

1 **Harpacticoid copepod response to epiphyte load variations in *Posidonia oceanica***

2 (L.) Delile, meadows.

3
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11 **Abstract**

12
13 We conducted a field experiment to assess the response of phytal harpacticoids to
14 nutrient-driven increases of epiphyte load in *Posidonia oceanica* meadows. First, we evaluated
15 differences in species richness, diversity and assemblage structure of phytal harpacticoids in *P.*
16 *oceanica* meadows with differing epiphyte loads. Second, we conducted a field experiment
17 where epiphyte load was increased through an in-situ addition of nutrients to the water column
18 and evaluated the responses of the harpacticoid assemblages. We predicted that there would be
19 changes in the harpacticoid assemblages as a result of nutrient-driven increases of epiphyte load,
20 and that these changes would be of a larger magnitude in meadows of low epiphyte load. Our
21 results show that the harpacticoid fauna (>500 μm) present in *P. oceanica* meadows in the Bay
22 of Palma comprised taxa which are considered phytal and other less abundant ones previously
23 described as sediment dwellers or commensal on other invertebrate species. Nutrient addition
24 had an overall significant effect on epiphyte biomass and on harpacticoid abundance, diversity
25 and assemblage structure possibly as a response to the increased resources and habitat
26 complexity provided by epiphytes. The abundance of dominant species at each location was
27 favoured by nutrient addition and in some cases correlated with epiphytic biomass, though
28 never strongly. This may indicate that structural complexity or diversity of the epiphytic cover
29 might be more important than the actual epiphytic biomass for the harpacticoid species

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

5

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

8

1 **Introduction**

2
3 Degradation of coastal areas due to human-induced eutrophication is one of the main
4 reasons causing seagrass decline worldwide (Burkholder et al., 2007; Waycott et al.,
5 2009). Excessive nutrient inputs have been invoked as being responsible of seagrass
6 die-back, mainly by stimulating the growth of drifting and epiphytic macroalgae (see
7 Burkholder et al., 2007 and references therein) that limit seagrass access to light and
8 nutrients and thus strongly reduce seagrass size and metabolism (Cornelisen and
9 Thomas, 2004; Ruiz et al., 2001).

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

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

5 Crustaceans are in general very sensitive to organic pollution due to their limited
6 anoxia tolerance which makes them good subjects for eutrophication monitoring (Blake
7 and Duffy, 2010; Korpinen et al., 2010). Among them, harpacticoid copepods are often
8 the most diverse and numerically dominant invertebrate group in phytal habitats (Hicks,
9 1985; Arroyo et al., 2004), and their importance as trophic link between primary and
10 secondary producers in benthic environments is now undisputed (e.g. Sogard, 1984;
11 Aarnio et al., 1996; Davenport et al., 2011; Jenkins et al., 2011). Harpacticoids respond
12 readily to increases in habitat complexity (Jenkins et al., 2002, Arroyo et al., 2006), and
13 organic matter content in the sediment (Gee and Warwick, 1985; Danovaro et al., 2002)
14 and in general, increases in epiphytic biomass, whether seasonal or episodic, are
15 paralleled by higher numbers and diversities of this taxon (Hall and Bell, 1993;
16 Rutledge and Fleeger, 1993). Harpacticoids are generally very motile: phytal species
17 can colonise seagrass blades at distances higher than 20m and reach ambient densities in
18 2-4 days (Bell & Hicks, 1991; Kurdziel & Bell, 1992), and their generation times can be
19 as short as 10-18 days, a normal development time of 2-3 months being common for
20 many species (Fleeger, 1979). A few families are morphologically adapted to live in the
21 phytal, showing in general, larger sizes than their interstitial counterparts (see Hicks and
22 Coull, 1983 for a review). In sediments, their spatial distribution is conditioned by the
23 patchy distribution of diatoms (Decho & Castenholz 1986; Sandulli and Pinckney
24 1999). They adapt their grazing rates and abundance to increases in microphytobenthos
25 (Montagna et al., 1995) controlling both microalgal biomass and their diel variations

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

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

25

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

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14

15 **Material and Methods**

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

25 In August 2008, six 1 m² plots were randomly established at each of the four
26 localities, using galvanized iron bars fixed at each corner (Figure 1). Plots were

