

## Epiphyte load on the seagrass *Posidonia oceanica* (L.) Delile does not indicate anthropogenic nutrient loading in Cabrera Archipelago National Park (Balearic Islands, Western Mediterranean)

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**SUMMARY:** The epiphyte load on the leaves of the seagrass *Posidonia oceanica* (L.) Delile was estimated at the end of summer in three sites and two depths (7 m and 17 m) of the Cabrera Archipelago National Park (Balearic Islands, Western Mediterranean) from 2004 to 2006 to evaluate if epiphyte load could be used as an indicator of anthropogenic nutrient loading in the shallow marine ecosystems of the park. Asymmetrical ANOVA was used to divide data variability into two components: the contrast between the site receiving direct anthropogenic inputs of nutrients and organic matter (Es Port) and the two control sites (Cala Santa María, Es Burri); and the variability between the control sites. The nitrogen concentration in *P. oceanica* leaves was higher in Es Port than in Cala Santa María and Es Burri but only at 7 m depth. There was no difference between the epiphyte load on *P. oceanica* shoots in Es Port and the sites without inputs at both 7 m and 17 m. However, the epiphyte load in Es Burri was higher than that in Cala Santa María at both depths. The results show that the epiphyte load on *P. oceanica* shoots was not a sensitive indicator of anthropogenic nutrient and organic matter loading in Cabrera Archipelago National Park.

**Keywords:** seagrass, epiphytes, nutrient loading, *Posidonia oceanica*, Western Mediterranean.

**RESUMEN:** LA BIOMASA DE EPÍFITOS DE LA ANGIOSPERMA MARINA *POSIDONIA OCEANICA* (L.) DELILE NO ES INDICADORA DE APORTES ANTROPOGÉNICOS DE NUTRIENTES EN EL PARQUE NACIONAL ARCHIPIÉLAGO DE CABRERA (ISLAS BALEARES, MEDITERRÁNEO OCCIDENTAL). – La biomasa de epífitos y el contenido en cenizas de los mismos, la biomasa foliar del haz, el contenido en nitrógeno de las hojas y la densidad de haces de la angiosperma marina *Posidonia oceanica* (L.) Delile se midieron en tres localidades y a dos profundidades (7 m, 17 m) del Parque Nacional Archipiélago de Cabrera (Islas Baleares, Mediterráneo Occidental) en los meses de septiembre del período 2004-2006 para evaluar el valor de la biomasa de epífitos como indicador de aportes antropogénicos de nutrientes a los ecosistemas del Parque. La variabilidad total de los datos se repartió por medio de un ANOVA asimétrico en dos componentes: el contraste entre la localidad receptora de aportes antropogénicos de nutrientes y materia orgánica (Es Port) y dos localidades control (Cala Santa María, Es Burri) por un lado, y la variabilidad entre las dos localidades control por otro. La concentración de nitrógeno en las hojas de *P. oceanica* fue superior en Es Port que en Cala Santa María y en Es Burri, pero sólo a la profundidad de 7 m. La biomasa de epífitos sobre las hojas de *P. oceanica* fue similar en Es Port y en las dos localidades control tanto a 7 m como a 17 m de profundidad. Sin embargo, la biomasa de epífitos en Es Burri fue superior a la de Cala Santa María en las dos profundidades estudiadas. Los resultados obtenidos muestran que la biomasa de epífitos sobre las hojas de *P. oceanica* no es un buen indicador de los aportes antropogénicos de nutrientes y materia orgánica en los ecosistemas costeros del Parque Nacional Archipiélago de Cabrera.

**Palabras clave:** angiospermas marinas, epífitos, aportes de nutrientes, *Posidonia oceanica*, Mediterráneo Occidental.

## INTRODUCTION

Seagrasses are the main plant group able to grow in unconsolidated substrata. They represent the main “hard substratum” in shallow sedimentary marine environments. Seagrasses support a species-rich community of epiphytic organisms composed of procariots and eucariotic micro- and macro-organisms (Borowitzka *et al.*, 2006). Compared to seagrasses the epiphytic community has a low biomass but its primary productivity can be of the same order of magnitude (Borowitzka and Lethbridge, 1989; Borowitzka *et al.*, 2006). Therefore, seagrass epiphytes can make a significant contribution to the flow of carbon and nutrients through the community (Young *et al.*, 2005; Borowitzka *et al.*, 2006; Lepoint *et al.*, 2007).

