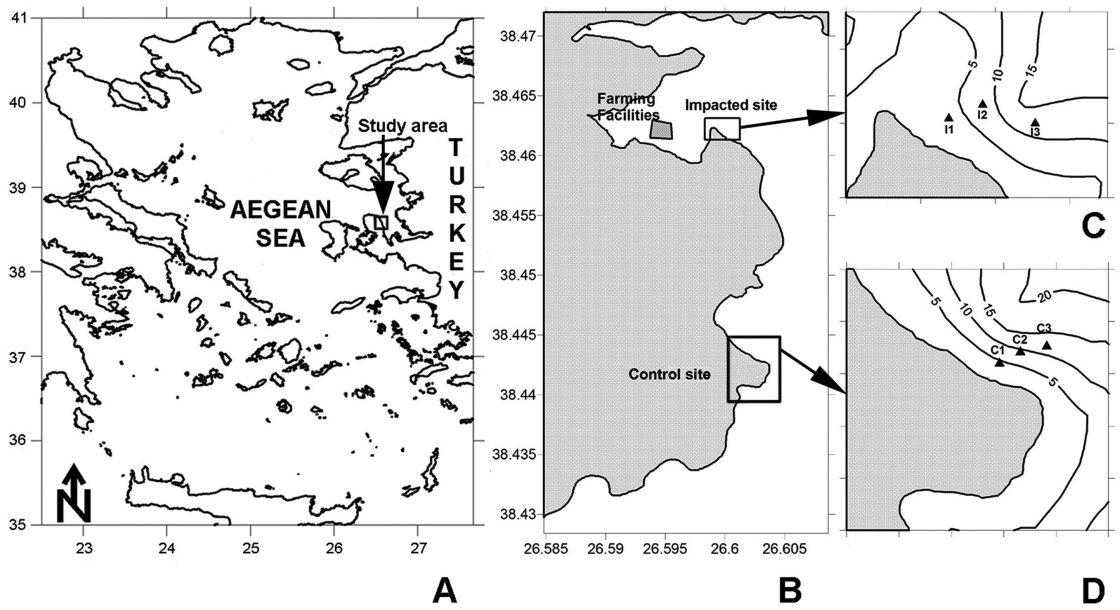


Fig. 1: Location of the study area.

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metrically following the sulfochromic oxidation method (Hach, 1988).

STATISTICA software was used for statistical analysis. Analysis of variance (3-way ANOVA) was used to test the hypothesis that the mean percentage coverage of bryozoan species differed between locations (impact and control), depth range (0-5 m; 5-10 m; 10-15 m) and leaf side (Front and Back). Cochran's C test was used before each analysis to check for homogeneity of variance and the data were log-transformed when necessary. The mean coverage values of frequently observed species on adult leaves (*Bantariella verticillata*, *Alcyonidium* sp., *Aetea truncata*, *Chorizopora brongniartii*, *Fenestrulina joannae*) were compared among stations and leaf sides by Kruskal-Wallis non-parametric tests when data were not normally distributed. Soyer's (1970) Frequency Index (F) was used for determining the frequency value for each bryozoan species as constant (F≥49%), common (49%>F≥25%) and rare (F<25%).

Results

The nutrient concentrations in the water column at the sampling stations are presented in Table 1. TIN, NO_2-N and NO_3-N were recorded with higher mean values at the control meadows. Concerning NH_4-N, higher concentrations were observed in the impacted area compared to the control site. Concentration reached a maximum value at station I2 where it was 3 times higher than the value recorded at station C1. Dissolved phosphorous concentrations (oPO_4-P) presented high mean values at the control meadow and the highest concentration was recorded at station C2. Additionally, maximum (0.56 ± 0.03 μM) and minimum (0.20 ± 0.02 μM) values of TPO_4-P were measured in the control areas. Chlorophyll-a concentrations displayed differences between meadows and the highest level were detected at the impacted station (0.10 ± 0.01 μg/l). Organic matter (%) values were significantly higher at the impacted sites (7.6-10.7%) compared to control stations.

