Harpacticoid copepod response to epiphyte load variations in *Posidonia oceanica* (L.) Delile, meadows.

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Abstract

We conducted a field experiment to assess the response of phytal harpacticoids to nutrient-driven increases of epiphyte load in *Posidonia oceanica* meadows. First, we evaluated differences in species richness, diversity and assemblage structure of phytal harpacticoids in *P. oceanica* meadows with differing epiphyte loads. Second, we conducted a field experiment where epiphyte load was increased through an in-situ addition of nutrients to the water column and evaluated the responses of the harpacticoid assemblages. We predicted that there would be changes in the harpacticoid assemblages as a result of nutrient-driven increases of epiphyte load, and that these changes would be of a larger magnitude in meadows of low epiphyte load. Our results show that the harpacticoid fauna (>500 µm) present in *P. oceanica* meadows in the Bay of Palma comprised taxa which are considered phytal and other less abundant ones previously described as sediment dwellers or commensal on other invertebrate species. Nutrient addition had an overall significant effect on epiphyte biomass and on harpacticoid abundance, diversity and assemblage structure possibly as a response to the increased resources and habitat complexity provided by epiphytes. The abundance of dominant species at each location was favoured by nutrient addition and in some cases correlated with epiphytic biomass, though never strongly. This may indicate that structural complexity or diversity of the epiphytic cover might be more important than the actual epiphytic biomass for the harpacticoid species

investigated, more species-specific studies being necessary to ascertain this and clarify the relationships between harpacticoids and epiphytes in seagrass meadows. To our knowledge, this is the first account of harpacticoid species associated with *Posidonia oceanica* leaves and the epiphytic community they harbour in the Mediterranean Sea.

Keywords: *Posidonia oceanica*, eutrophication, epiphyte biomass, harpacticoid copepods, environmental monitoring.

Introduction

Degradation of coastal areas due to human-induced eutrophication is one of the main reasons causing seagrass decline worldwide (Burkholder et al., 2007; Waycott et al., 2009). Excessive nutrient inputs have been invoked as being responsible of seagrass die-back, mainly by stimulating the growth of drifting and epiphytic macroalgae (see Burkholder et al., 2007 and references therein) that limit seagrass access to light and nutrients and thus strongly reduce seagrass size and metabolism (Cornelisen and Thomas, 2004; Ruiz et al., 2001).

Increases in epiphytic algal biomass are often accompanied by an enhancement of faunal abundance, particularly grazers and other organisms which are favoured by the expansion of habitable space and resources (Lewis & Hollingworth, 1982; Johnson and Scheibling, 1987; Castejón, 2011). Invertebrate responses to epiphytic biomass increases are often species-specific (Jaschinski & Sommer, 2011), since nutrient enrichment frequently results in the proliferation of opportunistic green algae and cyanobacteria (Coleman and Burkholder, 1994; Lerodiaconou and Laurenson, 2002), which are less preferred items or non-palatable for some grazers. In turn, invertebrates and particularly mesograzers inhabiting these macrophytic assemblages play a fundamental role in structuring the algal communities (Jernakoff and Nielsen, 1997; Duffy & Hay, 2000; Duffy and Harvilicz, 2001), and regulating the interaction between seagrasses and their epiphytes (Fong et al., 2000). Invertebrates are also an essential link between primary producers and higher trophic levels such as macroinvertebrates and ichtyofauna (Stoner, 1979; Edgar and Shaw, 1995; Jenkins et al., 2011). Alterations to the balance of these key-players caused by disturbances such as eutrophication may result in significant impacts to the dynamics of seagrass systems. Hence, it is fundamental to understand the interactions of seagrasses, epiphytes and grazers and

examine eutrophication-driven changes of trophic pathways, since they might be of primary importance for the maintenance of community structure and functioning in particularly vulnerable ecosystems such as seagrass meadows (Neckles et al., 1994; Valentine & Duffy, 2006; Heck & Valentine, 2007; Hughes et al., 2009).

Crustaceans are in general very sensitive to organic pollution due to their limited anoxia tolerance which makes them good subjects for eutrophication monitoring (Blake and Duffy, 2010; Korpinen et al., 2010). Among them, harpacticoid copepods are often the most diverse and numerically dominant invertebrate group in phytal habitats (Hicks, 1985; Arroyo et al., 2004), and their importance as trophic link between primary and secondary producers in benthic environments is now undisputed (e.g. Sogard, 1984; Aarnio et al., 1996; Davenport et al., 2011; Jenkins et al., 2011). Harpacticoids respond readily to increases in habitat complexity (Jenkins et al., 2002, Arroyo et al., 2006), and organic matter content in the sediment (Gee and Warwick, 1985; Danovaro et al., 2002) and in general, increases in epiphytic biomass, whether seasonal or episodic, are paralleled by higher numbers and diversities of this taxon (Hall and Bell, 1993; Rutledge and Fleeger, 1993). Harpacticoids are generally very motile: phytal species can colonise seagrass blades at distances higher than 20m and reach ambient densities in 2-4 days (Bell & Hicks, 1991; Kurdziel & Bell, 1992), and their generation times can be as short as 10-18 days, a normal development time of 2-3 months being common for many species (Fleeger, 1979). A few families are morphologically adapted to live in the phytal, showing in general, larger sizes than their interstitial counterparts (see Hicks and Coull, 1983 for a review). In sediments, their spatial distribution is conditioned by the patchy distribution of diatoms (Decho & Castenholz 1986; Sandulli and Pinckney 1999). They adapt their grazing rates and abundance to increases in microphytobenthos (Montagna et al., 1995) controlling both microalgal biomass and their diel variations

(Pace and Carman, 1996; Buffan-Durbau and Carman, 2000). These characteristics, added to their aforementioned importance in benthic trophic webs, suggests that harpacticoids might also be useful markers of eutrophication-driven changes in seagrass habitats, since they not only respond to the habitat complexity created by larger epiphytic algae but will also show variations in relation with increased microbial biomass induced by eutrophication. Despite this, and the fact that harpacticoids have proved a sensitive tool in sediment pollution studies (e.g.: Gee and Warwick, 1985; Coull and Chandler, 1992), and coral reef eutrophication monitoring (Snelgrove & Lewis, 1989), their specific use to assess eutrophication effects in macrophyte communities has seldom been attempted (but see Fleeger et al., 2008).

In the Balearic Islands (NW Mediterranean), *Posidonia oceanica* L. Delile is the dominant seagrass. Biomass and structure of the epiphytic community in *P. oceanica* have been reported to change seasonally (Mazzella & Ott, 1984; Ballesteros, 1987), mainly in response to seasonality of seagrass vegetative development, but also to increased nutrient availability during summer (Prado et al., 2008; Castejón et al., 2012). The increase of epiphyte load has been found to negatively affect *P. oceanica* shoot size (Apostolaki et al., 2011; Castejón et al., 2012) and to enhance consumption by macro-herbivores (Alcoverro et al., 1997; Prado et al., 2007), though responses of the mesograzer community have only recently been assessed (Castejón, 2011). To date, there are no published accounts of harpacticoid assemblages associated with *P. oceanica* despite the fact that Novak (1982) found them to be the year-round dominant meiobenthic taxon on the leaves of *P.oceanica* in the Gulf of Naples, and they provided the highest contribution to meiofaunal production (ca. 50%) in a *P. oceanica* meadow in the Ligurian Sea (NW Mediterranean; Danovaro et al., 2002).

The aim of this study was to assess the response of phytal harpacticoids to epiphyte overgrowth in *Posidonia oceanica* meadows. First, we evaluated differences in species richness, diversity and assemblage structure of phytal harpacticoids in *P. oceanica* meadows with different epiphyte load. Second, we conducted a field experiment where epiphyte load was increased through the addition of nutrients to the water column in those same meadows and evaluated the responses of the harpacticoid assemblages. We predicted that there would be changes in the harpacticoid assemblages as a result of nutrient-driven increases of epiphyte load, and that these changes would be of a larger magnitude in meadows of low epiphyte load, where presumably, epiphyte load increases would be highest. To our knowledge, this is the first account of harpacticoid species associated with *Posidonia oceanica* leaves and the epiphytic community they harbour in the Mediterranean Sea.

Material and Methods

The study was carried out in the Bay of Palma (Mallorca, Western Mediterranean), during summer (August – September), 2008. Four localities, two with high and two with low epiphytic load (g dry weight (DW) of epiphytes per g dry weight (DW) of leaves in a *P. oceanica* shoot; see Castejón, 2011, for details) were selected as sampling and experimental sites. Depth of the localities ranged between 5 and 6 m. The two localities with high epiphytic load (Cala Nova and Cala Estancia) were located at the innermost part of the Bay, while the two localities showing lower epiphytic loads (Cala Viñas and Enderrocat) were located closer to the mouth of the Bay, on either side of it (Figure 1).

In August 2008, six 1 m^2 plots were randomly established at each of the four localities, using galvanized iron bars fixed at each corner (Figure 1). Plots were

approximately 10 m apart from each other at all locations. Three plots received nutrient addition in the water column, while the other three served as control for the fertilization factor. A slow-release fertilizer (Osmocote ^{TM} N:P:K, 15:9:9 + 3MgO + trace elements) was employed as a source of nutrients (Heck et al., 2000; Prado et al., 2008), filling a 250 ml plastic diffuser which was placed 40 cm above the sediment, tied to one of the frames defining the plots, at the corner of each fertilized plot. The fertilizers were left for 42 days. Prior to the set-up of the experiment, to obtain an estimate of shoot density at each of the localities and initial samples of the faunal population associated to *P. oceanica* leaves, we randomly defined three 40 x 40 cm plots in the same areas where the experiments were later set up (i.e.: at all four locations, marked with a G.P.S.), counted the number of *P. oceanica* shoots present in each of them, and collected faunal samples using a suction sampling device with a 40 x 40 cm opening mouth and a collector bag made of 200µm mesh (see Buia et al., 2003 for a description of the device). This sampler allows the fauna of *P. oceanica* (fundamentally the leaves) to be aspirated, while not damaging the plants themselves. It is easily and quickly deployed over the selected sampling area and all fauna are directly sucked into a 200 µm mesh bag, minimizing the escape of vagile fauna. Once in the laboratory, samples were sieved with a 500 µm mesh and fixed in 4% buffered formalin to preserve them until processing. We used a 500 µm mesh because the study was initially focused on macrofauna. We decided to analyze the harpacticoid fauna in detail, given the high amount found in all samples. The high amount of large specimens collected, indicated that at least this fraction of the harpacticoids associated with *P. oceanica* was well represented. Finally, the above mentioned reasons of adequacy of this taxon as indicator of organic enrichment justified an attempt to explore their response to increases in epiphyte load.

