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Epiphyte presence and seagrass species identity influence rates of herbivory in Mediterranean seagrass meadows

Candela Marco-Méndez [a, b, *], Luis Miguel Ferrero-Vicente [a, b], Patricia Prado [c],
Kenneth L. Heck [d, e], Just Cebrián [d, e], Jose Luis Sánchez-Lizaso [a]

[a] Department of Marine Science and Applied Biology, University of Alicante, Carretera San Vicente del Raspeig s/n − 03690, Alicante, Spain
[b] Research Marine Centre of Santa Pola (CIMAR), Santa Pola City Council − University of Alicante, Spain
[c] IRTA. Aquatic Ecosystems, Sant Carles de la Ràpita. Ctra. Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain
[d] Dauphin Island Sea Laboratory, 101 Bienville Boulevard, Dauphin Island, AL 36528, USA
[e] Department of Marine Sciences, University of South Alabama, Mobile, AL 36688, USA

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ABSTRACT

Herbivory on Mediterranean seagrass species is generally low compared to consumption of some other temperate and tropical species of seagrasses. In this study we: (1) investigate the feeding preference of the two dominant Mediterranean seagrass herbivores, the sea urchin *Paracentrotus lividus* and the fish *Sarpa salpa,* on *Posidonia oceanica* and *Cymodocea nodosa* and (2) elucidate the role of epiphytes in herbivore choices. We assessed consumption rates by tethering seagrass shoots, and preferences by food choice experiments with the following paired combinations: 1) Epiphytized leaves of both *C. nodosa* vs. *P. oceanica* (CE vs PE); 2) Non-epiphytized leaves of *C. nodosa* vs. *P. oceanica* (CNE vs. PNE); 3) Epiphytized vs non-epiphytized leaves of *C. nodosa* (CE vs. CNE) and 4) Epiphytized vs non-epiphytized leaves of *P. oceanica* (PE vs PNE). We found that preference for *C. nodosa* was weak for *S. salpa*, but strong for *P. lividus*, the species responsible for most consumption at our study. Overall both herbivores showed preference for epiphytized leaves. The higher nutritional quality of *C. nodosa* leaves and epiphytes together with the high coverage and diversity of the epiphyte community found on its leaves help explain the higher levels of herbivory recorded on epiphyted leaves of *C. nodosa*. Other factors such as seagrass accessibility, herbivore mobility and size, and behavioral responses to predation risks, may also affect the intensity of seagrass herbivory, and studies addressing the interactions with these factors are needed to improve our understanding of the nature, extent and implications of herbivory in coastal ecosystems.

1. Introduction

Herbivory on seagrasses has been often thought to remove only a modest amount of leaf production (by approx. 10–15 %, Den Hartog, 1970; Thayer et al., 1984). However prior estimates of leaf consumption rates that were assessed using indirect methods, such as quantifying herbivore bite marks, are now known to underestimate seagrass consumption (e.g. Cebrián et al., 1996a) compared to the less frequently-used estimates provided by tethering experiments (e.g. Tomas et al., 2005a; Prado et al., 2007).

In the Mediterranean, seagrass meadows are dominated by *Posidonia oceanica* (L.) Delile (Prado et al., 2011), while *Cymodocea nodosa* (Ucria) Ascherson is commonly found in small patches within these meadows (Pérès and Picard, 1964). Herbivory rates on these seagrass species are generally low in comparison to some other seagrass species, although they may vary substantially (e.g., 2–57 % of *P. oceanica* leaf productivity, Cebrián et al., 1996a; Prado et al., 2007; 1–50 % of *C. nodosa* leaf productivity, Cebrián et al., 1996b). Although a few studies have used direct methods to estimate rates of seagrass herbivory (e.g. Kirsch et al., 2002; Tomas et al., 2005a; Prado et al., 2007), further reassessment of herbivory rates by direct methods is needed to evaluate the importance of herbivory for the ecological functioning of Mediterranean systems.

The sea urchin *Paracentrotus lividus* (Lam.) and the fish *Sarpa salpa* (L.) are the two main macroherbivores in the Western

* Corresponding author. Department of Marine Science and Applied Biology, University of Alicante, Carretera San Vicente del Raspeig s/n − 03690, Alicante, Spain.
E-mail address: candela.marco@ua.es (C. Marco-Méndez).

Mediterranean, and are commonly observed in shallow seagrass meadows and rocky bottoms (Verlaque, 1990). Prado et al. (2007) found that *S. salpa* accounted for 70% of the total leaf consumption by herbivores (approx. 40% of leaf production) with *P. lividus* accounting for the remaining 30% (approx. 17% of leaf production; Prado et al., 2007) in *Posidonia oceanica* meadows, although herbivory intensity can vary largely through space and time (Cebrián et al., 1996a,b; Prado et al., 2007, 2010; Steele et al., 2014). Factors such as plant availability and accessibility, plant nutritional quality, human pressure on herbivore populations, and herbivore recruitment and predation risk have all been shown to influence the intensity of herbivory on seagrasses meadows (Prado et al., 2008a, 2009, 2010). In addition, it has been shown that as many terrestrial plants and marine algae, seagrasses chemically deter herbivores using secondary metabolites, although inhibition varies among consumers (Vergés et al., 2007, 2011). Therefore seagrass−herbivore interactions are further complicated and studies are needed to improve our knowledge of the mechanisms controlling feeding decisions and consumption rates of major herbivores in seagrass ecosystems (Heck and Valentine, 2006).

