

Selective top-down control of epiphytic biomass by amphipods from *Posidonia oceanica* meadows: implications for ecosystem functioning

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ABSTRACT. Mediterranean *Posidonia oceanica* meadows shelter an important biomass and biodiversity of amphipod crustaceans that graze on epiphytes. However, their actual significance for ecosystem functional processes is hard to estimate, due to the lack of adequate data. Here, a field microcosm-based inclusion experiment was used to test if three of the dominant taxa of the amphipod community (*Apherusa chiereghinii*, *Dexamine spiniventris* and *Gammarus* spp.) could exert top-down control on seagrass leaf epiphytes. Influence of amphipod activity on nutrient availability for the host species was also investigated. All grazer taxa significantly reduced biomasses of erect macroalgae and erect sessile animals present on leaves. None of them consumed encrusting epiflora or epifauna. This selective top-down control could have important implications for the structure of the epiphytic community on leaves of *P. oceanica*, which is one of the most diverse and abundant of all seagrass species. Grazing activity of all taxa caused higher N content of seagrass leaves, likely through amphipod excretion and/or sloppy feeding. Since *P. oceanica* meadows often grow in oligotrophic zones where plant growth can be nutrient-limited, this N enrichment could enhance seagrass production. Overall, the ecological interaction between *P. oceanica* and amphipods could be seen as a facultative mutualistic relationship. Our results suggest that amphipod mesograzers are key-elements in some of the functional processes regulating these complex and yet endangered ecosystems, which are essential components of Mediterranean coastal zones.

KEY WORDS: Epiphyte grazing, mesograzers, Amphipoda, nutrient cycling, *Posidonia oceanica*

INTRODUCTION

Seagrasses are widespread foundation species, present in many coastal zones throughout the world. They form meadows that constitute key coastal ecosystems, and whose paramount ecological importance is widely recognized (DUARTE 2002; VALENTINE & DUFFY 2006). In several (putatively all) meadow ecosystems, the seagrass, the epiphytes that grow on it and the grazers able to consume either the seagrass or its epiphytes are linked by a complex and intricate interplay of reciprocal interactions and feedback loops, termed seagrass/epiphyte/grazer system (JERNAKOFF et al. 1996). Natural or anthropogenic fluctuations in this system

can influence many ecological processes, and ultimately impact the whole meadow functioning (VALENTINE & DUFFY 2006).

The Neptune grass, *Posidonia oceanica* (L.) Delile, is the most widespread seagrass of the Mediterranean Sea. This species is endemic to the Mediterranean and forms large, typically monospecific and fully submerged meadows from shallow depths to 45 meters. The complex tridimensional structure of these meadows offers a suitable habitat to hundreds of animal and plant species, as well as micro-organisms (BUIA et al. 2000). In addition, *P. oceanica* supports complex, elaborate food webs (VIZZINI 2009). As a result, *P. oceanica* meadows, which

cover up to 50000 km² (BETHOUX & COPIN-MONTÉGUT 1986), are biodiversity hotspots in the Mediterranean Sea.

P. oceanica is a large (leaf length up to 150 cm) and long-lived (leaf life span of 9–12 months) seagrass (GOBERT et al. 2006). These features allow the development of unique epiphytic communities (*sensu* BOROWITZKA et al. 2006; i.e. all organisms attached to the exterior surface of the plant). They are one of the most diverse and well-structured communities among all seagrasses, and can represent up to 40% of the foliar biomass (MAZZELLA et al. 1989). Epiphytes cover all parts of the plant (leaf and rhizomes) and include bacteria, fungi, protozoa, microalgae, macroalgae (mostly crustose and erect Rhodophyta and Phaeophyta), as well as encrusting or erect sessile invertebrates, mainly represented by bryozoans, hydrozoans and polychaetes (BUJA et al. 2000). The epiphytic cover is an essential compartment of Neptune grass meadows, and a key feature of *P. oceanica*-associated food webs. Since they have a higher nutritional quality and a better palatability than seagrass leaves or detritus, epiphytes are readily consumed by various animal taxa (LEPOINT et al. 2000; VIZZINI 2009).

