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Variability in patterns of macro-epiphytic leaf community of *Posidonia oceanica* in the Islands of Kuriate: Western coast of Tunisia

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ABSTRACT

Objective: To test the response of the epiphyte community structure and biomass of the *Posidonia oceanica* (*P. oceanica*) leaves to natural disturbance.

Methods: Sampling of *P. oceanica* was carried in winter and summer on three sites in Kuriate Islands (western coast of Tunisia) subject to different environments disturbances. Shoots of *P. oceanica* were preserved in seawater-formalin (5%) solution for macro-epiphytes species identification in the laboratory. The samples were examined for leaf surface per shoot and the coverage (expressed as a percentage of leaf surface) of each morphological group, then carefully scraped with a razor blade. Epiphytes and scraped leaves were oven-dried at 60 °C for 48 h. Biomass was expressed as g dry weight/shoot.

Results: The biomass and the percentage cover of macro-epiphytic leaves showed seasonal variation. The highest values of epiphytic leaves were detected in summer whereas the lowest values were registered during winter. ANOVA showed that Kuriate Islands functioned as a single ecosystem in terms assemblage of macro-epiphytic leaves since no significant variation was detected for biomass and percentage cover at the scale site. Our study showed that natural disturbance had no effect on the assemblage distribution and the biomass of macro-epiphyte on the leaves of *P. oceanica* between the scales of site, whereas variability at the smallest scale was detected. ANOVA showed that exposure to wind and current had no effect on the biomass of macro-epiphytes leaves.

Conclusions: Biomass and assemblages of macro-epiphytic leaves of *P. oceanica* were high in summer and homogenous between all sites investigated. Natural disturbances such as exposure to wind have no effect on the distribution and the biomass of epiphytes on the shallow meadow.

1. Introduction

Seagrasses are the main plant group able to grow in unconsolidated substrata, which support a species-rich community of epiphytic organisms composed of prokaryotes and eukaryotic micro- and macro-organisms[1,2]. Compared to seagrasses, the epiphytic community has a low biomass but its primary productivity can be of the same order of magnitude[2,3]. Therefore, seagrass epiphytes can make a significant contribution to the flow of carbon and nutrients through the community[2,4,5]. Epiphytes of seagrass play an important role in ecosystem functioning and they are

considered as an important food resource for many organisms[6,7]. Moreover, epiphytes of seagrass are considered as sensitive indicators of natural and anthropogenic disturbance. Changes in the abundance and composition of their assemblages occur in relation to variation in environmental conditions[8,9]. In addition, they provide an early warning of ecosystem change revealing first-order changes in organism function since molecular, biochemical, and/or cellular changes triggered by pollutants are measurable in cells, tissues, and/or cellular fluids[10,11]. In the Mediterranean Sea, *Posidonia oceanica* (*P. oceanica*) represents the most important seagrass in terms of productivity, distribution and habitat structuring. It provides substratum to a species-rich epiphytic community[12,13], which achieves maximum biomass between the end of spring and the end of summer[1,14]. Monitoring of epiphyte of *P. oceanica* beds is becoming a useful tool to test the health of coastal environment and several countries have developed programs to study the distribution and the characteristics of seagrass beds and their epiphyte[1,15-18]. The effect of different kinds of human-induced disturbances on

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seagrasses in Tunisian coast include such phenomena as reduced water clarity (e.g. sediment loading resuspension, eutrophication), direct mechanical damage (e.g. dredging and filling, propeller scarring) and the release of toxic compounds into coastal waters (e.g. oil spills, industrial discharge) has been discussed by Ben Brahim et al.[1,19] and Mabrouk et al.[20]. But no studies deal with the effect of natural disturbances such as exposure to wind, wave and tide, on the composition and the abundance of leaf epiphytes in *P. oceanica* meadows. In particular, small variations of these physical factors may limit growth and distribution of seagrass and their epiphytes standing in habitats under stress conditions of temperature and salinity[21,22]. The aim of this study was therefore to assess spatial and seasonal epiphyte distribution and biomass according to natural disturbance such as expose to wave, tide and wind. We thus compared the biomass and assemblages of macro-epiphyte of *P. oceanica* at a station exposed to natural disturbance to two sheltered stations and examined their spatial and seasonal variability using a hierarchical sampling design[23]. We expected that epiphyte load and percentage cover would be low on the sites exposed to northern wind on the two seasons since these physical factors were disadvantageous for recruitment and installation of epiphytes.