The abundance of the sea urchin *Paracentrotus lividus* in shallow habitats seem to be affected by refuge availability of predation refuges, with the highest densities recorded in rocky habitats (>50 to 100 ind m^{-2}) (Delmas and Régis, 1986; Delmas, 1992). In seagrass beds, *P. lividus* abundances are commonly higher in *Posidonia oceanica* (2−3 ind·m^{-2}; Boudouresque and Verlaque, 2001, 2013) than in *Cymodocea nodosa* (0.7 ind·m^{-2}; Fernandez and Boudouresque, 1997), which may be due to differing predation pressure (Traer, 1980), and/or to reduced meadow recruitment in the absence of significant rhizome structural complexity (Prado et al., 2009). Regarding *Sarpa salpa,* studies have shown similar densities in *P. oceanica* meadows (2.5 ind·m^{-2}; Guidetti, 2000; Guidetti and Bussotti, 2000, 2002) and rocky habitats (2.3 ind·m^{-2}; Guidetti, 2000), but lower densities appear to happen in *C. nodosa* meadows (0.22 ind·m^{-2}; Guidetti and Bussotti, 2000, 2002).

Feeding behavioral patterns and food preferences can also differ between both herbivores (Prado et al., 2011) causing different impacts on seagrass meadows. *S. salpa* potentially feeds on a wide range of macroalgae and seagrasses (Christensen, 1978; Havelange et al., 1997; Stergiou and Karpouzi, 2001) and may aim at maintaining a diverse diet to achieve the required nutrients (Goldenberg and Erzini, 2014). Factors such as seagrass abundance, habitat heterogeneity and patterns of movement are known to influence the grazing patterns of *S. salpa* (Prado et al., 2008a, 2011). In contrast, marine invertebrates living on the sea bottom, such the sea urchin *Paracentrotus lividus,* are less mobile but also have a diet commonly based on macroalgae and seagrasses although they are sometimes influenced by the limitation of food resources (Fernandez, 1990; Mazzella et al., 1992; Boudouresque and Verlaque, 2001, 2013).

Preferences and higher feeding rates of marine herbivores on diets with high nitrogen and protein content or with low amounts of structural components have been reported by some studies (Mariani and Alcoverro, 1999; Goecker et al., 2005), suggesting that herbivores will maximize the consumption of food items with higher nutritional and energy contents (Hughes, 1980). Seagrasses leaves may also have varying levels of structural carbohydrates (cellulose), which may affect food digestibility and absorption (e.g. Klumpp and Nichols, 1983). In fact, previous studies have suggested that differences in the nutritional quality among seagrass species could result in different levels of herbivory (Cebrián and Duarte, 1998; Prado et al., 2010). Epiphytes growing on the seagrass leaves have also been reported to have higher nutritional quality than leaves (e.g. Alcoverro et al., 1997a, 2000), which may lead to increased feeding selectivity by herbivores (Heck et al., 2006;

Marco-Méndez et al., 2012). Indeed, *Paracentrotus lividus* (Boudouresque and Verlaque, 2001, 2013; Tomas et al., 2005b) and *Sarpa salpa* (Verlaque, 1981, 1985) preferentially feed on the epiphytic flora of *Posidonia oceanica* leaves. These studies also suggested that not only the nutritional quality but also the composition of the epiphytic assemblages can strongly influence herbivore consumption through changes in the abundance of certain taxa or morphological groups (Thacker et al.,. 2001; Prado et al., 2008b; Marco-Méndez et al., 2012).

In this context, the aims of this study were to investigate the intensity of herbivory and food preferences by *Paracentrotus lividus* and *Sarpa salpa*, in a mixed seagrass-rocky habitat with the presence of *Posidonia oceanica* and *Cymodocea nodosa* in the Western Mediterranean and to elucidate the mediating role of epiphytes in determining herbivore choices. Both food choices and tethering experiments were carried out and were complemented with feeding behavioral observations in the field. In addition, the contribution of each food source to the diet was estimated by gut contents analyses and stable isotopes. Nutrient contents and epiphytic community analyses of the two seagrass species were investigated as potential explanatory variables for herbivore behavior. The results will contribute to further our understanding on the feeding behavior of these two herbivores as well as their role on the trophic functioning of Mediterranean seagrass beds.

2. Material and methods

2.1. Study site

The study site was located at Cabo de las Huertas (38° 21.264′N; 0° 24.207′W; Alicante, Spain; Fig 1). The site features a mixed seagrass-rocky habitat (depth range: 2−5 m; covered area: ~0.30 km^2) composed by inter-mixed small patches (of variable size) of seagrasses, macroalgae, unvegetated sandy substrate and unvegetated rocky substrate. The work was conducted in late summer, when epiphyte biomass show maximum values and herbivore pressure (as frequency of attack marks on leaves) by *Sarpa salpa* and *Paracentrotus lividus* is presumed to be greatest (Alcoverro et al., 1997b).

