

Variability in the structure of epiphytic assemblages of in relation to human interferences in the Gulf of Gabes, Tunisia

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Title: Variability in the structure of epiphytic assemblages of Posidonia oceanica in relation to human interferences in the Gulf of Gabes, Tunisia

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1 **Abstract**

2 In this study we evaluate whether the pattern of spatial variability of the macro-epiphytes 3 assemblages of leaves of *Posidonia oceanica* differed in relation to anthropogenic 4 interference in the Gulf of Gabes (southern coast of Tunisia). A hierarchical sampling design 5 was used to compare epiphytic assemblages at 5m depth in terms of abundance and spatial 6 variability at disturbed and control locations. The results indicate that the biomass and mean 7 percentage cover decreased at locations near the point of sewage outlet in comparison to 8 control locations. These losses were related to the distance from the source of disturbance. 9 This study revealed that the diversity is reduced in disturbed locations by the loss of biomass 10 and the mean percentage cover**,** explained by means of a multiple-stressor model which plays 11 an important role in the macro-epiphytes setting. It is urgent to propose the best management 12 plans to save the remaining *P. oceanica* meadow in the Gulf of Gabes and its associated 13 epiphytes. 14

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16 **Keywords**: Macro-epiphytes ; *Posidonia oceanica* ; disturbance ; spatial variability ; Gabès 17 Gulf.

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1 **1. Introduction**

2 Over the past 20 years, the Gulf of Gabes, on the southeast coast of Tunisia, has produced 3 more than 65% of the annual fish yield in Tunisia **(**Ben Mustapha, 1995; C.G.P., 1996), being 4 favoured by widespread seagrass beds widely recognized as key ecosystems in temperate and 5 tropical infra littoral habitats (Hemminga and Duarte, 2000; Boudouresque et al., 2009). 6 However, fish production is gradually decreasing parallel to the decline of littoral beds of the 7 endemic Mediterranean seagrass *Posidonia oceanica* (L.) Delile species as the coastal area of 8 the Gulf is now a threatened biotope mainly due to the pressure of anthropogenic expansion 9 and dumping of large quantities of phosphogypsum and other chemical products which 10 severely impacted benthic habitats ((Hamza-Chaffai et al., 1999, Bejaoui et al., 2004). The 11 loss of the native vegetable cover in the Gulf of Gabes is estimated at 90% with the *P.* 12 *oceanica* beds being replaced by the opportunistic and caulerpenyne-producing green algae 13 *Caulerpa prolifera* (Forsskål) Lamouroux in deeper zones (Hamza et al., 1995; Ramos-Espla 14 et al., 2000). The remaining *Posidonia* meadows cover the littoral fringes at depths ranging 15 between -3 and -4 m (Hattour et al., 1998). On the other hand, most biological studies of the 16 Gulf of Gabes have dealt with the distribution of phytoplankton (Drira et al., 2008; Bel 17 Hassen et al., 2009) zooplankton (Drira et al., 2009) ciliates (Kchaou et al., 2009; Hannachi et 18 al., 2009), demosponges (Ben Mustapha et al., 2003) while seagrass species and their 19 epiphytes which are known to be highly sensitive to environmental changes (Balata et al., 20 2007; Prado et al., 2008; Montefalcone, 2009) have been given limited attention. Epiphytes of 21 seagrass not only contribute significantly to the primary production of the meadow (Buia et 22 al., 1992; Nelson and Waaland, 1997; Duarte et al., 2004), but are also more sensitive to 23 environmental changes than the plant host (Delgado et al., 1999; Nesti et al., 2008; 24 Giovannetti et al., 2010). For example, various studies reported increases in epiphyte biomass 25 parallel with nutrient enrichment (Armitage et al., 2006; Neckles et al., 1993), eutrophication

