

Magnitude of herbivory in
Posidonia oceanica (L.) Delile
and factors responsible
for spatial variation



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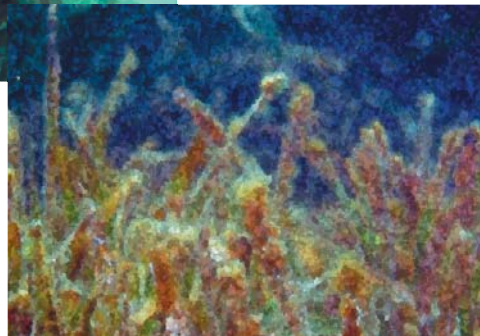
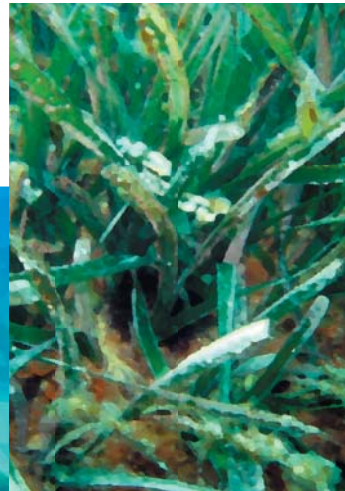
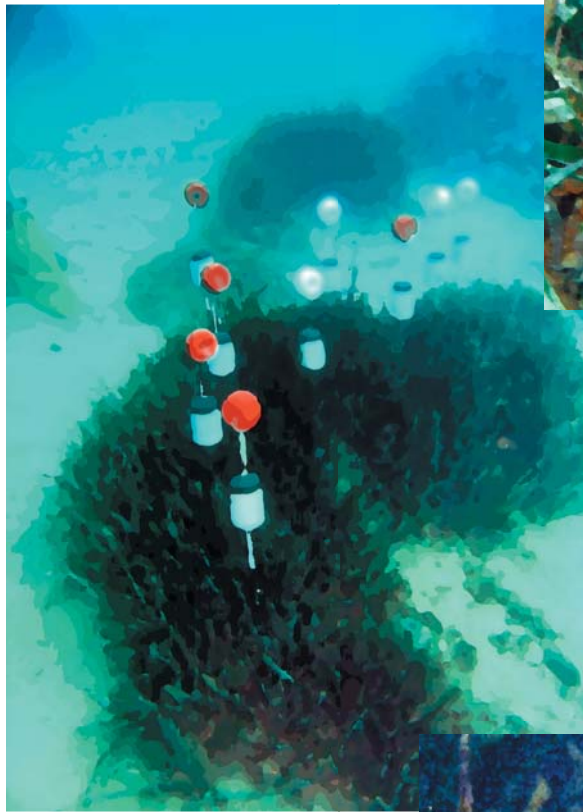
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Seasonal response of *Posidonia oceanica* epiphytic assemblages to nutrient increase

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RESUM:

L'efecte de la disponibilitat de nutrients en la comunitat d'epífits de *Posidonia oceanica* (L.) va ser investigat a una praderia oligotròfica, i posant especial atenció a la composició específica. L'experiment va consistir en el subministrament mensual de nutrients durant un any sencer. El subministrament de nutrients va tenir un efecte modest en la biomassa d'epífits (l'augment va ser detectat només durant els mesos d'agost i setembre), però va causar modificacions importants en l'estructura de les comunitats d'epífits de juny a setembre. Concretament, es va detectar una reducció inicial de la cobertura del briozou *Electra posidoniae* i una diversificació de les comunitats de Ceramials a la primavera i un increment dels feòfits durant l'estiu (*Sphacelaria cirrosa*, *Dictyota* spp.).

Altres variables, com per exemple la cobertura d'epífits, el contingut en nitrogen dels epífits o el quocient algues/ animal també van respondre al subministrament de nutrients durant l'estiu; però van mostrar diferents respostes en funció de la composició d'epífits. Contràriament, la composició hivernal, representada en la seva majoria per zoepífits i algues calcàries, no va respondre de cap manera al tractament. Els resultats d'aquest estudi suporten la hipòtesi que, en ecosistemes temperats, la comunitat d'epífits està en gran part controlada per la disponibilitat de nutrients des de finals de primavera fins finals d'estiu, no solament quantitativament sinó també en termes qualitius.



INTRODUCTION

Epiphytes contribute substantially to the seagrass ecosystems primary and secondary production (Morgan & Kitting 1984, Pollard & Kogure 1993), represent a significant part of their biodiversity (Mazzella et al. 1992, Jernakoff et al. 1996), and participate in important functional aspects (Sand-Jensen 1977, Walker & Woerkerling 1988).

The overall importance of epiphytes has been largely recognised, and has stimulated research on the factors controlling their abundance. Among them, light (Dalla Via et al. 1998, Lepoint et al. 1999), temperature (Neckles et al. 1993) and nutrients (Borum 1987, Frankovich & Fourqurean 1997) have been identified as the most important. Surprisingly for its relevance, much less attention has been paid to the control exerted by the taxonomic composition of the epiphytic community (but see Borum et al. 1984, Frankovich & Fourqurean 1997). Variability in the importance of epiphytes' effects has been largely associated to biomass variability, but it is most likely that epiphyte-mediated processes within seagrass beds are determined by the relative abundance of species and the existence of functional groups which provide specific ecosystems services. Epiphyte composition (e.g. encrusting versus erect forms) might demark considerably the respective shading capacities (Sand-Jensen 1977) thus having a great influence on the vitality of the seagrass host. Similarly, biogeochemical processes such as nitrogen fixation (Goering & Parker 1972) and recycling of calcium carbonate (Walker & Woerkerling 1988) are accomplished by specific taxa. Complex interactions may also arise from the synthesis of chemical and structural compounds by species of macroalgae (Hay et al. 1987, Hay 1996), modifying the feeding habits of certain grazers (Mazzella & Russo 1989, Jernakoff & Nielsen 1997, Gacia et al. 1999) which in turn are known to shape epiphytic communities (Alcoverro et al. 1997b).

Along with biomass patterns, changes in epiphyte composition are thought to be largely regulated by nutrient availability, especially in oligotrophic waters. In effect, unlike seagrasses that have two complementary sources of nutrients (i.e. water column and sediment pore water) algal epiphytes are highly dependent on column water nutrients (Williams &



Ruckelshaus 1993). Consequently, a direct relation between the seasonal fluctuations in water nutrient availability and the epiphyte biomass and composition (Borum 1985, 1987, Frankovicht & Fourqurean 1997, Wear et al. 1999, Beleggratis et al. 1999) is expected. However, it is often neglected that epiphyte communities are consisting on heterogeneous assemblages of both autotrophic and heterotrophic organisms (e.g. bryozoans, hydrozoans, ascidians, foraminifers) that may have different environmental requirements.

In the *Posidonia oceanica* ecosystems, the dominant seagrass species in the oligotrophic Mediterranean, epiphytes have been reported to change seasonally (Ben 1969, Mazzella & Ott 1984, Ballesteros 1987, Mazzella et al. 1989), but the influence of continuous nutrient supply on the diverse seasonal succession in community composition has not been properly investigated. The distinct seasonality in nutrient availability in the Mediterranean most likely plays a crucial role in structuring epiphyte community composition as a result of the high dependence of epiphytes on nutrients in the water column and the absence of storage capacity during unfavourable periods. If nutrients exert such control on epiphyte communities, we should expect to observe a significant response to enrichment (Silverstein et al. 1986), either in terms of increased biomass or in changes in epiphyte composition.