1 approximately 10 m apart from each other at all locations. Three plots received nutrient
2 addition in the water column, while the other three served as control for the fertilization
3 factor. A slow-release fertilizer (OsmocoteTM N:P:K, 15:9:9 + 3MgO + trace elements)
4 was employed as a source of nutrients (Heck et al., 2000; Prado et al., 2008), filling a
5 250 ml plastic diffuser which was placed 40 cm above the sediment, tied to one of the
6 frames defining the plots, at the corner of each fertilized plot. The fertilizers were left
7 for 42 days. Prior to the set-up of the experiment, to obtain an estimate of shoot density
8 at each of the localities and initial samples of the faunal population associated to *P.*
9 *oceanica* leaves, we randomly defined three 40 x 40 cm plots in the same areas where
10 the experiments were later set up (i.e.: at all four locations, marked with a G.P.S.),
11 counted the number of *P. oceanica* shoots present in each of them, and collected faunal
12 samples using a suction sampling device with a 40 x 40 cm opening mouth and a
13 collector bag made of 200µm mesh (see Buia et al., 2003 for a description of the
14 device). This sampler allows the fauna of *P. oceanica* (fundamentally the leaves) to be
15 aspirated, while not damaging the plants themselves. It is easily and quickly deployed
16 over the selected sampling area and all fauna are directly sucked into a 200 µm mesh
17 bag, minimizing the escape of vagile fauna. Once in the laboratory, samples were
18 sieved with a 500 µm mesh and fixed in 4% buffered formalin to preserve them until
19 processing. We used a 500 µm mesh because the study was initially focused on
20 macrofauna. We decided to analyze the harpacticoid fauna in detail, given the high
21 amount found in all samples. The high amount of large specimens collected, indicated
22 that at least this fraction of the harpacticoids associated with *P. oceanica* was well
23 represented. Finally, the above mentioned reasons of adequacy of this taxon as indicator
24 of organic enrichment justified an attempt to explore their response to increases in
25 epiphyte load.

1 Forty-two days after nutrient addition, samples from the fertilized and non-
2 fertilized plots were gathered. Five shoots of *P. oceanica* were collected, placed in an
3 individual plastic bag and carried to the laboratory, where they were stored frozen at -
4 20°C until processing. Epiphytes in all the leaves of each shoot were scraped off using a
5 razor blade and collected in preweighed Whatman GF/C glass fibre filters. Filters were
6 dried (60°C, 48 h) to determine epiphyte dry weight (g DW). Seagrass leaves were dried
7 (60°C, 48 h) to quantify the leaf biomass (g DW) of each shoot. The epiphyte load of
8 each *P. oceanica* shoot was expressed as epiphyte biomass per leaf biomass (g DW
9 epiphyte g DW leaf⁻¹). Samples of the epifaunal community (one 40 x 40 cm sample per
10 plot) were collected as during the August sampling, at each of the fertilized and non-
11 fertilized plots, and processed in the laboratory as above. Invertebrates from all samples
12 were sorted in the laboratory using a dissecting microscope, and all copepods further
13 identified using a compound microscope.

14

15 **Statistical analyses**

16 *Spatial and temporal variation in harpacticoid assemblage structure*

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

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

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

9

10 Changes following nutrient addition

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

18

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

22

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

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

5

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

13

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

18

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

25

26 *Relationship between epiphytic load and harpacticoid abundance and diversity*

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

8

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

10

11 **Results**

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

20 Among harpacticoids, the predominant species were *Porcellidium tenuicauda* Claus
21 1860, *Eudactylopus latipes* (Scott, T. 1893), *Metamphiascopsis hirsutus* (Thomson &
22 A. Scott, 1903) and *Eupelte gracilis* Claus, 1860, which together accounted for about
23 78% of the harpacticoid assemblage associated with *P. oceanica* at the 4 locations under
24 study (Table 1). In all locations, *Porcellidium tenuicauda* was the most abundant
25 harpacticoid species associated with *P. oceanica*.

1

2 Spatial and temporal variation

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

9

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

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

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

3 4 Changes following nutrient addition

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

14
15
16 Results from the SIMPER analysis conducted to identify which species accounted more
17 for these variations between localities are shown in Table 5. In general, the dominant
18 species showed variations between locations, and these accounted for major variations
19 between them: *Metamphiascopsis hirsutus* was much more abundant in Cala Viñas than
20 in the other locations, *Porcellidium tenuicauda* was more abundant in Cala Nova and
21 Enderrocat and *Eudactylopus latipes* was more abundant in Cala Estancia, while it was
22 absent in Enderrocat.

23
24 Results from the three-way ANOVA indicated significant differences in epiphyte load
25 between those localities assigned to high and low epiphyte load levels, as expected, and

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

21

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

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

5

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

22

23 Relationship between epiphyte load and harpacticoid abundance and diversity

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

1 correlated with epiphyte biomass (SR correlations, $p > 0,05$). As for diversity measures,
2 only the number of species (S) was significantly correlated with epiphyte biomass, all
3 other indexes showing no significant relationship with this variable (SR correlations,
4 $p > 0,05$).

5

6 **Discussion**

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

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

1 due to the aforementioned rise of the predominant species, but also to new colonizers
2 and the proliferation of opportunistic species such as *Tisbe* spp. Tisbids are common in
3 a wide variety of organically enriched environments (Fava and Volkmann, 1975; Hicks,
4 1980), and showed higher abundances in fertilized plots with respect to control ones in
5 our study (Table 1). The addition of species was particularly evident in Enderrocat, the
6 locality with low initial epiphyte load and the lowest initial number of species (5),
7 which were more than doubled (up to 15 species in fertilized samples *versus* 7 in control
8 ones) with nutrient addition. Here, species such as *Ambunguipes rufocinta*,
9 *Phyllothalestris mysis*, *Peltidium robustum* or *Dactylopusia tisboides*, which are also
10 normally associated with phytal habitats, appeared only after fertilization.