Nutrient availability has been considered to be a main source of variability in epiphyte biomass in seagrass communities (Borum, 1985; Tomasko and Lapointe, 1991; Frankovich and Fourqurean, 1997; Lapointe *et al.*, 2004), and epiphyte overgrowth an indicator of anthropogenic nutrient loading and a driver of seagrass loss (Tomasko *et al.*, 1996; Tomasko and Lapointe, 1991; Wear *et al.*, 1999; Lapointe *et al.*, 2004; Ralph *et al.*, 2006). However, mesocosm and *in situ* experiments show that low light and herbivory may hinder the development of epiphytes on seagrass leaves and uncouple epiphyte loads from nutrient availability (Tomasko and Lapointe, 1991; Neckles *et al.*, 1993; Williams and Ruckelshaus, 1993; Heck *et al.*, 2000). Although point sources of nutrients in Florida Bay were clearly associated with epiphytic overgrowth, the epiphyte load on seagrass leaves was weakly correlated to nutrient availability at the scale of the entire bay (Frankovich and Fourqurean, 1997).

*Posidonia oceanica* (L.) Delile is the main seagrass in the Mediterranean and provides substratum to a species-rich epiphytic community (Ballesteros, 1987; Mazzella *et al.*, 1989), which achieves maximum biomass between the end of spring and the end of summer (Ballesteros, 1987; Romero, 1988). The summer epiphytic community represents the last stage of its seasonal development and is dominated by encrusting Corallinaceae and opportunistic macroalgal species which can reach high cover (Ballesteros, 1987; Romero, 1988). Nutrient-addition experiments have shown that during summer the *P. oceanica* epiphytic community responds to the rise in the availability of nutrients with an increase in the

biomass of macroalgae, while the length of *P. oceanica* leaves and the size of shoots decrease (Leoni *et al.*, 2006; Prado, 2006). Summer appears, therefore, to be a sensitive season when nutrient loads may promote the development of macroalgal epiphytes and affect *P. oceanica* negatively. Similar to other seagrasses, low light availability (Ruiz and Romero, 2001) and herbivory (Alcoverro *et al.*, 1997; Ruiz *et al.*, 2001; Tomás *et al.*, 2005) may hinder the development of epiphytes on *P. oceanica* leaves.

We estimated the epiphyte load on the leaves of *Posidonia oceanica* at the end of summer in three sites of Cabrera Archipelago National Park (Balearic Islands, western Mediterranean) to evaluate if epiphyte load could be used as an indicator of anthropogenic nutrient loading in the shallow marine ecosystems of the park. We expected that epiphyte load would be higher at the site where the park facilities are located and which receives tourist and boat visits than at the other two sites where there is no resident human population and access to tourists and boats is not permitted.

## MATERIAL AND METHODS

The Cabrera Archipelago is located 9 km south of Mallorca (Balearic Islands, western Mediterranean) and is composed of a main island, Cabrera (11.5 km<sup>2</sup>), and 18 additional smaller islands (Servera, 1993). Except for the period from 1809 to 1814, when Cabrera was a Spanish army prison camp which received around 9000 people (cumulative), the human population inhabiting the archipelago has always been low (<40 persons, Frontera *et al.*, 1993). The archipelago was declared a national park in 1991 and since then boat access has been limited and restricted to the sheltered bay of Es Port in Cabrera Island. There are *Posidonia oceanica* meadows inside all Cabrera island bays and around the smaller islands towards the NE to a maximum depth of 35 m (Ballesteros *et al.*, 1993). Es Port is the only bay that receives direct inputs of nutrients and organic matter from the resident population and park visitors.