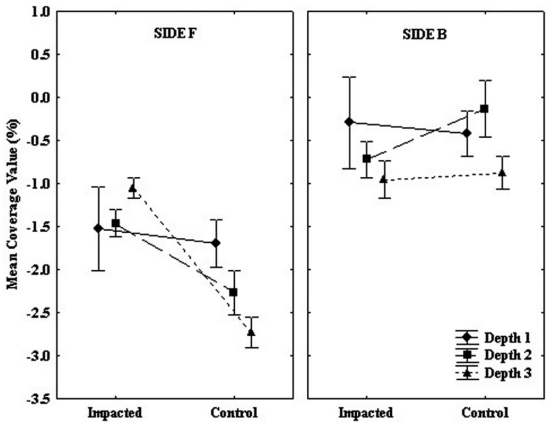


Fig. 2: Mean percentage cover (vertical bars denote 0.95 confidence intervals) of epiphytic Bryozoan species at the control and impacted locations for leaf sides (F: Front; B: Back).

The results of ANOVA performed on the bryozoan coverage data are given in Table 2. Statistically significant differences were found between stations, sides and depths ($p<0.05$) regarding bryozoan coverage data. Bryozoan coverage was affected by abiotic factors such as depth, leaf side and location. Although *P. oceanica* leaves tend to be covered by different bryozoan species and their coverage also increased with depth on the front side of leaves at impacted locations, no statistically significant difference was revealed. Coverage of bryozoan species on the back side was always higher than that found on the front side for both locations. Moreover, the coverage on the back side showed a decreasing tendency with depth at the impacted area (Fig. 2).

Concerning sides, coverage did not show any significant variations in the 0-5 m depth range between impacted and control stations. Regarding the mean coverage value, differences became evident on the front sides and changed significantly with increasing depth range at the control locations, C2 and C3 (Fig. 2). Thus, the lowest mean coverage value on the leaves was determined at the 10-15 m depth range at the control site.

A total of 20 epibiont bryozoan species was identified on *Posidonia* leaves at the control and impacted meadows. A total of 19 species were determined at control sites. However, eight of these species were rare; namely, *Pherusella* sp., *Copidozoum tenuirostre*, *Bugula stolonifera*, *Bugula* sp., *Diplosolen obelia*, *Tubulipora* sp., *Monoporella nodulifera* and *Electra posidoniae*. Lower species richness was observed at the impacted locations where a total of 12 species were recorded. *Bowerbankia gracilis* was considered as a rare species at the impacted locations (Table 3). Nevertheless, 11 species were found at both localities where *B. verticillata*, *Alcyonidium* sp., *A. truncata*, *C. brongniartii*, *F. joannae* were frequently observed on the leaves of *P. oceanica*. Mean bryozoan coverage values (%) and species richness showed an increasing trend on the tip part (0-15 cm) of the leaves. This is probably due to the fact that the greatest epiphytic coverage occurs towards leaf tips and is correlated with leaf age (Trautman & Borowitzka, 1999). In contrast to the front side, coverage values increased downwards on the leaves at the back side. Although both sides of the leaves were covered extensively by *B. verticillata* at the impacted meadows, there was a significant difference in coverage on the front side between the two locations ($p<0.05$). The frequency index values of *B. verticillata* were constant on both sides of the leaves at the deepest station. This species preferred the first 15 cm from the apex of both sides of the leaves; however, a higher mean coverage value was determined on the front side (Table 4).