2. Materials and methods

2.1. Study area and sampling station

The study was carried out in the Islands of Kuriate which are two emergent shoals, located off the Bay of Khenis north-east of the Cape of Monastir and about 18 km from the Monastir City (Figure 1). They mainly include a small island (Qûrya Essaghira) about 70 ha and a larger island (Qûrya El Kabira) about 270 ha of area and perimeter of 6.9 km and a distance of about 2.5 km from the first[24]. These islands are home to a remarkable terrestrial and marine flora and fauna richness. These two Islands are characterized by a flat and low morphology not exceeding 4.5 m in the highest region, with multiple depression areas. The substrate is mainly composed of sandstone and carbonate rocks, covered by sand. The average rainfall is between the isohyets 400 mm and 500 mm and the average temperature is 20 °C. The temperature difference between the average of the coldest month (January) and warmest (August) is relatively moderate (15.4 °C)[25]. The *P. oceanica* meadow is well represented around the Kuriat Archipelago where it extends from 0 to 27 m[25-29]. On soft bottoms, these meadows cover almost the entire perimeter of the islands. The shoot densities vary between 600 and 700 shoot/m² with a lower cover (70%).

2.2. Sampling

Three sites in the greatest Island of Kuriate distant by 1.5 to 2 km from each other were chosen for the samplings. The site El Boret (35°47'53 N, 11°01'25 E) located on the west side of the Big Island Kuriate. In winter, it is windy permanently from the north side; during the summer, it is especially southern and south/east side windy. Given the shallow depth, this site is a shelter for fishermen during storms. Meadow of *P. oceanica* is on a rocky bottom at a

depth not exceeding 1 m. The meadow is dense and has tiger type. *P. oceanica* cords have a length of 1.5 to 3 m and take an east-west direction. In this meadow, a dense flora composed mainly of *Cymodocea nodosa*, *Caulerpa prolifera*, *Caulerpa racemosa*, *Padina pavonica* and *Cystoseira crinita* was associated.

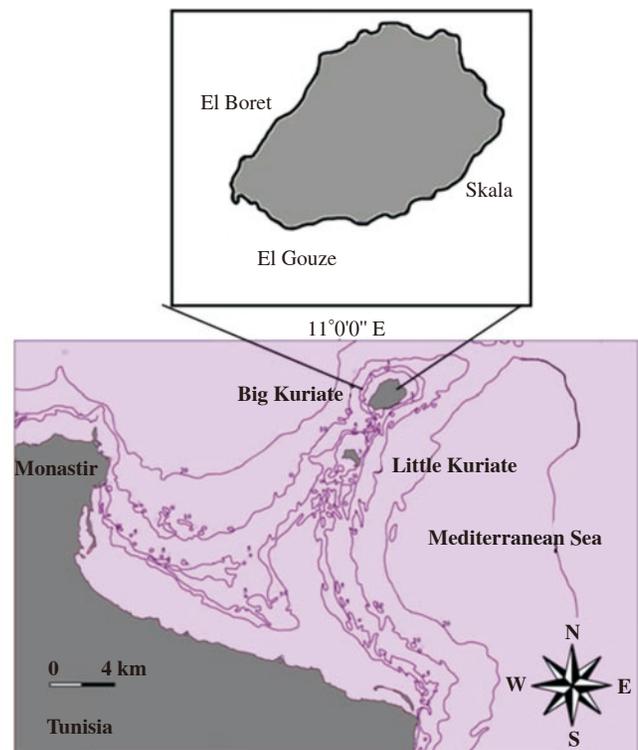


Figure 1. A map of the study area with a focus on sampling sites in the Kuriate Islands.