11 Conversely, nutrient enrichment in Cala Estancia did not cause an increase in
12 epiphyte load nor a response from the harpacticoid assemblage. Cala Estancia had,
13 originally, the most diverse harpacticoid assemblage, the highest epiphyte load, the
14 smallest *Posidonia* leaves and the most sparsely distributed shoots (Castejón, 2011).
15 Abundances of all other invertebrate taxa on unfertilized plots were also highest here,
16 and they also showed a decreasing trend with fertilization (Castejón, 2011). Cala
17 Estancia is at the innermost part of the bay and probably receives the steadiest nutrient
18 input from anthropogenic sources, representing a saturated stage where an increase in
19 nutrients would not trigger any further epiphyte growth or grazer response (Edgar,
20 1993; Edgar and Aoki, 1993). Higher turbidity levels or increased sedimentation rates at
21 this site, could be posing a stronger pressure on the *Posidonia* (explaining its reduced
22 shoot sizes and densities), the epiphytes and the harpacticoid assemblage than that
23 exerted by nutrient levels alone.

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

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

14 The species distribution found in our study need not reflect annual dominance
15 patterns, since our sampling and experimental times were confined to the summer
16 months, which coincide with the period of maximum epiphyte load (i.e.: maximum
17 abundance of resources). We did not analyze the specific composition of the epiphytic
18 assemblage, but changes in epiphytic assemblages associated with *P. oceanica* due to
19 nutrient enrichment, have been reported elsewhere (Prado et al., 2008; Balata et al.,
20 2010). In this sense, similar processes could have enhanced harpacticoid species
21 dominance linked to particular (increasing) epiphyte species in our study sites. Indeed,
22 nutrient enrichment is supposed to favour mainly encrusting corallines and filamentous
23 forms (Prado et al., 2008; Balata et al., 2010), which seem to be also the type of algae
24 mainly triggering harpacticoid responses to variations in epiphytic cover (Hall & Bell,
25 1993; Jarvis and Seed, 1996) though this reactions are often species-specific. Many

1 phytal species have been found associated with red algae (Lang, 1948), and particularly
2 *Eupelte gracilis* was found amidst coralline species in the Mediterranean (Monard,
3 1928). In our experiment, *E. latipes* seemed to respond more acutely to variations in
4 epiphyte biomass showing a significant rise in fertilized plots, particularly at locations
5 where it was not abundant prior to fertilization. This species has been found in tidal
6 pools (Lang, 1965; Tanaka and Hue, 1966) were ephemeral opportunistic algae abound,
7 together with *M. hirsutus* (Tanaka and Hue, 1966), which was also previously described
8 from seagrass habitats (Lang, 1948). It could be that these two are opportunistic species
9 that were abundant in our assemblages only because of the proliferation of epiphytes
10 during our study time. As a matter of fact, they were the only two species correlated
11 with epiphytic biomass. On the other hand, *P. tenuicauda* and *E. gracilis* showed no
12 correlation with epiphyte biomass, despite being more abundant in fertilized plots in
13 Cala Nova, where nutrient-driven epiphyte increases were stronger. *Porcellidium*
14 *tenuicauda*, was the dominant harpacticoid in our study, and is typically associated with
15 flat laminar algae (Lang, 1948; Huys et al., 1996), so it could be that its association was
16 more with the *P. oceanica* blades than with the macroalgal epiphytes. Its increase, as
17 well as that of *E. gracilis* could be related to increases in diatoms and microbes
18 associated with the *P. oceanica* leaves, which would also increase with fertilization.
19 This suggests that qualitative aspects of the epiphytes might be more important than
20 quantitative ones when explaining harpacticoid abundance and diversity patterns found
21 on enriched plots. Often algal morphology (as surface area or fractal dimension) has
22 been invoked as a better indicator of habitat provision than its biomass (or volume),
23 especially for smaller individuals as those comprising the meiofauna (Gee & Warwick,
24 1994). Algae with differing morphologies provide gradients of habitat complexity
25 which in turn offer varying degrees of protection, sediment retention, food provision in

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

4

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

21

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24

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 \pm 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 \pm 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 \pm 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 triangles), 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.