Bottom cover in the mixed seagrass-rocky habitat of study during this period was: 17 ± 3% *Caulerpa. prolifera;* 11 ± 3% *Posidonia oceanica*, 10 ± 2% *Cymodocea nodosa*, 7 ± 3% unvegetated sandy substrate; 4 ± 1% *Padina pavonica* and the remaining 51 ± 4% rocky substrate, which appears unvegetated or with occasional presence of coralline red macroalgae such as maërl (*Lithothamnion corallioides* and *Phymatolithon calcareum)* and *Jania rubens*. Seagrass shoot density was 2071 ± 291 shoots m^{-2} for *C. nodosa* and 584 ± 29 shoots m^{-2} for *P. oceanica* (Mean ± SE).
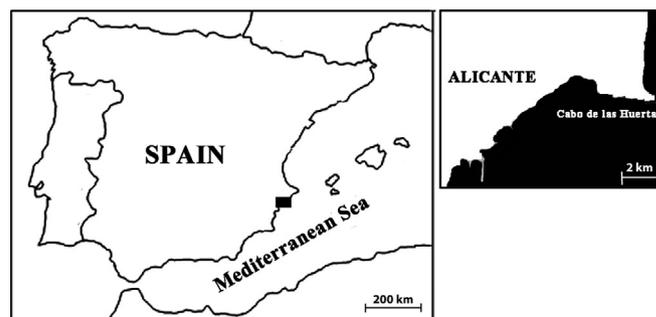

Fig. 1. Map of the study area, Cabo de las Huertas (Alicante, Spain).

2.2. Tethering experiments

Consumption rates of *Cymodocea nodosa* and *Posidonia oceanica* leaves were estimated with tethering experiments in the mixed seagrass-rocky habitat. We deployed one tethering line for each seagrass species within monospecific seagrass patches. Each tethering line consisted of 20 replicates, with one replicate corresponding to one 3-leaf shoot. Prior to deployment leaves were cut down to the same length to remove previous herbivore marks, their area measured, and tissues hole-punched at the base of the leaf to allow estimates of any growth during the experiment (Prado et al., 2007). The shoots were attached to pins using cable ties, and deployed in the field for a week by securing the pins to the bottom and tying them to a thin rope. After this period, shoots were collected and leaf area lost to urchins and *Sarpa salpa* consumption (which were different in the shape of scars produced by each species; Boudouresque and Meisnez, 1982) was measured to estimate consumption rates (cm^2 leaf area consumed per day).

2.3. Herbivore abundance and feeding observations

Sea urchin abundance was counted in 40 cm × 40 cm quadrats randomly deployed within the mixed seagrass-rocky habitat of study (n = 20). We further investigated feeding items being consumed by sea urchins by randomly collecting individuals (n = 86) from the mixed seagrass-rocky habitat and noting the resources attached to their oral side, that contained bite marks (e.g., *Posidonia oceanica*, *Cymodocea nodosa*, *Jania rubens* and *C. prolifera*). Individuals of *Sarpa salpa* were counted within the study area by divers using visual census within 50 m^2 transects (n = 18). In each transect we recorded number of individuals within a school of fish, the average size of individuals and the food items consumed when feeding.

2.4. Food choice experiments

Paired food choice experiments were conducted for both *Paracentrotus lividus* and *Sarpa salpa*. A total of 4 different food choice experiments was carried out: 1) *Cymodocea nodosa* epiphytized (CE) vs. *P. oceanica* epiphytized (PE); 2) *C. nodosa* non-epiphytized (CNE) vs. *P. oceanica* non-epiphytized (PNE); 3) *C. nodosa* epiphytized (CE) vs. *C. nodosa* non-epiphytized (CNE); and 4) *P. oceanica* epiphytized (PE) vs. *P. oceanica* non-epiphytized (PNE). For sea urchins, six 50 cm × 50 cm × 50 cm cages made of PVC and plastic mesh were haphazardly deployed on an unvegetated patch within the study area (2–3 m depth), each containing 3 sea urchin individuals and 3 seagrass replicates (n = 18). Each replicate of the different paired combinations tested was constituted by two different shoots, one of each food choice (e.g. CE vs PE), tied together. All sea urchins were starved during 24 h prior to being used in the experiment. All individuals used were mature (test diameter > 30 mm; Serafy, 1979), with approx. 50 mm test diameter, and were replaced with new individuals at the end of each paired food preference test. For *S. salpa*, we deployed tethering lines containing the paired combinations within an unvegetated patch (n = 18). Food choice experiments ran for four days and after this period, samples were collected and transported to the laboratory. Area lost from the tethers was processed in the same way as for the other tethering experiments.

2.5. Gut contents, stable isotope analyses and nutrient contents

Individuals of *Paracentrotus lividus* (test diameter without spines: 4.8 ± 0.1 cm) and *Sarpa salpa* (total length: 22.9 ± 0.6 cm) were collected randomly from the mixed segrass-rocky habitat of study for gut contents (n = 10), nutrient content (n = 5) and stable isotope analyses (SIA, n = 5). In the laboratory, the Aristotle's lantern in sea urchins and lateral muscle in fish were isolated for SIA and nutrient content analysis. Gut contents were extracted and food items separated under the microscope (i.e. *P. oceanica* green leaves, *P. oceanica* detritus, *C. prolifera*, *Jania rubens*, other macroalgae, and fauna). Each fraction was dried to constant mass at 60 °C.

Shoots of *Posidonia oceanica* and *Cymodocea nodosa* were randomly collected for SIA and nutrient content analysis. These samples included *P. oceanica* and *C. nodosa* epiphytized leaves, epiphyte unscraped (PE and CE) and non-epiphytized leaves, scraped free of epiphytes (PNE and CNE; n = 5), as well as their respective epiphytes (EC and EP; n = 5) which included both epifauna (heterotrophic metazoans) and epiflora (macroalgae). Samples were dried to constant mass at 60 °C and grounded to fine powder for determination of nutrient contents (C: N) and isotopic signatures (δ^{15}N and δ^{13}C). Analyses were carried out with an EA-IRMS (Thermo Finnigan) analyzer in continuous flow configuration at the Technical Unit of Instrumental Analyses (University of La Coruña). The average difference in isotopic composition between the sample and reference material ($\delta_{\text{sample-standard}}$, expressed in ‰) corresponds to:

$$[(R \text{ sample} - R \text{ standard})/R \text{ standard}] \times 1000 = \delta_{\text{sample-standard}}$$

where *R* sample is the ^{13}C/^{12}C or ^{15}N/^{14}N ratio in the sample; *R* standard is the ^{13}C/^{12}C or ^{15}N/^{14}N ratio for the reference material (i.e. CaCO$_3$ from belemnite (PBD) for δ^{13}C and atmospheric nitrogen for δ^{15}N measurements) calibrated against an internal standard (i.e. Atropina, IAEA and/or UGS).