1 (Balata et al., 2008; Frankovich et al., 2009) and water quality (Meric et al., 2005). 2 Differences in the spatial heterogeneity of epiphytes (Piazzi et al., 2004; Martínez-Crego et 3 al., 2010) and shifts in species composition (Nesti et al., 2008; Fourqurean et al., 2010) have 4 also been observed. It is widely known that the distribution of epiphytic assemblages of *P.* 5 *oceanica* both on leaves (Van der Ben., 1971; Mazzella et al., 1989) and rhizomes (Piazzi et 6 al., 2002; Balata et al., 2008) can change, but these changes have not yet been explored on a 7 horizontal scale in the Gulf of Gabes. Differences in the spatial heterogeneity of epiphytes 8 (Piazzi et al., 2004) and shifts in species composition (Nesti et al., 2008; Fourqurean et al., 9 2010; Martínez-Crego et al., 2010) have also been observed. Several findings have provided 10 evidence that epiphytes are patchy at 1 to 10 cm (Kendrick and Burt, 1997; Jernakoff and 11 Neilsen, 1998) and > kilometer scales, (Vanderklift and Lavery 2000, Lavery and Vanderklift 12 2002), other studies have indicated that epiphytes may be uniform at relatively small spatial 13 scales (e.g., 0.1 m Vanderklift and Lavery 2000; <10 m, Saunders et al., 2003, Piazzi et al., 14 2004). Moore et al. (2006) reported that epiphytes biomass is uniform at the scale < 100 m 15 transect. Variability in leaves epiphytic biomass at the scale of meters may be relatable to 16 differences in shoot density (Gambi et al., 1989), and to impacts of water movements on both 17 settlement and recruitment of propagules (Trautman and Borowitzka, 1999; Vanderklift and 18 Lavery, 2000). It is, therefore, of importance to undertake a study of the effects of urban 19 pressure on *P. oceanica* dynamics under the impact of human activities versus control 20 conditions. To our knowledge, only the flowering and fructification of *P. oceanica* along the 21 coastline of the Gulf of Gabes has been the subject of studies in recent years (Hattour et al., 22 1998). As part of a comprehensive study intended to understand the dramatic decline of the 23 endemic Mediterranean seagrass *P. oceanica* in the Gulf of Gabes, we analyzed, for the first 24 time in this ecosystem, the structure and patterns of spatial variability of leaf macro-epiphytic 25 assemblages and tested their relationships with anthropogenic disturbance. We attempted to

1 answer the questions 1) how vulnerable are the leaf macro-epiphytic assemblages to these 2 environmental pressures? 2) Does epiphyte biomass increase parallel with organic and 3 nutrient enrichment and which members of the epiphytic community should be considered as 4 most sensitive species? And 3) Does anthropogenic disturbance modify the spatial scales at 5 which variability of the epiphytic communities is highest/lowest or also influences the 6 partition of total variability among spatial scales? We, thus, compared epiphytic assemblages 7 between locations exposed to urban and industrial effluents and control locations, and 8 examined their variability at spatial scales ranging from cm to km, using a hierarchical 9 sampling design (Underwood, 1994; Chapman et al., 1995).

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11 **2. Material and methods**

12 *2.1. Study area*

13 The Gulf of Gabes is located between 35° N and 33° N and extends from "Ras kapoudia" at 14 the 35° N parallel level to the Tunisian-Libyan border (Fig. 1). It shelters various islands 15 (Kerkennah and Djerba) and lagoons (Bougrara and El Bibane). The climate is dry (average 16 precipitation: 210 mm year⁻¹) and sunny with strong easterly winds. The tide is semidiurnal, 17 with a maximum range of about 2 m.

18 *2.2. Sampling*

19 Because the *P. oceanica* epiphytic community reaches its optimum seasonal development in 20 summer (Romero, 1988) fieldwork was conducted in July-August 2006. Four locations distant 21 30 km from each other were chosen for the samplings. The depth of all stations was 5 m and 22 temperature and salinity ranges were 27°C-30°C and 38-40 P.S.U., respectively. Two 23 locations were disturbed by a sewage discharge: Ghannouch (34° 30' 523'' N 10° 54' 743'' E) 24 and Zarrat (33º 43'417'' N 10º 20' 486'' E) labeled herein respectively, D1 and D2. The other

1 two: Hassar (34° 44' 290'' N 11° 08' 590'' E) and Ajim (33° 45' 112'' N 10° 42' 992'' E) were 2 control locations, located north and south of the disturbed area and labeled respectively, C1 3 and C2. Within each location (D1, D2, C1, C2), three sites were randomly selected, 500-600 4 m apart, and within each site two sub-sites were also randomly selected. Each sub-site was 5 randomly divided into three plots, each plot then being divided into three quadrats 20 cm 6 apart. We examined variability at tens of centimeter-scale by collecting samples from the 7 same quadrat. This quadrat was 20 cm \times 20 cm and divided into a grid of 10 cm \times 10 cm sub-8 quadrats.