In this study, we experimentally test the hypothesis of the control of nutrients on the composition of the seagrass epiphytic community. In particular, we examine the response of epiphyte composition to nutrient availability and how this response interacts with the natural cycles of nutrient availability. To this end, we conducted a year-long nutrient addition experiment in a *Posidonia oceanica* (L.) Delile meadow living in oligotrophic conditions, and we assessed the changes induced in the epiphytic community taxonomic composition.

MATERIALS AND METHODS

Study site and experimental design

The study area was located in Fenals cove, in the northeast Mediterranean Spanish coast (41° 41' N, 003° 18' E). Water temperature in the area usually oscillates seasonally from 13 to 23°C (Cebrián et al. 1996), although the summer of the sampling period was exceptionally hot (up to 26°C in July). The area is also characterized by relatively low annual water column levels of nutrients averaging $0.96 \pm 0.07 \mu\text{M}$ for nitrate and $0.29 \pm 0.04 \mu\text{M}$ for phosphate (Cebrián et al. 1996).

The experiment was carried out in a shallow (8 m depth) *Posidonia oceanica* meadow, in which vegetated and sand patches alternate. We selected 12 seagrass patches of equivalent area (2-3 m²), at least 10m apart one from each other, and assigned 6 of them, randomly chosen to the enrichment treatment, and remaining the other 6 as controls. The density of shoots was high enough (1050 ± 112 shoots m⁻²; Alcoverro et al. 2001) to minimise any possible effect resulting from repetitive shoot sampling.

Water nutrient enrichment was performed monthly for one year, using a combination of di-Potassium Hydrogen Phosphate anhydrous, Sodium nitrate and Ammonium chloride (Panreac) and OSMOCOTE universal fertilizer (ratio N-P-K: 14-13-13) as sources of nutrients (Heck et al. 2000). At each enrichment event, a combination of 6 nutrient and 3 Osmocote containers were deployed per plot after sample collection. No decreased water clarity in the form of either particulate matter or phytoplankton blooms was observed in or near the plots.

Sample collection and processing

Each month six shoots per plot were collected prior to the fertilization treatment, and transported to the laboratory in an icebox in order to preserve plants and epibiota. Three of the shoots were used for epibionts identification; only the oldest leaf was examined as representative of the shoots epiphytic and sessile epifauna assemblages (Vanderklift & Lavery



2000, Ballesteros personal communication). Nevertheless, in instances where the oldest leaf was too short (i.e. less than 10 cm), the second oldest leaf was also considered, to ensure that we examined equivalent leaf areas in all shoots. Percentage cover of large encrusting species was first estimated visually, and epibionts were then removed with a razor blade and identified to species level under a microscope. A cover of 0.1% was assigned to small species that were found once per sample (present species according to Ballesteros 1987). The cover of erect species was estimated visually considering the 0.1% surface occupied by present species as a reference.

The three remaining shoots were used to determine leaf length and width, and the presence of bite marks (fish or sea urchin; Boudouresque & Meinesz 1982) or broken apices. Epiphytes were then removed with a razor blade (Dauby & Poulicek 1995) and leaves and epiphytes were dried at 60°C for 48 h, weighed separately, and ground to a fine powder with a mortar. These samples were analysed with an EA 1108 CHNS-O Carlo Erba Analyser (Serveis Científico-Tècnics, Universitat de Barcelona) to determine their nitrogen and carbon content.

Data analyses

Univariate analyses. The significance of the effects of nutrient treatment (fixed factor), plot (random factor) and month (fixed factor) on species richness, alpha-diversity, epiphytic biomass and seagrass and epiphyte nutrient content (dependent variables) were assessed with a 3-way analysis of variance. Treatment was orthogonal with month, and plot nested within treatment. ANOVA assumptions of homogeneity of variances and normality were tested by Cochran's test and Kolmogorov-Smirnov distribution-fitting test of the residuals, respectively. Appropriate transformations were used when necessary. However, it has to be reminded that the F statistic is known to be robust against violations of these assumptions (Underwood 1981). For all analysis, the critical level of significance was fixed at $p < 0.05$. Student-Newman-Keuls post hoc comparisons were used whenever necessary.



Multivariate analyses. Patterns of spatial and temporal variation in epiphytic assemblages were investigated using the PRIMER software package (Clarke & Warwick 1994, Carr 1997). Untransformed leaf cover values for each species were first used to build a Bray-Curtis (BC) dissimilarity matrix. Non-metric multidimensional scaling (n-MDS) was then used to examine differences among months and between treatments. Analysis of similarities (ANOSIM; Clarke 1993) was also conducted to test whether differences in species composition and abundance observed in n-MDS results were significant. As ANOSIM does not allow testing for more than two factors, we first conducted a two-way nested ANOSIM (Clarke & Green 1988) to test for monthly differences between treatments and among plots. A separated 2-way orthogonal analysis was conducted to test for differences between treatments and among months.

RESULTS

Epiphytic taxa

A total of 114 epibiontic species were found over all months and treatments. Taxa included: diatoms (tube dwelling species), cyanobacteria (2 species), Rhodophyta (57 species), Phaeophyta (15 species), Chlorophyta (8 species), hydrozoans (15 species), bryozoans (13 species), ascidians (2 species) and one kamptozoon (Entoprocta) and were similar to those reported to occur seasonally in the same area (Ballesteros 1987, García-Rubies 1987). A detailed list with species encountered during the study is available at <http://www.ceab.csic.es/es/plantilla.html>.

Univariate analyses

Species richness. Seasonality was the most important source of variance in species richness. Minimum values were attained in autumn, after leaf fall (4.3 ± 1 [S.E] species per leaf) and maximum in spring (16.6 ± 2.4 [S.E] species per leaf in May 2004), when the typical epiphytic community with *Giraudia sphacelariodes* and *Castagnea* spp. developed.



Enrichment caused a significant increase in the number of species per leaf, but only in summer, i.e. from June to September 2003 and again in June 2004 (16.3 ± 1.8 [S.E] and 8.4 ± 1.9 [S.E] species per leaf in nutrient and control plots, respectively; Table 1a, SNK; $p < 0.05$; Fig. 1a). This difference was mostly caused by the increase in the number of species of red algae, particularly Ceramiaceae, represented by small individuals occurring occasionally.

α - diversity. Alpha diversity showed similar patterns of seasonal variation as species richness. Enriched plots showed significantly higher values of diversity from June to September 2003 and in June 2004 (mean values for that period of c.a. 1.6 ± 0.2 [S.E] and 0.9 ± 0.2 [S.E] in nutrient and control plots, respectively, Table 1b, SNK; $p < 0.05$). However, a relative decrease was observed for both treatments in August-September resulting from an increased dominance of a few algae species (i.e. *Sphacelaria cirrosa*, *Dictyota* spp., Fig. 1b).