2.6. Epiphytic community

For epiphytic community analysis we collected the oldest leaves on shoots of *Posidonia oceanica* and *Cymodocea nodosa* (n = 8), which includes the epiphyte community accumulated during the entire life span of the leaf (Cebrián et al., 1999; Prado et al., 2008b). Epiphytic cover (%) of leaf surface was estimated visually, and then, organisms were scraped off gently for identification to genus level under the microscope. Finally epiphytes were dried to a constant mass at 60 °C for biomass determination.

2.7. Data analyses

Results from food choice experiments were analyzed with paired t-tests. Differences in consumption rates (i.e. tethering experiments), number of epiphytic taxa and epiphyte cover and biomass between *Posidonia oceanica* and *Cymodocea nodosa* leaves were analyzed with standard t-tests. Differences in isotopic signatures (δ^{15}N and δ^{13}C) and nutrient contents (C: N molar ratio) among food resources were tested with a one-way ANOVA with 6 levels (PE, PNE, CE, CNE, EC and EP). Student-Newman-Keuls post-hoc tests were used to single out significant groupings. ANOVA assumptions of normality and homogeneity of variance were assessed with the Kolmogorov–Smirnov and the Cochran's C-test, respectively. When assumptions were not met, the level of significance was set at 0.001 in order to reduce the possibility of committing Type I error (Underwood, 1997). Epiphytic assemblages were investigated with n-MDS ordination (presence-absence transformation, Bray–Curtis similarity index), ANOSIM and SIMPER available in the PRIMER-E v.6 software package (Clarke and Warwick, 1994, 2001).

3. Results

3.1. Herbivore densities and feeding observations

The average abundance of *Paracentrotus lividus* and *Sarpa salpa* in the mixed seagrass-rocky habitat was 19.1 ± 2.6 and 0.5 ± 0.1 ind·m^{-2}, respectively. Food items attached to the oral side of *P. lividus* indicated that 23.3% of the individuals were feeding on *Posidonia oceanica*, 15.1% on *Jania rubens*, 8.1% on *Padina pavonica*, 7% on *Cymodocea nodosa*, 3.5% on *C. racemosa var. cylindracea*, and 3.5% on *C. prolifera* at the time of collection, with the remaining 39.5% not feeding. For *S. salpa*, observations showed 44.9% of the individuals were feeding on *C. prolifera*, 41.4% on *P. oceanica*, and 2.2% on *C. nodosa*, and 11.4% were not feeding at the time of observation.

3.2. Tethering experiments

Leaf consumption rates measured with tethering experiments did not vary significantly between seagrass species (F = 4.3; t = −1.2; *df* = 34.2; p = 0.22; Fig. 2). *P. lividus* was responsible for 96% of the leaf consumption recorded for *Posidonia oceanica*, and *Sarpa salpa* only contributed 4%. All the consumption recorded for *Cymodocea nodosa* was due to *P. lividus*.

3.3. Food choice experiments

Paracentrotus lividus displayed higher consumption rates of CE vs. PE (t = −3.575, *df* = 17, p = 0.002, Fig. 3A) and CNE vs. PNE (t = −3.699, *df* = 17, p = 0.002, Fig. 3B). For both seagrass species, consumption was also higher in the presence of epiphytes (CE vs. CNE: t = −3.660, *df* = 17, p = 0.002, Fig. 3C; PE vs. PNE: t = 2.563, *df* = 17, p = 0.020, Fig. 3D). The results for *Sarpa salpa* appeared qualitatively to follow a similar pattern to that observed for *P. lividus*, at least qualitatively, although significant differences were only found for the experiment CNE vs. PNE (t = 2.426; *df* = 17; p = 0.027; Fig. 3F). All other comparisons were not significantly different (CE vs. PE: t = 2.087; *df* = 17; p = 0.052; Fig. 3E; CE vs. CNE: t = 2.018; *df* = 17; p = 0.060; Fig. 3G; PE vs. PNE: t = 1.837; *df* = 17; p = 0.084; Fig. 3H).

3.4. Gut contents

Gut contents of *Paracentrotus lividus* were mostly much digested detritus which was difficult to identify, decayed and green leaves of

Fig. 2. Tethering results showing leaf loss of each seagrass species (cm^2 d^{-1}) by *P. lividus* and *S. salpa* herbivory.

Posidonia oceanica, and a small amount of algae and fauna. In contrast, gut contents of *Sarpa salpa* mostly corresponded to *P. oceanica* and *C. prolifera* with a low contribution of other algal species (Fig. 4).