Amphipods (Arthropoda, Malacostraca) are, alongside gastropods and polychaetes, one of the dominant groups of vagile invertebrates found in *P. oceanica* meadows (GAMBI et al. 1992). They form an abundant and diverse community, whose dominant taxa graze on epiphytes (LEPOINT ET AL. 2000; VIZZINI et al. 2002) with species-specific dietary preferences (MICHEL et al. in press). Since many fishes rely on them as prey (BELL & HARMELIN-VIVIEN 1983; PINNEGAR & POLUNIN 2000), amphipods constitute an important trophic link to higher trophic levels. However, the ecological significance of these trophic links at the scale of the meadow ecosystem, as well as their functional implications, remain unclear.

In a number of other temperate seagrass systems, amphipod mesograzers (*sensu* BRAWLEY 1992; i.e. organisms whose body size

is larger than that of a copepod, but smaller than 2.5 cm) can exert top-down control on epiphytic assemblages (HOWARD 1982; NECKLES et al. 1993; JERNAKOFF & NIELSEN 1997; DUFFY & HARVILICZ 2001). By doing so, they can release the seagrass from competition for nutrients and/or light, and have positive, indirect effects on seagrass biomass (DUFFY et al. 2001; MYERS & HECK 2013), production (NECKLES et al. 1993), or density (WHALEN et al. 2013). Moreover, mesograzers are able, through direct or indirect interactions, to act as regulators and to dampen impacts of environmental changes on meadow ecosystems (e.g. ALSTERBERG et al. 2013). In *P. oceanica* meadows, gastropods have received some attention (GACIA et al. 2009), but no data exist concerning the influence of epiphyte/amphipod trophic relationships on meadow ecosystem functioning. This limits insights about the actual ecological role of these potentially important mesograzers.

In this context, the objectives of this study were 1) to quantify the impact of amphipod feeding on the epiphytic cover of the leaves of *P. oceanica* and 2) to investigate potential indirect effects of amphipods on their seagrass host. To achieve these goals, we tested the impact of grazer inclusion on biomass of epiphytic functional groups and C/N ratios of *P. oceanica* leaves using in situ microcosms. To account for potential interspecific differences, experiments were focused on three of the dominant species of the community, i.e. *Apherusa chierighinii* Giordani-Soika, 1949, *Dexamine spiniventris* (Costa, 1853) and *Gammarus aequicauda* (Martynov, 1931). These species display contrasting feeding habits and, taken together, they represent about 60% of the total amphipod abundance in Calvi Bay (MICHEL 2011; MICHEL et al. in press).

Neptune grass meadows, like most seagrass ecosystems worldwide, are currently threatened by human activities (DUARTE 2002). Through this work, our ultimate goal is to put the trophic relationship between leaf epiphytes and amphipod mesograzers in the wider context of meadow functioning, and therefore to improve

the knowledge of ecological interactions among this remarkably important, yet endangered, ecosystem.

MATERIALS AND METHODS

Experiments were carried out in Calvi Bay (western Mediterranean Sea, north-western Corsica, France). *Posidonia oceanica* meadows cover about 50% of this bay, and reach depths of nearly 40 m. Meadows of Calvi Bay are mostly characterized by a continuous extension, and show important foliar biomass and production (BAY 1984; GOBERT et al. 2003). Work was undertaken by scuba diving in the surroundings of the STARESO research station (University of Liège). A circular (radius: 10 m, center coordinates: 42°34'46" N, 8°43'32" E) experimental site was set up in a continuous meadow zone. Depth of the experimental site ranged from 9.5 to 11 m. Meadow density at site depth was 314 ± 121 shoots.m⁻² (mean \pm SD of 45 measurements).