The study was performed in September of 2004, 2005 and 2006, which is the time of year when the *Posidonia oceanica* epiphytic community reaches its maximum seasonal development (Ballesteros, 1987; Romero, 1988), and when the anthropogenic nutrient loads and the magnitude of the response of

the epiphytic community to them are assumed to be maximal. We chose three sites which are all located in Cabrera Island: Es Port (EP), Cala Santa María (SM), and L'Olla-Es Burri (EB). The *P. oceanica* meadow in SM is in good condition in terms of vegetative growth and shoot demography while that in EP is in decline (Marbà *et al.*, 2002). The content of organic matter, the rate of sulphate reduction, and the total pool of reduced sulfides are higher in EP sediments than in SM sediments (Holmer *et al.*, 2003). The residence time of water in EP and SM is 11 days and 4 days respectively. This difference, together with the high anthropogenic loading of organic matter and nutrients in EP compared to SM, is considered to be the cause of the different statuses of *P. oceanica* in the two sites (Marbà *et al.*, 2002). EB is an open bay similar to SM but it faces to the south east and has no direct anthropogenic inputs of nutrients and organic matter. Given that both the epiphyte load and its timing change with depth (Ballesteros, 1987; Romero, 1988; Mazzella *et al.*, 1989; Lepoint *et al.*, 1999) we selected two depths (7 m and 17 m) at each of the three sites to perform the study. Six sampling stations were then selected: EP7 (39°8.687'N, 2°55.920'E), EP17 (39°8.810'N, 2°55.860'E), SM7 (39°9.005'N, 2°56.964'E), SM17 (39°9.156'N, 2°56.891'E), EB7 (39°8.971'N, 2°57.859'E), and EB17 (39°8.888'N, 2°57.929'E).

The density of *Posidonia oceanica* shoots was estimated from the counts of the number of shoots present inside 400 cm<sup>2</sup> quadrats haphazardly placed inside the meadow. The number of quadrats counted in each site, depth and year varied between 5 and 15. The high variability of the shoot counts led us to pool the data obtained during the three years to increase the number of replicates ( $24 \leq n \leq 37$ ). No sea urchins were observed at any of the stations during the 2004 and 2005 samplings. In 2006 we estimated the density of sea urchins by counting the sea urchins inside forty 2500 cm<sup>2</sup> quadrats haphazardly placed inside the *P. oceanica* meadow at each of the sampling stations; no sea urchins were found in any of the quadrats.

Five *Posidonia oceanica* shoots were haphazardly collected along each of two 10 m long transects, which were 30 m apart, in order to estimate the leaf shoot mass and the epiphyte load of the shoots ( $n = 10$ ) at each station and sampling event. The epiphytes in all the leaves of each shoot were scraped using a razor blade and collected in pre-weighed Whatman GF/C glass fibre filters which were dried

(60°C, 48 h) to estimate their dry weight. The leaves were then dried (60°C, 48 h) to estimate the mass of each shoot. Epiphyte load was calculated as the biomass of epiphytes in the leaf shoot divided by the biomass of the leaf shoot (mg epiphyte DW g<sup>-1</sup> leaf DW). The dried epiphytes of 6 out of the 10 shoots collected at each station and sampling event were burned (450°C, 6 h) to determine their ash content. Similarly, a sub-sample of dried leaves of 6 out of the 10 shoots collected at each station and sampling event was used to determine the concentration of nitrogen in the leaves using a CNH elemental analyser. We determined the ash content of the epiphytes to evaluate if there were major differences in the proportion of encrusting coralline to filamentous algae in the epiphytic community in the different study sites. The nitrogen concentration in *P. oceanica* leaves was used as a relative indicator of nitrogen and nutrient loads available to them.

Asymmetrical analysis of variance (pp. 415-418, Underwood, 1997) was used to evaluate if leaf nitrogen, shoot density, leaf shoot biomass, epiphyte load, and the ash content of the epiphytes were different between the anthropogenically disturbed site (Es Port) and the two control sites (Cala Santa María, Es Burri) during the three-year study period. Asymmetrical ANOVA divided the variability of the whole dataset into two components: the contrast between the disturbed site and the two control sites (fixed factor), and the variability between the control sites (random factor, nested in the control level). Except for leaf nitrogen, Cochran's C tests indicated that the data needed to be transformed to homogenise the variances before performing ANOVAs. The statistical analyses were performed using STATISTICA software (StatSoft, Inc., 2005).