3.5. Stable isotope analyses

Both isotopic signals showed significant differences among food items (One way ANOVA, p < 0.001; Fig. 5A; Table 1). The δ^{15}N values recorded for the consumers (*Sarpa salpa*: 12.9 ± 0.5‰ and *Paracentrotus lividus* 10.2 ± 0.1‰) were the highest observed. These values lay much closer to both types of epiphytes, which included both macroalgae and metazoans (EC: 6.8 ± 0.1‰, EP: 6.8 ± 0.3‰) than to epiphytized (PE: 4.5 ± 0.1‰; CE: 6.6 ± 0.1‰) and non-epiphytized leaves (PNE: 4.4 ± 0.1‰; CNE: 6.5 ± 0.1‰). The δ^{13}C signals recorded for *S. salpa* (-16.2 ± 0.4‰) and *P. lividus* (-15.8 ± 0.2‰) were also closer to epiphytes (EC: -17.1 ± 0.2‰ and EP: -15.9 ± 0.2‰) than to epiphytized (PE: -13.3 ± 0.1‰; CE: -11.0 ± 0.2‰) and non-epiphytized leaves (PNE: -13.8 ± 0.2‰; CNE: -9.6 ± 0.1‰); (Fig. 5A).

3.6. Nutrient contents in seagrass leaves and epiphytes

The two herbivores showed the lowest C: N molar ratios (*Paracentrotus lividus*: 1.07 ± 0.01; *Sarpa salpa*: 1.06 ± 0.04). For dietary items, there were significant differences among C:N molar ratios, with the highest values found for seagrass leaves and the lowest values for epiphytes (Fig. 5B; Table 1).

3.7. Epiphytic community

No significant differences were detected in leaf epiphyte biomass per unit leaf area between the two seagrass species (CE: 1.26 ± 0.22 mg DW·cm^{-2}; PE: 0.79 ± 0.03 mg DW·cm^{-2}; t = 1.805, *df* = 18, p = 0.088). However, we found significant differences in leaf epiphytic cover (t = 6.611, *df* = 14, p = 0.000) and in the number of epiphytic taxa (t = 3.608, *df* = 14, p = 0.003) between the two species, with values being higher on *Cymodocea nodosa* (67.5 ± 3.6%; 0.91 ± 0.16 taxa cm^{-2}) than in *Posidonia oceanica* (32.4 ± 3.9%; 0.32 ± 0.03 taxa cm^{-2}).

n-MDS ordination of epiphytic taxa also displayed distinctive groupings for *Cymodocea nodosa* and *Posidonia oceanica* (ANOSIM: Global R = 0.623, p = 0.001; Fig. 6). SIMPER analyses indicated an average of similarity between *C. nodosa* (CE) and *P. oceanica* (PE) of 69.49%, with *Myriactula gracilis* (Archaeplastida) *Botryllus* sp. (Metazoa) and *Ectocarpus confervoides* (Stramenopiles) contributing the most to this dissimilarity (10.06%, 8.78%, and 7.92% respectively). The average similarity among *C. nodosa* leaves was 59.33% and was mainly due to *Ceramium* sp. (Archaeplastida) and to *Myriactula gracilis* (Archaeplastida) with contributions of 28.05% and 22.64%, respectively. The average of similarity among *P. oceanica* leaves was 48.27% mainly due to *Botrillus* sp. (Metazoa) and to *Cladophora* sp. (Archaeplastida) with contributions of 17.56% and 15.84%, respectively.

4. Discussion

This study evaluated herbivory impacts in a mixed shallow meadow of *Posidonia oceanica* and *Cymodocea nodosa* using direct methods. Although *P. oceanica* has been commonly reported to be subjected to heavy herbivore pressure in shallow meadows (Prado et al., 2007, 2008a), our results showed that herbivory on *C. nodosa* is also important. *P. lividus* was responsible for the consumption of both seagrasses at our study site, which contrasts with previous studies in *P. oceanica* meadows, where herbivory was mostly due to
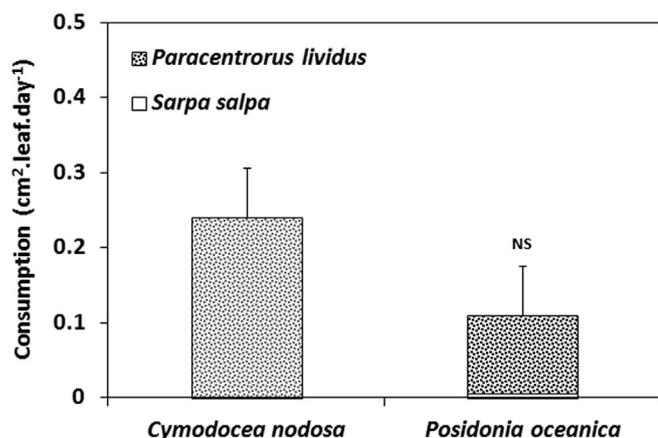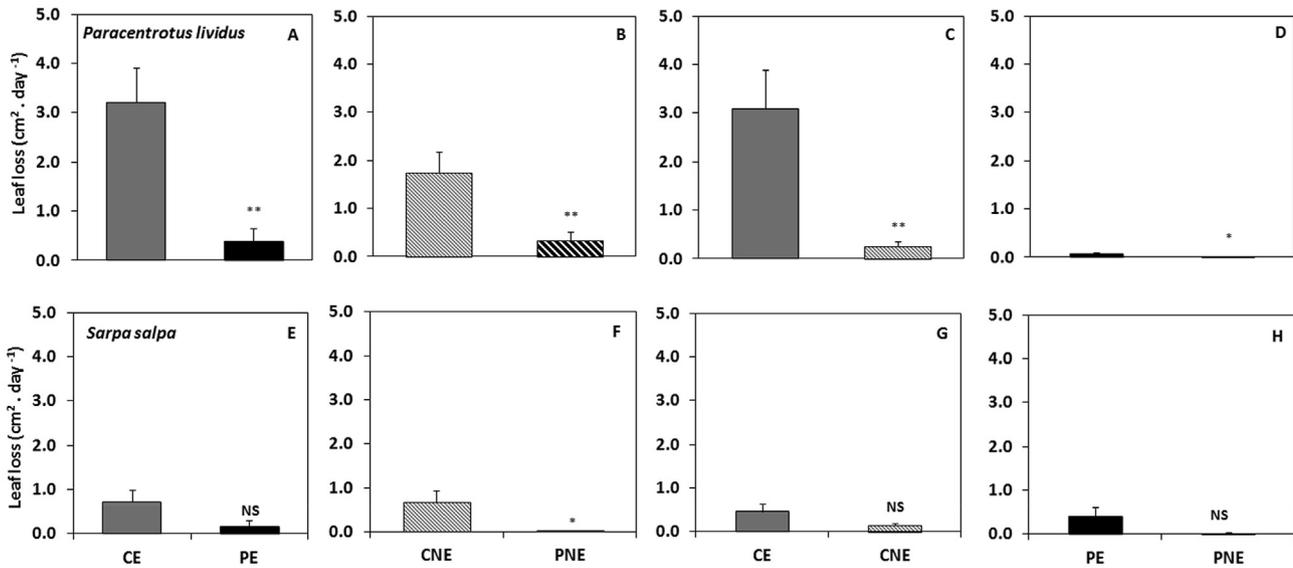
Fig. 3. Leaf loss (cm² d⁻¹) to *P. lividus* (A, B, C, D) and *S. salpa* (E, F, G, H) during paired food preference experiments. A, E. *C. nodosa* epiphytized (CE) vs. *P. oceanica* epiphytized (PE); B, F. *C. nodosa* non-epiphytized (CNE) vs. *P. oceanica* non-epiphytized (PNE); C, G. *C. nodosa* epiphytized (CE) vs. *C. nodosa* non-epiphytized (CNE); D, H. *P. oceanica* epiphytized vs. *P. oceanica* non-epiphytized. Mean ± SE. *p < 0.05; **p < 0.01; ***p < 0.001; NS = not significant results.