In situ microcosms were set up in this site, directly in the *P. oceanica* meadow. They consisted of 400- μ m nylon mesh cylinders (20 cm diameter X 180 cm length). Terminal portions (last 15 cm) of each end were made of elastic fabric, to facilitate microcosm opening, closing and sealing. To place microcosms, a patch of circa 10 *P. oceanica* shoots was randomly selected. Vagile fauna was eliminated by gently shaking the seagrass leaves, in order to cause grazer displacement without destroying the epiphytic cover. Each microcosm was then placed around the leaves. The bottom elastic part was tied around the rhizomes of the shoots, so that amphipods only had access to the foliar stratum. Microcosms were sealed as tight as possible using large plastic cable ties. In addition, each microcosm was anchored to the ground using 2 metal stakes. A float was attached to the top part to ensure adequate position of the microcosm in the water column. Four treatments were considered: one control without grazers, and three others, each containing a single grazer

taxon. Each treatment was replicated twice, giving a total of 8 microcosms. In addition, a procedural control consisting of a patch of 10 shoots without microcosm was realized, to ensure that the microcosm itself had no effect on the epiphyte community or the seagrass, notably through shading.

Amphipods were sampled using light traps which were modified after those described by MICHEL et al. (2010). Each live animal was identified through direct observation and photographs. The accuracy of these identifications was checked at the end of the experiment. All identifications were correct in the cases of *Apherusa chierighinii* and *Dexamine spiniventris*. However, a minor proportion (about 5%) of animals considered as being *Gammarus aequicauda* actually belonged to the morphologically close *Gammarus crinicornis* Stock, 1966 or *Gammarus subtypicus* Stock, 1966. Consequently, they will be referred to as “*Gammarus* spp.” over the course of this article.

Body size differed across grazer taxa. Specimens of *A. chierighinii* (total body length 5.48 ± 1.17 mm; mean \pm SD) were much smaller than those of *D. spiniventris* (total body length 9.89 ± 1.59 mm; mean \pm SD) or *Gammarus* spp. (total body length 12.41 ± 2.59 mm; mean \pm SD). To account for these differences, different grazer population sizes were used (50 individuals for *A. chierighinii*, 20 individuals for *D. spiniventris* and *Gammarus* spp.). These populations respectively correspond to amphipod densities of 707 and 283 ind.m⁻², and are within the range commonly encountered in Calvi bay (87–1028 ind.m⁻²; STURARO et al. 2015). In all cases, only individuals that could clearly be identified as adults were selected.

Amphipods were added to the corresponding microcosms on 9 June 2009 for one replicate of each treatment, and on 10 June 2009 for the other replicate. During the course of the experiment, maintenance dives were performed twice a week to ensure the metal stakes remained in place, and to gently scrub off the epiphytes that developed

on the microcosm mesh with a soft brush. The experiment ended after 21 days. At this stage, all *P. oceanica* shoots were cut at the rhizome level, and the microcosms were brought back to the laboratory unopened for processing.

Each seagrass shoot ($n = 7$ to 11, according to the microcosm) was processed separately. *P. oceanica* leaves were checked for grazing marks, and their epiphytes were scraped under a binocular microscope, using a scalpel blade. They were separated into four functional groups according to LEPOINT et al. (2007): erect algae (also referred to as “erect epiflora”), encrusting algae (= “encrusting epiflora”), erect animals (= “erect epifauna”) and encrusting animals (= “encrusting epifauna”). Seagrass tissues, epiphytes and grazers were oven-dried at 60°C for 72 h, and their biomass was subsequently determined using an analytical balance (AX105 DeltaRange, Mettler-Toledo, Greifensee, Switzerland). Reproducibility range of successive weighings was ± 0.04 mg.

The basal portions (first 5 cm) of each seagrass leaf blade were cut. All leaf fragments originating from the same shoot were grouped together and ground to a homogeneous powder. Carbon and nitrogen contents of seagrass leaves were determined using a NA1500 elemental analyzer (Carlo Erba, Milano, Italy). Glycine (Merck, Darmstadt, Germany) was used as a standard for elemental contents measures. Analytical precision was 2% of the relative content of samples (i.e. 0.6% for a sample containing 30% of a given element). C/N ratios were calculated using relative organic C and N contents, both expressed in percentage of total dry mass.