## RESULTS

The density of *Posidonia oceanica* shoots at 7 m depth was twice that at 17 m depth (Fig. 1). Shoot density at the 7 m depth was higher in SM ( $726 \pm 48$  shoots m<sup>-2</sup>, mean  $\pm$  1 standard error) than in EB ( $501 \pm 34$  shoots m<sup>-2</sup>) but no significant difference was found between these two control sites and EP ( $538 \pm 36$  shoots m<sup>-2</sup>). Shoot density at 17 m depth varied between  $245 \pm 16$  shoots m<sup>-2</sup> in EP and  $312 \pm 22$  shoots m<sup>-2</sup> in SM; there were no significant differences between the sites (Table 1).

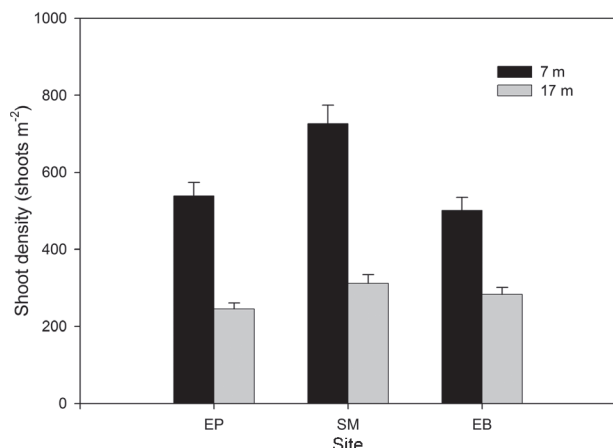


FIG. 1. – Shoot density (shoots m<sup>-2</sup>) of *Posidonia oceanica* at three sites (EP: Es Port; SM: Cala Santa María; EB: Es Burri) and two depths (7 m, 17 m) in each site in Cabrera Archipelago National Park (Balearic Islands, Western Mediterranean) in 2004-2006. Error bars represent +1SE.

The concentration of nitrogen in the leaves of *Posidonia oceanica* ranged between  $0.7 \pm 0.03\%$  of leaf dry weight (DW) in EP17 in 2004 and  $1.3 \pm 0.03\%$  of leaf DW in EP7 in 2005 (Fig. 2). Leaf nitrogen was higher in the disturbed site (EP: 1.0% to 1.3% of leaf DW) than in the other two sites (SM, EB: 0.8% to 1.0% of leaf DW) at 7 m depth (Table 1); no differences were detected, however, between any of the sites at 17 m depth (0.7% to 1.0% of leaf DW).

Leaf shoot biomass of *Posidonia oceanica* was lower in EP (from  $0.40 \pm 0.07$  g DW shoot<sup>-1</sup> to  $0.49 \pm 0.05$  g DW shoot<sup>-1</sup>) than in SM (from  $0.58 \pm 0.05$  g DW shoot<sup>-1</sup> to  $0.84 \pm 0.12$  g DW shoot<sup>-1</sup>) and EB (from  $0.51 \pm 0.07$  g DW shoot<sup>-1</sup> to  $0.80 \pm 0.11$  g DW shoot<sup>-1</sup>) at both depths, 7 m and 17 m (Fig. 2). No significant differences were found in the leaf shoot biomass between the two control sites at any of the depths (Table 1).