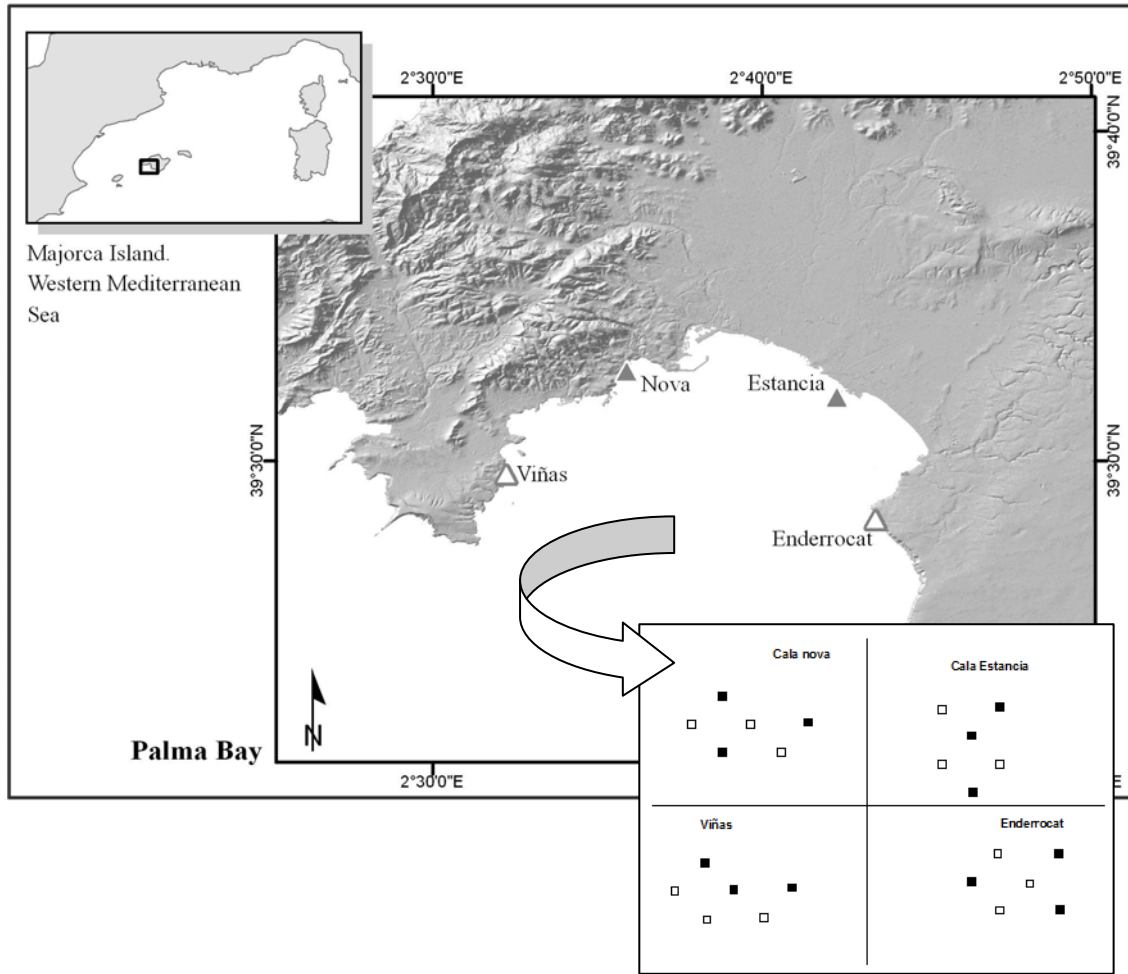
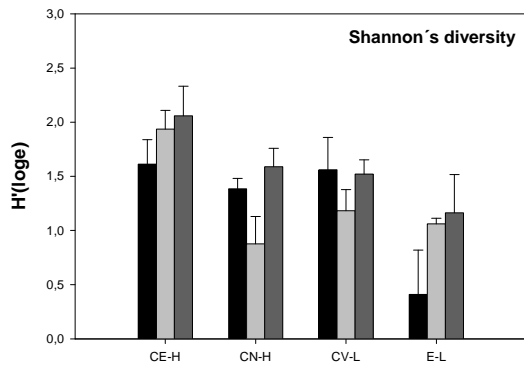
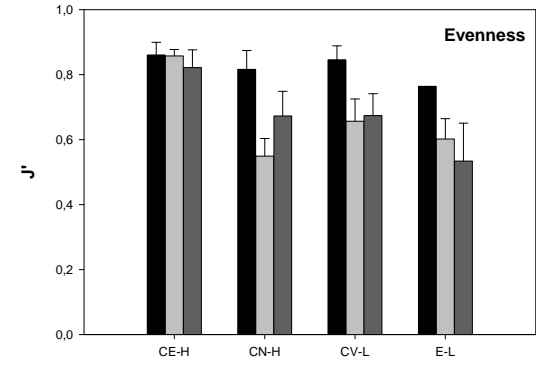
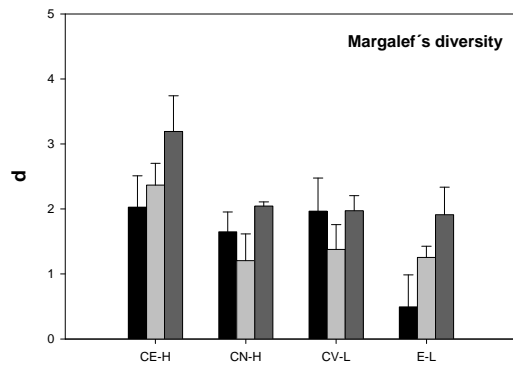
