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Epiphytes and nutrient contents influence *Sarpa salpa* herbivory on *Caulerpa* spp vs. seagrass species in Mediterranean meadows.

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Abstract: Mediterranean seagrass ecosystems are endangered by increased colonization of *Caulerpa* species, which may replace them, affecting key ecosystem processes. The fish *Sarpa salpa* (L.) is one of the main macroherbivores in the western Mediterranean seagrass meadows which is known to feed on a wide range of macroalgae such as *Caulerpa* species. In order to elucidate if this consumption could minimize the spread of invasive species, during summer-autumn 2012, we investigate the importance of *S. salpa* herbivory pressure on *C. prolifera* and *C. cylindracea* compared to *Posidonia oceanica* and *Cymodocea nodosa* in a mixed meadow. A combination of field experiments and dietary analyses were used to investigate consumption rates, dietary contributions, and feeding preferences for the different macrophytes, including the role of epiphytes and nutrient contents in mediating fish herbivory. In summer, *C. nodosa* was the most consumed macrophyte (12.75 ± 3.43 mg WW·d⁻¹), probably influenced by higher fish densities, higher nutritional quality of leaves and epiphytes, and by differences in epiphyte composition. Feeding observations suggest that fish may have a variable diet, although with a consistent selection of mixed patches with *C. nodosa* and *C. prolifera*. Indeed, food choice experiments suggest that when seagrass leaves are not epiphytized, fish prefer feeding on *C. prolifera*. Gut content and stable isotopic analyses supported the dietary importance of epiphytes and *C. prolifera* but also suggested that *C. cylindracea* could occasionally be an important food item for *S. salpa*. Our results highlight the role of epiphytes in *S. salpa* feeding decisions but also suggest that *C. nodosa* and *C. prolifera* may have an important contribution to fish diet. The variability in *S. salpa* diet confirm the need to carry out multiple approach studies for a better understanding of its potential influence over different macrophytes species.

**Key words**: Food choice, *Sarpa salpa*, *Cymodocea nodosa*, *Posidonia oceanica*, *Caulerpa prolifera*, *Caulerpa cylindracea*, epiphytes, nutrients
INTRODUCTION

Mediterranean seagrass meadows are dominated by *Posidonia oceanica* (L.) Delile (Den Hartog 1970, Thayer et al. 1984), while *Cymodocea nodosa* (Ucria) Ascherson is commonly found in small patches within these meadows (Pérès & Picard 1964). Herbivory rates on these seagrass species are extremely variable according to the available literature (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). This variability in estimated herbivory has been suggested to be partly a consequence of the different methods employed for quantification (Tomas et al. 2005a). Previous estimates of leaf consumption rates were assessed using indirect methods, such as quantifying herbivore bite marks, which 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). Direct methods have shown that, in some instances, grazing can be heavy and determine the structure and distribution of temperate macrophyte assemblages (e.g. Tomas et al. 2005a, b, Taylor & Schiel 2010). In addition, these works also provide evidence that herbivory can be highly variable through space and time, displaying different patterns of defoliation between meadows and/or seasons (Prado et al. 2007, 2010, Steele et al. 2014). Nevertheless, further studies are required to compare herbivore impacts on different macrophyte species, including the two main seagrass species and abundant macroalgae, and to determine the role of food preferences in the ecological functioning of mixed Mediterranean seagrass meadows.

Mediterranean seagrass ecosystems are endangered by increased colonization by *Caulerpa* species, which may replace them; consequently affecting key ecosystem functions and services (Hendriks et al. 2010). Among the main *Caulerpa* living in the Mediterranean, only the chlorophyte *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, is endemic. It develops
in shallow subtidal waters, co-occurring with the seagrasses *C. nodosa* and *P. oceanica* (Vergara et al. 2012, Marco-Méndez et al. 2015). The green alga *Caulerpa cylindracea* (Sonder) [formerly *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque; (hereinafter, *C. cylindracea*, according to Belton et al. 2014, Marín-Guirao et al. 2015)], originally described from southwestern Australia, has rapidly spread throughout the western Mediterranean during the last 20 years (Verlaque et al. 2000, 2003). The alga has successfully colonized a wide variety of soft and hard substrata, including dead *Posidonia oceanica* rhizomes or “matte” (tough, lignified roots and rhizomes admixed with sediment; Boudouresque & Meisnez 1982) and *C. nodosa* meadows (Vazquez-Luis et al. 2008).