The epiphyte load on *Posidonia oceanica* leaves varied between  $115 \pm 18$  mg epiphyte DW g<sup>-1</sup> leaf DW in SM17 in 2005 and  $500 \pm 177$  mg epiphyte DW g<sup>-1</sup> leaf DW in EB7 in 2006 (Fig. 2). Epiphyte load in EP was not different (from  $134 \pm 23$  mg epiphyte DW g<sup>-1</sup> leaf DW to  $365 \pm 32$  mg epiphyte DW g<sup>-1</sup> leaf DW) from that in the control sites both at 7 m depth and 17 m depth. However, the epiphyte load in EB (from  $244 \pm 52$  mg epiphyte DW g<sup>-1</sup> leaf DW to  $500 \pm 177$  mg epiphyte DW g<sup>-1</sup> leaf DW) was higher than that in SM (from  $115 \pm 18$  mg epiphyte DW g<sup>-1</sup> leaf DW to  $198 \pm 25$  mg epiphyte DW g<sup>-1</sup> leaf DW) at both depths (Fig. 2, Table 1).

The concentration of ash in leaf epiphytes ranged from  $81.8 \pm 1.4\%$  of epiphyte DW in EB7 in 2005 to  $88.3 \pm 0.9\%$  of epiphyte DW in EP17 in 2004 (Fig. 1). Similarly to epiphyte load, the ash content of the epiphytes was not different between EP (from 84.1

TABLE 1. – Results of an asymmetrical analysis of variance to evaluate if leaf nitrogen, shoot density, leaf shoot biomass, epiphyte load, and the ash content of the epiphytes were different in a disturbed site (Es Port) and two control sites (Cala Santa María, Es Burri) at two depths (7 m, 17 m) in Cabrera Archipelago National Park (Spain) from 2004 to 2006. DW: dry weight. Significant sources of variation are indicated by asterisks:\*\*\*, P < 0.05; \*\*\*\*, P < 0.005. §Data were log<sub>e</sub>-transformed. #Data were arcsin-transformed.

| Source of variation   | Depth = 7 m               |         |    | Depth = 17 m |          |         |    |        |         |
|---|---------------------------|---------|----|--------------|----------|---------|----|--------|---------|
|   | SS                        | d.f.    | MS | F            | SS       | d.f.    | MS | F      |         |
| Leaf nitrogen<br>(% leaf DW)                                  | Disturbed vs. Control = D | 0.6769  | 1  | 0.6769       | 22.1***  | 0.0208  | 1  | 0.0208 | 1.0     |
|   | Sites (D)                 | 0.0306  | 1  | 0.0306       | 0.9      | 0.0205  | 1  | 0.0205 | 1.1     |
|   | Residual                  | 1.7178  | 51 | 0.0337       |          | 0.9427  | 51 | 0.0185 |         |
|   | Total                     | 2.4253  | 53 |              |          | 0.9841  | 53 |        |         |
| Shoot density§<br>(shoots m <sup>-2</sup> )                   | Disturbed vs. Control = D | 0.222   | 1  | 0.222        | 0.1      | 0.299   | 1  | 0.299  | 2.9     |
|   | Sites (D)                 | 2.045   | 1  | 2.045        | 12.5***  | 0.100   | 1  | 0.100  | 0.4     |
|   | Residual                  | 15.440  | 94 | 0.164        |          | 23.484  | 91 | 0.258  |         |
|   | Total                     | 17.719  | 96 |              |          | 23.893  | 93 |        |         |
| Leaf shoot biomass§<br>(g DW shoot <sup>-1</sup> )            | Disturbed vs. Control = D | 3.6270  | 1  | 3.6270       | 153.5*** | 2.2502  | 1  | 2.2502 | 29.3*** |
|   | Sites (D)                 | 0.0236  | 1  | 0.0236       | 0.1      | 0.0766  | 1  | 0.0766 | 0.5     |
|   | Residual                  | 18.4469 | 87 | 0.2120       |          | 11.9812 | 87 | 0.1377 |         |
|   | Total                     | 22.0975 | 89 |              |          | 14.3080 | 89 |        |         |
| Epiphyte load§<br>(mg epiphyte DW g <sup>-1</sup><br>leaf DW) | Disturbed vs. Control = D | 0.3375  | 1  | 0.3375       | 0.05     | 0.2053  | 1  | 0.2053 | 0.02    |
|   | Sites (D)                 | 7.1206  | 1  | 7.1206       | 34.6***  | 8.8041  | 1  | 8.8041 | 33.2*** |
|   | Residual                  | 17.9185 | 87 | 0.2060       |          | 23.0821 | 87 | 0.2653 |         |
|   | Total                     | 25.3765 | 89 |              |          | 32.0916 | 89 |        |         |
| Epiphyte ash#<br>(% epiphyte DW)                              | Disturbed vs. Control = D | 0.0084  | 1  | 0.0084       | 0.5      | 0.0042  | 1  | 0.0042 | 0.2     |
|   | Sites (D)                 | 0.0180  | 1  | 0.0180       | 7.7**    | 0.0199  | 1  | 0.0199 | 9.6***  |
|   | Residual                  | 0.1188  | 51 | 0.0023       |          | 0.1056  | 51 | 0.0021 |         |
|   | Total                     | 0.1452  | 53 |              |          | 0.1297  | 53 |        |         |