Sarpa salpa (~70% of total annual losses) and lesser extent to *P. lividus* (the remaining 30%) (Prado et al., 2007). This notable herbivory by *P. lividus* evidenced in our study may be explained by the high abundance of sea urchins in this mixed seagrass-rocky habitat (19 ind m⁻²) which differs notably from the abundances commonly reported in *P. oceanica* (2–3 ind m⁻²; Boudouresque and Verlaque, 2001, 2013) and *C. nodosa* meadows (approx. 0.7 ind m⁻²; see Fernandez and Boudouresque, 1997). The high availability of rocky substrate (approx. 51% of coverage) that provided shelter from predation probably influenced these densities (Ebling et al., 1966; Boudouresque and Verlaque, 2001, 2013). On the other hand, the lower *S. salpa* densities (0.49 ind m⁻²), which were more typical of *C. nodosa* mixed meadows (0.22 ind m⁻²; see Guidetti and Bussotti, 2002) than of those reported for *P. oceanica* meadows or rocky habitats (~2.5 ind m⁻²; Guidetti, 2000), explain the low observed herbivory by this species.

Food choice experiments revealed a slight preference for *Cymodocea nodosa* by *Sarpa salpa* whereas it was strongly evident for *Paracentrotus lividus*. In the presence of epiphytes, *P. lividus* showed an approx. 8 times higher consumption of *C. nodosa* than *Posidonia oceanica*, and values also remained higher when epiphytes were removed. Conversely to the general pattern (Prado et al., 2007, 2008a), *S. salpa* displayed lower consumption rates than *P. lividus* in all paired food choice tests, and experiments were only clearly conclusive about the preference for *C. nodosa* when epiphytes were removed. Although this preference for *C. nodosa* has been previously reported for *P. lividus* (Boudouresque and Verlaque, 2001, 2013) this is the first time that preference for *C. nodosa* has been reported for *S. salpa*. Yet, multiple preference experiments may be necessary to unravel food preferences of consumers. For instance, Goldenberg and Erzini (2014) conducted an aquarium experiment with adult *S. salpa* and the seagrasses *C. nodosa*, *Z. marina* and *Z. noltii*, and their results pointed to *Z. noltii* as the preferred food. For epiphytes, the preference of *P. lividus* for coated leaves confirms previous results suggesting their influence on sea urchin food-preferences and consumption rates (e.g. Greenway, 1995; Marco-Méndez et al., 2012). Yet, the consistent selectivity of both consumers for *C. nodosa* over *P. oceanica* even when epiphytes were
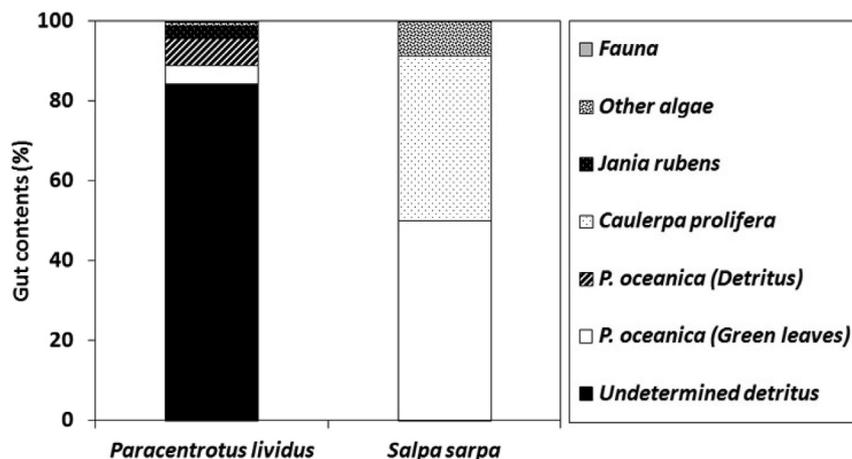