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Table 1. Physico-chemical characteristics of the stations

Parameters	I1	I2	I3	C1	C2	C3
DO (mg/l)	7.76±0.06	7.38±0.03	7.61±0.11	7.70±0.20	7.70±0.10	7.56±0.06
Temperature (°C)	22.1	21.7	22	22.1	21.8	22
o.PO_4-P (µM)	0.06±0.00	0.08±0.01	0.09±0.02	0.08±0.01	0.14±0.05	0.08±0.00
NO_{3-}N (µM)	0.39±0.02	0.45±0.03	0.53±0.09	0.55±0.07	1.06±0.46	1.07±0.01
NO_2-N (µM)	0.03±0.01	0.07±0.01	0.03±0.01	0.07±0.01	0.08±0.00	0.04±0.01
NH_4-N (µM)	1.11±0.31	1.55±0.17	1.30±0.23	0.55±0.13	0.57±0.11	0.46±0.21
TIN (µM)	1.31±0.29	1.36±0.14	1.54±0.32	1.17±0.06	1.71±0.36	1.57±0.20
T.PO_4-P (µM)	0.40±0.09	0.34±0.12	0.36±0.05	0.37±0.06	0.56±0.03	0.20±0.02
Reak.Si (µM)	1.02±0.18	1.3±0.00	1.27±0.04	1.55±0.15	1.2±0.20	1.55±0.06
Chl.-a (µg/l)	0.09±0.01	0.10±0.01	0.09±0.01	0.07±0.01	0.07±0.01	0.08±0.00
Organic matter (%)	10.7	8.3	7.6	3.2	6.1	7.3

Table 2. The results of three-way ANOVA based on mean percentage cover of bryozoan species on front and back surfaces of Posidonia oceanica leaves collected from different depths at impacted and control stations [df: Degree of freedom. SS: Sum of square. MS: Mean square. F: F-value. p: Probability of null hipothesis. ($p < 0.05$)].

	df	SS	MS	F	*p*
Depth	2	16.0	8.0	9.9	.000*
Stations	1	13.3	13.3	16.4	.000*
Side	1	161.4	161.4	199.1	.000*
Depth*Stations	2	18.7	9.3	11.5	.000*
Depth*Side	2	7.3	3.6	4.5	.011*
Stations*Side	1	30.3	30.3	37.4	.000*
Depth*Stations*Side	2	11.5	5.7	7.1	.001*
Error	838	679.3			

Table 3. Frequency index (F) values of the taxa identified in the epiphytic assemblages on both sides of leaves (F>49: Constant; 25≤F≤49: Common; F<25: Rare)

STATIONS	I1		I2		I3		C1		C2		C3	
SPECIES	FRONT	BACK	FRONT	BACK	FRONT	BACK	FRONT	BACK	FRONT	BACK	FRONT	BACK
Pherusella sp.											1.03	
Alcyonidium sp.	69.23	63.64	16.00	13.24			14.29	13.33	36.17	35.71	28.87	20.99
Nolella gigantea			2.40	1.47	8.68	10.00					1.03	3.70
Bowerbankia gracilis				4.41	1.37							
Mimosella gracilis			4.80		2.74	4.29					3.09	2.48
Bantariella verticillata	30.77	9.09	44.80	32.35	69.41	50.00	38.10	31.11			31.96	23.46
Aetea truncata		9.09	8.80	8.82	13.24	21.43		6.67	6.38			1.24
Electra posidonia											1.03	
Copidozoum tenuirostre									4.26		3.09	4.94
Monoporella nodulifera										3.57		
Beania hirtissima				1.47							1.03	
Beania mirabilis			0.80	1.47			9.52	2.22			1.03	4.94
Bugula stolonifera												1.24
Bugula sp.												2.48
Fenestrulina joannae		9.09	0.80	4.41			16.67	11.11	29.79	28.57	20.62	17.28
Chorizopora brongniartii			16.00	22.06	2.74	10.00	16.67	26.67	14.89	25.00	7.22	17.28
Diplosolen obelia								2.22				
Tubulipora sp.									2.13	3.57		
Lichenopora radiata			0.80				2.38	6.67	2.13			
Cyclostome (sp.)		9.09	5.60	8.82	2.28	4.29	4.76		4.26	3.57		

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Table 4. Mean percentage cover of the frequently observed bryozoan species on each fragment of the leaves (A, B, C, D, E, F, G and H) at both sides (F:Front; B:Back).