Cover. Minimum seasonal values were detected in October 2003 (35.3 ± 6.2 [S.E] %) resulting from the seasonal leaf fall and the development of a few pioneer species (e.g. *Fosliella* spp., *Myrionema magnusii* and the zooepiphytes *Sertularia perpusilla* and *Electra posidoniae*), while maximum were registered in May (95 ± 2.4 [S.E] %) coinciding with the largest annual animal abundance (*Sertularia perpusilla* and *Electra posidoniae*) and the development of the algal community (e.g. *Sphacelaria cirrosa*, *Giraudia sphacelariodes*, *Castagnea* spp., and Ectocarpaceae). Nutrient enrichment had a significant effect on cover, causing a significant decrease in June 2003 (63.8 ± 4.3 [S.E] and 84.9 ± 4.9 [S.E] %, enriched and control respectively, Table 1c, SNK; $p < 0.05$; Fig. 1c) largely due to the quick decline of the bryozoan *Electra posidoniae* in fertilized plots. In contrast, the opposite trend was observed in August-September, with an increase of epiphyte cover in nutrient plots (85.5 ± 6.2 [S.E] and 43.4 ± 6.3 [S.E] % leaf cover in nutrient and control plots respectively; Table 1c, SNK; $p < 0.05$; Fig. 1c) as a result of the development of the algae *Sphacelaria cirrosa* and *Dictyota* spp.



Table 1. Results of 3-factor ANOVA for species richness, α -diversity, cover and epiphytic biomass. Significant differences are indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ANOVA Source of variation	a) Species richness			b) α -diversity			c) Cover			d) Epiphytic Biomass			
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Treatment = T	1	1550	314.58	0.0000*	14.088	237.75	0.0000*	4359	139.02	0.0000*	0.0027	17.11	0.0020*
Plot = P (T)	10	4.9	1.93	0.0419*	0.059	1.4	0.1806	31	1.03	0.4195	0.0002	0.66	0.7650
Month = M	9	559.7	151.48	0.0000*	14.682	254.25	0.0000*	10351	227.66	0.0000*	0.1888	990.24	0.0000*
T x P (T)													
T x M	9	150.7	40.77	0.0000*	1.360	23.55	0.0000*	3645	80.17	0.0000*	0.0059	30.78	0.0000*
P (T) x M	90	3.7	1.45	0.0141*	0.058	1.36	0.0327*	45	1.49	0.0087*	0.002	0.81	0.8827
T x P (T) x M													
Cochran's test			C= 0.10			C= 0.06				C= 0.04			C= 0.08
Transformation			-			-				-			$\sqrt[3]{x}$

Table 2. Results of 3-factor ANOVA for leaf length, nitrogen content in *Posidonia oceanica* and epiphytes and Algae/ Animal ratio. Significant differences are indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ANOVA Source of variation	a) Leaf Length			b) Algae/Animal ratio			c) N in <i>Posidonia</i>			d) N in epiphytes			
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Treatment = T	1	13.34	4.98	0.0498*	95871	952.16	0.0000*	3.666	267.54	0.0000*	0.171	2.28	0.1622
Plot = P (T)	10	2.68	1.76	0.0690	101	1.17	0.3100	0.035	2.61	0.0051*	0.0075	1.72	0.0765
Month = M	9	273	115.14	0.0000*	67629	840.64	0.0000*	9.924	724.22	0.0000*	18.843	271.60	0.0000*
T x P (T)													
T x M	9	4.25	1.79	0.0803	40748	506.51	0.0000*	0.1737	12.67	0.0000*	1.028	14.81	0.0000*
P (T) x M	90	2.37	1.56	0.0043*	80	0.94	0.6344	0.0296	12.16	0.0000*	0.069	1.59	0.0029*
T x P (T) x M													
Cochran's test			C= 0.04			C= 0.07				C= 0.05			C= 0.09
Transformation			-			-				-			-



Biomass. Seasonality was again the main source of variability, with minimum values in October 2003 (0.016 ± 0.002 [S.E]) and a peak in May 2003 and 2004 (0.18 ± 0.024 [S.E]) resulting from the large cover and weight of the bryozoan *Electra posidoniae* and the hydrozoan *Sertularia perpusilla*. The effect of enrichment on epiphytic biomass was only significant during August-September 2003 (0.06 ± 0.009 [S.E] and 0.013 ± 0.005 [S.E] g per shoot in nutrient and control plots respectively; Table 1d, SNK; $p < 0.05$; Fig. 1d) and coincided with the bloom of *Sphacelaria cirrosa*, and to a lesser extent, of *Dictyota* spp.

Length of oldest leaf. Seasonal variation was the main source of variance, with short leaves during leaf fall in October 2003 (10 ± 1.4 [S.E] cm) and long leaves during the productive period in May-June 2003 (17.3 ± 0.9 [S.E] cm). In contrast, enrichment resulted in a slightly lower size of leaves in enriched plots in July compared to the control plots, probably as a result of major consumption by herbivores (13.53 ± 1.9 [S.E] cm and 13.9 ± 1.8 [S.E] cm, respectively in nutrient and control plots) but displayed no differences during the rest of the study period (Table 2a, SNK; $p < 0.05$; Fig. 1e).

Algae-Animal ratio (per cover). Maximum values were obtained in August-September (c.a. 179.8 ± 22.5 [S.E], Fig. 1f), caused by the large cover of a few algae species (i.e. *Sphacelaria cirrosa* and *Dictyota* spp.). This ratio reached its minimum in March-May (c.a. 32.4 ± 9.1 [S.E], Fig. 1f) due to the abundance of the bryozoan *Electra posidoniae*. Enrichment caused an increase in the algal dominance from July to September (127.9 ± 16.7 [S.E] and 22.4 ± 6.2 [S.E] in nutrient and control plots, respectively; Table 2b, SNK; $p < 0.05$; Fig. 1f). The increase in the abundance of *Sphacelaria cirrosa* accounted for most of these differences (see above).