Common chemical components such as phenolics (Mariani & Alcoverro 1999; Verges et al. 2007, 2011) are known to deter feeding in algae and terrestrial plants mediating plant–herbivore interactions (Orians et al. 2002; Taylor et al. 2002; Vergés et al. 2007; 2011). In particular, *Caulerpa* species contain different levels of caulerpenyne (Jung et al. 2002), a secondary metabolite that acts as a feeding deterrent that inhibits the growth of microorganisms and is toxic to larvae and adults of potential herbivores (Lemée et al. 1996, Ricci et al. 1999). This chemical deterrence and the consequential lower palatability has been often considered one of the main causes for *Caulerpa* species invasion success (Sant et al. 1996). However, recent studies suggest that Mediterranean herbivores have evolved the capability to tolerate this secondary metabolite (Cornell & Hawkins, 2003), allowing them to consume large quantities of *Caulerpa* spp (Cebrian et al. 2011; Tomas et al. 2011a,b, Marco-Méndez et al. 2015). Since fish generally have higher mobility and greater consumption rates than invertebrate herbivores, they have been hypothesized to be able to limit the spread of introduced algae (e.g. Weijerman et al. 2008, Vermeij et al. 2009). According to this, fish herbivory pressure on *Caulerpa* species could eventually benefit seagrass species by reducing
the proliferation of these species and their negative impact on the dynamics of Mediterranean seagrass meadows (Ruitton et al. 2005).

The fish *Sarpa salpa* (L.) is one of the main macroherbivores in the western Mediterranean, and is commonly observed in shallow seagrass meadows and rocky bottoms (Verlaque 1990) feeding on a wide range of macroalgae and seagrasses (Havelange et al. 1997). This species has been reported to account for 70% of the total leaf consumption of *P. oceanica* (Prado et al. 2007) and is known to ingest large quantities of *Caulerpa* species such as *C. prolifera* (Marco-Méndez et al. 2015) and *C. cylindracea*, providing at least some resistance to invasion of native assemblages (Tomas et al. 2011b). In general, studies point to higher feeding activity of *S. salpa* in summer to accumulate reserves for the winter period, when fish eat less and adults prepare for reproduction (Peirano et al. 2001). However, *S. salpa* herbivory pressure seems to vary greatly over space and time (Prado et al. 2007, 2010, Steele et al. 2014). It is also influenced by other factors such as macrophyte availability and accessibility, habitat heterogeneity, nutritional quality, human pressure on herbivore populations, herbivore recruitment, predation and patterns of movement (Prado et al. 2008 a, b, 2011).

Preferences and feeding rates of marine herbivores may be driven by enhanced nitrogen and protein content, epibiotic load, or lower amounts of chemical and structural components (Mariani & Alcoverro, 1999, Vergés et al. 2007, 2011). Varying levels of structural carbohydrates in seagrass leaves (cellulose), may affect food digestibility and absorption (e.g. Klumpp & Nichols, 1983) and differences in nutritional quality among seagrass species or between seagrasses and epiphytes could result in different levels of herbivory (Alcoverro et al. 1997b, Cebrián & Duarte, 1998, Prado et al. 2010). Furthermore, it has been shown that secondary metabolites of both macroalgae and seagrasses chemically deter herbivores, although inhibition varied between consumers (Vergés et al. 2007, 2011).
Given that several factors could be involved in the complex seagrass–herbivore interactions, studies require combined experimental approaches and dietary analyses integrating temporal variability in resource acquisition. Among methods used to quantify dietary contributions, stomach content analysis is the most accurate, although it applies to very short time periods and requires extensive sampling (Legagneux et al. 2007). In contrast, more recent techniques, such as stable isotopes (reviewed in Kelly 2000), provide useful complementary and time-integrative methods in dietary studies (Marco-Méndez et al. 2012), based on the premise that consumers’ tissues will resemble the long-term isotopic composition of the diet (Fry & Sherr 1984, Minagawa & Wada 1984).