Inter-treatment differences of measured parameters were tested using analysis of variance followed by multiple comparison procedures. Since Shapiro-Wilk normality tests revealed that several datasets did not follow a Gaussian distribution, data were log-transformed. Individual shoot measurements were analyzed through nested 1-way ANOVA using “treatment” as a fixed factor and “microcosm” as a random

factor nested within treatment. When differences among treatments were present, they were explored using Tukey’s HSD post-hoc test. All statistical analyses were conducted using JMP 9.0.0 (SAS Software, Cary, U.S.A.).

RESULTS

Survival rate was low for *Apherusa chieraghinii* (18%; final grazer density 127 ind.m⁻²), but much higher for *Dexamine spiniventris* (80%; final grazer density 226 ind.m⁻²). It was 115% in *Gammarus* spp. (final grazer density 325 ind.m⁻²), suggesting that animals reproduced over the course of the experiment. All microcosms, including control treatments, were contaminated with non-amphipod invertebrates (gastropods or copepod crustaceans), indicating that the defaunation step may not have been sufficient. However, biomass of these undesired animals was always low (less than 5% of amphipod grazer biomass) and was comparable across treatments. It was therefore assumed that their impact was negligible in regard to changes

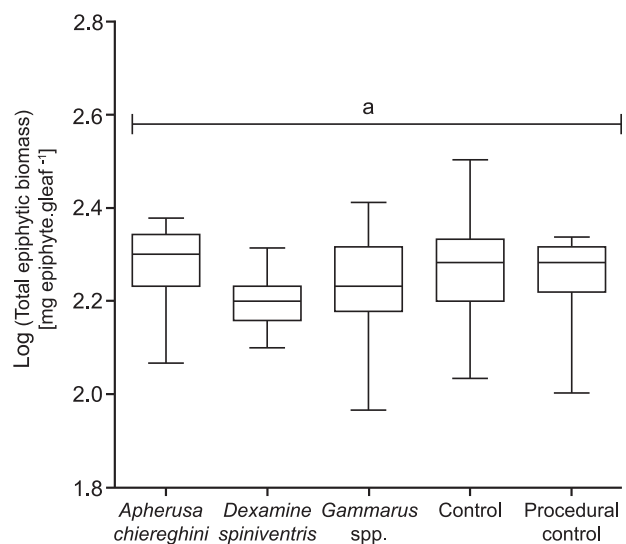


Fig. 1. – Biomass of total epiphytes in each treatment at the end of the grazing experiment, expressed in mg of epiphytes per gram of *Posidonia oceanica* leaf. Central black bars represent medians, box limits are upper and lower quartiles, and error bars represent the full range of the data (minimum-maximum). Different letters indicate statistically different groups (1-way ANOVA & Tukey’s HSD post-hoc test, $p < 0.05$).

caused by introduced amphipods. No unplanned amphipod grazers were observed.

At the end of the experiment, the total biomass of epiphytes present on *Posidonia oceanica* leaves (Fig. 1) was similar across treatments (1-way ANOVA, $F_{4,73} = 1.70$, $p = 0.3167$), suggesting presence of grazers had no significant effect on the epiphytic community as a whole. However, functional group-specific trends were present (Fig. 2). Grazer presence had no effect on encrusting algae biomass (Fig. 2a; 1-way ANOVA, $F_{4,73} = 1.60$, $p = 0.3489$), nor on encrusting animals biomass (Fig. 2b; 1-way ANOVA, $F_{4,73} = 0.57$, $p = 0.6993$). On the other hand, biomass of erect algae (Fig. 2c)

differed across treatments (1-way ANOVA, $F_{4,73} = 41.38$, $p = 0.0032$). It was significantly lower in all grazed treatments than in the “control” and “procedural control” ones (Tukey’s HSD post-hoc test, $p < 0.05$ in each case; Fig. 2c). The situation was similar for erect epifauna (Fig. 2d), whose biomass tended to be lower when amphipods were present (1-way ANOVA, $F_{4,73} = 64.36$, $p = 0.0008$). As for erect epiflora, this trend was significant for all three grazed treatments (Tukey’s HSD post-hoc test, $p < 0.05$ in each case; Fig. 2d).