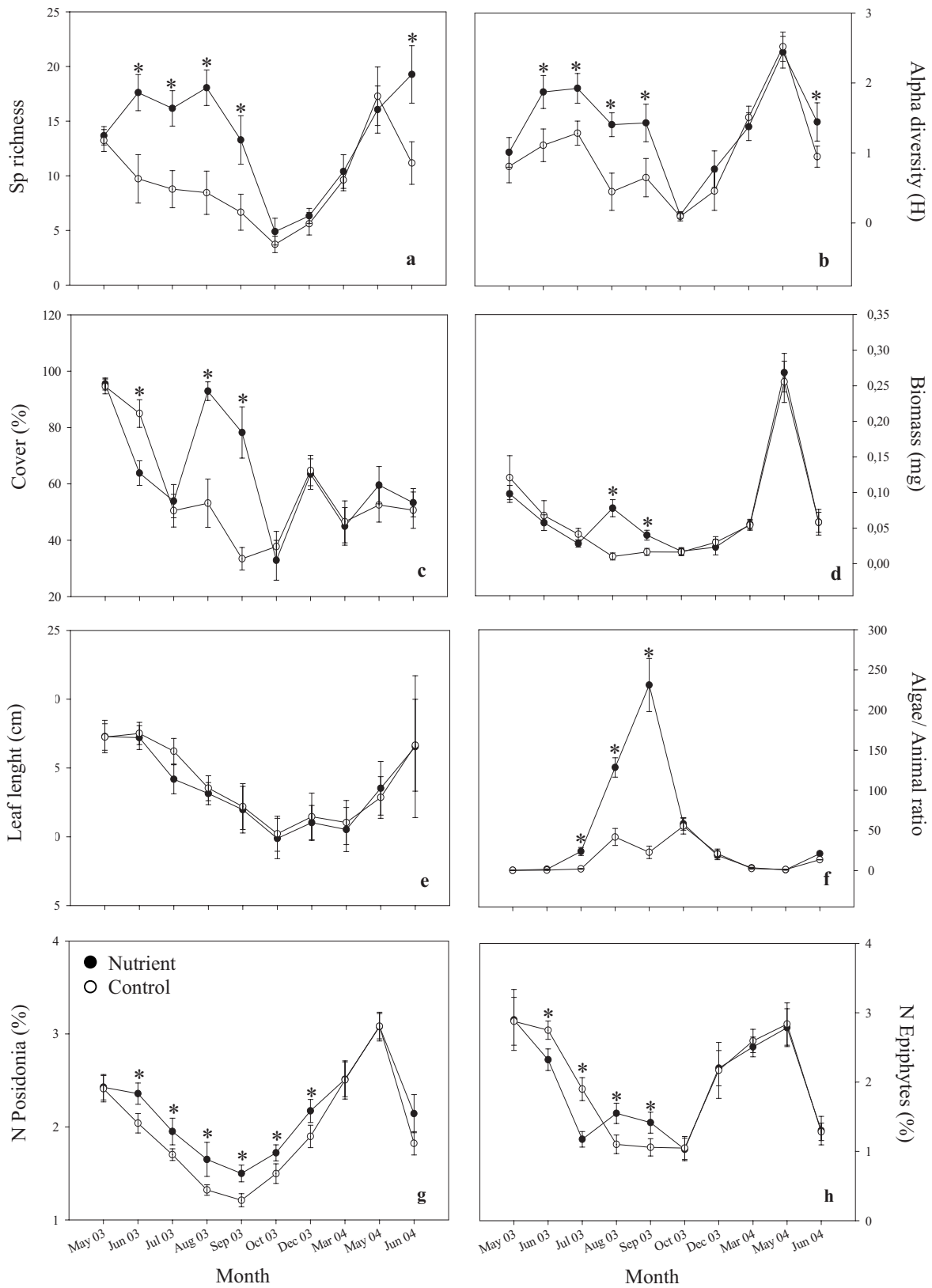


Fig. 1. Monthly trends in species richness, alpha diversity, epiphytic biomass, epiphytic cover, leaf length and nitrogen content of *Posidonia oceanica* for both nutrient and control plots. * In SNK statistically significant result.

Nitrogen content in *Posidonia oceanica*. Seasonal variability followed the typical pattern, with highest values being recorded in May (3.1 ± 0.1 [S.E] %), and lowest during September-October (1.3 ± 0.07 [S.E] %). Fertilization resulted in a significant increase in the N content of *P. oceanica* from June to December 2003 and then again in June 2004 (1.6 ± 0.1 [S.E] % and 1.9 ± 0.14 [S.E] % in nutrient and control plots, respectively; Table 2c, SNK; $p < 0.05$; Fig. 1g).

Nitrogen in epiphytes. Seasonal trends were similar to those of *Posidonia oceanica* leaves, with highest values in October (1 ± 0.2 [S.E] %) and lowest in May (2.8 ± 0.3 [S.E] %). Enrichment produced significant differences between treatments throughout the summer (Table 2d, SNK; $p < 0.05$; Fig. 1h). However, earlier animal decrease in nutrient plots during June-July resulted in higher N content in control plots (1.7 ± 0.1 [S.E] % and 2.3 ± 0.1 [S.E] % in nutrient and control plots, respectively). In contrast, the mid- to late-summer algal community (August-September), exhibited higher N content in nutrient (1.5 ± 0.1 [S.E] %) than in control plots (1.1 ± 0.2 [S.E] %).

Multivariate analyses

Posidonia oceanica epiphytic assemblage composition was influenced by season and nutrients (two-way crossed ANOSIM, $R = 0.984$, $p = 0.001$ and $R = 0.268$, $p = 0.001$ for differences among months and between treatments, respectively, Fig. 2). Communities did not respond to fertilization immediately after the initiation of the experiment (May 2003, n-MDS ordinations, Fig. 3), but from June to September the composition of the epiphytic assemblage differed largely between nutrient and control plots (i.e. global R values close to 1, n-MDS ordinations, Fig. 3). This difference decreased during fall, and disappeared in winter, and communities began to diverge again in spring (May and June 2004), although less markedly than the previous year. Variability among plots was generally small (i.e. Global R-values close to zero, Table 3), except for June, July and October 2003, indicating a certain



degree of patchiness. For all n-MDS ordinations, generally low stress values indicated a good overall representation of assemblages' patterns.

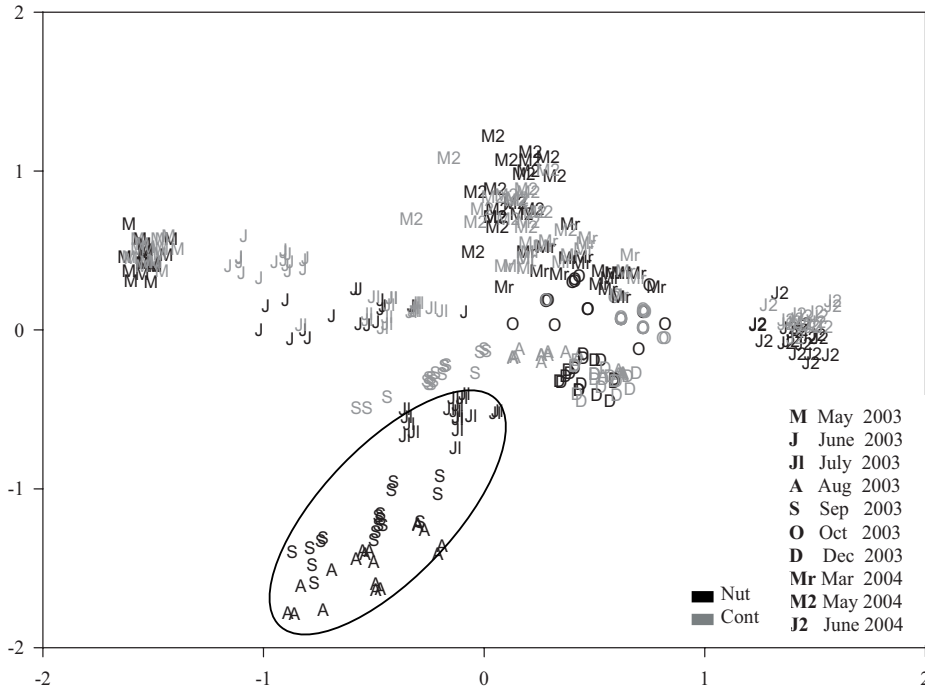


Fig. 2. n-MDS ordination showing differences found between months and treatments. The effect of enrichment in assemblages composition during the summer period is encircled.

Table 3. Results of two-way nested ANOSIM based on dissimilarity matrices derived from untransformed leaf cover values of epiphytic species. a) Differences among replicate plots and b) differences between treatments at each month of the experiment. Significant differences are indicated in bold. R: ANOSIM statistic; *p*: probability level.