The aims of this study were to compare the importance of *S. salpa* herbivory on *Caulerpa* species vs. seagrasses in a mixed meadow, and to elucidate if this consumption could eventually control the spread of invasive species. With these aims we investigated summer and autumn abundances and consumption rates of *S. salpa* on *P. oceanica, C. nodosa, C. prolifera* and *C. cylindracea*, as well as their potential relationship with temporal changes in the abundances of those macrophytes in a western Mediterranean mixed meadow. In addition, we investigated whether *S. salpa* feeding preferences, epiphytes presence and nutrient content in macrophytes could explain herbivory pattern observed in the field. To this end, a combination of field experiments and dietary analyses were used to investigate consumption rates, dietary contributions, and feeding preferences for the different macrophyte species, including the role of epiphytes and nutrient contents in mediating herbivory by *S. salpa*. We hypothesize that: 1) *S. salpa* abundances and consumption rates will be higher in summer than in autumn influenced by higher fish abundances and macrophytes availability; 2) Epiphytes and macroalgae will have lower C:N ratios than seagrasses which will trigger higher herbivory pressure and 3) *Caulerpa* species abundance could being responding not only to seasonal changes but to *S. salpa* herbivory pressure.
MATERIAL AND METHODS

Study site

The study site was located at Cabo de Santa Pola (38° 12'34.56"N, 0° 30'31.55"W, western Mediterranean) in a mixed habitat (depth range: 2-4 m; study area: ~0.75 km$^2$) formed by intertwined patches of variable size of *Posidonia oceanica*, *Cymodocea nodosa*, *Caulerpa prolifera*, unvegetated sandy substrate, and rocky substrate covered by *Caulerpa cylindracea* and, other macrophyte species to a lesser extent (e.g. *Cystoseira compressa*; *Dylophus sp*; *Enteromorpha compressa*; *Jania rubens*, *Padina pavonica* and *Halopteris scoparia*). *C. cylindracea* was first recorded in 2002 at a site located around ten km north of the study area, where it colonized soft sediments and dead matte of *P. oceanica*. Two months later, it was detected on the rocky platform of our study area (Pena-Martín et al. 2003). Currently, this nonindigenous alga occurs in extensive areas of ecologically important rocky bottoms, as well as on sandy and muddy substrates, and on dead matte of *P. oceanica* (Marín-Guirao et al. 2015). It also occurs intermixed with *C. nodosa* in seagrass meadows, with a patchy distribution (Vazquez-Luis et al. 2008).

The main aims of this study were to compare the importance of *S. salpa* herbivory on *Caulerpa* species vs. seagrasses in a mixed meadow, to identify mediating factors influencing herbivory and to elucidate if this consumption could eventually control the spread of invasive species. To achieve these goals, we investigated *S. salpa* abundances and consumption rates on *P. oceanica*, *C. nodosa*, *C. prolifera* and *C. cylindracea* and their potential relationship with temporal changes in those macrophytes abundances in a western Mediterranean mixed meadow. The study was carried out in two randomly selected locations (A and B) 2-3 km apart (Fig. 1) and in two different times during 2012, summer (July-August) and autumn (September-October). In summer macrophytes biomasses are expected to be higher in
response to temperature, nutrients and light conditions (Alcoverro et al. 1997b) and the feeding activity of *S. salpa* to be more intense in order to accumulate reserves for the winter period (Peirano et al. 2001). In autumn (September-October), both macrophytes biomasses (Alcoverro et al. 1997b) and *S. salpa* feeding activity are expected to decrease before reaching their minimum in winter, when fish eat less and adults prepare for reproduction (Peirano et al. 2001).