The site El Gouze (35°47'09 N, 11°01'50 E) is located on the southern shores of the great Kuriate. At this level, there is a circulation of sea current between the two islands Kuriate (personal observation and fishermen). This site is characterized by a dense *Posidonia* seagrass with different form: tiger, plain and fragmented with a large abundance of *Caulerpa racemosa* in association; there is also the presence of *Cymodocea nodosa*, *Caulerpa prolifera*, *Halimeda tuna*, *Padina pavonica* and *Cystoseira crinita*.

The site Skala (35°47'42 N, 11°02'37 E), located at the east side of the large Kuriate, is protected in winter of the action of the dominant northern winds while in summer, it is subjected to the action of the wind from the south side. *P. oceanica* meadow is very dense and takes different forms: tiger, isolated tufts and plain meadow. The depth is of the order of 2 m. In this meadow, a dense flora of *Caulerpa prolifera*, *Padina pavonica*, *Cymodocea nodosa* and *Caulerpa racemosa* was associated. Within each site, two subsites were randomly selected. Each subsite was also randomly divided into three plots, each plot then being divided into four quadrats 20 cm apart. We examined variability at tens of centimeter-scale by collecting samples from the same quadrat.

2.3. Data collection

Five shoots were randomly collected on both seasons from each quadrat during scuba diving and preserved in seawater-formalin (5%) solution for species identification in the laboratory. The samples were

examined for leaf surface per shoot and the coverage (expressed as a percentage of leaf surface) of each morphological group which was estimated with a binocular lens, then carefully scraped with a razor blade[30]. Epiphytes and scraped leaves were oven-dried at 60 °C for 48 h before weighing. Biomass was expressed in g dry weight/shoot[31,32].

2.4. Data analysis

Permutational multivariate analysis of variance (PERMANOVA) [33] was used to test the hypothesis that the biomass and the structure of epiphytic assemblages differed between seasons and to evaluate variability at different spatial scales[34]. PERMANOVA gives the permutation *P*-value and also the Monte Carlo asymptotic *P*-value for each test it performs. When very few permutations are possible, the Monte Carlo *P*-value should be given preference[33]. We used 999 random permutations for the test at an α -level of 0.05[35]. The analysis consisted of a 4-way model with subsites (two levels) being nested within sites (three levels), plots (three levels) being nested within subsites, quadrats (four levels) being nested within plots. Sites, subsites, plots and quadrats were random in the analysis. The data were transformed where necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's *C*-test). Variance components of all assemblages were calculated for seasons and for all spatial scales.

3. Results

The biomass of epiphytic leaves of macro-epiphytes showed seasonal variation. The high values were detected during the summer (Figure 2). The highest value of epiphytic leaves biomass [(0.602 ± 0.220) g dry weight/shoot] was detected in summer whereas the lowest value [(0.417 ± 0.200) g dry weight/shoot] was registered during winter. The biomass of macro-epiphytic leaves showed a significant seasonal variability (Table 1). No significant difference at the scale site, subsite and plot was detected, while significant difference at the smallest scale quadrat was revealed and the seasonal effect was obvious on the sites since significant interaction was observed.

The assemblage of epiphytes showed seasonal variation almost similar to all taxa except for Hydrozoa (Figure 3 and Table 2). The percentage of coverage of Bryozoa was higher in summer than in winter except for the site El Gouze. Algae proliferated for all sites in summer than in winter. Incrusted Annelida showed an even distribution between seasons and between sites, whereas the percentage cover of Hydrozoa was homogenous for all seasons and sites.