ANOSIM	a) Within treatments		b) Between treatments	
	R statistic	<i>p</i>	R statistic	<i>p</i>
May 2003	0.171	5.6	-0.05	70.1
June 2003	0.272	0.1	1	0.2
July 2003	0.364	0.1	1	0.2
August 2003	0.104	12.4	1	0.2
September 2003	0.131	6.3	1	0.2
October 2003	0.273	0.3	0.298	3.7
December 2003	0.043	28.1	0.133	10.6
March 2004	-0.027	58.8	0.124	15..4
May 2004	-0.012	51.9	0.471	0.2
June 2004	0.064	16.9	0.602	0.2



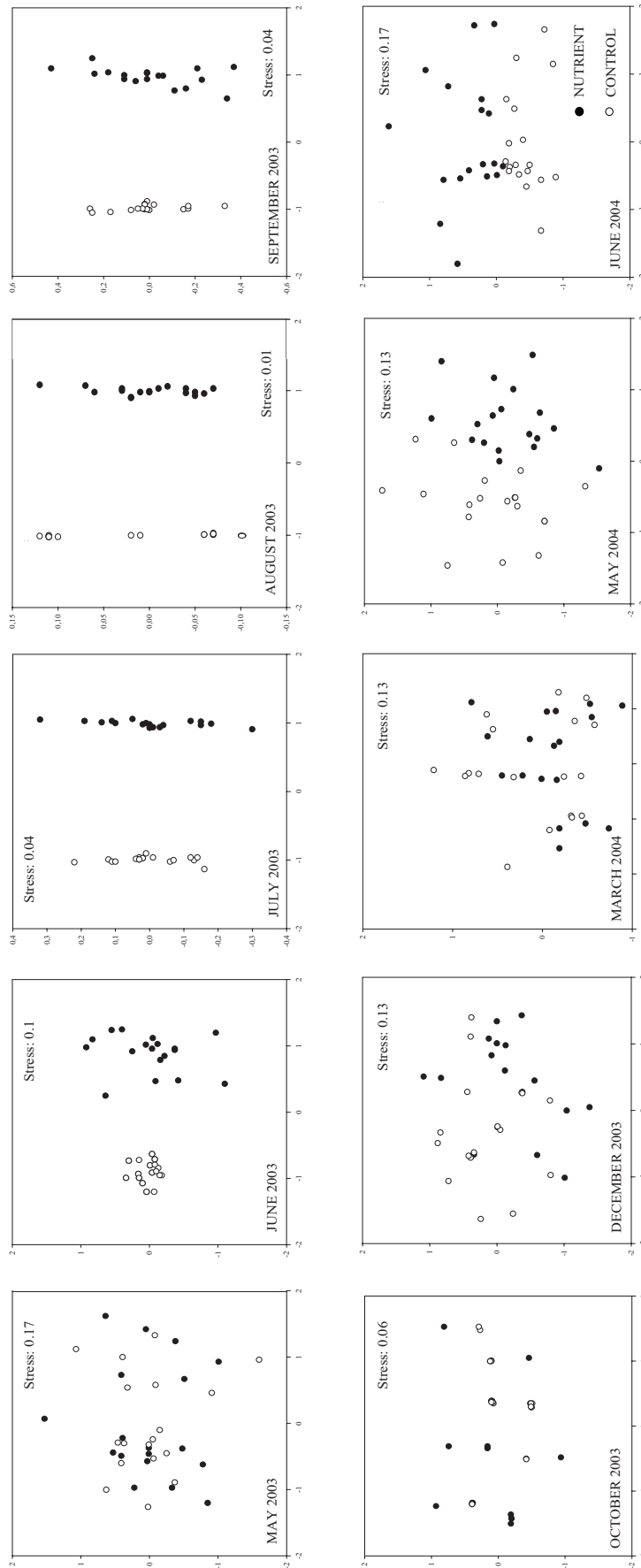


Fig. 3. n-MDS plots indicating differences between treatments at each study month.



DISCUSSION

Nutrient enrichment noticeably altered the epiphytic community inhabiting leaves of *Posidonia oceanica*. In addition to changes in epiphytic species richness, diversity, biomass, cover and epiphyte nutrient content, a drastic change in the taxonomic composition of epiphyte assemblages was detected. This indicates the strong and direct dependence of epiphytic community on water nutrient availability, not just in quantitative terms as it had been previously documented (Borum 1985, 1987, Cambridge et al. 1986, Williams & Ruckelshaus 1993, Frankovicht & Fourqurean 1997, Delgado et al. 1999, Wear et al. 1999, Drake & Dobbs 2003), but also in qualitative and structural terms.

Experimental addition of nutrients caused an increase in both species richness and diversity, confirming previous findings (Wear et al. 1999, Bokn et al. 2002, Beleggratis et al. 1999, Hillebrand 2003), which was due to the occurrence of large numbers of fleshy macroalgae with little contribution to the overall biomass. In fact, biomass only increased in August by the growth of ephemeral species that spread over the leaves in nutrient plots and dominated the epiphytic community. Under conditions of increased nutrient availability, the development of fast growing macroalgae is usually stimulated since they have higher demands of nutrients per biomass unit (Pedersen & Borum 1996). However, this takes place only when other factors or resources such as light and temperature are at their optima, thus explaining the pattern of higher biomass values in early spring prior nutrients become limiting (Jacobs et al. 1982, Borum et al. 1984).

Monthly enrichment was not able to disrupt the patterns of seasonal succession identified in earlier studies (Ben 1969, Mazzella & Ott 1984, Ballesteros 1987, Romero 1988, Mazzella et al. 1989, Alcoverro et al. 1997b), indicating that factors other than nutrient availability (e.g. light and temperature conditions, seasonal hydrodynamics or seasonal changes in host characteristics) may also have an important role structuring epiphytic assemblages. In winter, *Posidonia oceanica* the epiphytic community is dominated by hydrozoans, bryozoans and slow-growing, encrusting algae, and this winter assemblage



remains unchanged despite the fertilising treatment. This is due to two main reasons. On the one hand, zoepiphytes are mostly suspension feeders, not directly affected by nutrients and generally showing different requirements than macroalgae in relation to environmental features such as light, temperature and hydrodynamics (Gili et al. 1984, Pomar 2001, Lippert et al. 2001). On the other hand, encrusting algae have relatively low nutrient and light demands (Dethier & Steneck 2001) and are therefore also unlikely to respond to changes in nutrient availability, while winter dim light conditions do not allow the blooming of fast-growing algae. In contrast, the response of such fast-growing algae occurred in spring-summer when light conditions tend to favour growth but nutrients are scarce (Pedersen & Borum 1996, Lotze et al. 1999). The sharp decrease in animal abundance in enriched plots between mid-spring and early summer (see Fig. 1f), is probably the consequence of algal overgrowth on the surfaces of leaves (i.e. nutrient mediated competition, Ocito et al. 2001). Similarly, the bloom of *Sphacelaria cirrosa* was caused by the coupling of nutrient availability and water temperature (or incident light) which may have favoured germination (Lotze et al. 1999) and/ or growth of species of Sphacelariales which may have higher ammonium and nitrate specific uptake rates than the plant and the remaining epiphytic community (Lepoint et al. 2004).

Our results have important implications for a better understanding of the relationship between nutrients and epiphytes in seagrass ecosystems inhabiting oligotrophic waters. For example, parameters such as epiphyte biomass or nutrient content have been used to detect or evaluate eutrophication (Kirkman 1996, Frankovich & Fourqurean 1997). Seagrass decline coinciding with nutrient discharge and epiphytic loads has been reported to occur permanently in other temperate systems (Cambridge et al. 1996, Walker & McComb 1992, Moore et al. 1996). In the *Posidonia oceanica* ecosystem such univariate measures are, in general, good descriptors, but should be interpreted with some caution because of the significant mediating influence that seasonality wields on the power of detecting a nutrient response. Nutrient content of epiphytes responded to nutrient fertilization positively (August-September) or negatively (June-July) depending on the period because of the strong influence

of seasonal changes in composition, and the rapid species turnover. Epiphyte biomass was not substantially changed by nutrient enrichment except in August as most of the changes were occurring at the level of species composition, and seasonal forcing (e.g. host dynamics, light temperature, nutrients and so on) was the main factor driving this variable. In contrast, epiphyte composition responds to nutrient enrichment unequivocally throughout the nutrient limiting period (from June to September) reinforcing the view that species composition is the best descriptor of eutrophication, with the caveat that this difference is only detected in spring-summer, when the extent of nutrient limitation is higher and marine communities are more sensitive to changes in nutrient concentrations (Perez et al. 1991, Alcoverro et al. 1997a,b). Importantly though, seasonality wielded a lesser mediating influence on seagrass enrichment, as indicated by monthly differences in seagrass nutrient content between treatments. This effect was long lasting, with similar extent of differences between treatments through most of the study period.