Forty-two days after nutrient addition, samples from the fertilized and non-fertilized plots were gathered. Five shoots of *P. oceanica* were collected, placed in an individual plastic bag and carried to the laboratory, where they were stored frozen at -20ºC until processing. Epiphytes in all the leaves of each shoot were scraped off using a razor blade and collected in preweighed Whatman GF/C glass fibre filters. Filters were dried (60ºC, 48 h) to determine epiphyte dry weight (g DW). Seagrass leaves were dried (60ºC, 48 h) to quantify the leaf biomass (g DW) of each shoot. The epiphyte load of each *P. oceanica* shoot was expressed as epiphyte biomass per leaf biomass (g DW epiphyte g DW leaf^{-1}). Samples of the epifaunal community (one 40 x 40 cm sample per plot) were collected as during the August sampling, at each of the fertilized and non-fertilized plots, and processed in the laboratory as above. Invertebrates from all samples were sorted in the laboratory using a dissecting microscope, and all copepods further identified using a compound microscope.

Statistical analyses

Spatial and temporal variation in harpacticoid assemblage structure

We first wanted to investigate whether there would be changes in harpacticoid assemblage structure depending on the level of epiphyte load (high, low) present at each locality and whether there would be differences between the assemblages found in August, and September that would illustrate the natural temporal change occurring at each of the locations. We did this by running a Permanova analysis (Anderson, 2005), using three fixed factors: epiphyte load (H=high; L=low), locality, nested in epiphyte load (H: CE = Cala Estancia, CN = Cala Nova; L: CV = Cala Viñas and E = Enderrocat), and sampling date (A = August, S = September), and constructing a triangular matrix on square-root-transformed data using Bray-Curtis similarities. The

analysis was run conducting an unrestricted permutation of the raw data, without replacing distances with their ranks, and using 4999 permutations.

We then examined variations in diversity of the harpacticoid assemblage between localities and sampling dates by calculating univariate measures of harpacticoid copepod fauna (i.e.: Number of individuals (N), number of species (S), Margalef´s diversity (d), Shannon-Wiener diversity (H') and Pielou´s evenness (J')), and conducting a three way ANOVA with epiphyte level, locality (nested in epiphyte level), and sampling date as factors.

Changes following nutrient addition

Following the previous analysis, we wanted to know if the addition of nutrients into the water column would cause changes in the epiphyte load and in the harpacticoid assemblages found at each locality and if these changes would be different depending on whether these locations had originally high or low epiphyte loads. To do so, we conducted another Permanova test, this time using the factors epiphyte load and locality (nested in epiphyte load), as above, and nutrient addition (C = non-fertilized, F = fertilized), and running the test under the same premises as before.

Permutational tests of multivariate dispersion (PERMDISP, Anderson, 2004), were used to check the homogeneity in the average dissimilarities of samples from the central location point, whenever results from Permanovas were significant.

Variations in epiphyte biomass in the plots (g DWof epiphytes per plot – 40x40 cm -) and in the abundance of the total, and dominant harpacticoid species (number of individuals per plot – 40x40 cm) with nutrient addition at each locality were

investigated by means of a three-way ANOVA with the same factors as above. Epiphyte biomass per plot was calculated as the mean epiphyte biomass (g DW of epiphytes) per shoot in each plot and multiplied by the mean number of shoots per plot counted in each locality during the August sampling.

To investigate whether nutrient addition and variations in epiphytic load had any bearing in diversity of the harpacticoid assemblage, we conducted a three-way ANOVA on the same diversity indexes used above, comparing their variation between fertilized and non-fertilized plots at all locations. Factors were again epiphyte load, location (nested in epiphyte load), and nutrient addition. Given the sensitivity of all these indexes to sample size, we also compared diversity under the different treatments at each location using k-dominance curves (Lambshead et al., 1983).

In all cases involving an ANOVA, normality and homoscedasticity of the data were checked with the Shapiro-Wilkins and Cochran tests, respectively and data were log transformed in those cases in which these assumptions were not met. Pair-wise differences between samples were investigated by means of Tukey´s HSD test.

The species responsible for major differences among localities were identified by means of a SIMPER analysis, which was performed on the original data matrix after square-root transforming the data using Primer 6.0 (Plymouth Marine Laboratory Inc.). In all occasions in which it was used, the square – root transformation was chosen to down-weight the importance of highly abundant species, hence taking both common and rare species into account when comparing treatments.

Relationship between epiphytic load and harpacticoid abundance and diversity

Finally, to investigate whether variations in total harpacticoid number, abundance of the predominant species, diversity and species richness could be linked to variations in epiphyte biomass in the plots, we carried out a series of correlation analyses between these variables. Since we expected the relationship between harpacticoid abundance and epiphyte biomass to be monotonic but not necessarily linear, we conducted Spearman rank correlations between epiphyte biomass per plot and the total abundance of harpacticoids and that of the predominant species, per plot.

All univariate analyses were done using STATISTICA 7.0 StatSoft, Inc.

Results

The harpacticoid fauna (>500 µm) present in *P. oceanica* meadows in the bay of Palma comprised taxa which are considered phytal and other less abundant ones which have been previously described as sediment dwellers or commensal on other invertebrate species (Table 1). Harpacticoids (48.52%) dominated the copepod assemblage together with Calanoids (49.57%), though it is likely that the latter were present in the water column and inadvertently sampled. Calanoids were only very abundant at Enderrocat, harpacticoids predominating at all other locations (Table 1). Cyclopoids and Siphonostomatoids were also present, but in much lower numbers (Table 1).

Among harpacticoids, the predominant species were *Porcellidium tenuicauda* Claus 1860, *Eudactylopus latipes* (Scott, T. 1893), *Metamphiascopsis hirsutus* (Thomson & A. Scott, 1903) *and Eupelte gracilis* Claus, 1860, which together accounted for about 78% of the harpacticoid assemblage associated with *P. oceanica* at the 4 locations under study (Table 1). In all locations, *Porcellidium tenuicauda* was the most abundant harpacticoid species associated with *P. oceanica.*

Spatial and temporal variation

The Permanova detected significant differences in the harpacticoid assemblage structure between localities with High and Low epiphyte loads (Table 2), but also between localities with the same epiphyte load level (Pair-wise comparisons, Table 2). This analysis also detected differences between sampling dates but no effects of the interaction between factors (Table 2). No differences in dispersion of the samples were detected for any of the factors (Permdisp, $p>0,05$).

The three-way ANOVA indicated significant differences between sampling dates for the overall abundance of harpacticoids, which were more abundant in September than in August, but not for any of the other diversity indexes. However, there was a significant interaction between locality and date, for Shannon´s diversity, and while at Cala Estancia and Enderrocat diversity increased from August to September, the trend was reversed in Cala Nova and Cala Viñas, where the values of this index were lower in September (Table 3, Figure 2). Only H'(loge) was significantly different between epiphyte loads, being higher at those localities with high epiphyte load (Table 3, Figure 2). On the other hand, the ANOVA showed significant differences between localities for Margalef´s and Shannon´s diversity. Both indexes were significantly higher at Cala Estancia than Enderrocat according to Tukey´s HSD comparisons (Figure 2).

As regards the predominant harpacticoid species, only *Porcellidium tenuicauda* and *Metamphiascopsis hirsutus* showed significant differences between sampling dates, both being more abundant in September than in August (Figure 4, Table 3). *M. hirsutus* was also significantly more abundant at Cala Viñas than any of the other locations, while *Eudactylopus latipes* was significantly more abundant at Cala Estancia (Figure 4,

Table 3). The latter species was significantly more abundant at high epiphyte load levels than at locations with a low original epiphyte cover (Figure 4, Table 3).

Changes following nutrient addition

In this case, the Permanova showed significant differences in harpacticoid assemblage structure between epiphyte load levels, localities and between plots in which nutrients were added and non-fertilized ones (Table 4), but no interactions between any of the factors were significant, indicating that all localities responded in the same way to fertilization. Again, pair-wise comparisons between localities nested in each epiphyte load level also indicated significant differences between them, signifying an overall difference between localities, beyond variations in the original epiphyte load present in them (Table 4). Once again, no differences in dispersion of the samples were detected for any of the factors (Permdisp, $p>0,05$).

Results from the SIMPER analysis conducted to identify which species accounted more for these variations between localities are shown in Table 5. In general, the dominant species showed variations between locations, and these accounted for major variations between them: *Metamphiascopsis hirsutus* was much more abundant in Cala Viñas than in the other locations, *Porcellidium tenuicauda* was more abundant in Cala Nova and Enderrocat and *Eudactylopus latipes* was more abundant in Cala Estancia, while it was absent in Enderrocat.

Results from the three-way ANOVA indicated significant differences in epiphyte load between those localities assigned to high and low epiphyte load levels, as expected, and

also between fertilized and non-fertilized plots (Table 6). Total harpacticoid abundance and that of *E. latipes* and *E. gracilis*, also showed a significant interaction effect between locality and fertilization level (Table 6, Figures 2, 4). However, only Cala Nova showed significant higher numbers of harpacticoids between fertilized and unfertilized plots in pair-wise comparisons (Figure 2). Of the predominating species, only *E. gracilis* showed a significantly higher abundance after fertilization in Cala Nova, in Tukey´s pair-wise comparisons. Total harpacticoid abundance was also significantly affected by fertilization, copepod numbers being higher, in general, in fertilized plots than in unfertilized ones (Table 6, Figure 2). Locality played an important role in the abundance of the various predominant species (Table 6). For example, *Eudactylopus latipes* was not found in Enderrocat at all, while it was quite abundant at all other sites. *Metamphiascopsis hirsutus* was significantly more abundant at Cala Viñas than all other locations, and *Porcellidium tenuicauda* was significantly more abundant at Cala Nova and Enderrocat than at Cala Estancia (Figure 4, Table 6). *E. latipes* showed the same trend as epiphytic biomass, being more abundant in high epiphytic load localities than in those with low epiphytic load, in fertilized than in non-fertilized plots, and showing variations in its abundance trends depending on which locality was examined (i.e.: a decrease in fertilized plots in Cala Estancia, but an increase in Cala Viñas and Cala Nova, though only the latter was significant in Tukey post-hoc comparisons).

As regards diversity measures, species richness showed a significant effect of nutrient addition, species number increasing in fertilized plots (Table 6). Margalef´s diversity index, Pielou´s evenness and Shannon´s diversity also showed significant variations between localities with low and high epiphyte loads, and among localities nested in

these epiphyte loads: Cala Estancia was significantly different from all others in the case of Margalef´s and Shannon´s indices and from Cala Nova and Enderrocat for Pielou´s evenness (Table 6, Figure 2). No interaction between factors was detected for these variables.

The k-dominance curves (Figure 5), showed different patterns for the various study sites. While in Cala Estancia the most diverse assemblages were the September ones, compared to the initial plots sampled in August, comparisons between the two former treatments was not possible due to the fact that their curves intersected. This would also compromise interpretation of the Shannon´s diversity and Pielou´s evenness results (Lambshead et al., 1983), provided differences between fertilized and non-fertilized plots would have been detected. In Cala Nova, the curves corresponding to initial and fertilized plots were superimposed, and suggested a higher diversity of these assemblages than those belonging to non-fertilized September plots. The former two curves followed a sigma shape which is typical of undisturbed sites, while the curve corresponding to non-fertilized plots was typical of assemblages dominated by very few species, as was the case in Cala Viñas for both fertilized and non-fertilized plots (September). Here, more diverse assemblages were found in initial plots (August). Finally, the situation was again different in Enderrocat, where fertilized plots were the most diverse, followed by unfertilized controls and initial plots, which followed almost the same trend.