No seagrass grazing seemed to occur in any of the amphipod-containing microcosms, as no grazing marks or other damage to seagrass

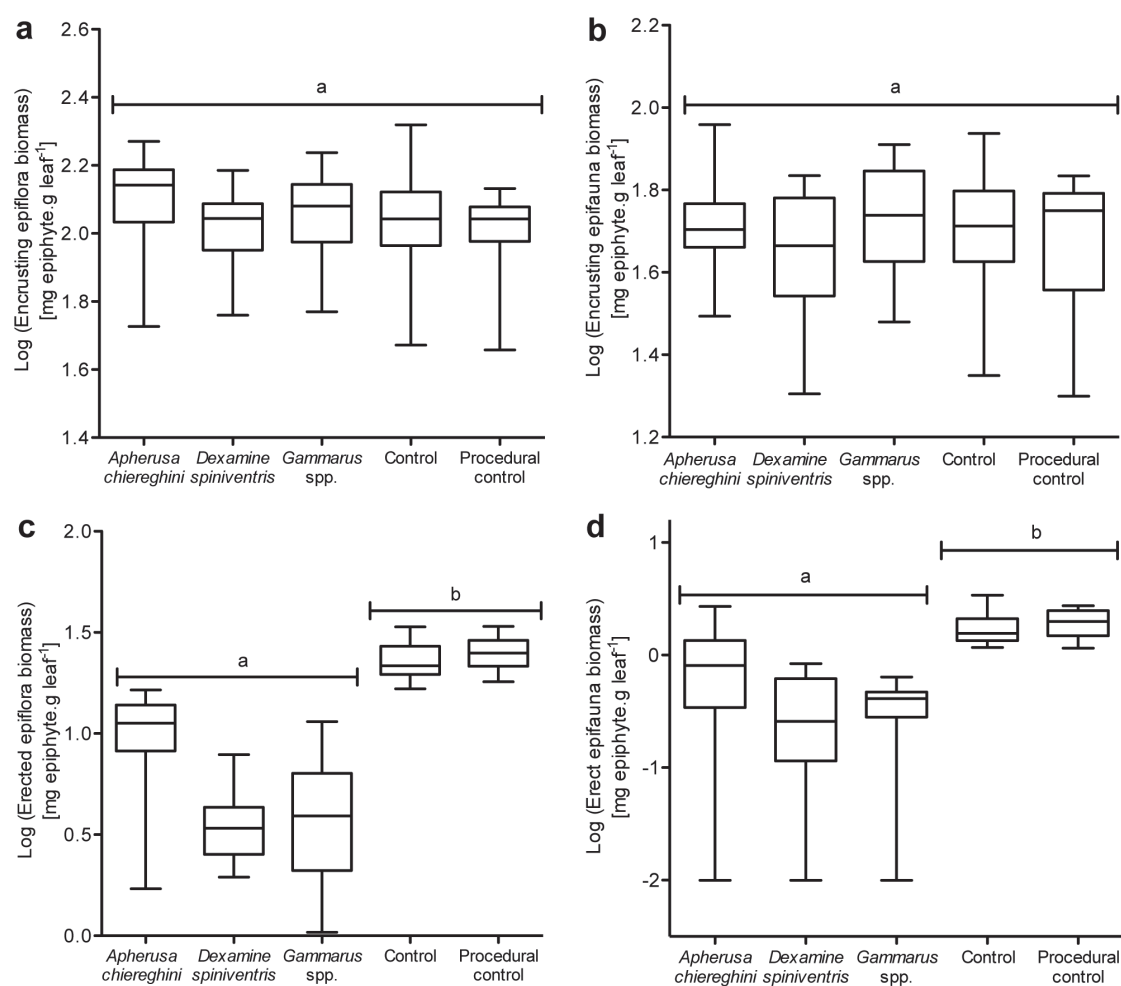


Fig. 2. – Biomass of (a) encrusting algae, (b) encrusting animals, (c) erect algae and (d) erect animals in each treatment at the end of the grazing experiment, expressed in mg of epiphytes per gram of *Posidonia oceanica* leaf. Central black bars represent medians, box limits are upper and lower quartiles, and error bars represent the full range of the data (minimum-maximum). Different letters indicate statistically different groups (1-way ANOVA & Tukey’s HSD post-hoc test, $p < 0.05$).

leaves were noted. Grazer presence had an effect on the C/N ratio of *P. oceanica* leaves (1-way ANOVA, $F_{4,73} = 1041.46$, $p < 0.0001$; Fig. 3). It was significantly lower in treatments containing grazers than in both control conditions (control and procedural control; Tukey's HSD post-hoc test, $p < 0.05$ in each case). These lower C/N ratios were linked with higher N content of seagrass leaves, as carbon content was similar in all treatments (data not shown).

No significant effect of the "microcosm [treatment]" factor was detected for any of the performed comparisons (Tukey's HSD post-hoc test, $p > 0.05$ in each case), indicating that none of the analyzed parameters varied across the two microcosms of a single treatment.

DISCUSSION

Amphipods from *Posidonia oceanica* meadows had inconspicuous effects on their host's epiphytic cover. While no effects on total biomass of the epiphytic community, or on the one of crustose morphotypes were seen, the standing stocks of