9 *2.3. Data collection*

10 The density of *P. oceanica* shoots was estimated from 216 replicates present within 100 cm² 11 sub-quadrats inside each meadow. Five shoots were randomly collected from each sub-12 quadrat during SCUBA diving and preserved in seawater-formalin (5%) solution for species 13 identification in the laboratory. Leaf length corresponds in fact to the average evaluated from 14 each meadow. The samples were examined for leaf surface per shoot and the coverage 15 (expressed as a percentage of leaf surface) of each morphological group which was estimated 16 with a binocular lens, then carefully scraped with a razor blade (Libes, 1986). Epiphytes and 17 scraped leaves were oven-dried at 60 °C for 48 h before weighing (Alcoverro et al., 1997; 18 Lepoint et al., 1999).

19 *2.4. Data analysis*

20 Multivariate analysis (MPMANOVA) was used to test the hypothesis that the structure of 21 epiphytic assemblages differed between disturbed and control locations and to evaluate 22 variability at different spatial scales (Anderson, 2001). The analysis consisted of a 6-way 23 model with sites (three levels) being nested within locations (two levels), subsites (two levels) 24 being nested within sites, plots (three levels) being nested within subsites, quadrats being 25 nested within plots and sub-quadrats (four levels) being nested within quadrats. Sites,

1 subsites, plots and quadrats were random whereas the contrast of disturbed versus control and 2 the disturbed were fixed. All data were transformed to $\ln (x + 1)$ to remove heterogeneity of 3 variances (after Cochran's *C*-test). SIMPER analysis (Clarke, 1993) was used to identify the 4 percentage contribution of each species to the Bray-Curtis dissimilarity between the average 5 of the disturbed and the control locations. A two-dimensional nMDS (non-metric 6 multidimensional scaling), based on the centroids of the sites of the four locations, was used 7 for a graphical representation of the data for leaf epiphytic assemblages.

8 Variance components of all assemblages were calculated for each location and for all spatial 9 scales. A discriminant analysis was used for classifying the different meadows into one 10 among possible *K* classes. These classes were identified by linear combinations of the 11 variables maximizing the homogeneity of each class. The test Wilks' lambda was employed to 12 test whether there are differences between the means of identified groups of subjects among a 13 combination of dependent variables.

14 Epiphyte biomass was standardized by shoot biomass to be able to compare epiphyte load 15 among locations. This is necessary because a significant positive correlation was found 16 between epiphyte biomass and leaf biomass (Fig. 2), allowing us to express epiphyte biomass 17 as g_{dw} epiphytes / g_{dw} of leaves. Differences in epiphytic biomass and the percentage cover of 18 the main phyla or morphological groups were analyzed through 6-way ANOVA with the 19 same factors and levels used in the multivariate analysis. Student-Newman-Keuls (SNK) test 20 was employed for a posteriori multiple comparisons of means.

21 **3. Results**

22 A total of 49 taxa were identified in the epiphyte assemblages of control locations: 20 23 Macroalgae (11 Rhodophyta, 6 Cyanobacteria, 2 Chlorophyta, 1 Heterokontophyta), 11 24 Bryozoa, 9 Cnidaria (Hydrozoans), 3 Annelida, 2 Tunicata and 3 Porifera. A total of 32 taxa 25 were identified in the epiphyte assemblages of disturbed locations: 13 Macroalgae (7

1 Rhodophyta, 4 Cyanobacteria, 1 Chlorophyta, 1 Heterokontophyta), 9 Bryozoa, 8 Cnidaria 2 (Hydrozoans), 1 Annelida and 1 Tunicata (Appendix 1). In control locations, leaf assemblages 3 were dominated by filamentous algae belonging to the genera *Polysiphonia*, *Ceramium* and 4 *Antithamnion;* common algal species were also present such as *Dasycladus vermicularis*, 5 *Cladophora* sp., *Griffithsia opuntoides*, *Neomonospora* sp. and *Laurencia obtusa*. Among 6 animals, the Bryozoans *Alcyonidium* and *Electra Posidoniae* dominated the community. The 7 Tunicata *Botryllus schlosseri* and *Clavelina lepadiformis* were numerically dominant. Among 8 the Cnidaria the most abundant species were *Dynamina cavolinii*, *Monotheca* sp. and 9 *Aglaophenia* sp. For encrusting Annelida the genus *Spirorbis* was the dominant genus, and for 10 the Porifera the genus *Ircinia* dominated the Porifera assemblage. In disturbed locations, leaf 11 assemblages were dominated by filamentous algae belonging to genera *Antithamnion* and 12 *Polysiphonia*; other common algal species were also present such as *Laurencia obtusa*, 13 *dictyota dichotoma* and *Ceramium gracillimum*. Among Bryozoa the genera *Alcyonidium*, 14 *Scrupocellaria* and *Bowerbankia* were the most abundant. The Cnidaria *Dynamena* and 15 *Obelia* were the most abundant genera. For the encrusting Annelida, the genus *Spirorbis* 16 dominated the assemblage.