		A (0-5cm)		B (5-10cm)		C (10-15cm)		D (15-20cm)		E (20-25cm)		F (25-30cm)		G (30-35cm)		H (35-40cm)	
SPECIES	STA-TIONS	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B
B. verticillata	**I1**	0.543		0.700			1.543										
B. verticillata	**I2**	2.009	0.291	2.195	0.599	0.634	0.783		0.957								
B. verticillata	**I3**	2.572	0.193	3.467	0.588	3.487	0.690			1.232	1.877						
B. verticillata	**C1**	3.724	0.324	1.209	0.692	1.198	0.939	0.536	1.154	0.381							
B. verticillata	**C3**	0.374	0.141	0.207	0.294	0.150	0.350	0.255	0.374		0.524	0.056	1.462				
A. truncata	**I1**		0.418														
A. truncata	**I2**	0.459	0.359	0.342	0.390	0.439	0.732										
A. truncata	**I3**	0.502	0.218	1.150	0.416	0.245	0.656	0.420	0.607		0.980	0.871	0.636				
A. truncata	**C1**		0.286		0.534												
A. truncata	**C2**	0.242		0.346		1.169											
A. truncata	**C3**				0.209												
Alcyonidium sp.	**I1**		0.511	0.174	0.905	0.126											
Alcyonidium sp.	**I2**	0.156	0.292	0.240	0.487	0.065	0.653	0.090									
Alcyonidium sp.	**C1**	0.076	0.379	0.144	0.550	0.055	0.877		1.079					0.072			
Alcyonidium sp.	**C2**	0.393	0.585	0.132	0.866	0.181	1.234	0.064	2.421	0.189							
Alcyonidium sp.	**C3**	0.086	0.250	0.414	0.357	0.068	0.425	0.060	0.847	0.097	0.524	0.090	0.586	0.024	0.808	0.020	
C. brongniartii	**I2**	0.312	0.325	0.091	0.500	0.122	0.947		0.984	0.032		0.036					
C. brongniartii	**I3**	0.030	0.176	0.246	0.392		0.495	0.036	0.496		0.945				0.966		
C. brongniartii	**C1**	0.119	0.360	0.122	0.758	0.121	0.856	0.060									
C. brongniartii	**C2**	0.079		0.086	0.843	0.100	0.814		0.901	0.077							
C. brongniartii	**C3**	0.093	0.159		0.277	0.057	0.614		0.437	0.059	0.501				0.654		
F. joannae	**I1**				0.855												
F. joannae	**I2**	0.161	0.308		0.619		0.877										
F. joannae	**C1**		0.347	0.055	1.200	0.118	0.873	0.137		0.122							
F. joannae	**C2**	0.290	0.436	0.137	0.695	0.056	0.691				1.153	0.046					
F. joannae	**C3**	0.283	0.158	0.199	0.414	0.028	0.523		0.698		0.823		0.627		0.733		

A. truncata was rare on both leaf sides and appears to have a preference for the 15-40 cm intervals, especially at the control location (Table 4). *Alcyonidium* sp. showed significant differences ($p<0.05$) between leaf sides and covered more extensively the back sides of leaves collected from I1 where it was constant (Table 3). The species showed a settlement preference on the upper parts (0-15 cm) of the front side of leaves while exhibiting a higher colonizing ability in the lower parts of the back sides, particularly at the deepest station C3.

C. brongniartii was seen at both locations and its settlement was affected by leaf side ($p<0.05$). The coverage of this species was more intense on the back side of leaves and was common to stations C1 and C2. Additionally, *F. joannae* was also a common species at the control station (C2) where its coverage was higher between the 0-15 cm intervals. However, it was represented with lower mean coverage values on the leaves at the impacted meadows. The mean coverage of *F. joannae* showed important differences between leaf sides ($p<0.05$) and was higher on the proximal parts of the back side.