Relationship between epiphyte load and harpacticoid abundance and diversity

Only the abundances of *E. latipes* and *M. hirsutus* showed a significant correlation with epiphyte biomass (Figure 6), though correlation values were not very high. Neither total harpacticoid abundance nor that of *E. gracilis* or *P. tenuicauda* were significantly

correlated with epiphyte biomass (SR correlations, p>0,05). As for diversity measures, only the number of species (S) was significantly correlated with epiphyte biomass, all other indexes showing no significant relationship with this variable (SR correlations, p>0,05).

Discussion

Nutrient enrichment in our study was followed by an increase in harpacticoid species richness and a rapid proliferation of the dominant species at each locality. This caused variations in diversity to be more subtle, due to reduced evenness in fertilized locations, which masked the increase in species number following fertilization and increased epiphyte loads. This seems to be partly in accordance with ecological theory, which predicts that under conditions of rapid population growth (i.e.: increased resources), dominant species will predominate more rapidly than when population growth rates of all species are lower (i.e.: under reduced resources) (Huston, 1979), and has been previously shown for phytal harpacticoids (Hicks, 1980). Moreover, the effect of epiphyte load and nutrient addition on harpacticoid abundance, species richness and diversity, varied among locations, the initial level of epiphyte load present in the *Posidonia* blades, having a bearing on harpacticoid response.

Eutrophication is supposed to cause an initial increase in diversity (or when nutrient enrichment is kept at moderate levels) but a long-term loss of species and colonization by opportunistic fast growing species (Isaksson and Pihl, 1992; Norkko and Bonsdorff, 1996; Raffaelli et al., 1998; Tagliapietra et al., 1998). The duration of our experiment precluded the identification of the latter processes since we examined variation between plots one month after nutrient addition. Despite this, changes in assemblage structure as a result of fertilization could already be discernible, probably

due to the aforementioned rise of the predominant species, but also to new colonizers and the proliferation of opportunistic species such as *Tisbe* spp. Tisbids are common in a wide variety of organically enriched environments (Fava and Volkmann, 1975; Hicks, 1980), and showed higher abundances in fertilized plots with respect to control ones in our study (Table 1). The addition of species was particularly evident in Enderrocat, the locality with low initial epiphyte load and the lowest initial number of species (5), which were more than doubled (up to 15 species in fertilized samples *versus* 7 in control ones) with nutrient addition. Here, species such as *Ambunguipes rufocinta*, *Phyllothalestris mysis*, *Peltidium robustum* or *Dactylopusia tisboides*, which are also normally associated with phytal habitats, appeared only after fertilization.

Conversely, nutrient enrichment in Cala Estancia did not cause an increase in epiphyte load nor a response from the harpacticoid assemblage. Cala Estancia had, originally, the most diverse harpacticoid assemblage, the highest epiphyte load, the smallest *Posidonia* leaves and the most sparsely distributed shoots (Castejón, 2011). Abundances of all other invertebrate taxa on unfertilized plots were also highest here, and they also showed a decreasing trend with fertilization (Castejón, 2011). Cala Estancia is at the innermost part of the bay and probably receives the steadiest nutrient input from anthropogenic sources, representing a saturated stage where an increase in nutrients would not trigger any further epiphyte growth or grazer response (Edgar, 1993; Edgar and Aoki, 1993). Higher turbidity levels or increased sedimentation rates at this site, could be posing a stronger pressure on the *Posidonia* (explaining its reduced shoot sizes and densities), the epiphytes and the harpacticoid assemblage than that exerted by nutrient levels alone.

The general higher abundances of harpacticoids observed in fertilized plots in our study could be explained by an increased colonization from adjacent patches or by

the proliferation of the populations already “inhabiting” them. Generation times of harpacticoids in phytal habitats have been found to be around 1 month, and may be reduced under fertilization conditions (Hall & Bell, 1993; Song et al., 2010), their populations showing a younger age structure and a higher percentage of ovigerous females (Fleeger et al., 2008). In fact, we found an increased representation of copepodites of *Eudactylopus latipes* and *Metamphiascopsis hirsutus* in fertilized plots in Cala Nova and Cala Viñas, respectively, which could indicate an increase in the population occurring concomitantly with the colonization from the surrounding meadow. Increases in copepodid stages of other species (unidentified thalestrid copepodites appeared also in some fertilized plots) could have been overlooked due to the mesh size used in the laboratory (500 μm), through which many of these smaller individuals, together with the nauplii, may have passed. Ovigerous females of the four dominant species were not counted, but could be observed in all treatments.

The species distribution found in our study need not reflect annual dominance patterns, since our sampling and experimental times were confined to the summer months, which coincide with the period of maximum epiphyte load (i.e.: maximum abundance of resources). We did not analyze the specific composition of the epiphytic assemblage, but changes in epiphytic assemblages associated with *P. oceanica* due to nutrient enrichment, have been reported elsewhere (Prado et al., 2008; Balata et al., 2010). In this sense, similar processes could have enhanced harpacticoid species dominance linked to particular (increasing) epiphyte species in our study sites. Indeed, nutrient enrichment is supposed to favour mainly encrusting corallines and filamentous forms (Prado et al., 2008; Balata et al., 2010), which seem to be also the type of algae mainly triggering harpacticoid responses to variations in epiphytic cover (Hall & Bell, 1993; Jarvis and Seed, 1996) though this reactions are often species-specific. Many

phytal species have been found associated with red algae (Lang, 1948), and particularly *Eupelte gracilis* was found amidst coralline species in the Mediterranean (Monard, 1928). In our experiment, *E. latipes* seemed to respond more acutely to variations in epiphyte biomass showing a significant rise in fertilized plots, particularly at locations where it was not abundant prior to fertilization. This species has been found in tidal pools (Lang, 1965; Tanaka and Hue, 1966) were ephemeral opportunistic algae abound, together with *M. hirsutus* (Tanaka and Hue, 1966), which was also previously described from seagrass habitats (Lang, 1948). It could be that these two are opportunistic species that were abundant in our assemblages only because of the proliferation of epiphytes during our study time. As a matter of fact, they were the only two species correlated with epiphytic biomass. On the other hand, *P. tenuicauda* and *E. gracilis* showed no correlation with epiphyte biomass, despite being more abundant in fertilized plots in Cala Nova, where nutrient-driven epiphyte increases were stronger. *Porcellidium tenuicauda*, was the dominant harpacticoid in our study, and is typically associated with flat laminar algae (Lang, 1948; Huys et al., 1996), so it could be that its association was more with the *P. oceanica* blades than with the macroalgal epiphytes. Its increase, as well as that of *E. gracilis* could be related to increases in diatoms and microbes associated with the *P. oceanica* leaves, which would also increase with fertilization. This suggests that qualitative aspects of the epiphytes might be more important than quantitative ones when explaining harpacticoid abundance and diversity patterns found on enriched plots. Often algal morphology (as surface area or fractal dimension) has been invoked as a better indicator of habitat provision than its biomass (or volume), especially for smaller individuals as those comprising the meiofauna (Gee & Warwick, 1994). Algae with differing morphologies provide gradients of habitat complexity which in turn offer varying degrees of protection, sediment retention, food provision in

the form of diatom and bacteria accumulation etc. to the various harpacticoid taxa inhabiting them (Hicks, 1977a; Hicks, 1980), and accumulations of particular taxa, as those registered here could respond to increases in specific algal species.

In conclusion, our results show that differing levels of epiphyte load have a bearing on harpacticoid assemblage structure, and that variations in epiphyte biomass induced by nutrient addition cause further changes in the abundance of the dominant species and on species distribution, depending also on the location under study. On the other hand, our results suggest that harpacticoid species response to epiphyte development due to nutrient addition may be more linked to changes in the composition of the various epiphytic species than to direct biomass changes in epiphytic load. Further studies are necessary to evaluate the specific response of these epiphyte-harpacticoid interactions, as well as the implications they may have for overall species diversity under eutrophication. Nonetheless, the rapid response to nutrient-driven changes in epiphyte biomass shown in our experiment, suggests that harpacticoids may well serve as indicator organisms in eutrophication-monitoring studies in macrophytic systems. On the other hand, the differing situations encountered at the various locations sampled in our study highlight the strength of spatial variation in seagrass dynamics and the importance of conducting correct spatial replication when attempting to explain patterns of disturbance-effected changes in vulnerable and impacted habitats.

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References

Aarnio, K., Bonsdorff, E., Rosenback, N., 1996. Food and feeding habits of juvenile flounder, *Platichthys flesus* (L.), and turbot, *Scophthalmus maximus* L., in the Aaland archipelago, northern Baltic Sea. *Journal of Sea Research*, **36**, 311–320.

Alcoverro T., Duarte, C.M., Romero, J. (1997) The influence of herbivores on *Posidonia oceanica* epiphytes. *Aquatic Botany*, **56**, 93-104.

Anderson, M.J. 2004. PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland, New Zealand.

Anderson, M.J. 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand.

Apostolaki E. T., Holmer, M., Marbà, N., Karakassis, I. (2011). Epiphyte dynamics and carbon metabolism in a nutrient enriched Mediterranean seagrass (Posidonia oceanica) ecosystem. *Journal of Sea Research*, **66**, 135-142.

Arroyo N.L., Maldonado, M., Perez-Portela, R. & Benito, B., 2004. Distribution patterns of meiofauna associated with a sublittoral Laminaria bed in the Cantabrian Sea (northeastern Atlantic). *Marine Biology,* **144**, 231-242.

Arroyo N.L., Maldonado, M., Walters, K. (2006) Within- and between-plant distribution of harpacticoid copepods in a North-Atlantic bed of *Laminaria ochroleuca*. *Journal of the Marine Biological Association of the U.K.*, **86**, 309–316.

Arunachalam M., Nair N.B. (1988) Harpacticoid copepods associated with the seagrass *Halophila ovalis* in the Ashtamundi Estuary, south-west coast of India. *Hydrobiologia* **167/168**: 515-522.

Balata, D. Piazzi, L., Nesti, U., Bulleri, F., Bertocci, I. (2010) Effects of enhanced loads of nutrients on epiphytes on leaves and rhizomes of *Posidonia oceanica. Journal of Sea Research*, **63**, 173–179.

Ballesteros E. (1987) Estructura i dinamica del poblament algal de les fulles de *Posidonia oceanica* (L.) Delile als herbeis de Tossa de mar (Girona). Bulleti *de la Institucio* catalana d'Historia natural, **54**, 13–30.

Bell S.S., Hicks, G.R.F. (1991) Marine landscapes and faunal recruitment: a field test with seagrasses and copepods. *Marine Ecology Progress Series,* **73**, 61-68.

Blake R.E, Duffy, J.E. (2010) Grazer diversity affects resistance to multiple stressors in an experimental seagrass ecosystem. *Oikos* **119,** 1625–1635.

Buffan-Dubau E., Carman, K.R. (2000) Diel feeding behavior of meiofauna and their relationships with microalgal resources. *Limnology and Oceanography*, **45**, 381–395.