17 There was a difference between the two disturbed locations; the location Ghannouch was 18 more affected than the location Zarrat, the number of species and the mean percentage cover 19 were reduced for the majority of taxa. We noted the absence of *Aetea truncata*, *Micropora* 20 *complanata*. Filamentous algae, namely *Ceramium tenuissimum*, *Neomonospora* sp. and 21 *Dasya* sp. were absent in the location of Ghannouch.

22 The high mean value of shoot density (1022 \pm 54.23 shoot m⁻²) was recorded in the site 1 of 23 Hassar location (C1), whereas the lowest mean value (119.5 \pm 17.67 shoot m²) has been 24 detected in site 1 of Ghannouch location (D1) (Fig. 3). The lowest mean value of the leaf 25 surface area (205.28 \pm 5.56 cm² shoot $^{-1}$) was recorded in the site 1 of the location Ghannouch

(D1), whereas the hightest mean value (467.28 \pm 5.82 cm² shoot ⁻¹) was detected in the site 2 2 of the location Ghannouch (D1) (Fig. 4).

3 The hightest mean value of leaf length $(59.68 \pm 7.87 \text{ cm})$ was recorded in the site b of the 4 control location Hassar (C1), whearas le lowest mean value (29.2 \pm 4.87 cm) was observed in 5 the site a of the disturbed location Ghannouch (D1) (Fig. 5).

6 ANOVA analysis showed that for density, leaf surface area and leaf length there was a 7 significant difference between (i) locations and (ii) between the contrast 'disturbed vs. 8 control' locations. For leaf surface area and leaf length, no significant difference could be 9 detected in the disturbed and the control locations separately. The difference was not 10 significant for shoot density among the control locations (Table 1). The disturbed location D1 11 has a lower mean epiphyte biomass (g_{dw} epiphytes / g_{dw} of leaves) than the location D2; this 12 value ranging between 0.02 ± 0.07 and 0.1 ± 0.08 g_{dw} epiphytes /g_{dw} of leaves at D1, and 13 between 0.25 ± 0.07 and 0.38 ± 0.09 g_{dw} epiphytes /g_{dw} of leaves in the location D2. In the 14 control location C1, the mean epiphyte biomass varied between 0.46 ± 0.01 and 0.63 ± 0.03 15 g_{dw} epiphytes /g_{dw} of leaves and between 0.33 ± 0.17 and 0.38 ± 0.19 g_{dw} epiphytes /g_{dw} of 16 leaves in the location C2 (Fig. 6).

17 Epiphyte biomass vs. leaf biomass displayed a fairly similar relationship in all locations, 18 suggesting that the leaf biomass pattern and hence the leaf surface availability was an 19 important factor governing the epiphytic biomass pattern as also reported by Borowitzka et al. 20 (2006) (Fig. 2). The two control locations C1 and C2 had the upper slope whereas the 21 disturbed locations present the lower what strongly suggests that anthropogenic disturbance 22 affects epiphyte load (Test of homogeneity of slope between control and disturbed locations; 23 $F_{(n=3)} = 430.07$; p = 0.00002). Analyses of variance indicate a wide variability in the epiphytic 24 biomass among locations, sites, subsites and quadrats, whereas variation at the scale plot was 25 insignificant (Table 2). Variation between the two disturbed locations was important for both

1 the largest and the smallest scales (quadrat), whereas significant difference was detected only 2 at the subsite and quadrat scale for the control locations. The structure of epiphytic 3 assemblage of leaves differed between the contrast of disturbed vs. control locations (Table 4 3), while no significant difference was observed among disturbed and control location 5 separately. Significant difference was observed in particular at the smallest scales plots and 6 quadrats. Epiphytes were generally abundant on leaves of *P. oceanica* (Fig. 7). Algae, 7 bryozoans and encrusting Annelida were common and abundant at all locations. Also 8 hydrozoans were widespread, but their percentage cover was generally low. The two 9 disturbed meadows were different from each other with an extra variability in percentage 10 covered in hydrozoans. Four morphological groups of organisms were sufficiently abundant 11 to be included in univariate analyses. For example, and concerning the percentage cover of 12 filamentous algae and hydrozoans, we recorded a significant difference between (i) locations 13 when considered separately and between (ii) the contrast 'disturbed vs. control locations'. For 14 encrusting Annelida a significant difference was detected only for the scale locations when 15 considered separately, whereas, no significant difference was found for the contrast 'disturbed 16 vs. control locations'. For bryozoans, there was no significant difference between these two 17 scales (Table 4). On a scale site, a significant variability was detected for encrusting Annelida. 18 At the scale subsite, a significant variability was detected for algae, whereas at the scale plot, 19 variability was detected for all parameters. For the scale quadrat, a difference was significant 20 for all variables but no difference was found between the percentage covers of disturbed and 21 control locations.