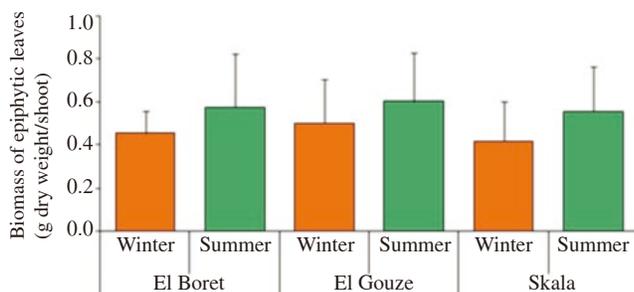


Figure 2. Variability in mean biomass of epiphytic leaves (\pm SD g dry weight/shoot) of *P. oceanica* on the three sites of Kuriate Islands during the winter and summer.

Table 1

Results of the PERMANOVA on biomass of macro-epiphytic leaves of *P. oceanica* in the Islands of Kuriate.

Source of variation	df	MS	F	P(perm)	P(MC)
Se	1	9017.74	80.62	0.001	0.001
Si	2	1174.88	3.28	0.141	0.090
Su(si)	3	358.23	0.19	0.917	0.978
Pl(su(si))	12	1863.64	0.96	0.494	0.517
Rp(pl(su(si)))	36	1922.51	6.55	0.001	0.001
Se × Si	2	509.13	4.55	0.107	0.045
Se × Su(si)	3	111.86	1.00	0.420	0.437
Se × Pl(su(si))	12	111.54	0.64	0.827	0.872
Se × Rp(pl(su(si)))	36	175.14	0.59	0.997	0.998
Residual	324	293.34			
Cochran's C-test		C = 0.578 .ns			
Transformation		Ln(x + 1)			

Se: Seasons; Si: Site, Su: Subsite; Pl: Plot; Rp: Replicat; df: Degree of freedom; MS: Mean square; P(perm): Probability; P(MC): Probability of Monte Carlo. Significant probability is on bold.

ANOVA showed that Kuriates Islands functioned as a single ecosystem in terms assemblage of macro-epiphytic leaves since no significant variation was detected at the scale site. Significant variability is raised at the scales subsite and replicat for the percentage of coverage of all macro-epiphytic leaf analyzed.

Biomass of epiphytic leaves and the percentage cover of the algae, Bryozoa, Hydrozoa and incrustated Annelida showed a different spatial variability (Table 3). For algae, incrustated Annelida and Hydrozoa, the largest variability of the percentage cover was detected at the scale subsite and replicat, while for the Bryozoa, the greatest variability was detected at the scale subsite. For the biomass of epiphytic leaves, most of the variability was revealed at the scale quadrat.

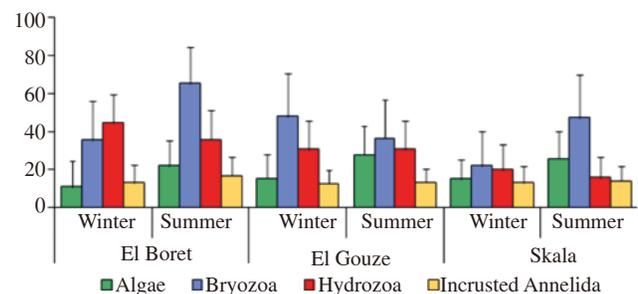


Figure 3. Mean percentage cover of the macro-epiphyte groups at the three nested sites in Kuriate Islands.

Table 3

Variance of macro-epiphytic biomass of leaves and percentage cover of algae, Bryozoa, Hydrozoa, incrustated Annelida in the Islands of Kuriates.