Buia M.C., Gambi, M.C., Dappiano, M. (2003) I sistemi a fanerogame marine. In: Gambi M.C., Dappiano M. (Editors). Manuale di Metodologie di campionamento e studio del benthos marino mediterraneo. *Biologia Marina Mediterranea*, **19** (Suppl.), 145-198.

Burkholder J.M., Tomasko D.A., Touchette B.W. (2007) Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, **350**, 46-72.

Castejón, I. 2011. Grazing on the epiphytic community of *Posidonia oceanica* (L.) Delile: An assessment of its relevance as a buffering process of eutrophication effects. PhD Thesis. Universidad de las Islas Baleares, Spain. 165pp.

Castejón-Silvo I., Terrados, J., Domínguez, M., Morales-Nin, B. 2012. Epiphyte response to in situ manipulation of nutrient availability and fish presence in a *Posidonia oceanica* (L.) Delile meadow, *Hydrobiologia*, DOI 10.1007/s10750-012-1190-1

Cornelisen, C.D., Thomas, F.I.M. (2004) Ammonium and nitrate uptake by leaves of the seagrass *Thalassia testudinum*: impact of hydrodynamic regime and epiphyte cover on uptake rates. *Journal of Marine Systems*, **49**, 177–194.

Coull B.C., Chandler, G.T. (1992) Pollution and meiofauna. Field, laboratory and mesocosm studies. *Oceanography and marine biology*, **30**, 191-271.

Danovaro R., Gambi C., Mirto S. (2002) Meiofaunal production and energy transfer efficiency in a seagrass *Posidonia oceanica* bed in the Western Mediterranean. *Marine Ecology Progress Series*, 234, 95-104.

Davenport J., Ezgeta-Bali, D., Peharda, M., Skeji, S., Nincevi c-Gladan, Z., Matijevi, S. (2011) Size-differential feeding in Pinna nobilis L. (Mollusca: Bivalvia): Exploitation of detritus, phytoplankton and zooplankton. *Estuarine Coastal and Shelf Science*, **92**, 246-254.

Decho A.W., Castenholz R.W. (1986) Spatial patterns and feeding of meiobenthic harpacticoid copepods in relation to resident microbial flora. *Hydrobiologia*, **131**, 87-96.

Duffy J.E., Hay, M. E. (2000) Strong impacts of grazing amphipods on the organization of a benthic community. *Ecological Monographs*, **70**, 237–263

Duffy J.E., Harvilicz, A.M. (2001) Species-specific impacts of grazing amphipods in an eelgrass-bed community. *Marine Ecology Progress Series*, **223,** 201-211.

Edgar G.J., Aoki M. (1993) Resource limitation and fish predation: their importance to mobile epifauna associated with Japanese *Sargassum*. *Oecologia*, **95**, 122-133.

Edgar G.J., Shaw, C. (1995) The production and trophic ecology of shallow-water fish assemblages in southern Australia. III. General relationships between sediments, seagrasses, invertebrates and fishes. *Journal of Experimental Marine Biology and Ecology*, **194**, 107-131.

Fleeger, J.W. (1979) Population dynamics of three estuarine meiobenthic harpacticoids (Copepoda) in South Carolina. *Marine Biology*, **52**, 147-156.

Fleeger, J.W., Johnson, D.S., Galván, K.A., Deegan, L.A. (2008) Top-down and bottom-up control of infauna varies across the saltmarsh landscape. *Journal of Experimental Marine Biology and Ecology*, **357**, 20-34.

Fong C.W., Lee, S.Y., Wu, R.S.S. (2000) The effects of epiphytic algae and their grazers on the intertidal seagrass *Zostera japonica. Aquatic Botany*, **67**, 251-261.

Hall M.O., Bell S.S. (1993) Meiofauna on the seagrass *Thalassia testudinum*: population characteristics of harpacticoid copepods and associations with algal epiphytes. *Marine Biology*, **116**, 137-146.

Heck K.L., Pennock J.R., Valentine J.F., Coen L.D., Sklenar S.A. (2000) Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnology and Oceanography*, **45**, 1041–1057

Heck K.L., Valentine J.F. (2007) The primacy of top-down effects in shallow benthic Ecosystems. *Estuaries and Coasts*, **30**, 371-381.

Hicks G.R.F. (1977a) Species associations and seasonal population densities of marine phytal harpacticoid copepods from Cook Strait. *New Zealand Journal of Marine and Freshwater Research*, **11**, 621–643.

Hicks, G.R.F. (1980) Structure of phytal harpacticoid copepod assemblages and the influence of habitat complexity and turbidity. *Journal of Experimental marine Biology and Ecology*, **44**, 157-192.

Hicks G.R.F. (1985) Meiofauna associated with rocky shore algae. In: Moore, P.G., Seed, R. (Eds.), The Ecology of Rocky Coasts. Hodder & Stoughton, London, pp. 36–56.

Hicks G.R.F., Coull, B.C., (1983) The ecology of marine meiobenthic harpacticoid copepods. Oceanography and Marine Biology Annual Review, **21**, 67–175.

Hughes, A.R., Williams, S.L., Duarte, C.M., Heck, K.L., Waycott, M. (2009) Associations of concern: declining seagrasses and threatened dependent species. Frontiers in Ecology and the Environment, **7 (5)**, 242-246.

Huston M. (1979) A general hypothesis of species diversity. *American Naturalist*, **113**, 81-101.

Isaksson I., Pihl, L.(1992) Structural changes in benthic macrovegetation and associated epibenthic faunal communities. *Netherlands Journal of Sea Research*, **30**, 131–140.

Jarvis S.C., Seed R. (1996) The meiofauna of *Ascophyllum nodosum* (L.) Le Jolis: characterization of the assemblages associated with two common epiphytes. *Journal of Experimental Marine Biology and Ecology*, **199**, 249-267.

Jaschinski S., Sommer U. (2011) How do nutrient conditions and species specific identity influence the impact of mesograzers in eelgrass-epiphyte systems? *Marine Biology*, **158**, 193-203.

Jenkins G.P., Walker-Smith G.K., Hamer P.A. (2002) Elements of habitat complexity that influence harpacticoid copepods associated with seagrass beds in a temperate bay. *Oecologia*, **131**: 598-605.

Jenkins G.P., Syme A., Macreadie P.I. (2011) Feeding ecology of King George whiting *Sillaginoides punctatus* (Perciformes) recruits in seagrass and unvegetated habitats. Does diet reflect habitat utilization? Journal of Fish Biology, **78**, 1561-1573.

Jernakoff, P. and Nielsen, J. (1997) The relative importance of amphipod and gastropod grazers in *Posidonia sinuosa* meadows. *Aquatic Botany*, **56**, 183–202.

Johnson S.C., Scheibling, R.E. (1987) Structure and dynamics of epifaunal assemblages on intertidal macroalgae *Ascophyllum nodosum* and *Fucus vesiculosus* in Nova Scotia, Canada. *Canadian Journal of Zoology*, **65**, 129-141.

Korpinen S., Jormalainen, V., Pettay, E. (2010) Nutrient availability modifies species abundance and community structure of Fucus-associated littoral benthic fauna. *Marine Environmental Research*, **70**, 283-292.

Kurdziel J.P., Bell, S.S. (1992) Emergence and dispersal of phytal-dwelling meiobenthic copepods. Journal of Experimental Marine Biology and Ecology, **163**, 43-64.

Lambshead P.J.D., Platt, H.M., Shaw, K.M. (1983) The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *Journal of Natural History*, **17**, 859-874.

Lang K. (1948) Monographie der Harpacticiden. 2 vols., pp. 1682. Lund: Hakan Ohlsson.

Lang K. (1965) Copepoda harpacticoidea from the Californian Pacific Coast. Kungl. Svenska Vetenskapsakademiens Handlingar, Fjärde Serien. Band 10. Nr 2. 560pp.

Lewis J.B., Hollingworth C.E. (1982) Leaf epifauna of the Seagrass *Thalassia testudinum*. *Marine Biology*, **71**, 41-49.

Mazzella L., Ott, J. (1984) Seasonal changes in some features of *Posidonia oceanica* (L.) Delile leaves and epiphytes at different depths. In: Boudouresque CF, de Grissac AJ, Olivier J (eds) Proceedings of the international workshop on *Posidonia oceanica* beds, Vol 1. GIS Posidonie Publ, Marseilles, p 119–127.

Monard, A. (1928) Les harpacticoides marins de Banyuls. *Arch. Zool. exp. gén*. **67**, 59.

Neckles H.A., Wetzel, R.L., Orth, R.J. (1993) Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina*) dynamics. *Oecologia*, **93**, 285-295.

Norkko A., Bonsdorff, E. (1996). Altered benthic prey availability due to episodic oxygen deficiency caused by drifting algal mats. *PSZN I: Marine Ecology*, **17**, 355–372.

Novak R. (1982) Spatial and seasonal distribution of the meiofauna in the seagrass *Posidonia oceanica*. *Netherlands Journal of Sea Research*, **16**: 380-388.

Pace M.C., Carman, K.R. (1996) Interspecific differences among meiobenthic copepods in the use of microalgal food sources. *Marine Ecology Progress Series*, **143**, 77–86.

Prado P., Alcoverro, T. Martínez-Crego, B., Vergés, A. Pérez-Llorens, J.L., Romero, J. (2007). Macrograzers strongly influence patterns of epiphytic assemblages in seagrass meadows. *Journal of Experimental marine Biology and Ecology*, **350**, 130-143.

Prado P., Alcoverro, T., Romero, J. (2008) Seasonal response of Posidonia oceanica epiphyte assemblages to nutrient increase. *Marine Ecology Progress Series*, **359**, 89-98.

Raffaelli D., Raven J.A., Poole, L.J. (1998) Ecological impact of green macroalgal blooms. *Oceanography and Marine Biology Annual Review*, **36,** 97–125.

Ruiz, J.M., Perez, M., Romero, J. (2001) Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. *Marine Pollution Bulletin*, **42**, 749–760.

Rutledge P. A., Fleeger, J.W. (1993) Abundance and seasonality of meiofauna, including harpacticoid copepod species associated with stems of the Salt-Marsh cord grass, *Spartina alterniflora. Estuaries*, **16 (4)**, 760-768.

Sandulli R., Pinckney, J. (1999) Patch sizes and spatial patterns of meiobenthic copepods and benthic microalgae in sandy sediments: a microscale approach. *Journal of Sea Research*, **41(3),** 179-187.

Snelgrove P.V.R., Lewis, J.B. (1989) Response of a coral-associated crustacean community to eutrophication. *Marine Biology*, **107**, 249-257.

Song S.J., Ryu J., Khim J.S., Kim W., Yun S.G. (2010) Seasonal variability of sommunity structure and breeding activity in marine phytal harpacticoid copepods on *Ulva pertusa* from Pohang, east coast of Korea. *Journal of Sea Research*, **63**, 1-10.

Stoner, A.W. (1979) Species-specific predation on amphipod crustacea by the pinfish *Lagodon rhomboides*: Mediation by macrophyte standing crop. *Marine Biology*, **55(3),** 201-207.