In summary, nutrient availability in the *Posidonia oceanica* seagrass meadow studied controls epiphytic assemblages from spring to autumn, while during the winter period other factors seem to determine community composition and abundance. The life period of epiphytes is completely determined by seagrass leaf fall, and does not allow species to employ long-term strategies like nutrient storage during the favourable periods. As a result, epiphyte communities perfectly mirrors nutrient availability in the column water during the period of nutrient limitation, and constitute an ideal indicator of nutrient availability during that period. No nutrient thresholds have been identified in this study for detrimental interactions between epiphytes and the plant host. This will require a separate study with increased nutrient enrichments. Until this is achieved and considering the importance of nutrients on the control of *P. oceanica* epiphyte composition a precautionary approach is recommended for the possible consequences of nutrient discharges in oligotrophic seagrass meadows.



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Supplemental Table 1. Species list of the macroalgae and sessile epifauna found growing over the oldest leaf of *Posidonia oceanica*

		M-03		J-03		JI-03		A-03	
		N	C	N	C	N	C	N	C
Bacillariophyceae									
1	Tube dwelling diatoms	0.35	0.16	0.07	0	0	0	0	0
Cyanophyceae									
2	<i>Lyngbya meneghiniana</i>	0	0	0.011	0	0.022	0.011	0.072	0.011
3	<i>Lyngbya sordida</i>	0	0	0	0.005	0.011	0.016	0.022	0.044
Rodophyceae									
4	<i>Acrochaetium leptonema</i>	0	0.066	0	0	0	0	0	0.005
5	<i>Acrosorium venulosum</i>	0	0.011	0.011	0.11	0	0	0	0
6	<i>Acrothamnion preissii</i>	0	0	0	0	0	0	0	0
7	<i>Aglaothamnion brodiaei</i>	0	0	0	0	0	0	0	0
8	<i>Aglaothamnion tenuissimum</i>	0	0	0.033	0.005	0.005	0.005	0.033	0.011
9	<i>Aglaothamnion furcellariae</i>	0	0	0	0	0	0	0	0.005
10	<i>Aglaothamnion tripinnatum</i>	0	0	0.005	0	0.005	0	0.011	0.005
11	<i>Anotrichium barbatum</i>	0	0	0	0	0	0	0	0
12	<i>Antithamnion cruciatum</i>	0.21	0.13	0.53	0.016	0.20	0.016	0.022	0.011
13	<i>Antithamnion decipiens</i>	0	0.11	0.033	0	0	0	0.027	0.005
14	<i>Apoglossum ruscifolium</i>	0.016	0	0	0	0	0	0	0
15	<i>Botryocladia</i> sp.	0	0	0	0	0	0	0	0
16	<i>Callithamnion tetragonum</i>	0.005	0	0	0	0	0	0	0
17	<i>Ceramium ciliatum</i>	0	0	0	0	0.005	0	0	0.055
18	<i>Ceramium circinatum</i>	0	0	0	0	0	0	0	0
19	<i>Ceramium diaphanum</i>	0	0	0	0	0	0	0	0
20	<i>Ceramium flaccidum</i>	0.055	0.027	0.016	0.011	0.11	0.038	0.1	0.044
21	<i>Ceramium</i> gr. <i>rubrum</i>	0	0	0	0	0	0	0	0
22	<i>Ceramium</i> sp. (juvenile)	0	0	0.005	0	0	0	0	0
23	<i>Ceramium strictum</i>	0.14	0.19	0.027	0.022	0.072	0.066	0.027	0.05
24	<i>Champia parvula</i>	0	0	0.011	0	0	0	0	0
25	<i>Chondria capillaris</i>	0	0	0	0	0	0	0.011	0
26	<i>Chylocladia verticillata</i>	0.038	0.17	0.077	0.05	0.088	0.005	0.17	0
27	<i>Colaçonema daviesii</i>	1.22	0.93	0.072	0.044	0.094	0.027	0.044	0.05
28	<i>Compsothamnion thuyoides</i>	0	0	0	0	0.011	0	0.005	0
29	<i>Crouania attenuata</i>	0	0	0	0	0	0	0	0

Seasonal response of *Posidonia oceanica* epiphytic assemblages to nutrient increase

	S-03		O-03		D-03		Mr-04		M-04		J-04	
	N	C	N	C	N	C	N	C	N	C	N	C
	Bacillariophyceae											
1	0	0	0	0	0	0	0.18	0	1.22	0.21	0.055	0
	Cyanophyceae											
2	0.016	0.055	0	0	0.005	0.005	0	0	0.005	0	0.022	0
3	0.016	0.011	0.005	0	0	0	0	0	0	0.005	0.005	0.011
	Rodophyceae											
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0.055	0.44	0	0.39	0.51	0
6	0	0	0	0	0	0	0	0	0.16	0.005	0	0
7	0	0	0	0	0	0	0	0	0.005	0.011	0	0
8	0.033	0.011	0	0	0.011	0	0.033	0.011	0.016	0.016	0.027	0.011
9	0.016	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0.011	0.027	0.28	0	0
11	0.005	0	0	0	0	0	0	0	0	0	0	0
12	0.022	0.011	0.005	0	0	0	0.005	0.005	0.005	0.005	0.072	0.005
13	0.022	0.005	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0.005	0	0.016	0
15	0	0	0	0	0	0	0	0	0	0	0.005	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0.027
18	0	0	0	0	0	0	0	0	0	0.005	0	0
19	0	0	0	0	0	0	0	0	0	0	0.011	0.005
20	0.05	0.038	0.005	0	0	0	0.033	0.016	0.033	0.022	0.083	0.027
21	0	0	0	0	0	0	0.011	0.005	0.011	0.016	0	0
22	0	0	0.005	0	0	0	0.027	0.027	0.033	0.055	0.033	0.011
23	0	0	0	0	0	0	0	0	0	0.005	0.005	0.005
24	0	0	0	0	0	0	0	0	0	0	0	0.005
25	0	0	0.005	0	0	0	0	0	0	0	0	0.005
26	0.33	0	0	0	0	0.005	0.027	0.022	0	0.005	0.005	0.011
27	0.011	0	0.005	0	0	0	0.016	0.022	0.038	0.077	0.005	0.005
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0.022	0.005