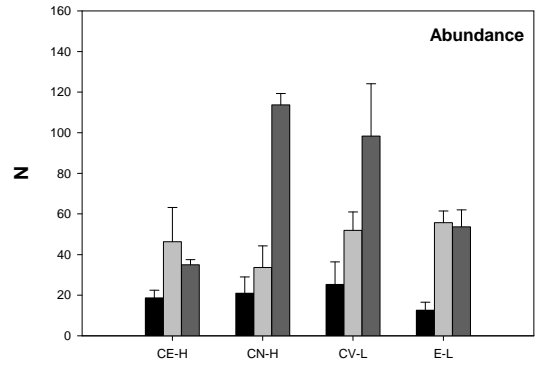
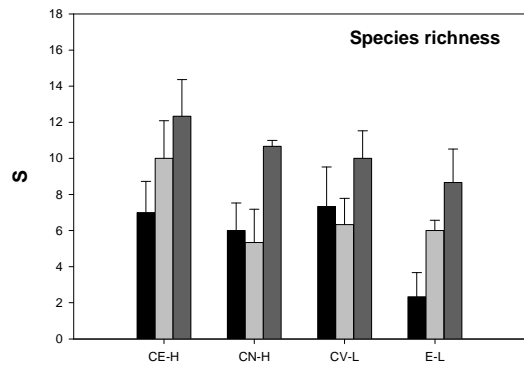
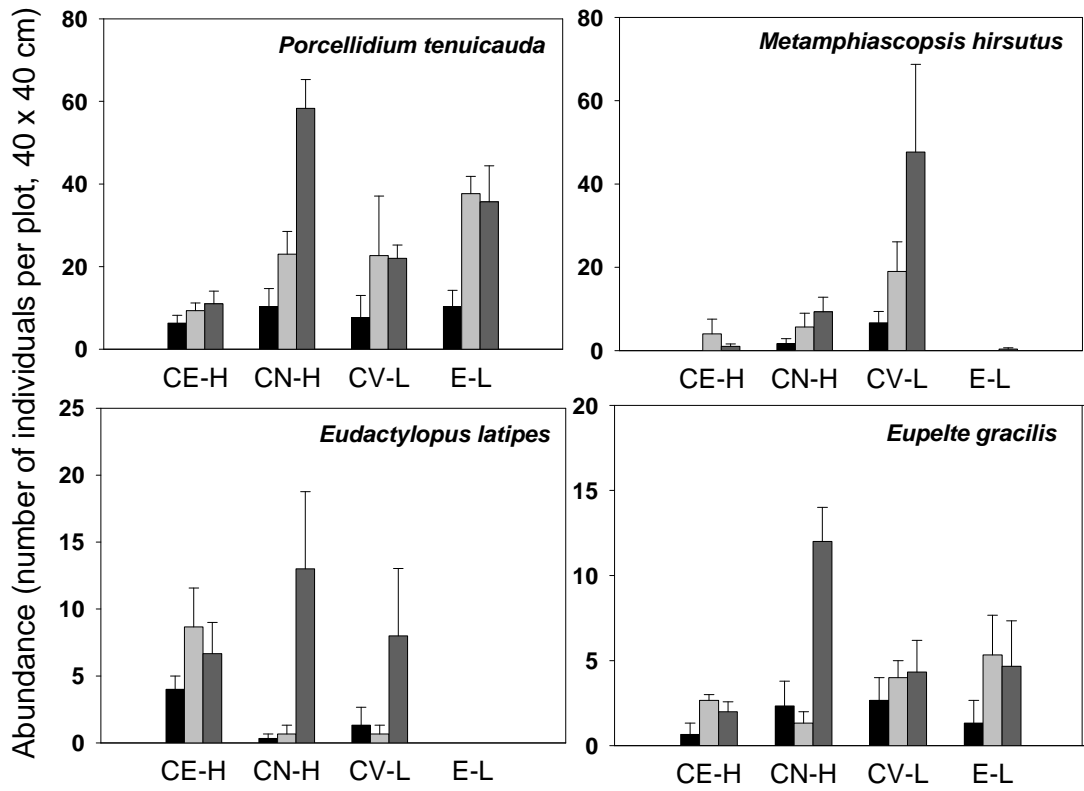
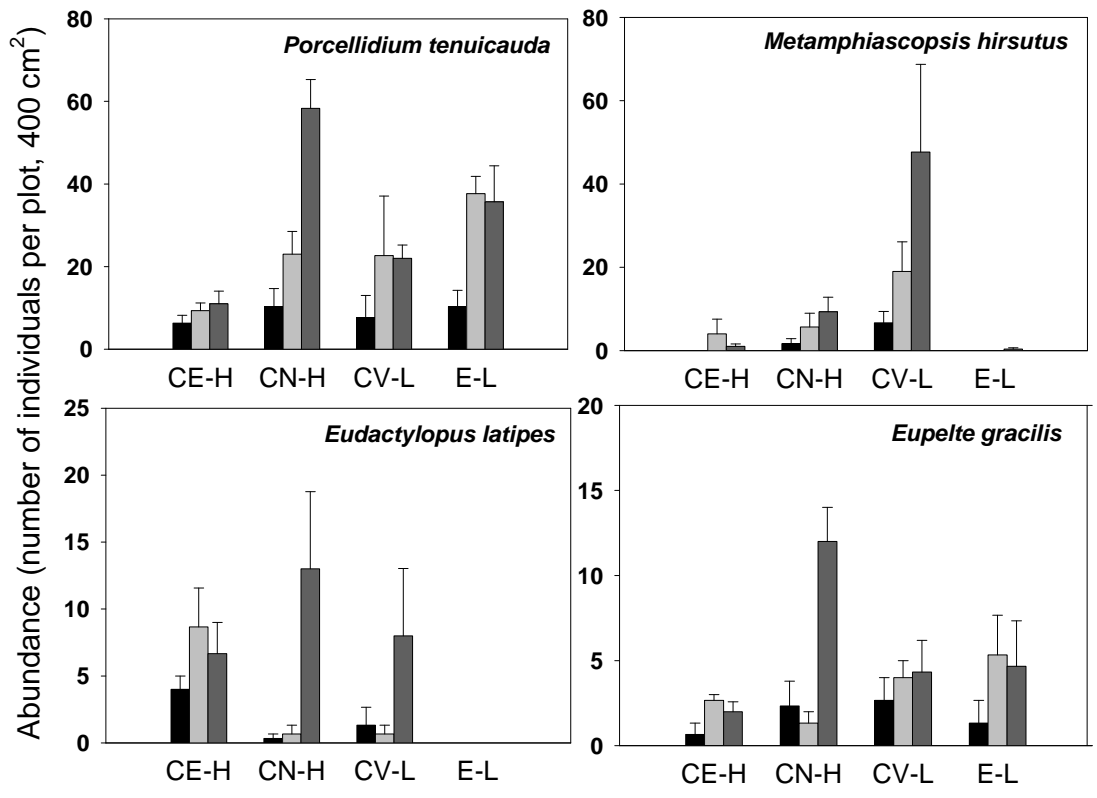