erect epiphytes were lower in the presence of any of the three grazer taxa. This was the case for algae but also for sessile animals. Depletion of epiphytic micro- or macroalgae by amphipods occurs in a number of temperate and subtropical seagrass systems. Experimental discrepancies, alongside differences in biology and life history of amphipods, result in the scattering of amphipod grazing impacts over a broad spectrum (HUGHES et al. 2004). Strong, marked effects are common. In some cases, exclusion of amphipods can cause an increase of over 400% of epiphytic biomass (e.g. CAINE 1980; WHALEN et al. 2013). In this study, impacts were less drastic, as amphipods consumed 50 to 90% of erect algal biomass. This effect is nonetheless more marked than those recorded for other species in different meadows, where amphipods can have moderate and/or low effects on epiphytic abundance (see JASCHINSKI & SOMMER 2008; COOK et al. 2011). Consumption of sessile animals by amphipods, although apparently less generalized, also occurs in other systems. Amphipod grazers from *Zostera marina* meadows feed on erect bryozoans and tunicates, but do not seem to consume the crustose species (DUFFY & HARVILICZ 2001; DOUGLASS et al. 2007). Several of these amphipod taxa can also prey on juvenile bay scallops (*Argopecten irradians*) during their early life stages, when they live on the *Z. marina* blades (LEFCHECK et al. 2014).

None of the amphipod grazers seemed to consume encrusting epiphytes. This is consistent with widely observed trends of resistance of crustose algae to herbivory (POORE et al. 2012). Here, it could be linked with the feeding mechanism of the studied amphipods. All three taxa, like most herbivorous amphipods, use the typical feeding mode of gammarid amphipods. It involves cutting fragments through an initial bite from the mandible's incisor process before triturating and crushing them with the mandibular molar process. Food pieces are then gathered and brought to the mouth for ingestion (BELLAN-SANTINI 1999). Crustose morphotypes are not easily accessible to this type of feeding, and amphipods might therefore