22 nMDS ordination of leaf assemblages at the four locations showed a clear separation between 23 the centroids based on sites of each control and disturbed locations (Fig. 8). The species 24 responsible for the difference between disturbed and control locations changed. For example, 25 the cover percentages of the algae *Antithamnion* sp.*, Polysiphonia elongata, Ceramium*

1 *tenuissimum and Ceramium codii*, the bryozoans such as *Micropora complanata* and the 2 hydrozoan *Dynamena cavolinii* increased on leaves in the control locations, though some 3 species showed similar trends in these locations (Table 5).

4 Biomass, epiphytic leaf assemblages and the four abundant morphological groups of epiphyte 5 displayed different responses to the spatial variability among different scales at the disturbed 6 and the control locations (Table 6). Variance components indicated that most of the variability 7 occurred not only at the plot scale but also between locations and quadrats, with the exception 8 of biomass that displayed larger variation at the quadrat scale in disturbed locations.

9 Discriminate analysis, for both disturbed and control locations showed that there was a 10 strongly significant variation including all the factors of the *P. oceanica* meadows. Wilks' 11 lambda showed a high variability between locations for biomass, hydrozoans and density, and 12 high variability within locations especially for bryozoans (Table 7).

13 The projection on the first factorial design crossing the first two factorial discriminant axes 14 corresponding to the first two discriminating linear functions as in table 8, allows a 15 description and a classification of the characterized variables (Fig. 9). The first axis shows 16 discrimination between the two control and the two disturbed locations. The second axis 17 divides locations in three groups: the control location C1, the crossing of the two disturbed 18 locations, and the control location C2.

19 **4. Discussion**

20 The multivariate analysis illustrates a decrease in seagrass vitality (i.e. shoot density, leaf 21 surface area and leaf length) in disturbed versus control locations, thus indicating the inability 22 of disturbed meadows to withstand increasing urban interferences. Cancemi et al. (2003) and 23 Balestri et al. (2004) reported that the decline of the phenologic parameters used to assess the 24 seagrass bed vitality among the disturbed locations they sampled is function of the distance 25 from the disturbance source as compared to control locations. In fact, the mean biomass in the

1 inner Ghannouch location (D1) was lower than that recorded close to sewage outlet at Zarrat 2 location (D2). Similar results were reported by others (Short and Wyllie-Echeverria, 1996; 3 Leriche et al., 2006). We suspect the water column stability and the quality of sewage as 4 probably being the most important factor affecting the growth of *P. oceanica* in the coastal 5 disturbed area of the Gulf of Gabes. This is supported by at least two observations. First, the 6 disturbed locations D1 and D2, which have a muddy bottom, were mechanically turbulent 7 from the constant input of sewage outlet, conveying uniformity throughout the shallow water 8 column (Soussi and Mammoun, 1992; Zaouali, 1993), and therefore probably reducing leaf 9 photosynthetic activity (Mascaro et al., 2009). Decreases in shoot density and leaf length 10 related to decreases in light environment have already been reported from inner disturbed 11 locations (Ruiz and Romero, 2003). In contrast, Giovannetti et al. (2006) found no difference 12 in the shoot density, the Conservation Index and the epiphyte coverage between the control 13 (marine protected area) and the disturbed (urbanized area) locations examined in their study. 14 Only a shift in the epiphyte species composition was observed in the urban meadows 15 subjected to the increase of the sewage outfalls during summer, which showed a considerable 16 development of the brown algae. Indeed, Terrados et al. (2008) found no difference in shoot 17 density between the control and the disturbed locations that they sampled. However, these 18 authors studied locations that received inputs of nutrients and organic matter from only the 19 resident population **(**Giovannetti et al., 2006) and park visitors **(**Terrados et al., 2008), 20 whereas the Gulf of Gabes is exposed to a plethora of toxic inputs. For example, studies here 21 have reported high phosphogypsum concentration (10 g I^{-1}) at the sewage outlet, which spread 22 over more than 60 km² (Bejaoui et al., 2004). Dissolution of phosphogypsum was carried out 23 by releasing cadmium and fluorine in sea water (Bejaoui et al., 2004), and cadmium has also 24 been shown to be toxic to clams in the Gulf of Gabes (Hamza-Chaffai et al., 1999; Smaoui-25 Damak et al., 2003, 2006). In the control locations Hassar and Ajim, *P. oceanica* beds seemed