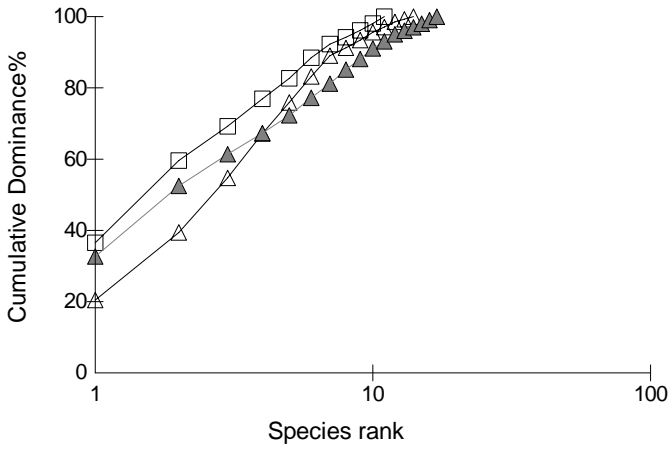
Figure 1



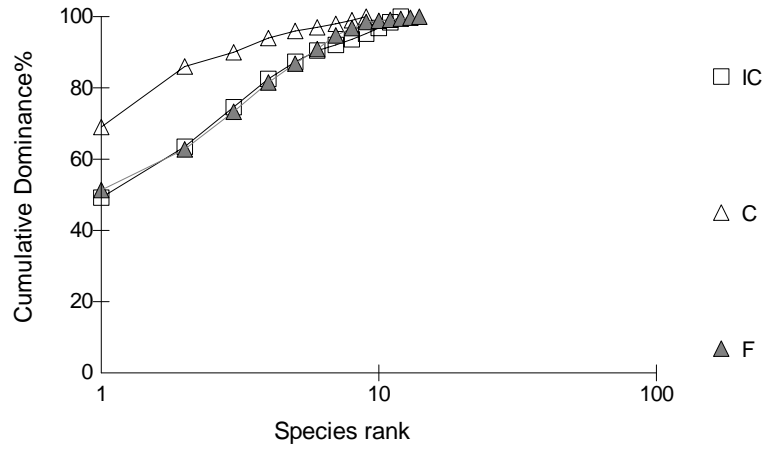




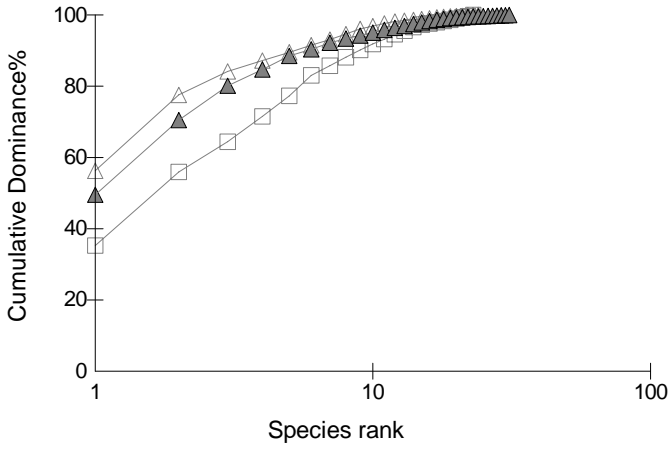
Cala Estancia - High epiphyte load