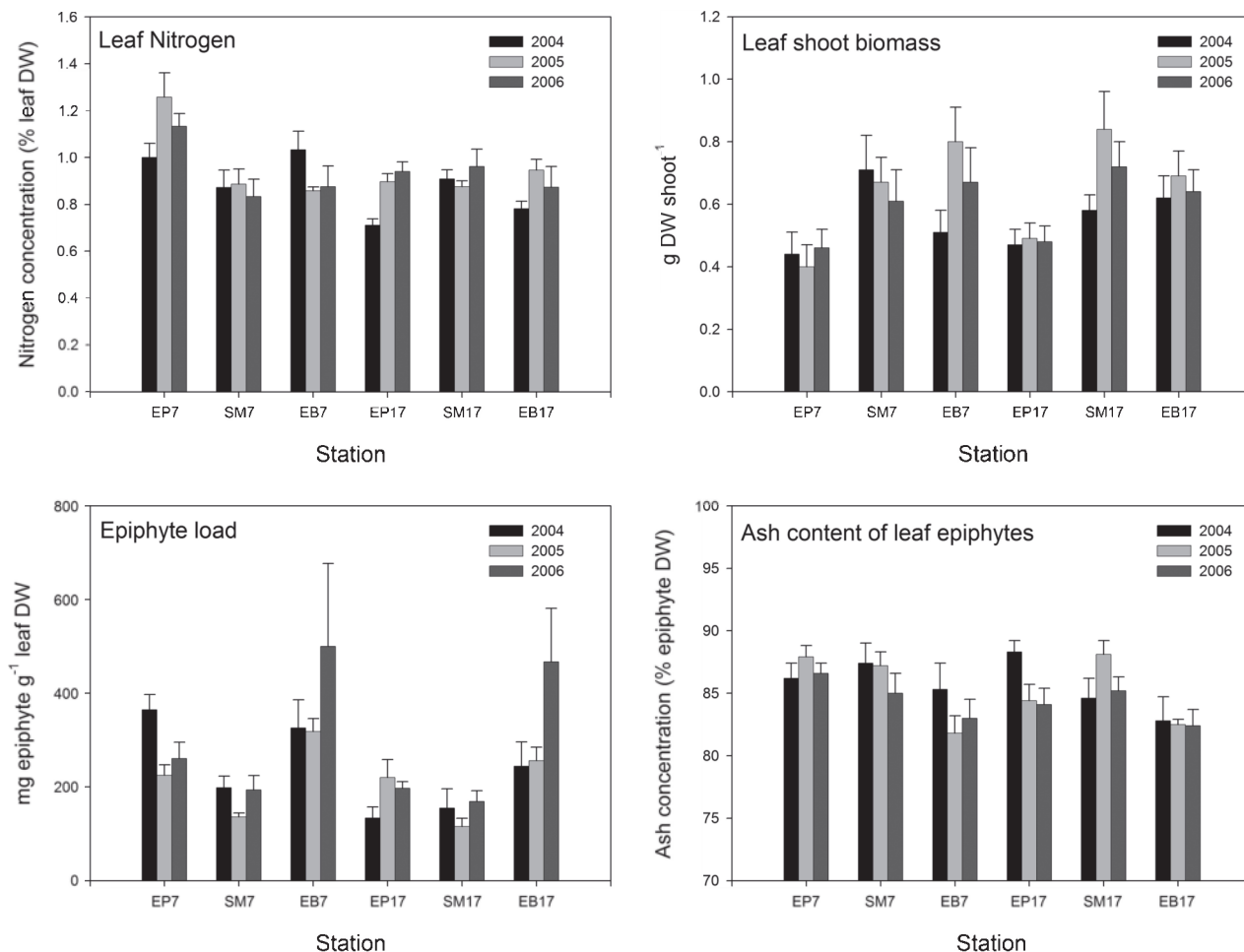


Fig. 2. – Concentration of nitrogen in the leaves (as percentage of leaf dry weight, DW), leaf shoot biomass (g DW shoot<sup>-1</sup>), epiphyte load (mg epiphyte DW g<sup>-1</sup> leaf DW), and the ash content of leaf epiphytes (as percentage of epiphyte DW) of *Posidonia oceanica* at three sites (EP: Es Port; SM: Cala Santa María; EB: Es Burri), two depths (7 m, 17 m) in each site, and three years (2004, 2005, 2006) in Cabrera Archipelago National Park (Balearic Islands, Western Mediterranean). Error bars represent +1SE.

± 1.3% of epiphyte DW to 88.3 ± 0.9% of epiphyte DW) and the two control sites (Table 1) but it was lower in EB (from 81.8 ± 1.4% of epiphyte DW to 85.3 ± 2.1% of epiphyte DW) than in SM (from 84.6 ± 1.6% of epiphyte DW to 88.1 ± 1.1% of epiphyte DW) at both depths.

## DISCUSSION

Anthropogenic inputs of nutrients and organic matter in Cabrera Archipelago National Park seem to increase the nitrogen availability at shallow depths only. That there was no difference between the epiphyte load on *Posidonia oceanica* leaves at 7 m depth at the disturbed site and the two control sites suggests that epiphyte load is not a good indicator of anthropogenic nutrient inputs in the park. This result is consistent with previous studies (Piazzi