Source of variation	Algae	Bryozoa	Hydrozoa	Incrusted Annelida	Biomass of epiphytic leaves
Season	5.45	6.13	0.00	0.00	11.60
Site	0.00	15.46	8.43	0.00	0.00
Subsite	29.77	20.99	52.42	30.56	0.00
Plot	0.00	7.98	0.00	8.87	2.50
Quadrat	39.60	14.40	33.42	46.38	45.50
Residual	25.19	34.86	5.73	14.19	40.41

Table 2ANOVA on mean percentage cover of macro-epiphytes of *P. oceanica* during summer and winter.

Source of variation	Algae					Bryozoa				Hydrozoa				Incrusted annelida			
	df	MS	F	P(perm)	P(MC)	MS	F	P(perm)	P(MC)	MS	F	P(perm)	P(MC)	MS	F	P(perm)	P(MC)
Se	1	4660.5	9.89	0.04	0.02	4729.92	16.9	0.01	0.00	561.95	3.39	0.16	0.13	1620.66	11.09	0.05	0.02
Si	2	798.5	0.24	0.66	0.90	1649.94	0.71	0.52	0.60	8630.67	1.23	0.38	0.35	250.56	0.05	0.92	0.99
Su(si)	3	3280.2	7.96	0.00	0.00	2324.51	4.61	0.02	0.01	6977.98	26.29	0.00	0.00	5366.32	5.07	0.01	0.01
Pl (su(si))	12	411.7	1.12	0.36	0.35	504.13	1.62	0.12	0.07	265.32	0.34	0.99	0.99	1057.34	0.81	0.64	0.72
Rp (pl(su(si)))	36	365.3	2.18	0.00	0.00	311.51	2.03	0.00	0.00	772.39	8.21	0.00	0.00	1312.44	5.97	0.00	0.00
Se × Si	2	810.9	1.72	0.32	0.25	3463.61	12.4	0.01	0.00	126.01	0.76	0.55	0.54	381.05	2.61	0.21	0.13
Se × Su(si)	3	471.1	1.37	0.29	0.26	278.66	1.77	0.18	0.14	165.71	3.15	0.07	0.04	146.16	0.42	0.77	0.83
Se × Pl(su(si))	12	324.7	0.68	0.78	0.83	157.61	1.02	0.45	0.46	52.55	0.38	0.98	0.99	351.63	1.33	0.23	0.21
Sa × Rp(pl(su(si)))	36	505.6	3.02	0.00	0.00	153.71	1.00	0.45	0.48	138.04	1.46	0.02	0.02	264.22	1.20	0.17	0.16
Residual	324	167.2				153.24				94.00				219.93			
Cochran's C-test		C = 0.320 .ns				C = 0.376 .ns				C = 0.399 .ns				C = 0.430 .ns			
Transformation		Ln(x + 1)				Ln(x + 1)				Ln(x + 1)				Ln(x + 1)			

Se: Season; Si: Site; Su: Subsite; Pl: Plot; Rp: Replicat; df: Degree of freedom; MS: Mean square; P (per): Probability; P (MC): Probability of Monte Carlo. Bold numbers indicate significant effects.