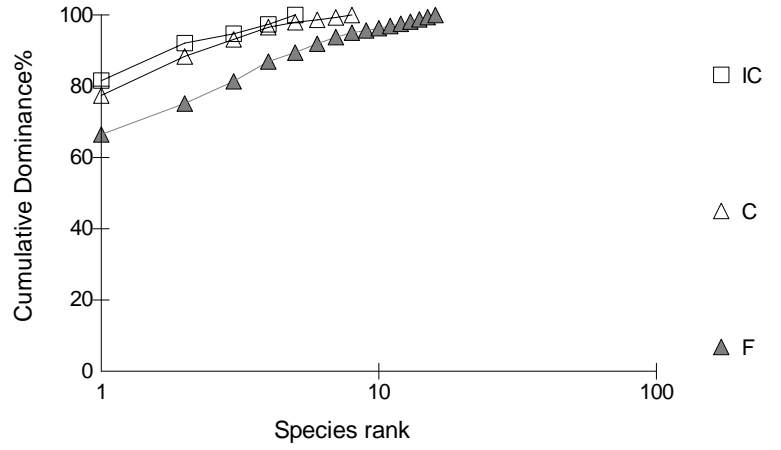
Cala Nova - High epiphyte load

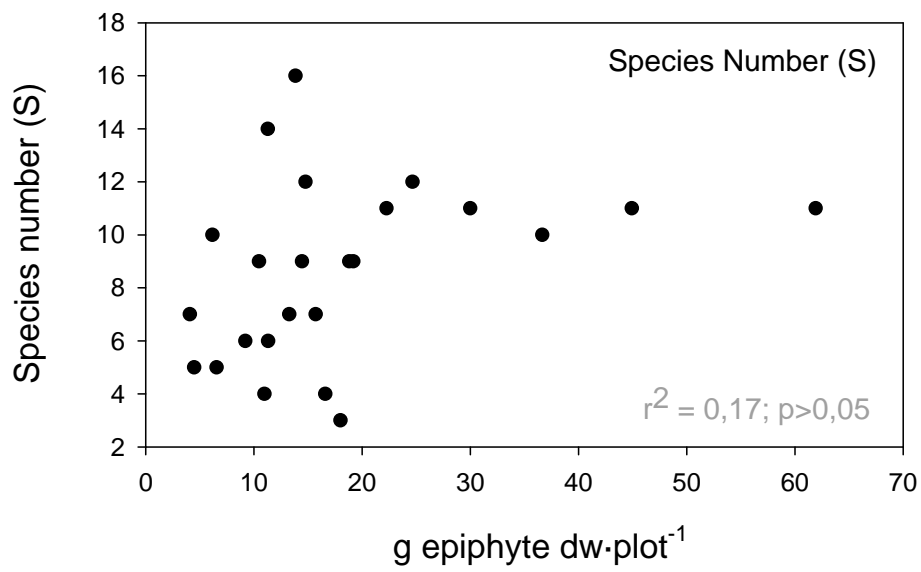
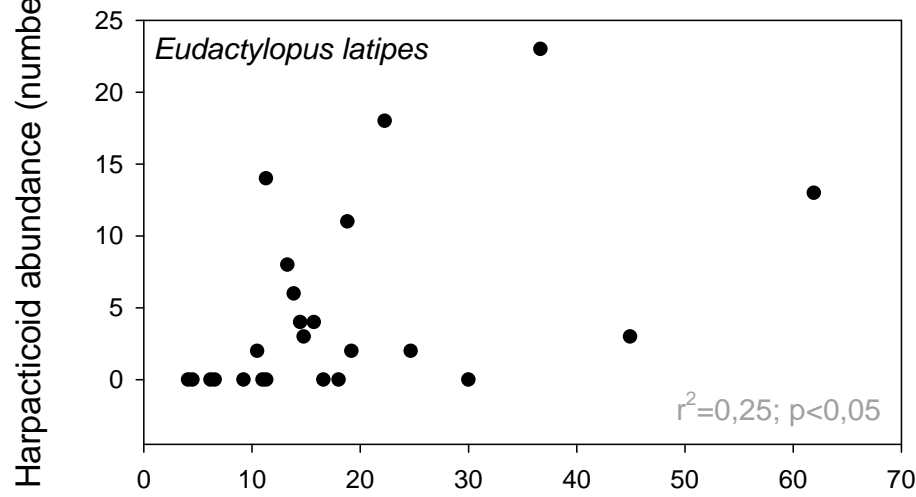
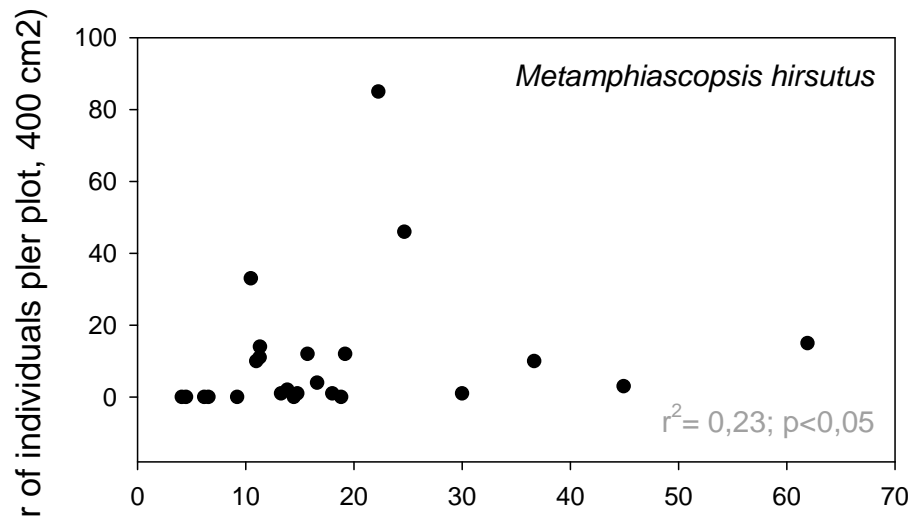