*et al.*, 2004), which showed that the epiphytic cover of *P. oceanica* shoots was the same in locations affected and unaffected by chemical factories and urban waste. The low biomass of *P. oceanica* shoots in Es Port, compared to that in Cala Santa María and Es Burri, cannot be attributed therefore to nutrient-driven epiphytic overgrowth. The nitrogen content of *P. oceanica* leaves at this depth was, however, a sensitive indicator of anthropogenic nutrient inputs in the park. The value of seagrass leaf nitrogen as an indicator of anthropogenic nitrogen loading in the coastal zone has been demonstrated in other locations (McClelland and Valiela, 1998; Lepoint *et al.*, 2008).

Direct anthropogenic inputs of nutrients and organic matter in Cabrera Archipelago National Park do not increase the availability of nitrogen at 17 m depth and do not promote the accumulation of epiphytic biomass on *Posidonia oceanica* shoots at this

depth. However, similarly to what was found at 7 m depth, the biomass of *P. oceanica* shoots at 17 m depth was lower in Es Port than in the control sites. This result supports the contention that the comparatively low biomass of *P. oceanica* shoots in Es Port cannot be attributed to nutrient-driven epiphytic overgrowth. Previous assessments of the status of *P. oceanica* in the park showed that leaf production and the net recruitment of shoots are lower in Es Port than in Cala Santa María (Marbà *et al.*, 2002). However, we did not find differences in shoot density between Es Port and the control sites. Organic matter loading and sulphide accumulation in the sediment have been suggested to be responsible for the differences in vegetative development of *P. oceanica* in Es Port and Cala Santa María (Marbà *et al.*, 2002).