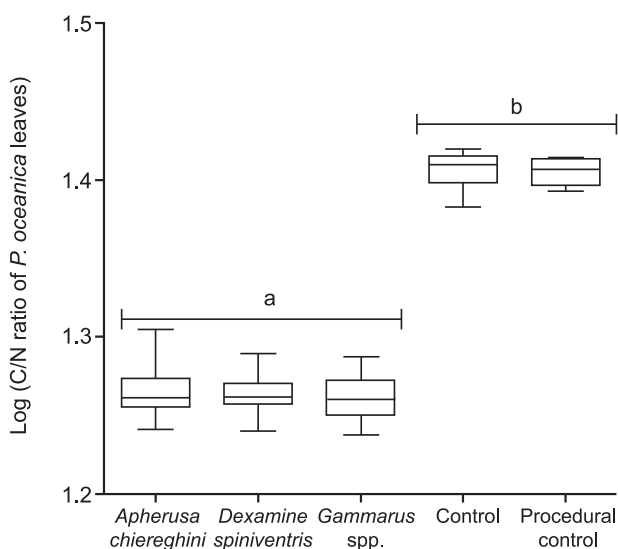


Fig. 3. – C to N ratio of *Posidonia oceanica* leaves in each treatment at the end of the grazing experiment. Central black bars represent medians, box limits are upper and lower quartiles, and error bars represent the full range of the data (minimum-maximum). Different letters indicate statistically different groups (1-way ANOVA & Tukey's HSD post-hoc test, $p < 0.05$).

simply be unable to consume them. Preferential consumption of erect epiphytes has important implications for the role of amphipod grazers in *P. oceanica* meadows. Their selective grazing pressure may be one of the processes involved in the structuring of the epiphytic cover of seagrass leaves. Discriminatory removal of certain taxa through grazing can indeed relieve the non-consumed species from competition for space, nutrients and/or light, and therefore allow their development and in turn modify the whole epiphytic community structure (JERNAKOFF et al. 1996; JASCHINSKI et al. 2010). On *P. oceanica* leaves, epiphytic biomass is at its lowest in winter. Organisms start to grow during spring. The fast-growing erect brown algae typically dominate the community in spring and early summer (May/June). Crustose epiphytes, such as red coralline algae, are present all year round, but become more and more abundant as the epiphytic cover develops. They are the dominant organisms in late summer, when epiphytic coverage and specific diversity are maximal (MAZZELLA et al. 1989; CEBRIAN et al. 1999; LEPOINT et al. 2000). Amphipods could play a part in this process. By grazing on erect algae, they could limit their biomass, and indirectly favor growth of crustose algae. In doing so, they would participate in the balance between the two epiphytic morphotypes, and allow the epiphytic community to fully develop, and reach its maximal diversity.

Amphipods are not the only mesograzers to impact epiphytic communities in Neptune grass meadows. Gastropods can indeed consume 54 to 70% of the total epiphytic biomass present on *P. oceanica* leaves (GACIA et al. 2009). Moreover, in *P. oceanica* meadows, the studied amphipods only consume macroepiphytes (MICHEL et al. in press) and only feed on erect morphotypes, while gastropods can use their radula to scrape the surface of the leaves and consume microepiphytes (mostly diatoms and bacteria; PEDUZZI 1987; MAZZELLA & RUSSO 1989; GACIA et al. 2009) and, to a lesser extent, crustose macroepiphytes (MAZZELLA & RUSSO 1989). The complementarity of feeding modes could lead to synergetic effects of these two

grazer taxa on the epiphytic communities, as biodiversity of grazer assemblages can, through horizontal interactions, modulate their influence on other compartments of the ecosystem. (DUFFY et al. 2001; DUFFY et al. 2003).