Cala Viñas - Low epiphyte load



Enderrocat - Low epiphyte load





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

<i>Sunaristes sp.</i>							0,33±0,5 7					
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					
<i>Tisbe spp.</i>	0,33±0,5 7	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
<i>Typhlamphiascus sp.</i>									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±84,6 0	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±0,5 7			0,66±1,15	0,66±1,15	2±3,46	0,3±0,57	0,66±0,5 7	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	
<i>Eudactylopus</i>	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	
<i>Eupelte</i>	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	
<i>Porcellidium</i>	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	
<i>Metamphiascopsis</i>	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	
<i>P. tenuicauda</i>	8,89	30,56	12,46
<i>E. latipes</i>	6,44	4,67	23,83
<i>M. hirsutus</i>	1,67	5,56	33,11
<i>P. sarsi</i>	0	3,44	41,97
<i>L. minor</i>	3,44	0,33	49,40
CE & CV, average dissimilarity = 60			
	CE-H	CV-L	
<i>M. hirsutus</i>	1,67	24,44	19,3
<i>P. tenuicauda</i>	8,89	17,44	29,87
<i>E. latipes</i>	6,44	3,33	38,76
<i>P. sarsi</i>	0	2,89	46,70
<i>L. minor</i>	3,44	0,33	53,72
CN & CV, average dissimilarity = 48,84			
	CN-H	CV-L	
<i>P. tenuicauda</i>	30,56	17,44	17,84
<i>M. hirsutus</i>	5,56	24,44	34,96
<i>E. latipes</i>	4,67	3,33	44,93
<i>E. gracilis</i>	5,22	3,67	53,59
CE & E, average dissimilarity = 63,03			
	CE-H	E-L	
<i>E. latipes</i>	6,44	0	15,67
<i>P. tenuicauda</i>	8,89	27,89	29,96
<i>L. minor</i>	3,44	0,44	38,09
<i>O. linearis</i>	3,22	0,56	45,94
<i>E. gracilis</i>	1,78	3,78	53,07
CN & E, average dissimilarity = 52,22			
	CN-H	E-L	
<i>P. tenuicauda</i>	30,56	27,89	16,91
<i>M. hirsutus</i>	5,56	0,11	31,03
<i>E. gracilis</i>	5,22	3,78	41,87
<i>P. sarsi</i>	3,44	1,33	50,32
CV & E, average dissimilarity = 59,78			
	CV-L	E-L	
<i>M. hirsutus</i>	24,44	0,11	25,72
<i>P. tenuicauda</i>	17,44	27,89	42,61
<i>E. gracilis</i>	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*	p<0,05
	Locality, L(E)	0,536	2	0,268	9,67	0,002*	
	Fertilization, F	0,421	1	0,421	15,19	0,001*	
	E*F	0,001	1	0,001	0,05	0,832	p<0,01
	L(E*F)	0,083	2	0,042	1,5	0,252	
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*	
<i>Eudactylopus</i>	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*	
<i>Eupelte</i>	Epiphyte load, E	0,033	1	0,033	0,579	0,458	P<0,05
	Locality, L(E)	0,12	2	0,06	1,063	0,369	
	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*	
<i>Metamphiascopsis</i>	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	
<i>Porcellidium</i>	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).