Tagliapietra D., Pavan, M. Wagner, C. (1998) Macrobenthic community changes related to eutrophication in Pallude della Rosa (Venetian Lagoon, Italy). *Estuarine Coastal and Shelf Science* **47**, 217–226.

Tanaka O., Hue, J.S. (1966) Preliminary report on the copepods found in the tide pool along the north-west coast of Kyushu. Proceedings of the Symposium on Crustacea. Part 1: 57-73. Symp. Ser. Mar. biol. Assoc. India No. 2 1965 [1966]

Valentine J.F., Duffy, J.E. (2006) The central role of grazing in Seagrass Ecology. In: A.W.D. Larkum et al., (eds.), Seagrasses: Biology, Ecology and Conservation, pp. 463-501. Springer. Netherlands.

Waycott M., Duarte, C.M., Carruthers, J.B. Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqueau, J.W., Heck Jr., K.L., Hughes, R. Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L. (2009) Accelerating loss of seagrasses across the globe threatens coastal systems. *PNAS*, **106(30)**, 12377-12381.

FIGURE CAPTIONS

Figure 1. Map of the Bay of Palma indicating the position of the four locations used in our experiment. Empty triangles indicate locations with a low initial epiphyte load, grey triangles indicate high initial epiphyte loads. The panel on the low right hand corner shows the disposition of experimental plots at each of the study sites. White squares indicate non-fertilized plots and black squares fertilized ones. Distance between plots was 10m.

Figure 2. Species richness (number of species per plot), abundance (number of individuals per plot) and diversity indexes (mean ± st. error) of harpacticoids at the four locations under study in August, initial (black bar), September non-fertilized (light grey bar), and September fertilized (dark grey). CE = Cala Estancia, CN = Cala Nova, CV = Cala Viñas, E = Enderrocat. H= high epiphyte load, L = low epiphyte load.

Figure 3. Epiphyte load (mean ± st. error) - upper panels – and epiphyte biomass – lower panels – of *Posidonia oceanica* in the 4 locations under study. Results of the preliminary survey performed in July (Castejón, 2011) when localities were assigned to High (striped bars) or Low epiphyte load (empty bars) are given in the left panels. Right panels present results of nutrient addition experiments in September (empty bars for non-fertilized plots and grey bars for those in which nutrients were added. CE = Cala Estancia, CN = Cala Nova, CV = Cala Viñas, E = Enderrocat. H= high epiphyte load, L = low epiphyte load.

Figure 4. Abundance (mean ± std. error) of the dominant harpacticoid species at the four locations under study in August (black bar), September non-fertilized (light grey bar), and September fertilized (dark grey). CE = Cala Estancia, CN = Cala Nova, CV = Cala Viñas, E = Enderrocat. H= high epiphyte load, L = low epiphyte load.

Figure 5. *K-dominance* cumulative curves based on harpacticoid copepod species abundances for the four locations under study in August, initial control (IC, white squares), September non-fertilized control (C, white traingles), and September fertilized (F, dark grey triangles).

Figure 6. Relationship between harpacticoid diversity and abundance and epiphyte biomass. Spearman rank correlations between the abundance of *M. hirsutus, E. latipes*, species number (S) and epiphyte biomass are indicated.

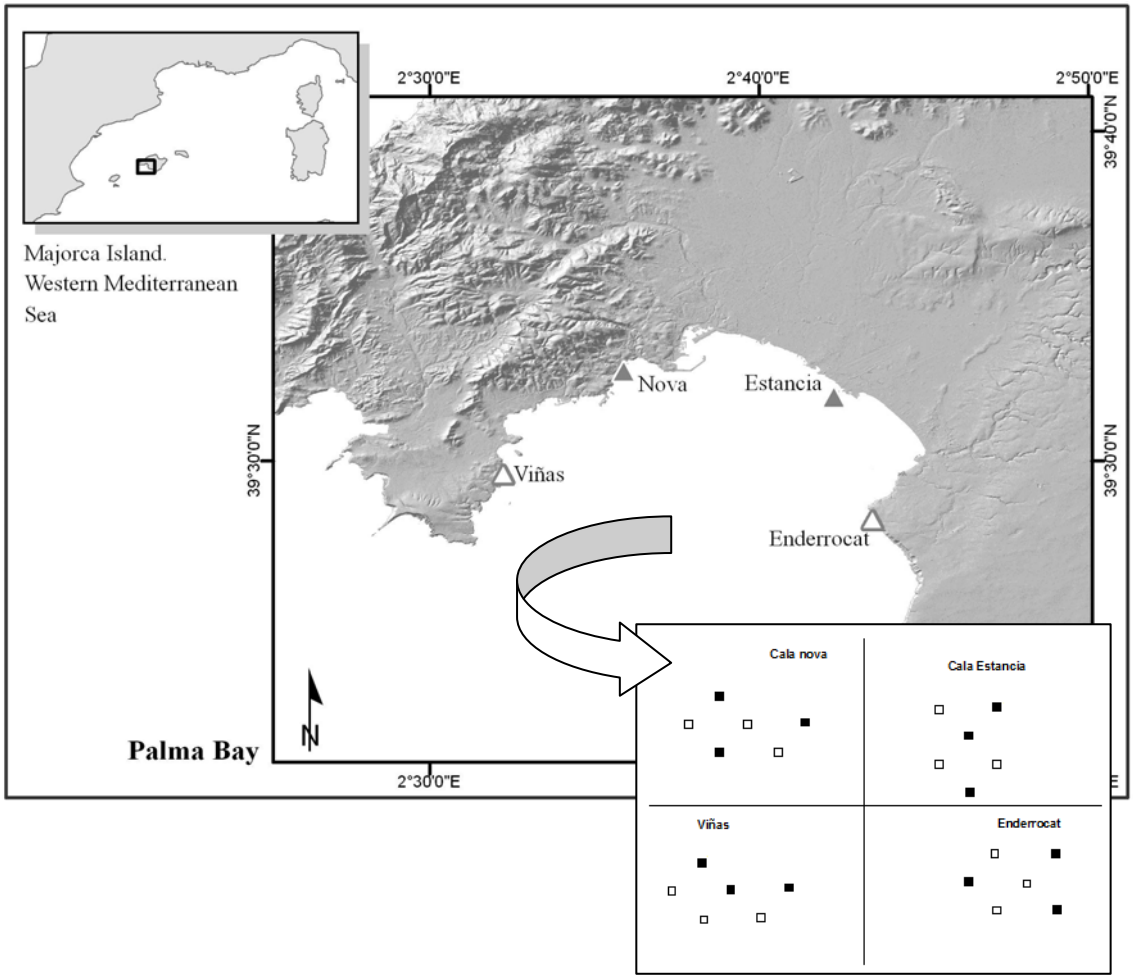
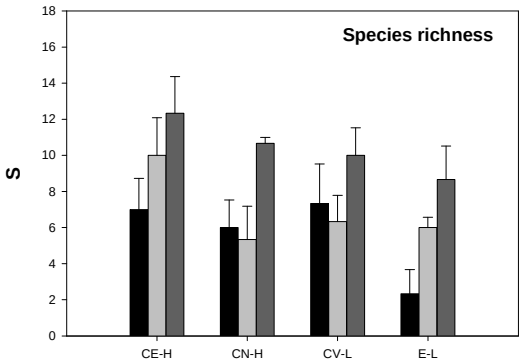
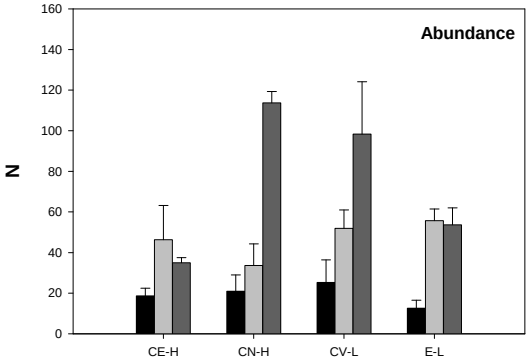
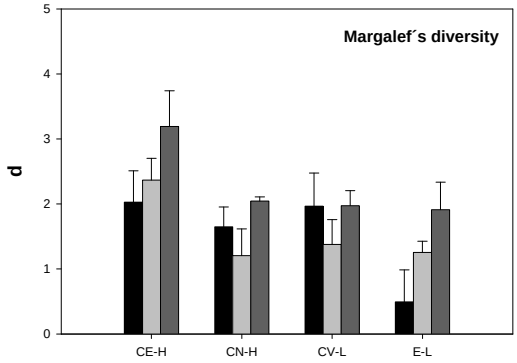
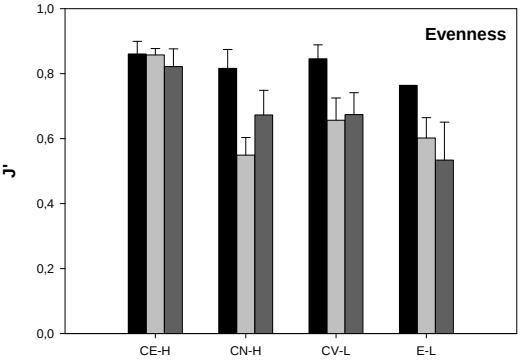

Majorca Island.
Western Mediterranean
Sea
2°30'0"E
2°40'0"E
2°50'0"E
39°40'0"N
39°30'0"N
Nova
Estancia
Viñas
Enderrocat
Palma Bay
N
Cala nova
Cala Estancia
Viñas
Enderrocat