30	<i>Dasya hutchinsiae</i>	0.022	0	0.027	0	0.088	0	0	0
31	<i>Dasya</i> sp.	0	0	0	0	0	0	0.022	0
32	<i>Dudresnaya verticillata</i>	0.027	0.038	0.11	0.2	0	0	0	0
33	<i>Erythrotrichia carnea</i>	0.066	0.072	0.1	0.022	0.055	0.005	0.061	0.011
34	<i>Falkenbergia rufolanosa</i> - stadium	0.055	0.072	0.066	0.083	0.061	0.016	0.083	0.044
35	<i>Herposiphonia tenella</i>	0	0	0	0	0.027	0	0.005	0
36	<i>Heterosiphonia crispella</i>	0	0	0	0	0	0	0.34	0.005
37	<i>Hydrolithon farinosum</i> + <i>Pneophyllum</i> spp.	4.55	4.66	13.38	13.05	14.166	13.05	9.77	30.27
38	<i>Jania rubens</i> var. <i>corniculata</i>	0	0.011	0.005	0.016	0.005	0	0.005	0.016
39	<i>Laurencia</i> sp. (juvenile)	0	0	0	0	0	0.011	0.15	0.033
40	<i>Laurencia minuta</i>	0	0	0	0	0	0	0.038	0
41	<i>Laurencia</i> sp.	0	0	0	0	0	0	0	0
42	<i>Lejolisia mediterranea</i>	0	0	0	0	0	0	0	0
43	<i>Lomentaria chylocradiella</i>	0.14	0	0	0.011	0.005	0	0.005	0
44	<i>Lomentaria ercegovicii</i>	0	0.022	0.16	0.011	0.055	0.038	0.038	0
45	<i>Lomentaria verticillata</i>	0	0	0	0	0	0	0	0
46	<i>Lomentaria</i> sp.	0	0	0	0	0	0	0	0
47	<i>Myriogramme distromatica</i>	0	0	0	0	0	0	0	0
48	<i>Nitophyllum micropunctatum</i>	0	0.016	0.1	0.005	0.011	0	0.022	0
49	<i>Porphyra</i> sp.	0	0.005	0	0	0	0	0	0
50	<i>Plocamium cartilagineum</i>	0.022	0.027	0.005	0.022	0.027	0	0	0
51	<i>Polysiphonia</i> spp. (4 siphons)	0	0.016	0.01	0	0.033	0.005	0.08	0.011
52	<i>Polysiphonia furcellata</i>	0	0	0	0	0	0	0	0
53	<i>Porphyrostromium boryanum</i>	0	0	0	0	0	0	0	0
54	<i>Rhodophyllis divaricata</i>	0	0	0.033	0	0	0	0	0
55	<i>Spermothamnion flabellatum</i>	0.011	0	0	0.005	0.016	0.005	0	0
56	<i>Stylonema alsidii</i>	0.016	0.005	0.066	0.005	0.072	0.011	0.022	0.005
57	<i>Stylonema cornu-cervi</i>	0	0	0.011	0	0	0	0	0
58	<i>Titanoderma pustulatum</i>	0	0	0	0	0	0	0	0
59	Unidentified Ceramiales	0	0	0.011	0	0.005	0	0	0.005
60	Unidentified Delesseriaceae	0.005	0.011	0.011	0.005	0.027	0	0	0
Phaeophyceae									
61	<i>Aglaozonia parvula</i>	0	0	0.033	0.022	0.061	0.066	0.016	0
62	<i>Cladosiphon cylindricus</i> + <i>irregularis</i>	0.23	0	0.23	0.14	0.64	0.016	0.2	0.011
63	<i>Dictyota dichotoma</i> + <i>linearis</i>	0	0	0	0	0.25	0	9.66	0.055
64	<i>Ectocarpus siliculosus</i>	1.43	1.34	0.75	0.21	0.45	0.011	0.044	0.011
65	<i>Feldmania</i> sp.	0	0	0	0	0	0	0	0



Seasonal response of *Posidonia oceanica* epiphytic assemblages to nutrient increase

30	0.22	0	0	0	0	0	0	0.011	0.38	0	0.18	0.005
31	0	0	0	0	0	0	0	0.005	0.005	0.005	0.044	0.011
32	0	0	0	0	0	0	0	0.016	0.038	0.19	0.1	0.038
33	0.061	0.027	0	0	0.005	0.005	0.038	0.038	0.072	0.055	0.083	0.016
34	0.41	0.061	0	0	0.016	0.005	0.055	0.027	0.066	0.05	0.11	0.05
35	0.066	0.005	0	0.005	0	0.005	0.005	0	0	0	0	0.011
36	3.29	0.31	0.038	0	0.26	0.016	0	0	0	0	0	0
37	9.16	10.38	20.55	25.83	32.77	36.38	23.33	21.38	15.11	14.55	42.14	42.44
38	0.027	0	0	0	0.011	0.005	0	0	0	0.011	0.011	0
39	0	0	0	0	0	0	0	0	0	0	0.055	0.016
40	0.005	0.038	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0.022	0.016	0.005	0
42	0.005	0	0	0	0	0	0	0	0	0	0	0.005
43	0	0	0	0	0	0	0	0	0.95	0.28	0.033	0
44	0	0	0	0	0	0	0	0	0	0.027	0.33	0.055
45	0	0	0	0	0	0	0	0	0.18	0.066	0.005	0
46	0	0	0	0	0	0	0	0	0.055	0	0	0
47	0	0	0	0	0	0	0.011	0.005	0.022	0.32	0.21	0.027
48	0.027	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0.005	0.011	0.016	0	0	0
50	0	0	0	0	0	0	0	0	0.016	0	0.011	0.005
51	0.18	0.011	0.016	0	0	0	0.005	0.005	0.005	0.011	0.061	0.033
52	0	0	0	0	0	0	0	0	0.005	0	0	0
53	0	0	0	0	0	0	0	0	0	0.016	0	0
54	0.027	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0.005	0	0	0.005	0.005	0	0	0.005	0.038	0.005
56	0.033	0.011	0.005	0	0.005	0	0.061	0.094	0.1	0.1	0.1	0.088
57	0	0	0	0	0	0	0.055	0.044	0.1	0.1	0.1	0.094
58	0	0	0	0	0.111	0	0	0	0.111	0.461	1.88	1.305
59	0	0	0.033	0.005	0	0.005	0	0	0.005	0	0	0
60	0	0	0	0	0	0	0.011	0	0.077	0.011	0.011	0.005
Phaeophyceae												
61	0.005	0	0	0	0.011	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0.011	0.083	0.53	0.13
63	3.66	0.022	0	0	0	0	0	0	0	0	0.055	0
64	0	0	0	0	0	0	0	0	0.19	0.17	0.011	0
65	0	0	0	0	0	0	0	0	0	0.11	0	0