1 healthy and no death was observed (Hattour et al., 1998; Ramos-Espla et al., 2000; Ben 2 Brahim et al., 2007). Also, since *P. oceanica* is very sensitive to low salinity in Tunisian 3 coastal regions such as the Gulf of Tunis (Northern Tunisia) (Ben Alaya, 1972), fresh waters 4 from sewage may also have lowered water salinity, thus affecting meadow growth too, but to 5 a lesser extent.

6 In terms of epiphytic leaf biomass, we also found the lowest mean biomass at the inner 7 disturbed Ghannouch location (D1). As previously mentioned for the meadow biomass, 8 increases in water turbidity in disturbed locations are also detrimental to leaf epiphytic 9 biomass since light is restricted (Cebrian et al., 1999). A similar general loss of epiphyte 10 biomass and the regression of *P. oceanica* meadow according to disturbance level has also 11 been reported (Cambrige and McComb, 1984; Guidetti, 2001; Piazzi et al., 2004). In addition, 12 macrograzers are known to feed preferentially on leaf tips where maximum epiphyte biomass 13 is reached (Alcoverro et al., 1997; Ruiz and Romero, 2003; Peterson et al., 2007). This may 14 have accounted, at least partially, for the natural loss of the epiphyte biomass both in 15 disturbed and control locations in the Gulf of Gabes. This decrease in epiphyte biomass was 16 also associated with a reduction of species sharing the epiphyte total biomass (49 species in 17 disturbed location vs 32 species in control locations, see Appendix). Our findings are also 18 supported by those of Mannino et al. (2010) who found a well-structured algal assemblage 19 close to the sea (with low anthropogenic pressure) compared to the locations subjected to 20 human disturbance. We, therefore, propose that in the Gulf of Gabes the spectrum of existing 21 conditions was narrow and thus unfavorable for the coexistence of a high number of species. 22 This is most likely due to toxicity from several compounds such as Cd (10-15 ppm, Soussi et 23 al., 1992) and, as previously mentioned, phosphogypsum with a sedimentary section reaching 24 50 cm in Ghannouch location. In addition, concentrations in hydrocarbon were relatively high 25 (between 882 μ g g⁻¹ and 4087 μ g g⁻¹) compared to those recorded from other coastal

1 Mediterranean sediments (Zaghden et al., 2005). The spatial distribution of these polluting 2 elements is favored by dominant winds (west-east in summer and north-east and south-west in 3 winter) which permanently induced not only the mixing of sediment but also the 4 remobilization of the surface deposits (Soussi et al 1992). Others have also reported a 5 decrease in the vitality of the *Posidonia* meadow due to trace metal pollution (Pergent-Martini 6 et Pergent, 2000; Ben Chikha, 2009). Furthermore, animals on leaves exhibited different 7 responses to disturbance. For example, encrusting Annelida and Bryozoa displayed a reduced 8 amount of variation in mean cover percentage at large spatial scales; in fact, the increase of 9 the relative dominance in mean cover near the sewage outlet indicates a functional change in 10 the assemblage related with the increase of the trophic component of micropredators 11 (Fraschetti et al., 2006). On the other hand, the sewage causes a significant decrease in terms 12 of mean cover of Hydrozoans as previously reported by Cifuentes et al. (2007). The 13 hydrozoan *Gonothyraea gracilis* and the two bryozoans *Amathia lendigera* and *Beania* 14 *hirtissima* (Bryozoa) were presumably the most sensitive species to environmental stress as 15 they were absent in the disturbed locations. We infer that this decrease is probably related to 16 the water turbulence close to the discharge which affected the availability of potential preys 17 such as zooplankton and particulate matter (Wahl, 1989; McKinney and Jackson, 1989; Ben 18 Brahim et al., unpublished). In addition, bryozoans and hydrozoans showed different 19 behavioral patterns related to the degree of disturbance, with bryozoans seeming more 20 adapted than hydrozoans to increased disturbance (McKinney and Jackson, 1989). Obviously, 21 further studies are needed, for example by comparing the response of different epiphytes taxa, 22 in order to elucidate the resilience of each group of epiphytes against disturbance levels and 23 their strategy facing disturbed conditions. The present study showed that the variation in both 24 the spatial pattern and abundance of macroalgal species, rather than their presence/absence, 25 are indicative of disturbance. For example, the assemblage of epiphytic algae dominated by