Discussion

Nutrient concentration is potentially a limiting factor and can determine the variability of epiphytic assemblages at spatial scale (Balata *et al*., 2008; Martínez-Crego *et al.*, 2008; Ruiz *et al.*, 2009). According to our results, the mean values of NO_2-N, PO_4-P, and TPO_4-P in the water column increased moderately in the control meadows where the mean value of NO_3-N was almost two times higher. In contrast, NH_4-N was found 2.5 times higher in the impacted meadows. At the stations selected near fish farming activity, NH_4-N concentrations have been found notably high during various studies (Pitta *et al*., 2006; Mantzavrakos *et al*., 2007; Balata *et al*., 2008). Nitrogen, mainly in the form of ammonium, is an excretion product of fish. In Neofitou & Klaoudatos (2008), the maximum values of NH_4-N (1.64 μM) were measured in the vicinity of fish farm stations in autumn. In Livorno and around Gorgona Island, higher values of NH_4-N were noted in potentially impacted meadows close to the suspended cages. However, there were no detectable differences between reference and impacted meadows as regards

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P concentrations (Balata *et al*., 2008). Regarding our study results, mean oPO_4-P values varied between 0.08 µM and 0.1 µM in impacted and control meadows, respectively. The The oPO_4-P values(0.48-0.61µM) reported by Ruiz *et al.* (2001) were found higher compared with the concentrations measured during this study.

Direct measurement of water column nutrients is generally ineffective due to the transportation by water flow (Koch, 2001), plant uptake (Lee *et al.*, 2004) and adsorption by particulate sediment (Koch, 2001). Nitrogen uptake of *P. oceanica* leaves is hindered by the increasing epiphyte load close to the sites enriched by nutrient inputs (Lepoint *et al*., 2007; Martínez-Crego *et al.*, 2008; Prado *et al*., 2008; Apostolaki *et al*., 2012). Therefore, the epiphyte community has been used as an early warning indicator of stress occurred in the meadows and their environment (Leoni *et al*., 2006; Balata *et al*., 2008).

The structure of the epiphytic bryozoan assemblages of leaves differed between impacted and control locations, leaf side and depth ($p<0.05$). Species richness was low at the impacted location. This situation can be explained by water quality parameters as well as several natural factors; local hydrodynamic flows (Cornelisen & Thomas, 2004), sea grass features (Kocak *et al*., 2011), larval availability and the role of biotic interactions such as herbivore pressure (Trautman & Borowitzka, 1999; Borowitzka *et al*., 2006; Prado *et al*., 2007; Ruiz *et al*., 2009). The spatial distribution of epiphytes on seagrass leaves is influenced by leaf morphology and growth rate, leaf age, leaf side, water flow over the canopy and canopy height (Dalla Via *et al*., 1998; Cebrián *et al*., 1999; Trautman & Borowitzka, 1999; Alcoverro *et al*., 2004). The leaves of *P. oceanica* do not provide stable substrates compared with other sorts of hard substrates i.e. surface of rocks or panels (Balata *et al*., 2008; Giovannetti *et al*., 2010). A shift in the epiphyte species composition was particularly observed in algal diversity in the meadows, which were subjected to the sewage outfalls, (Balata *et al*., 2010; Giovannetti *et al*., 2010).