Leaf nitrogen and leaf shoot biomass were not different between Es Burri and Cala Santa María, the two control sites that do not receive direct anthropogenic inputs of nutrients and organic matter. However, the epiphyte load was higher in Es Burri than in Cala Santa María, which once again suggests that the epiphyte load on *Posidonia oceanica* leaves in Cabrera Archipelago National Park is not driven by anthropogenic nutrient loading. The epiphytic community in Es Burri might be different from that in Cala Santa María, as a lower ash content of the epiphytes in Es Burri suggests that the proportion of encrusting corallines in the epiphytic community at this site is lower than that in Cala Santa María. There seemed to be more red macroalgae *Lophocladia lallemandii* (Montagne) F. Schmitz in the *Posidonia oceanica* epiphytic community in Es Burri than in Cala Santa María and Es Port (personal observation) and this probably contributed significantly to the epiphyte load of *P. oceanica* at that site. *L. lallemandii* is considered an invasive species in the western Mediterranean (Boudouresque and Verlaque, 2002) and has spread over several areas in the Balearic Islands (Patzner, 1998; Ballesteros, 2006). *L. lallemandii* is able to invade healthy *P. oceanica* meadows and its presence is associated with reductions in the size of *P. oceanica* shoots, the sucrose content of the rhizome, and the percentage of living shoots (Ballesteros *et al.*, 2007). A high presence of *L. lallemandii* in the epiphytic community of *P. oceanica* would be, if confirmed by further studies, a matter of great concern for the fate of this seagrass species in Cabrera Archipelago National Park.

Correlative (Alcoverro *et al.*, 1997; Ruiz *et al.*, 2001) and experimental (Tomás *et al.*, 2005) evi-

dences show that herbivores may hinder the development of epiphytes on *P. oceanica* leaves. Sea urchin grazing seems to be more important than fish grazing in controlling the biomass of *P. oceanica* epiphytes (Tomás *et al.*, 2005). We did not assess differences in herbivore pressure between our study sites but sea urchins were not found at any of the sampling events. Although we cannot exclude the possibility that the differences in epiphyte load between the study sites might be driven by differences in fish grazing, we do not consider that herbivory contributed significantly to the between-site variability of epiphyte load.

The size of *Posidonia oceanica* shoots has also been suggested to significantly contribute to between-site variability of epiphyte load (Alcoverro *et al.*, 1997). Our results do not support this contention because (1) significant differences in leaf shoot biomass between the disturbed and control sites were not associated with differences in epiphyte load between these sites, and (2) significant differences in epiphyte load between the control sites were not associated with significant differences in leaf shoot biomass.

Exposure to waves and currents is considered to have a negative effect on the accumulation of epiphyte biomass on seagrass leaves (Borowitzka *et al.*, 2006) and it might contribute to between-site variability of epiphyte load on *Posidonia oceanica* shoots in Cabrera Archipelago National Park. Leaf movement has a strong negative effect on the biomass of epiphytic algae on *Posidonia sinuosa* Cambridge and Kuo shoots (Lavery *et al.*, 2007). Both Cala Santa María and Es Port face the north but the mouth of the latter is much smaller than that of the former, which makes Es Port a more sheltered site than Cala Santa María. Indeed, Es Port bay is the only site within Cabrera Island that has been used as a harbour. The two control sites, Cala Santa María and Es Burri, are open bays which are more exposed to waves than Es Port. If wave exposure significantly (and negatively) contributed to the accumulation of epiphyte biomass on *P. oceanica* shoots in the study sites we would expect that the epiphyte load would be higher in Es Port than in the control sites. However, we found no difference in epiphyte load between Es Port and Cala Santa María and Es Burri at both 7 m and 17 m depths. Tethered (i.e., motionless) and un-tethered (i.e., mobile) leaves of *P. sinuosa* accumulated the same amount of epiphyte biomass after eight weeks in locations with very different degrees of wave exposure (Lavery *et al.*, 2007). Whether dif-

ferences in epiphyte load between Cala Santa María and Es Burri are driven by differences in wave exposure in the two bays is a question that requires additional studies.

In summary, between-site variability of epiphyte load on *Posidonia oceanica* shoots in the Cabrera Archipelago National Park might be determined by differences in species composition of the epiphytic community, herbivore pressure or wave exposure but our results show that differences in nutrient availability do not make a significant contribution to this variability. Hence, epiphyte load was not a sensitive indicator of anthropogenic nutrient and organic matter loading within the park.

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