1 the Rhodophyta (order Ceramiales) tends to decrease in the two disturbed locations D1 and 2 D2. In this context, filamentous algae, especially the Ceramiales**,** may be considered the most 3 sensitive to disturbance. In other Mediterranean regions, Dictyotales were the dominant 4 species and grew abundantly in the disturbed sampled areas (Balata et al., 2007). On the other 5 hand, our results showed that the decrease of Ceramiales algae was paralleled with the 6 decrease of the green algae *Dasycladus vermicularis*, the cyanobacteria *Lyngbia* sp.*, Rivularia* 7 sp.*, Gloeotrichia* sp., *Phormidium* sp. and the red algae *Dasya* sp.*, Griffithsia* sp. and 8 *Pleonosporium* sp., a decrease also paralleled with increases of anthropogenic disturbance. 9 Brown algae such as *Myrionema orbiculare* and *Giraudia sphacelarioides* are considered 10 characteristic of epiphyte community of *P. oceanica* leaves and have been already reported 11 along the northern Tunisian coast (Ribera et al., 1992). In many Mediterranean regions, *M.* 12 *orbiculare* was abundant (Giovannetti et al., 2006; Pardi et al., 2006; Sliskovic et al., 2010). 13 However, along the Tunisian coast this taxon was detected only in Bechateur which is located 14 in northern Tunisia (Menez and Mathieson, 1981). In the present study (southern Tunisia) 15 brown algae such as *M. orbiculare*, *G. sphacelarioides* were absent and only the species 16 *Dictyota dichotoma* was recorded. We infer that this absence might be related to the high 17 level of disturbance in the Gulf of Gabes. Our suggestion may be supported by the study of 18 Giovannetti et al. (2006) at Prelo cove in the Ligurian Sea (NW Mediterranean Sea) showing 19 that some brown algae such as *Myrionema orbiculare, Giraudia sphacelarioides and* 20 *Fenestrulina johannae* did not play a significant role in the epiphytic community structure, 21 whereas some Rhodophyta such as *Hydrolithon-Pneophyllum* spp. were the main epiphytes 22 that were present throughout the year at all stations. In control locations, leaf epiphytes 23 biomass of *P. oceanica* were most variable at the small scales investigated (among plots), 24 whereas variation at the intermediate scale (among sites, subsites) was negligible. In this 25 study, hierarchical sampling designs enabled an identification of homogeneity of epiphyte

1 assemblage distribution for the majority of functional groups and biomass among the scale 2 site (500-600 m), while heterogeneity is displayed at large scales among regions and at small 3 scales among plots. Our study supports the results of Pardi et al. (2006), and Piazzi et al. 4 (2004), in terms of variability of epiphyte load on *Posidonia oceanica* which was greatest at 5 the extremes of the spatial extents they investigated, with patchiness greatest at the among-6 shoot level (i.e. within quadrats), and at sites separated by a few hundred meters. Moore and 7 Fairweather (2006) and Balata et al. (2007) also considered that the epiphytes biomass within 8 the 100 m long transect sampled was homogenous, and more generally, Jernakoff and Neilsen 9 (1997) and Balestri et al. (2004) indicated that patchiness in epiphytes as well as in seagrass 10 morphology is significant over a range of scales (typically cm to km).

11 **5. Conclusion**

12 Our study illustrated a spatial variability related to anthropogenic disturbance for (i) the 13 phenologic parameters of the plant host *P. oceanica*, such as shoot density, leaf length and 14 leaf surface area in the disturbed locations, for (ii) the mean percentage cover of targeted 15 organisms and for (iii) the epiphytic biomass on leaves of *P. oceanica*.