Fig. 4. Percent of the different food items found in the gut contents of *P. lividus* and *S. salpa* (%).
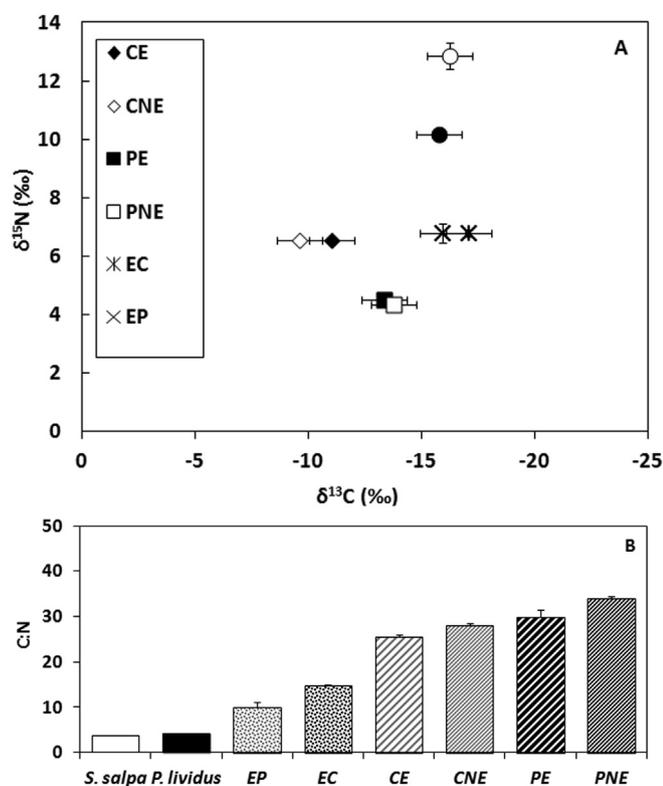
Fig. 6. n-MDS ordination of epiphyte taxa found on *C. nodosa* (CE) and *P. oceanica* leaves (PE). Mean ± SE. *p < 0.05; *p < 0.01; ***p < 0.001; NS = not significant.

Fig. 5. A. δ^{15}N and δ^{13}C signatures of consumers (*P. lividus* and *S. salpa*) and food items, including combined values for epiphytized leaves (CE, CNE, PE, PNE, EC and EP); **B**. C: N molar ratios in leaves and epiphytes. Mean ± SE. *p < 0.05; **p < 0.01; ***p < 0.001; NS = not significant results.

removed indicates that not only epiphytes but other factors inherent to plant features are also involved. Among these factors, differences between seagrasses in nutrients, chemical and structural defenses and epibiotic load, can influence herbivore preferences (Verges et al., 2007, 2011). In this study chemical and structural defences were not measured although Verges et al. (2011) showed that they can strongly influence *S. salpa* and *P. lividus* preferences.

Previous studies have also suggested that higher herbivory on *Cymodocea nodosa* over *Posidonia oceanica* could be related to higher specific growth rates which, in turn, could be due to enhanced nutritional quality in fast -growing species (Cebrián and Duarte, 1998). In our study, the higher C:N values recorded in non-epiphytized seagrass leaves of *C. nodosa* also suggests that preference could be related to the higher nutritional quality of this species. In addition, C:N ratios in both seagrass species (epiphytized and non-epiphytized leaves) were higher than in epiphytes supporting the hypothesis that the presence of epiphytes can increase
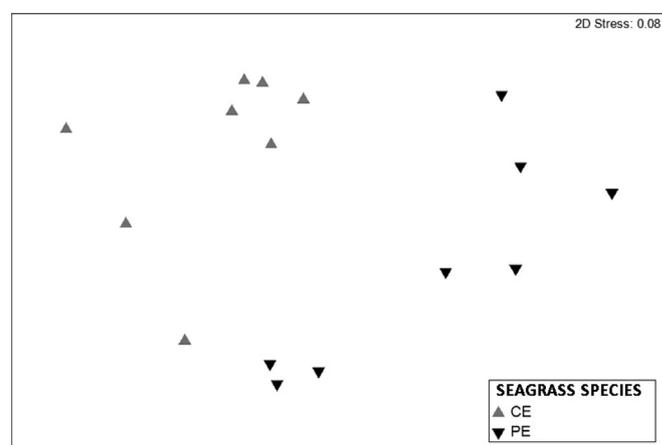
seagrass consumption rates and mediate herbivores preferences (Marco-Méndez et al., 2012) because of their higher nutritional value (e.g. Alcoverro et al., 1997b, 2000). In all, epiphytized leaves of *C. nodosa* showed higher nutritional value than epiphytized leaves of *P. oceanica* due to the higher quality of its leaves plus the increased nutritional value by the presence of epiphytes and may be involved in the observed food preferences.