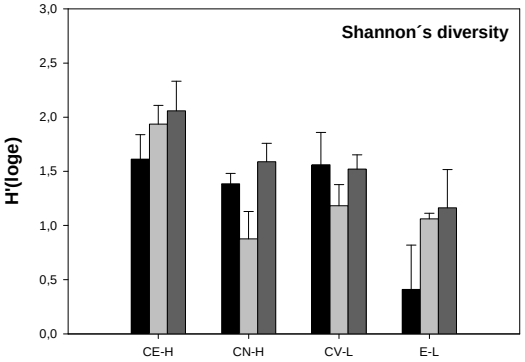
Figure 1

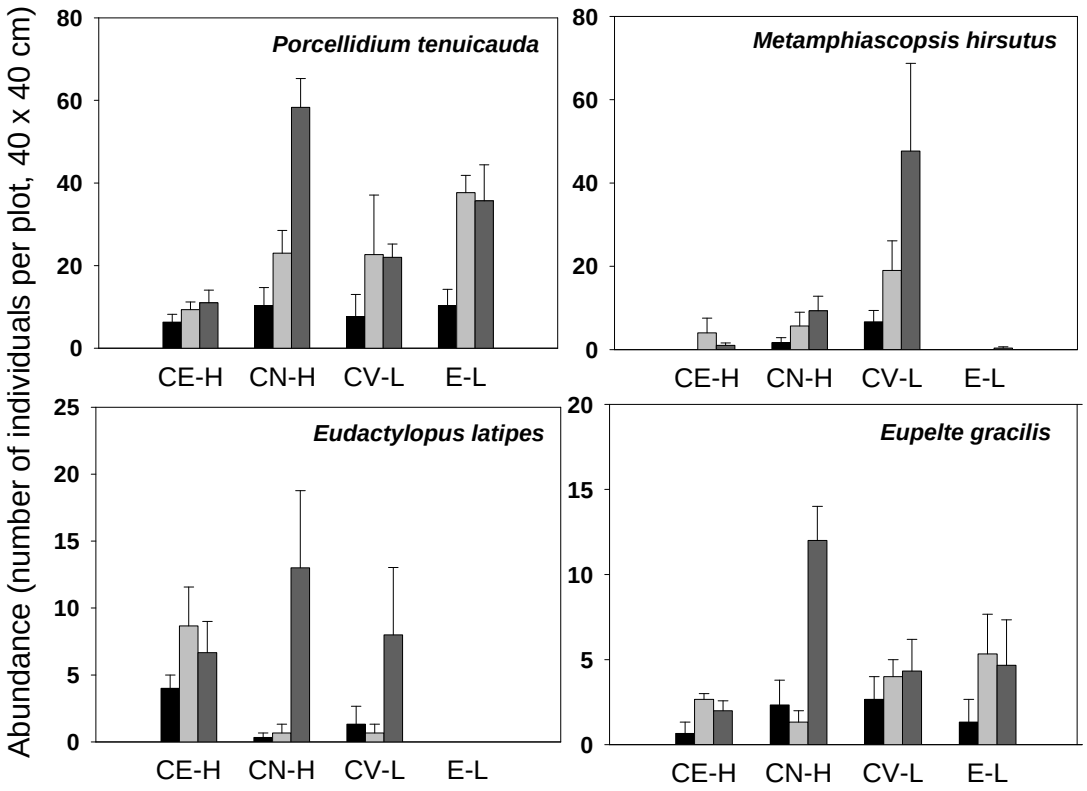

Species richness
S
0
2
4
6
8
10
12
14
16
18
CE-H
CN-H
CV-L
E-L

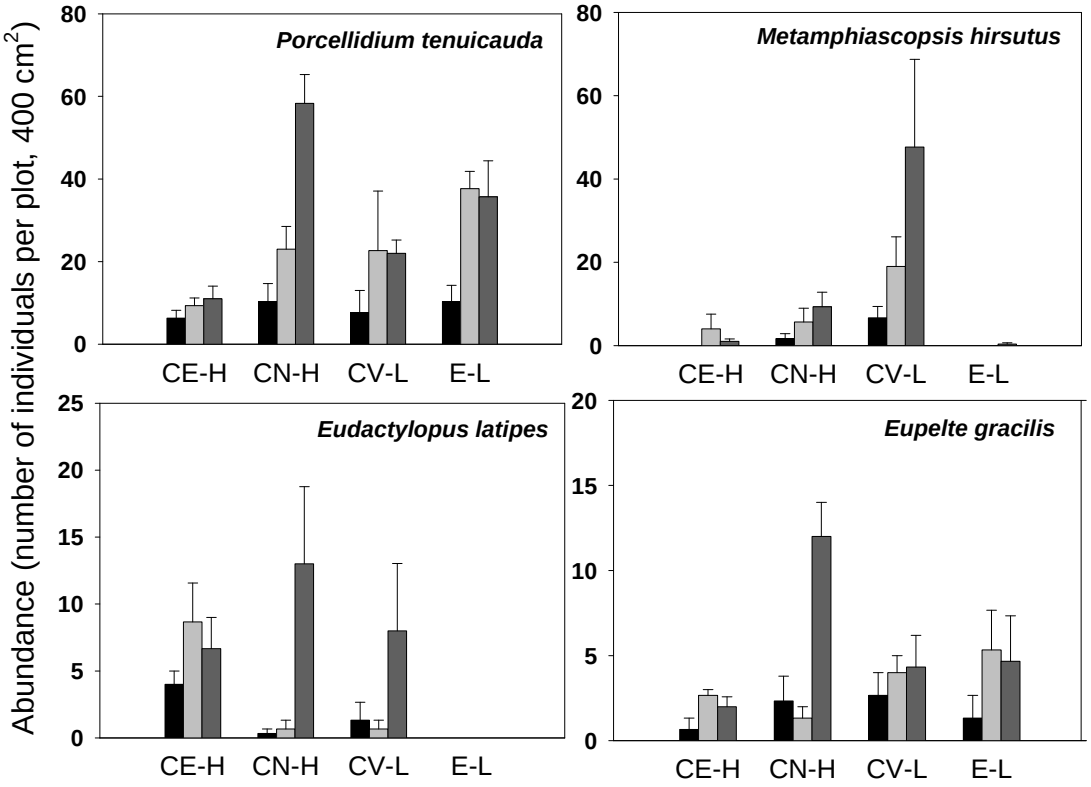

Abundance
N
0
20
40
60
80
100
120
140
160
CE-H
CN-H
CV-L
E-L

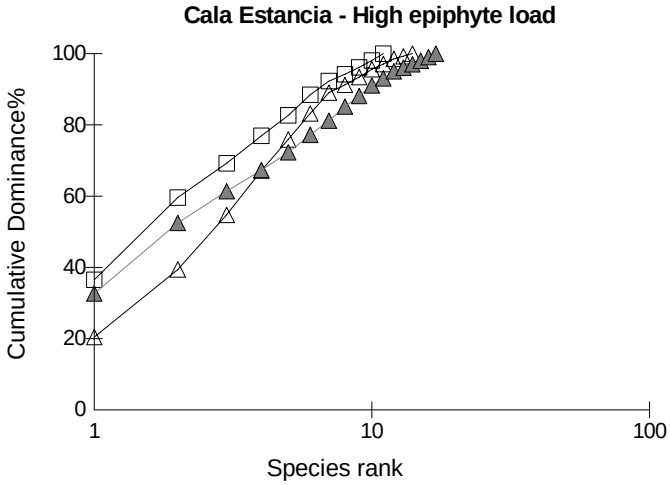

Margalef´s diversity
d
0
1
2
3
4
5
CE-H
CN-H
CV-L
E-L

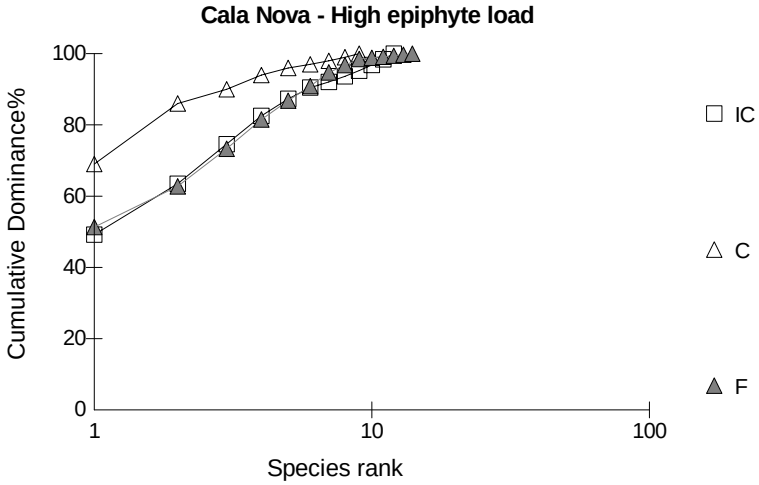

Evenness
J'
0,0
0,2
0,4
0,6
0,8
1,0
CE-H
CN-H
CV-L
E-L

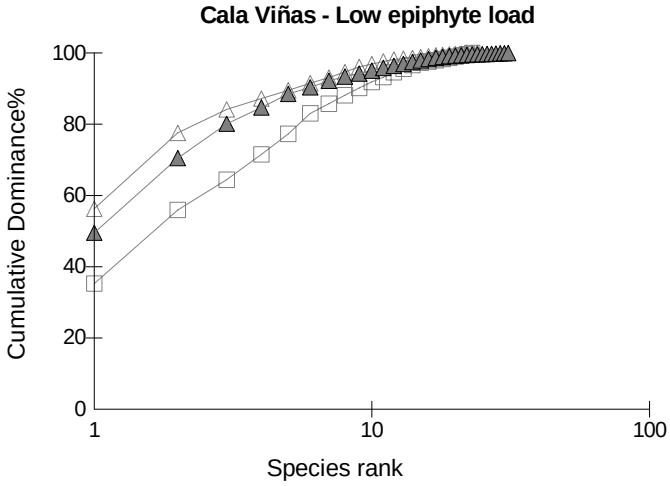

Shannon´s diversity
H'(loge)
0,0
0,5
1,0
1,5
2,0
2,5
3,0
CE-H
CN-H
CV-L
E-L

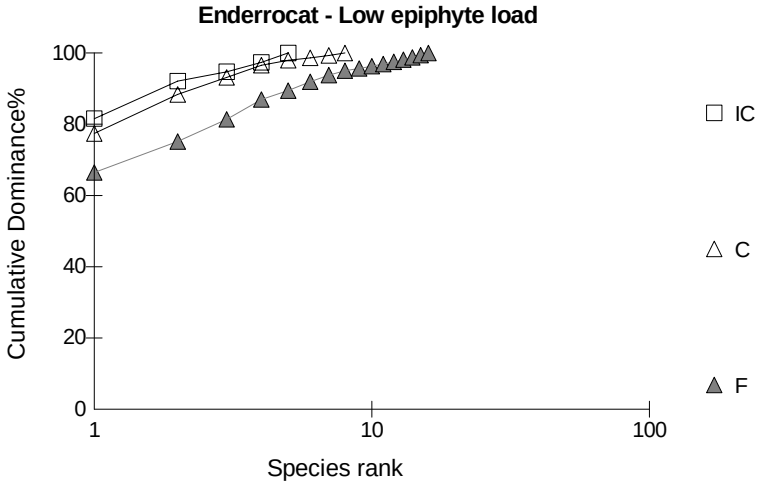

Abundance (number of individuals per plot, 40 x 40 cm)
Porcellidium tenuicauda
Metamphiascopsis hirsutus
Eudactylopus latipes
Eupelte gracilis
CE-H
CN-H
CV-L
E-L
0
20
40
60
80
5
10
15
25