66	<i>Giraudia sphacelarioides</i>	0.54	1.022	0.305	0.405	0.038	0.011	0.055	0.022
67	<i>Halopteris filicina</i>	0.011	0	0	0.011	0.005	0	0	0.011
68	<i>Halopteris scoparia</i>	0.011	0	0	0	0	0	0	0
69	<i>Kuckuckia spinosa</i>	0.34	0	0	0	0	0	0	0
70	<i>Kuetzingiella battersii</i>	0	0	0	0	0.038	0.011	0.005	0
71	<i>Myrionema magnusii</i>	1.66	19.33	21.83	19.05	26.66	20.77	20.77	18.38
72	<i>Sphacelaria cirrosa</i>	0.57	0.14	1.88	0.14	8.56	0.23	51.9	0.17
73	Unidentified Dictyotaceae	0	0	0	0	0	0	0	0
74	Unidentified Ectocarpales	0	0	0	0	0	0	0	0
75	<i>Zonaria tournefortii</i>	0	0	0	0	0.011	0	0.016	0
Chlorophyceae									
76	<i>Bryopsis</i> sp. (juvenile)	0	0	0	0	0.005	0	0.011	0
77	<i>Cladophora dalmatica</i>	0	0	0	0	0.005	0	0.041	0.011
78	<i>Cladophora laetevirens</i>	0	0	0	0	0.011	0.011	0.061	0.033
79	<i>Cladophora</i> sp.	0.005	0	0	0	0	0.005	0.016	0
80	<i>Enteromorpha multiramosa</i>	0.005	0	0.005	0	0.011	0	0.122	0
81	<i>Halicystis parvula</i>	0	0	0	0	0	0	0.005	0
82	<i>Ulvella</i> sp.	0	0	0	0	0	0	0.005	0
83	Unidentified Ulvaceae	0	0	0	0	0	0	0	0
Hydrozoans									
84	<i>Campanularia asymmetrica</i>	0	0	0	0	0	0.005	0	0
85	<i>Cladocoryne floccosa</i>	0	0.011	0	0	0	0	0.05	0.15
86	<i>Clytia hemisphaerica</i>	0.26	0.33	0.33	0.24	0.11	0.044	0	0
87	<i>Cordylophora pusilla</i>	0	0	0	0	0.005	0	0	0
88	<i>Cuspidiella</i> sp.	0	0	0	0	0	0	0.005	0
89	<i>Dynamena cornicina</i>	0	0	0	0	0	0	0	0
90	<i>Halecium nanum</i>	0	0	0.11	0.11	0	0.11	0	0.011
91	<i>Halecium pusillum</i>	0	0.027	0.055	0.038	0.022	0.011	0	0
92	<i>Hebella</i> sp.	0	0	0	0	0	0	0.005	0
93	<i>Obelia geniculata</i>	0.11	0	0.005	0	0	0	0	0
94	<i>Plumularia obliqua posidoniae</i>	0.16	0.033	0.36	0.016	0	0.027	0.022	0.011
95	<i>Sertularia perpusilla</i>	0.54	1.23	0.094	0.16	0.23	0.18	0.17	0.077
96	<i>Sertularia distans</i>	0	0	0	0	0	0	0	0
97	<i>Stylactis inermis</i>	0	0	0	0	0	0	0	0
98	Unidentified Hydrozoan	0.0011	0.022	0.011	0.033	0	0	0	0.005



Seasonal response of *Posidonia oceanica* epiphytic assemblages to nutrient increase

66	0	0	0	0	0	0	0	0	0.016	0.1	0.17	0
67	0	0	0	0	0	0	0.005	0	0.011	0.005	0.027	0
68	0	0	0	0	0	0	0	0	0.016	0.011	0.022	0
69	0	0	0	0	0	0	0	0	0	0.005	0	0
70	0	0	0.005	0	0	0	0	0.011	0	0	0	0
71	26.11	20.77	11.61	11.22	26.77	25.16	10.83	11.16	12.72	11.77	3.38	2.19
72	35.94	0.1	0	0	0.027	0	0	0	0.044	0.033	0.405	0.088
73	0	0	0	0	0	0	0	0	0	0	0.6	0.15
74	0	0	0	0	0	0	0	0	0.071	0.061	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyceae												
76	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0.011	0
78	0.016	0	0	0	0	0	0	0	0	0.005	0.005	0
79	0.016	0.005	0	0	0	0	0	0	0	0	0	0
80	0.038	0	0	0	0	0.005	0	0	0	0.011	0	0
81	0	0	0	0	0	0	0	0	0.005	0	0	0
82	0	0.005	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0.011	0.005	0.005	0.011	0.011	0
Hydrozoans												
84	0	0	0.011	0.1	0.116	0.311	0	0	0	0	0	0
85	0.038	0.011	0.15	0	0	0	0.14	0.22	0.705	0.944	0.016	0.038
86	0	0	0	0	0.45	0.38	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0.111	0.094	0.22	0.777	0.083	0
90	0	0	0	0	0	0	0	0	0.22	0.166	0	0
91	0	0	0	0	0	0	0	0	0	0.061	0	0
92	0	0	0	0.027	0	0	0	0	0	0	0	0
93	0.044	0	0	0.061	0	0	0	0	0.28	0	0	0
94	0.077	0.088	0.027	0.22	0.427	0.277	0.205	0.011	0.83	1.405	0.111	0.027
95	0.066	0.15	0.133	0	0.955	0.872	3.55	5.55	18.16	11.55	1.52	3.38
96	0	0	0.011	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0.005	0
98	0.022	0.016	0	0	0	0	0	0	0	0	0	0



Bryozoans									
99	<i>Aetea sica</i>	0	0	0	0	0	0	0	
100	<i>Amathia pruvoti</i>	0	0	0	0	0	0	0	
101	<i>Chorizopora brongniartii</i>	0.055	0	0.011	0	0.97	0.044	0.19	0.022
102	<i>Collarina balzaci</i>	0	0	0.005	0	0	0	0	
103	<i>Diaperoecia</i> spp.	0	0	0	0	0.033	0	0.005	0
104	<i>Disporella hispida</i>	0.05	0.061	0.005	0	0.22	0.37	0.17	0.36
105	<i>Electra pilosa</i>	0.11	0.055	0.98	0.833	0	0	0.005	0
106	<i>Electra posidoniae</i>	63.6	63.3	21.1	48.88	1.09	15.16	0.09	0.48
107	<i>Fenestrulina joannae</i>	0	0	0.011	0	0	0.033	0.033	0
108	<i>Lichenophora</i> spp.	0	0	0	0	0	0	0	0
109	<i>Mimosella verticillata</i>	0.088	0.13	0.027	0.066	0.36	0.016	0	0
110	<i>Scruparia ambigua</i>	0	0.022	0.005	0.011	0.016	0.005	0	0
111	Unidentified Bryozoan	0	0.011	0.005	0.005	0.016	0.016	0	0.011
Ascidians									
112	<i>Botryllus schlosseri</i>	0.61	0.44	0	1.94	0	0	0	0
113	Unidentified Didemnidae	0	0	0.27	0	0	0	0	0.11
Kamptozoans									
114	Unidentified Kamptozoan	0	0	0	0	0	0	0	0



Seasonal response of *Posidonia oceanica* epiphytic assemblages to nutrient increase

Bryozoans												
99	0	0	0	0	0	0	0	0	0	0	0.016	0.011
100	0	0	0	0	0	0	0	0	0	0	0.016	0
101	0.005	0.055	0	0	0.061	0.005	0.13	0.066	0.22	0	0.1	0.038
102	0	0	0	0	0	0	0.016	0	0	0.011	0	0
103	0	0	0	0.022	0	0	0.016	0.016	0.061	0.011	0.011	0.038
104	0.066	0.29	0.033	0	0.083	0.038	0	0.044	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0.72	0.25	5.88	7.11	6.38	7.55	0.36	0.083
107	0.016	0.005	0	0	0	0	0	0	0	0	0	0
108	0.011	0.038	0	0	0	0	0	0	0	0	0.011	0.016
109	0.044	0.011	0	0	0.066	0.038	0.016	0.005	0	0.005	0.011	0
110	0	0	0	0	0	0	0	0.005	0.011	0.005	0	0
	0	0	0.011	0	0	0.011	0	0	0	0	0	0
Ascidians												
112	0	0	0	0	0	0	0.027	0	0.41	0.25	0	0
113	0	0	0	0	0	0	0	0	0	0	0.14	0
Kamptozoans												
114	0	0	0	0	0	0	0	0	0.12	0	0.11	0