At the impacted site, twelve bryozoan species were determined. One of them, *B. gracilis*, was only observed at the impacted meadow and defined as a rare species according to frequency index values. Moreover, this species was found to be common in unhealthy sites and deep meadows (Ben Brahim *et al*., 2010; Martínez-Crego *et al*., 2010). In Port Kembla Harbour, the settlement rate of some marine fouling species was reduced by pollution which could also lead to a decline of *B. neritina*'s abundance (Moran & Grant, 1993). In the epiphyte community at the control site, *B. stolonifera* and *Bugula* sp. were observed only once, as it is a rare species like *D. obelia*, *E. posidoniae*, *M. nodulifera* and *Pherusella* sp., although *E. posidoniae* was a characteristic epiphyte on the leaves of *P. oceanica* (Balata *et al*., 2007). However, low frequency and coverage values in the depth range 10-15 m were noted for this species. *E. posidoniae* was also rarely found in a Chios sample (Hayward, 1974) and in the meadow near Bari and Brindizi in the Adriatic Sea. In the latter study, this particular situation was explained by substrate competition between this species and *Janua pagenstecheri* (Spirorbidae) (Occhipinti-Ambrogi, 1986). According to Ben Brahim *et al*. (2010), *E. posidoniae* was reported as a dominant species at a control location on the southern coast of Tunisia. In this study, *B. verticillata*, *Alcyonidium* sp., and *F. joannae* were widely distributed both in impacted and control meadows. The minimum and maximum frequency index values of rare species, which were observed only one side of leaves collected from one meadow during the examination period were 1.03 for *E. posidoniae* and *Pherusella sp.* and 3.57 for *M. nodulifera*, respectively. Some widely distributed species, such as *Alcyonidium* sp., *C. brongniartii*, *F. joannae, B. verticillata,* and *A. truncata* were presented as rare, common or constant. *Alcyonidium* sp. and *A. truncata* were also observed on the leaves collected from impacted and control locations in the Gulf of Gabes (Ben Brahim *et al*., 2010). In the impacted meadow, higher mean coverage values of *B. verticillata* were observed on almost every leaf sample. These high values were particularly determined from the front side of the leaves. *C. brongniartii* was a rare species in the impacted location while its frequency was found to be common or rare in the control meadow. This species did not show any important variation within stations. However, its coverage changed significantly between leaf sides ($p<0.05$). According to both number of colonies and covered area, *C. brongniartii* was the dominant species found in epiphytic communities in Chios (Hayward, 1974).

In this study, on the back side, bryozoan colonization was denser and also spread to the lower parts of the leaves. This can be explained by the effect of shade on bryozoan dominance in epiphytic community structures (Fitzpatrick & Kirkman, 1995). For coastal bryozoan species, light is an important factor for larval release (Hayward & Ryland, 1998) and according to Pisano & Balduzzi (1985), more abundant and richer bryozoan assemblages were found on the back side of panels submerged near Portofino (Italy).

Leaf length and shoot density are significantly influenced by epiphytic species composition (Martínez-Crego *et al*., 2008) and they are important characteristics for evaluating the health status of *Posidonia oceanica* meadows. In the control meadow, seagrass features such as adult leaf length, adult leaf surface area, and leaf area index showed an increasing trend towards deeper stations. However, the shoot density was an exception and it inclined to decrease along the depth gradient at both sites. (Kocak *et al.*, 2011). The differences in the structure of epiphytic assemblages along the depth gradient have been emphasized by various studies (Nesti *et al*., 2009; Martínez-Crego *et al*., 2010). The variations based on species composition and coverage data were more de-

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scriptive than total bryozoan coverage data. According to Lemmens *et al*. (1996), the density of total bryozoan species did not demonstrate significant differences between selected sites. According to our findings, locality, leaf side and leaf part can influence the coverage value of epiphytic bryozoan species. Five frequently observed species (*B. verticillata*, *A. truncata*, *Alcyonidium sp*., *C. brongniartii* and *F. joannae*) displayed a strong preference for the distal part (0-15 cm) of both sides of *Posidonia* leaves. However, the restricted distribution of the species on the distal part of the leaves was related with their settlement time, which had occurred before the rapid growth period of the plant (Hayward, 1974). Our study shows that *B. verticillata* could be an appropriate indicator for the detection of early anthropogenic disturbance. Moreover, *B. verticillata* showed statistically significant differences between sides and locations, while other frequently observed species showed only significant side differences, with the exception of *A. truncata*. As a consequence, some epiphytic bryozoan species and their coverage values on both sides of adult leaves are able to respond to changes in environmental conditions. Further investigations are necessary to clarify individual species response to anthropogenic disturbance.

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