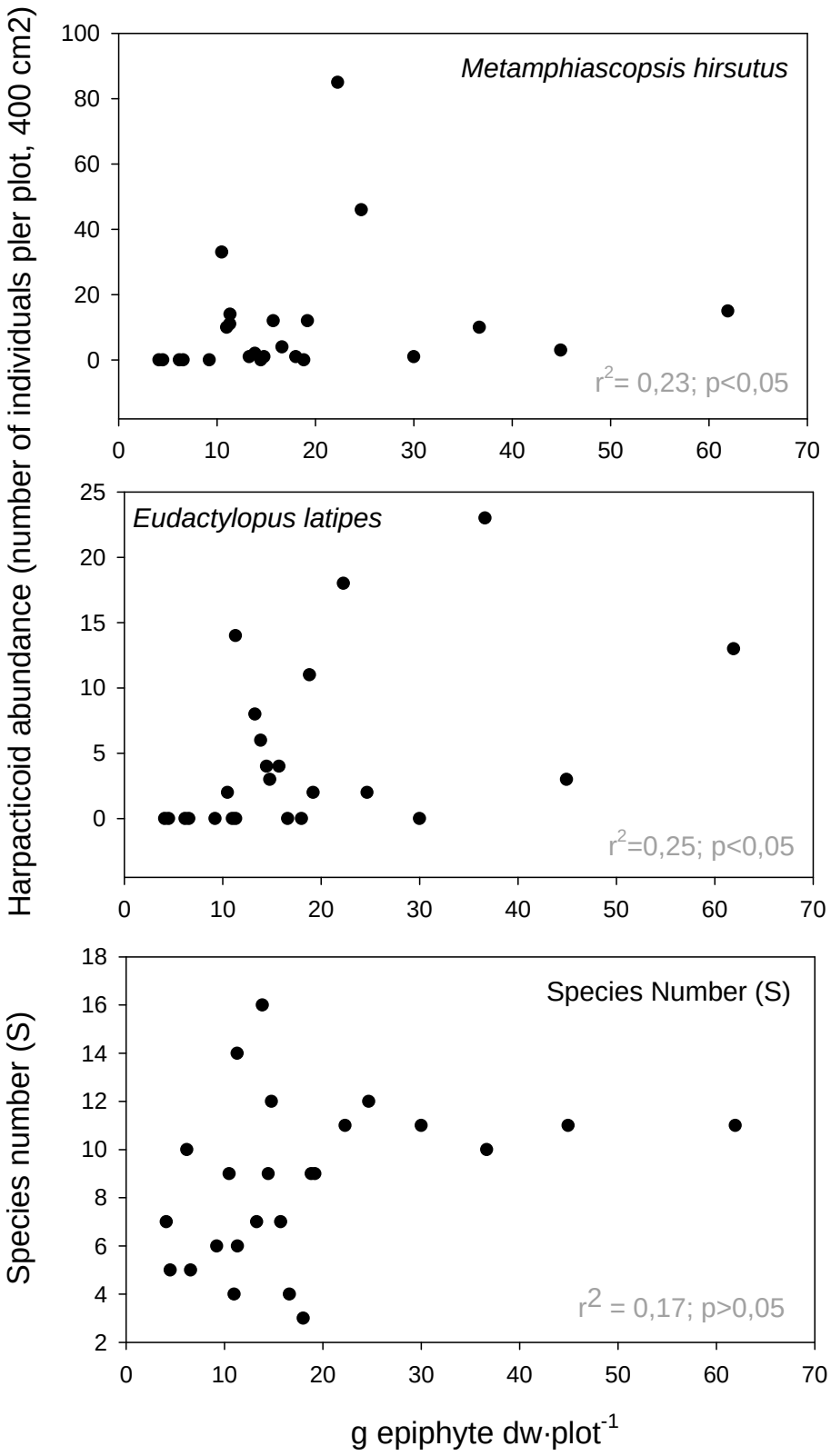

Abundance (number of individuals per plot, 400 cm2)
Porcellidium tenuicauda
Metamphiascopsis hirsutus
Eudactylopus latipes
Eupelte gracilis
CE-H
CN-H
CV-L
E-L
0
20
40
60
80
5
10
15
25


Cala Estancia - High epiphyte load
Cumulative Dominance%
100
80
60
40
20
0
1
10
100
Species rank


Cala Nova - High epiphyte load
Cumulative Dominance%
100
80
60
40
20
0
1
10
100
Species rank
IC
C
F


Cala Viñas - Low epiphyte load
Cumulative Dominance%
100
80
60
40
20
0
1
10
100
Species rank


Enderrocat - Low epiphyte load
Cumulative Dominance%
100
80
60
40
20
0
1
10
100
Species rank
IC
C
F


Harpacticoid abundance (number of individuals pler plot, 400 cm2)
Metamphiascopsis hirsutus
r2= 0,23; p<0,05
Eudactylopus latipes
r2=0,25; p<0,05
Species number (S)
Species Number (S)
r2 = 0,17; p>0,05
g epiphyte dw·plot-1

	Cala Nova			Cala Estancia			Enderrocat			Cala Viñas		
Date x Fert	AC	SC	SF	AC	SC	SF	AC	SC	SF	AC	SC	SF
Harpacticoida	21±13,85	33,33±18	113±9,86	24,66±18,14	51,66±16,50	96,33±44	18,33±6,35	45,6±28,8	33,6±4,04	12,66±6,8	48,66±9,01	53,66±14,5
*Ambunguipes rufocinta**	0,33±0,57	0,33±0,57	0,33±0,57	0,66±0,57			1±1	0,3±0,57	1±1			0,3±0,57
Canthocamptidae sp									0,3±0,57			
copepodites unident.								1±1,73				
*Dactylopusia tisboides**				0,33±0,57	0,33±0,57				0,33±0,57			3±2
Amphiascopsis cinctus			2,3±1,53									
*Eudactylopus latipes**	0,33±0,57	0,66±1,15	13±10	1,3±2,3	0,6±1,15	8±8,71	4±1,73	8,6±5,03	6,66±4,04			
*Eupelte gracilis**	2,3±2,51	1,33±1,15	12±3,46	2,6±2,3	4±1,73	4,3±3,21	0,6±1,15	2,6±0,57	2±1	1,33±2,30	5,33±4,04	4,66±4,61
Laophonte cornuta									0,3±0,57	0,33±0,57		0
Longipedia coronata	0,33±0,57		0,33±0,57					3,3±3,05	1,6±1,15			0,33±0,57
Longipedia sp. 1									0,6±1,15		0,66±1,15	0,66±1.15
Longipedia minor	0,33±0,57	0,33±0,57	0,33±0,57	0,33±0,57	0,33±0,57	0,33±0,57	1,66±2,88	5,6±5,03	3±2		0,33±0,57	1±1
Longipedia sp.								1±1				
*Metamphiascopsis hirsutus**	1,66±2,08	5,66±5,68	9,3±6,02	6,6±4,72	19±12,28	47,6±36,52		4±6,08	1±1			0,3±0,57
Orthopsyllus linearis	0,66±1,15			0,33±0,57	0,33±0,57	0,33±0,57	1±1	7±6,93	1,66±1,15	0,33±0,57		1,33±2,31
Orthopsyllus sp. 2				0,33±0,57			0,33±0,57		0,66±1,15			
*Peltidium robustum**	0,33±0,57		4,33±3,05			0,33±0,57						0,33±0,57
*Peltidium sp.**				1	0,33±0,57		0,33±0,57	0,66±1,15				0,33±0,57
*Phyllothalestris mysis**		0,33±0,57	2±1	0,33±0,57		3,66±2,51	1,33±1,53	0,66±1,15	1,33±1,52			0,33±0,57
*Phyllothalestris sp.**	0,33±0,57				1±1,73	2,6±2,51						
*Porcellidium fimbriatum**	1±1,73										0,33±0,57	0,33±0,57
*Porcellidium sarsi**	3±2	1,33±1,52	6±1,73	2,33±2,08	2,66±1,15	3,66±3,78				0,33±0,57	2,33±0,57	1,33±1,15
*Porcellidium tenuicauda**	10,33±7,50	23±9,54	58,33±12,05	7,66±9,29	22,66±24,94	22±5,57	6,33±3,21	9,3±3,21	11±5,29	10,33±6,8	37,66±7,23	35,66±15,17
*Scutellidium sp.**								0,33±0,57	0,33±0,57			

Sunaristes sp.								0,33±0,57				
Tetragonicipitidae sp.									0,33±0,57			
Thalestridae copepodites indet.*			0,33±0,57			0,33±0,57						
Thalestridae sp.*			0,33±0,57		0,33±0,57	0,33±0,57						
Thalestris sp. 1*						0,66±1,15	0,33±0,57					
Tisbe spp.		0,33±0,57	4,66±2,88	0,33±0,57	0,33±0,57	2±1	1±1	1±1	1±1		1,66±1,52	3,33±3,21
Typhlamphiascus sp.									0,33±0,57			
Calanoida	1±1	4,3±3,2	1,6±1,15	5±3,46	1±1,73	1,3±1,52	3±2,64	41±26,96	48,6±33,20	11,33±9,86	198,6±84,60	248,33±151,56
Cyclopoida				1,33±2,3		1±1,7		2,3±2,5	3,6±4			0,33±0,57
Siphonostomatoida		0,33±0,57		0,66±1,15	0,66±1,15	2±3,46	0,3±0,57	0,66±0,57	1,33±0,57		7±2	
S	13	11	15	16	13	17	13	17	20	6	10	18
N	66	114	346	95	160	302	62	269	262	72	763	907
d- Margalef´s Diversity	2,86	2,11	2,39	3,29	2,36	2,80	2,90	2,86	3,41	1,17	1,36	2,49
J' Pielou Evenness	0,71	0,56	0,64	0,80	0,58	0,62	0,81	0,69	0,58	0,59	0,33	0,25
H – Shannon´s Diversity	1,83	1,35	1,73	2,22	1,49	1,74	2,07	1,95	1,74	1,06	0,77	0,72

Table 1. Copepods (>500µm) associated with *Posidonia oceanica* leaves at four localities in the Bay of Palma (Majorca, Western Mediterranean) and various treatments under study (AC = August initial; SC = September non-fertilized; SF = September fertilized). Abundance (mean ± S. deviation of number of individuals per plot; n=3) and diversity measures for each location/treatment are provided. Shaded locations are those with a high initial epiphyte load as compared with white ones, with a low initial epiphyte load. * = typical phytal taxa.

Table 2. Results of the Permanova evaluating spatiotemporal differences in harpacticoid copepod assemblages among high and low epiphyte load localities in August and September. Pair-wise comparisons between localities nested in each epiphyte load level are also provided. P(perm) or P(MC) values are given depending on the amount of unique values obtained in Monte Carlo permutations (see Anderson, 2005 for details). E = epiphyte load; L = locality; D = sampling date. Significant results are highlighted in bold.

Source	df	SS	MS	F	P(perm)
Epiphyte load, E	1	2436,3021	2436,3021	**2,2466**	**0,048**
Locality, L(Epiphyte load)	2	12156,8584	6078,4292	**5,6051**	**0,0002**
Sampling Date, D	1	3048,8438	3048,8438	**2,8115**	**0,0124**
E*D	1	950,7180	950,7180	0,8767	0,5206
L(E)*D	2	2942,2439	1471,1220	1,3566	0,2132
Residual	16	17350,9894	1084,4368		
Total	23	38885,9556			
				t	**P(MC)**
Cala Estancia *vs* Cala Nova				**2,2061**	**0,0018**
Cala Viñas *vs* Enderrocat				**2,2638**	**0,0048**

Table 3. Results of the three-way ANOVA evaluating spatiotemporal differences of harpacticoid abundance and diversity among high and low epiphyte load localities in August and September. Significant differences are highlighted in bold. E = epiphyte load; L = locality; D = sampling date. C: Cochran´s C (only significant results, i.e.: non homogeneous, are indicated).