16 The hierarchical sampling designs used in this study similarly to other investigations dealing 17 with the spatial structuring of epiphytes-seagrass relations, pointed out a homogeneity in the 18 distribution of epiphyte assemblages for the majority of functional groups and biomass among 19 the scale site (500-600 m transect) while a heterogeneity was displayed at large scales among 20 regions and at small scales among plots. Seagrass beds separated by few hundred of meters in 21 both disturbed and control locations will apparently be subjected to unique environmental 22 conditions resulting in equally epiphytic leaves biomass and assemblages. *P. oceanica* 23 meadow and its associated epiphytes appear to be strongly affected by human pressure in the 24 Gulf of Gabes through sewage outlet (this study), in addition to other disturbances such as 25 trawling, mooring, fish farming and coastal pollution. The regression trend will significantly

1 increase unless there is efficient implementation of legal protection along with a reduction in 2 human interference. While restrictions on trawling have been introduced in Tunisia, this 3 practice being responsible for the loss of about 80% of the surface area of seagrass meadows 4 in the Gulf of Gabes (Zaouali, 1992), the legislation is not respected. In a recent review, many 5 authors regretted the lack of data in some Mediterranean regions, particularly indicating the 6 urgent need for studies on *P. oceanica* in North Africa (Ruiz et al., 2009). We, thus, believe 7 that our data may be, on a North African scale, not only worthwhile but also useful in 8 initiating other investigations. For example, the construction of "matte" by *P. oceanica*, a 9 feature unique among Mediterranean seagrasses and which may be useful for assessing the 10 regression of *P. oceanica* (Leriche et al., 2004; Boudouresque et al., 2009), should be further 11 addressed. Finally, it is urgent to propose the best management plans to save the remaining 12 20% of *P. oceanica* in the Gulf of Gabes and its associated epiphytes which have been 13 reported to be among the most productive marine and terrestrial ecosystems (Duarte and 14 Chiscano, 1999).

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Table 2. Analysis of variance on the epiphytic biomass on the leaves of *P. oceanica*. Bold numbers indicate significant effects.

Source of Variation	d.f	MS	$\mathbf F$	P
$Locations = L$	3	3.46×10^{7}	30.85	0.00
Disturbed vs. Controls (DC)	1	8.08×10^{7}	25.23	0.00
Among Disturbed $(=D)$	1	8.57×10^{6}	655.01	0.00
Among Controls $(=C)$	$\mathbf{1}$	1.44×10^{7}	6.48	0.06
Site (DC)	10	3.20×10^{6}	5.97	0.00
$\text{Site }(D)$	4	13097.61	0.25	0.89
Site (C)	$\overline{4}$	2.23×10^{6}	2.17	0.19
Subsite (Site (DC))	12	537886.63	4.834	0.00
Subsite $(Site (D))$	6	51026.48	1.94	0.11
Subsite (Site (C))	6	1.0×10^{6}	5.22	0.00
Plot (subsite (Site (DC)))	48	111265.64	1.05	0.40
$Plot$ (subsite (Site $(D))$)	24	26197.42	1.34	0.17
Plot (subsite $(Site(C))$)	24	196333.85	1.02	0.45
Quadrat (plot(subsite(Site (DC))))	144	105953.6	4.59	0.00
Quadrat (plot(subsite(Site(D))))	72	19465.36	4.58	0.00
Quadrat (plot(subsite(Site (C))))	$\overline{72}$	192441.83	4.59	0.00
Residual	648	23086.88		
Residual D	324	4243.37		
Residual C	324	41930.39		

Table 3. Non-parametric multivariate analysis of variance (NP-MANOVA) on epiphytic assemblage of *Posidonia oceanica*. Bold numbers indicate significant effects.

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Table 4. Analysis of variance on mean percentage cover of epiphytes of *Posidonia oceanica*.

Bold numbers indicate significant effects.

Table 5. Major species, ranked in order of importance, contributing to the average dissimilarities between the disturbed and the control locations as determined by similarity percentages (SIMPER).

Z

 $\sum_{i=1}^{n}$

Table 6. Variance components expressed as percentage calculated for the epiphytic biomass and the epiphytic assemblages on leaves in the disturbed and the control locations $(D =$ disturbed locations, $C =$ control locations).

Source of variation	Biomass		Whole assemblages		Filamentous algae		Bryozoans		Hydrozoans		Encrusting	
$(\%)$											Annelida	
	C	D	C	D		D	C	D	C	D	C	D
Location	8.75	8	θ	6.46	18.57	Ω	θ	Ω		$17.6 \t 11.36 \t 31.28$		24.55
Site	10.22	Ω	18.59	θ	$\mathbf{0}$	9.8	39.6	5.72	$\mathbf{0}$	12.04	13.94	$\overline{0}$
Subsite	0.00	3.56	θ	4.67	3.6	19.85	Ω	22.3	15.65	θ	9.14	Ω
Plot	22.03	12.82	81.41	88.87	77.83	70.35	60.4	72	66.75	76.6	45.66	75.45
Ouadrat	θ	26.83	$\mathbf{0}$	Ω	Ω	θ	Ω	θ	θ	0	0	θ

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