C/N ratios of basal portions of *P. oceanica* leaves were significantly lower in all grazed treatments. This was caused by a generalized trend towards N enrichment of growing host tissues when grazers were present. This enrichment could simply be an indirect effect of epiphyte consumption. Since epiphytic biomass decreases through grazing, nitrogen availability would be higher for the surviving organisms, leading to an apparent concentration effect. However, since leaf biomass exceeds by far erect macroalgae biomass, it is more likely that other, non-exclusive phenomena occur concomitantly. Grazing activity itself may directly enhance N cycling by processes such as excretion (fecal pellets and NH_4^+) and/or sloppy feeding. Excretion of either sessile (e.g. bryozoans; HURD et al. 1994) or vagile (BRACKEN et al. 2007) invertebrates can cause N enrichment in tissues of host seaweeds. In *Zostera marina* meadows, slow-moving gastropods can enhance N content of primary producers, while amphipod and isopod mesograzers fail to do so (JASCHINSKI & SOMMER 2010). This suggests that enrichment could only occur in the case of a tight association with seagrass leaves, and that dispersal and dilution of waste products would limit the fertilization effect in the case of highly motile and free-swimming crustaceans (JASCHINSKI & SOMMER 2010). Our results disagree with this hypothesis. The widely different general N availability in the two systems probably explains most of this difference. The Mediterranean Sea in general, and Calvi Bay in particular, are oligotrophic areas (LEPOINT et al. 2004), where plant growth can be limited by nutrient scarcity. Increase of nutrient supply through grazing could be more crucial there than in *Z. marina* meadows of the Baltic, and therefore cause stronger and more marked effects.

Nutrient additions have contrasting impacts on seagrass production (HUGHES et al. 2004). Since epiphytes are often able to use these nutrients more efficiently (higher uptake and growth rates) than the seagrass itself (LEPOINT et al. 2007), they tend to outgrow the seagrass, and can lead to seagrass death in some situations (BOROWITZKA et al. 2006). However, under top-down control of epiphytic growth by mesograzers, this effect is suppressed, and enhanced nutrient availability can have positive effect on seagrass production (HAYS 2005). Growth of *P. oceanica* can be enhanced by in situ nutrient fertilization (ALCOVERRO et al. 1997). In Calvi Bay meadows, low nutrient availability and constant grazing of fast-growing erect epiphytes by amphipods suggest that N enrichment could have a positive effect on seagrass growth.

Contrary to other grazer groups, crustaceans globally benefit seagrasses (POORE et al. 2012). However, the interaction between crustaceans and seagrasses can turn antagonistic. Some taxa (idoteid isopods, amphipod amphipods) graze directly on seagrass tissues when alternative food supplies are low (VALENTINE & DUFFY 2006). During our experiment, no grazing marks were observed. Moreover, under natural conditions, none of the dominant amphipods of *P. oceanica* meadows feed on their seagrass host (MICHEL et al. in press). The interaction has therefore no reason to become negative. Instead, amphipod mesograzers have two indirect, putatively positive effects on their seagrass host's production. First, through their feeding activity, they may release Neptune grass from competition for nutrients and/or light with faster-growing erect epiphytes. Second, through excretion and/or sloppy feeding, they may enhance nutrient cycling, and in turn boost seagrass production. The ecological interaction between *P. oceanica* and amphipod grazers could therefore be seen as a facultative mutualistic relationship, where amphipods would keep biomasses of fast-growing erect algal competitors at acceptable levels and supply nutrient for host growth, while the seagrass would provide trophic resources for amphipods, as well as a substratum and a shelter from predation (VALENTINE & DUFFY 2006).

Functional interactions among the seagrass/epiphyte/grazer system form a complex and entangled network, where multiple factors can directly or indirectly influence plant and animal components (JERNAKOFF et al. 1996). Unraveling the elaborate interactions between Neptune grass, epiphytes growing on its leaves and mesograzers inhabiting its meadows is a complicated task, and requires further work on many aspects. This study nevertheless presented results that constitute, to the best of our knowledge, the first direct, experimental evidence of the importance of amphipod grazers in tropho-functional relationships among *Posidonia oceanica* meadows. For this reason, it provides another step towards a better comprehension of this complex, pivotal, yet critically endangered, ecosystem.

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