	Effect	**SS**	**d.f.**	**MS**	**F**	**p**	**C**
Total harpacticoids	Epiphyte load, E	0,008	1	0,008	0,14	0,715	
	Locality, L(E)	0,056	2	0,028	0,51	0,611	
	Sampling date, D	**0,885**	**1**	**0,885**	**16,08**	**,001***	
	E*D	0,072	1	0,072	1,31	0,269	
	L(E)*date	0,054	2	0,027	0,49	0,619	
Eudactylopus	**Epiphyte load, E**	**0,831**	**1**	**0,831**	**15,38**	**,001***	
	Locality, L(E)	**1,506**	**2**	**0,753**	**13,94**	**,000***	
	Sampling date, D	0,023	1	0,023	0,43	0,519	
	E*D	0,059	1	0,059	1,1	0,31	
	L(E)*date	0,036	2	0,018	0,33	0,721	
Eupelte	Epiphyte load, E	0,152	1	0,152	1,515	0,236	
	Locality, L(E)	0,029	2	0,014	0,144	0,867	
	Sampling date, D	0,371	1	0,371	3,707	0,072	
	E*D	0,058	1	0,058	0,579	0,458	
	L(E)*date	0,244	2	0,122	1,216	0,322	
Porcellidium	Epiphyte load, E	0,027	1	0,027	0,242	0,63	p<0,05
	Locality, L(E)	0,575	2	0,288	2,54	0,11	
	Sampling date, D	**0,955**	**1**	**0,955**	**8,437**	**,010***	
	E*D	0,119	1	0,119	1,056	0,32	
	L(E)*date	0,032	2	0,016	0,143	0,868	
Metamphiascopsis	Epiphyte load, E	0,127	1	0,127	1,4	0,255	
	Locality, L(E)	**3,499**	**2**	**1,749**	**19,17**	**,000***	
	Sampling date, D	**0,586**	**1**	**0,586**	**6,42**	**,022***	
	E*D	0,064	1	0,064	0,7	0,415	
	L(E)*date	0,137	2	0,069	0,75	0,488	
Total species (S)	Epiphyte load, E	15,04	1	15,04	1,814	0,197	
	Locality, L(E)	45,42	2	22,71	2,739	0,095	
	Sampling date, D	9,37	1	9,37	1,131	0,303	
	E*D	0,04	1	0,04	0,005	0,944	
	L(E)*date	26,42	2	13,21	1,593	0,234	
Margalef´s diversity (d)	Epiphyte load, E	1,74	1	1,74	3,613	0,076	
	Locality, L(E)	**3,689**	**2**	**1,844**	**3,829**	**,044***	
	Sampling date, D	0,002	1	0,002	0,004	0,952	
	E*D	0,028	1	0,028	0,059	0,811	
	L(E)*date	1,832	2	0,916	1,902	0,182	
Pielou´s evenness (J')	Epiphyte load, E	1664	1	1664	3,974	0,064	p<0,001
	Locality, L(E)	**3336**	**2**	**1668**	**3,984**	**,039***	**p<0,001**
	Sampling date, D	1706	1	1706	4,075	0,061	p<0,001
	E*D	1679	1	1679	4,01	0,062	p<0,001
	L(E)*date	**3346**	**2**	**1673**	**3,997**	**,039***	
Shannon´s Diversity H'(loge)	**Epiphyte load, E**	**0,959**	**1**	**0,959**	**5,661**	**,030***	
	Locality, L(E)	**2,454**	**2**	**1,227**	**7,245**	**,006***	
	Sampling date, D	0,003	1	0,003	0,017	0,898	
	E*D	0,079	1	0,079	0,466	0,505	
	L(E)*date	**1,316**	**2**	**0,658**	**3,884**	**0,042***	

Table 4. Results of the Permanova investigating for variations in harpacticoid copepod assemblages among high and low epiphytic load localities with nutrient addition. Pair-wise comparisons between localities nested in each epiphyte load group are also provided. P(perm) or P(MC) values are given depending on the amount of unique values obtained in Monte Carlo permutations (see Anderson, 2005 for details). E = epiphyte load; L = locality, F = nutrient addition. Significant results are highlighted in bold.

Source	**df**	**SS**	**MS**	**F**	**P(perm)**
Epiphyte load, E	1	3608,1432	3608,1432	**5,4365**	**0,0004**
Locality, L(E)	2	9893,7075	4946,8538	**7,4536**	**0,0002**
Fertilization, F	1	2188,1047	2188,1047	**3,2969**	**0,0086**
E*F	1	505,8777	505,8777	0,7622	0,5932
L(E)*F	2	2348,7012	1174,3506	1,7694	0,0774
Residual	16	10619,0255	663,6891		
Total	23	29163,5598			
				t	**P(MC)**
Cala Estancia *vs.* Cala Nova				**2,46**	**0,0020**
Cala Viñas *vs.* Enderrocat				**2,58**	**0,0034**

Table 5. Results from the SIMPER analysis to identify species contributing most to differences between localities in pair-wise comparisons. Only contributions up to 50% cumulative percentage are represented. CE= Cala Estancia, CN= Cala Nova, E = Enderrocat, CV = Cala Viñas. H = high epiphyte load, L= low epiphyte load.

CE & CN, average dissimilarity= 59,31			
Species	Average abundance		Cumulative %
	CE-H	CN-H	
P. tenuicauda	8,89	30,56	12,46
E. latipes	6,44	4,67	23,83
M. hirsutus	1,67	5,56	33,11
P. sarsi	0	3,44	41,97
L. minor	3,44	0,33	49,40
CE & CV, average dissimilarity = 60			
	CE-H	CV-L	
M. hirsutus	1,67	24,44	19,3
P. tenuicauda	8,89	17,44	29,87
E. latipes	6,44	3,33	38,76
P. sarsi	0	2,89	46,70
L. minor	3,44	0,33	53,72
CN & CV, average dissimilarity = 48,84			
	CN-H	CV-L	
P. tenuicauda	30,56	17,44	17,84
M. hirsutus	5,56	24,44	34,96
E. latipes	4,67	3,33	44,93
E. gracilis	5,22	3,67	53,59
CE & E, average dissimilarity = 63,03			
	CE-H	E-L	
E. latipes	6,44	0	15,67
P. tenuicauda	8,89	27,89	29,96
L. minor	3,44	0,44	38,09
O. linearis	3,22	0,56	45,94
E. gracilis	1,78	3,78	53,07
CN & E, average dissimilarity = 52,22			
	CN-H	E-L	
P. tenuicauda	30,56	27,89	16,91
M. hirsutus	5,56	0,11	31,03
E. gracilis	5,22	3,78	41,87
P. sarsi	3,44	1,33	50,32
CV & E, average dissimilarity = 59,78			
	CV-L	E-L	
M. hirsutus	24,44	0,11	25,72
P. tenuicauda	17,44	27,89	42,61
E. gracilis	3,67	3,78	50,42

	Effect	SS	d.f.	MS	F	p	C
Epiphytes	**Epiphyte load, E**	**0,461**	**1**	**0,461**	**16,64**	**0,001***	
	Locality, L(E)	**0,536**	**2**	**0,268**	**9,67**	**0,002***	**p<0,05**
	Fertilization, F	**0,421**	**1**	**0,421**	**15,19**	**0,001***	
	E*F	0,001	1	0,001	0,05	0,832	
	L(E*F)	0,083	2	0,042	1,5	0,252	p<0,01
Total harpacticoids	Epiphyte load, E	0,056	1	0,056	1,9	0,187	
	Locality, L(E)	0,157	2	0,079	2,798	0,091	
	Fertilization, F	**0,232**	**1**	**0,232**	**7,856**	**0,013***	
	E*F	0,017	1	0,017	0,586	0,455	
	L(E*F)	**0,351**	**2**	**0,176**	**6,252**	**0,010***	
Eudactylopus	**Epiphyte load, E**	**1,515**	**1**	**1,515**	**20,32**	**0,000***	
	Locality, L(E)	**0,972**	**2**	**0,486**	**6,52**	**0,009***	
	Fertilization, F	**0,78**	**1**	**0,78**	**10,46**	**0,005***	
	E*F	0,006	1	0,006	0,08	0,785	
	L(E*F)	**2,025**	**4**	**0,506**	**6,79**	**0,002***	
Eupelte	Epiphyte load, E	0,033	1	0,033	0,579	0,458	
	Locality, L(E)	0,12	2	0,06	1,063	0,369	P<0,05
	Fertilization, F	0,151	1	0,151	2,66	0,122	
	E*F	0,202	1	0,202	3,569	0,077	
	L(E*F)	**0,589**	**2**	**0,294**	**5,2**	**,018***	
Metamphiascopsis	Epiphyte load, E	0,114	1	0,114	1,01	0,33	
	Locality, L(E)	**6,215**	**2**	**3,108**	**27,61**	**0,000***	
	Fertilization, F	0,082	1	0,082	0,73	0,406	
	E*F	0,054	1	0,054	0,48	0,497	
	L(E*F)	0,186	2	0,093	0,83	0,455	
Porcellidium	Epiphyte load, E	0,094	1	0,094	1,744	0,205	
	Locality, L(E)	**1,119**	**2**	**0,56**	**10,43**	**0,001***	
	Fertilization, F	0,126	1	0,126	2,356	0,144	
	E*F	0,042	1	0,042	0,783	0,389	
	L(E*F)	0,138	2	0,069	1,29	0,303	
Total species (S)	Epiphyte load, E	20,17	1	20,17	2,659	0,122	
	Locality, L(E)	32,17	2	16,08	2,121	0,152	
	Fertilization, F	**73,5**	**1**	**73,5**	**9,692**	**0,007***	
	E*F	0,67	1	0,67	0,088	0,771	
	L(E*F)	7,5	2	3,75	0,495	0,619	
Margalef's Diversity (d)	**Epiphyte load, E**	**1,967**	**1**	**1,967**	**5,246**	**0,036***	
	Locality, L(E)	**4,026**	**2**	**2,013**	**5,369**	**0,016***	
	Fertilization, F	**3,187**	**1**	**3,187**	**8,501**	**0,010***	
	E*F	0,064	1	0,064	0,17	0,686	
	L(E*F)	0.003	2	0,001	0,004	0,996	
Pielou´s evenness (J')	**Epiphyte load, E**	**0,071**	**1**	**0,071**	**4,877**	**0,042***	
	Locality, L(E)	**0,185**	**2**	**0,093**	**6,383**	**0,009***	
	Fertilization, F	0	1	0	0,034	0,855	
	E*F	0,007	1	0,007	0,499	0,49	
	L(E*F)	0,024	2	0,012	0,842	0,449	

Shannon´s Diversity H'(loge)	**Epiphyte load, E**	**0,884**	**1**	**0,884**	**6,196**	**0,024***
	Locality, L(E)	**1,928**	**2**	**0,964**	**6,756**	**0,007***
	Fertilization, F	0,61	1	0,61	4,272	0,055
	E*F	0,059	1	0,059	0,41	0,531
	L(E*F)	0,303	2	0,152	1,062	0,369

Table 6. Results of the three-way ANOVA investigating for variations in epiphyte biomass and harpacticoid abundance and diversity among high and low epiphytic load localities with nutrient addition. Significant differences are highlighted in bold. E = Epiphyte load; F = nutrient addition; L = locality. C: Cochran´s C (only significant, i.e.: non homogeneous results are indicated).