4. Discussion

The present study documented significant differences in biomass of macro-epiphytes and on the structure of epiphytic assemblages on leaves of *P. oceanica* between seasons. Both biomass and percentage cover of macro-epiphytic leaves show a seasonal variability with the high values detected in summer. The variability of assemblages and biomass founded in the present study agrees with those described for *P. oceanica meadows* in the southern coast of Tunisia by Ben Brahim *et al.*[25,36] and by Mabrouk *et al.*[20] in the region of Mahdia eastern coast of Tunisia, where biomass of macro-epiphytes on leaves of *P. oceanica* was high in summer and low in winter. Similar results were reported by Zakhama-Sraieb *et al.*[37] in Port El Kantaoui, Tunisia where summer appears a sensitive season marked by the high development and dominance by encrusting Corallinaceae and opportunistic macroalgal species which can reach high cover. Moreover, the epiphyte load and percentage cover of assemblages of different taxa were similar and no variability between the different sites was detected. The relationship between spatial patterns of macro-epiphytic leaves of *P. oceanica* and natural disturbance highlighted in the present study is not completely in agreement with patterns described in previous investigations carried out in different localities. In fact, Wahl *et al.*[2] observe that exposure to waves and currents is considered to have a negative effect on the accumulation of epiphyte biomass on seagrass leaves and it might contribute to between-site variability of epiphyte load on *P. oceanica* shoots. In our study, natural disturbance has no effect on the assemblage distribution and the biomass of macro-epiphyte on the leaves of *P. oceanica* between the scales site. The site El Boret marked as the most exposed site to northern wind didn't manifest any difference compared to the other protected sites. This situation suggests that the sector of Kuriate Islands is a region considered as homogeneous habitats. In contrast to other studies, epiphytic composition and abundance resulting from the interplay between bottom-up and top-down forces are mainly controlled by nutrient availability, physical constraints (hydrodynamic flows, sediment features) and by biological interactions (grazing by herbivores, dispersion, competition for nutrients, light and space)[38]. In addition to all these factors, the discharge of the products of several human activities

such as industrial effluents[39], mining wastes[40], fish farming[41,42], drilling fluids[43], sewage and agricultural runoff[16,44,45] or effluents from desalination plants[46] can alter the composition and abundance of the epibiota. Changes on the composition of epiphyte assemblages following nutrient enrichments have also been confirmed under controlled field experiments[47]. Our study also shows that natural disturbance such as exposition to permanent northern wind has no significant effect on the variability of the epiphytic community composition. This pattern found in shallow meadows was influenced by variability in the seagrass leaf length, which is often associated to natural processes such as herbivore pressure or hydrodynamism. Those natural processes are attenuate in deep meadows, where the fish *Sarpa salpa* and the sea urchin *Paracentrotus lividus*, the main macroherbivores of *P. oceanica*, are functionally absent[48]. Depth seems to be an important source of natural variability, which modifies the responses of the epiphytic community to the deterioration gradient. Epiphyte communities in deep meadows responded more evidently to differences in environmental quality than in shallow ones. Despite that the reduced number of shallow meadows sampled could have precluded the detection of clear patterns, some relevant functional differences between shallow and deep meadows seem to account for the different behaviour of their respective epiphytic communities. Moreover, variations at the largest scale (between sites) might reflect differences in the ecological setting at the localities, such as wave exposure, substrate sediment characteristics and/or biological impacts, such as grazing pressure or anthropogenic impacts. Factors such as physical disturbance, topographic complexity and nutrient availability[49,50] might operate on smaller scales to modify morphological epiphytes variables. Variations observed at the smallest scales (cm to m) are more difficult to explain, but they could be attributable to a defined nested components of variation[51] and/or differences in the microhabitat[36,49,50,52].

We highlight that natural heterogeneity in epiphyte composition at different spatial scales may interact with the environmental quality gradient, obscuring the interpretation of results and making their use difficult in monitoring programs[53]. While some epiphyte features did not respond to the environmental change gradient, they showed an important between-meadows variability or within-meadow

variability by the high values of mean square for all taxa as in Table 2. Therefore, sampling in deep meadows appears more adequate for a more cost-effective monitoring than that in shallow meadows. At a larger scale, geographic variability caused by differences among northern and southern meadows can also influence the epiphyte composition[47]. However, the north-south differences in epiphyte composition at smallest scales, can partially correspond to natural nutrient differences derived from the hydrodynamic bottom morphology and water motion influences in the region[54]. Finally, monitoring these epiphyte features in relatively deep meadows seem to reduce to a strict minimum natural sources of variability other than those associated to anthropogenic gradients. Therefore, they can be used in biomonitoring programs to extract ecologically-relevant information useful for environmental policy, and management goals. However, more research is still needed on the early warning capacity of this approach to give a possible predictive value.

Conflict of interest statement

We declare that we have no conflict of interest.

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