In addition, the epiphytic community structure also revealed important differences between seagrass species that might have further influenced herbivore preferences. *Cymodocea nodosa* displayed a higher epiphyte coverage and mean number of epiphytic leaf taxa. Variability in epiphytic biomass and species composition between seagrass species has been indicated to be influenced by multiple factors such as light conditions (Carruthers, 1994), nutrients (Prado et al., 2008b), and grazing (Prado et al., 2007b), but given that samples were collected from the same location, differences were more likely the resulting of shoot morphology and leaf age on patterns of epiphytic colonization (Knowles and Bell, 1998; Lavery and Vanderklift, 2002). Therefore, our study confirms the role of epiphytes in mediating seagrass consumption and preferences (e.g. Greenway, 1995; Marco-Mendez et al., 2012) especially for *Paracentrotus lividus* whose diet is indicated to be greatly supported by epiphytes (Tomas et al., 2005b).

In contrast to our results from food choice experiments, which pointed to *Cymodocea nodosa* as the most consumed and preferred food, feeding observations showed that both herbivores were feeding most frequently on *Posidonia oceanica*. For *Paracentrotus lividus,* most of the individuals were hidden within rocky shelters and passively caught drift food items without venturing into the meadow (Marco-Méndez, pers. observ.). In fact, when we analyzed gut contents of *P. lividus* we found a high fraction of undetermined detritus, with low contribution of *P. oceanica* and algae, and

Table 1
One-way ANOVA showing differences in δ^{15}N and δ^{13}C signatures and C:N ratio among food items. Significant differences are indicated: *p < 0.05, **p < 0.01, ***p < 0.001, ns: no significant.

Source of variation	$^{15}\delta$N				$^{13}\delta$C				C:N			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Sample type	5	6.8429	50.07	***	5	39.864	245.82	***	5	436.2017	138.59	***
Residual	24	0.1367			24	0.1622			24	3.1475		
Total	29				29				29			
SNK	EC = EP = CE=CNE > PE=PNE				CNE > CE > PE > PNE > EP > EC				PNE > PE = CNE > CE > EC > EP			
Transformation	NT				NT				NT			

surprisingly, no presence of *C. nodosa*. Macroalgae and seagrass are known to constitute the common diet of *P. lividus*, but some authors have suggested that preference for certain taxa may be altered by the relative availability of food resources, especially when they are limited (Fernandez, 1990; Mazzella et al., 1992; Boudouresque and Verlaque, 2001, 2013). Suspended organic particles have also been reported to be part of the diet of *P. lividus* (Verlaque and Nedelec, 1983; Frantzis et al., 1988; Bulleri et al., 1999) which we suspect could account for the 'undetermined detritus' found in their gut contents. Despite the limited number of guts analyzed, both our feeding observations and gut contents analyses suggest that the sea urchin diet was conditioned by the availability of drift material and low mobility from rocky shelters. The observed behavior suggests that sea urchins prioritize staying within their shelter to searching for their preferred food (Boudouresque and Verlaque, 2001, 2013). In late summer, when the experiment was carried out, *P. oceanica* leaves detach (Mateo et al., 2003) and floating detached leaves may become more available to sea urchins and constitute an important part of their diet (Boudouresque and Verlaque et al., 2001, 2013). On the other hand, enhanced sea urchin abundances in our study area might be the result of higher availability of rocky shelters, and higher levels of urchin herbivory may be elevated by the proximity of tethers to these shelters, which made the preferred food available to individuals.

For *Sarpa salpa*, the lower herbivory rates recorded (4% of *Posidonia oceanica* and no consumption of *Cymodocea nodosa*) were in agreement with low fish abundance (0.49 ind·m^{-2}). Analyses of fish stomach contents and feeding observations also pointed to *P. oceanica* and *C. prolifera* as the most important food items. These results are coherent with previous studies reporting *S. salpa* feeding on a wide range of macroalgae and seagrasses (Christensen, 1978; Havelange et al., 1997; Stergiou and Karpouzi, 2001). The large presence of *C. prolifera* in gut contents confirms *Caulerpa* spp. as an important diet item (see also Verlaque, 1990; Tomas et al., 2011). However, these results contrast with food choice experiments pointing to *C. nodosa* as the preferred food, and suggest that other factors were also driving feeding patters. Among potential explanatory variables, seagrass abundance, habitat heterogeneity and complexity, and the type of foraging movements are known to influence grazing impacts by *S. salpa* (Prado et al., 2008a, 2011). On the one hand, fish mobility across other sites with higher abundance of *P. oceanica* and *C. prolifera* within its home range (approx. 1 ha according to Jadot et al., 2002), may explain enhanced presence of these species within gut contents. On the other, given the similar availability of both seagrass species at the study site and the low fish abundance in the area, the slightly higher consumption of *P. oceanica* tethers might be just due to the arrival of a single school of fish, and detecting differences in consumption rates between seagrass species might have required longer than a week period.

In conclusion, preference for *Cymodocea nodosa* was weak for *Sarpa salpa* but strongly evident for *Paracentrotus lividus* which was primarily responsible for the consumption of both seagrasses. Preference for leaves coated by epiphytes vs. leaves without epiphytes, as well as their higher nutritional quality (e.g. Alcoverro et al., 1997b, 2000) confirms previous results that epiphytes strongly influence consumption rates and preferences (e.g. Marco-Méndez et al., 2012) because of their higher nutritional value. The higher nutritional quality of *C. nodosa* and the higher coverage and number of epiphytic taxa on its leaves appears to explain the higher herbivory of this species, at least for *P. lividus*. Our study also indicated the complexity of seagrass—herbivore interactions and suggested that final seagrass consumption rates are not only determined by food preferences, but also by factors that could influence herbivore behavior by changing their priorities such as predation risk and/or home-range mobility.

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