**Bottom characterization**

At each location (A and B) and study time (hereafter, T1: summer; T2: autumn) seagrasses shoot density (number per m$^2$) was measured by counting shoots in a 40 x 40 cm quadrant placed in three haphazardly selected patches within the mixed habitat. Percentage of bottom covered was estimated visually by scuba divers using a 25 meters tape measure (n = 3) and recording the length covered by the seagrasses and other substrates or macrophytes species (rock, sand, *Caulerpa prolifera*, etc.). Subsequently the data were expressed as percentages of bottom coverage (Sánchez-Lizaso 1993). Because of the heterogeneity of this mixed habitat three additional 20 x 20 cm quadrats were haphazardly selected at each study location and all macroalgae and seagrass within them carefully removed and placed in plastic bags. In the laboratory, all macrophytes were sorted into species, dried for 24 h at 80 °C, and weighed. Accordingly to standardized methods (Romero 1985, Alcoverro et al. 1995, Ruitton et al. 2005), we estimated the percent cover by each macrophyte species relative to the total weight of sample scraped off the rocky substrate. When during visual characterization two species were highly mixed in the same patch, we recorded it as: e.g. *C. nodosa* and *C. prolifera*.

**Fish abundances and feeding observations**
Individuals of *S. salpa* were counted by scuba divers using the visual transect census method (Harmelin-Vivien et al. 1985, Francour 1997). At each time and at each study location, visual censuses were carried out in two different days (T1: one day in July and another in August; T2: one day in September and another October) recording 16 censuses each day (n = 32 total censuses per time and location). All *S. salpa* encountered along haphazardly located 50 m$^2$ line transects were counted (ind. m$^{-2}$), and their average size (total length, TL) estimated. All fish counts were performed at the same time of the day (approximately between 10:00 and 13:00 h) to minimize possible variability due to differences in fish behaviour (Spyker & Van Der Berghe 1995).

The feeding activity of *S. salpa* was also recorded through visual observations. In this case, scuba divers recorded a total of 18 schools of fish feeding at each time and study location (each ca. 7 min in duration). On each occasion, we followed a school of fish and recorded the number of individuals within the school, their average size, and if they were swimming or feeding, in which case the food items consumed were recorded. The percentage of individuals swimming or feeding on the different items was estimated relative to the total of individuals observed.

**Tethering experiments**

Consumption rates of *C. prolifera, C. cylindracea, C. nodosa* and *P. oceanica* by *S. salpa* were estimated with tethering experiments deployed within monospecific patches at the two different times and locations of study (one tethering line per species, time, and location; i.e., a total of 4 tethering lines per macrophyte during the study). Each tethering line consisted of 18 replicates, with similar amounts of freshly collected macrophyte biomass (collected the morning of the experiment). Tethering lines consisted of floating replicates (by using small buoys) to avoid benthic invertebrate herbivores (e.g. sea urchins). Floating replicates were
tied to a thin cord and deployed in the field for a week; the lines were elevated a few centimeters from the bottom but integrated at the height of the surrounding vegetation, mixing them within the monospecific macrophyte patches. Each end of the line was secured to the bottom with rebar stakes. Controls for changes in wet weight unrelated to herbivory were simultaneously made to each of the tethering experiment performed during the study. For each species, 18 control replicates of identical portions of macrophyte (individually protected from herbivores by 0.5 cm² mesh cages) were deployed in the field during the same period (see Tomas et al. 2011b). To avoid any interference that fouling organisms could cause in the light entering the mesh, cages were checked every day and cleaned when needed. All replicates were cut down to remove previous herbivore marks and blotted dry of excess water before measuring initial and final wet weight (3 g wet weight per replicate). After a week, tethering and control replicates were collected and biomass consumption by *S. salpa*, whose bite marks are easily distinguishable (e.g. Tomas et al. 2005b), was estimated as [(Hi X Cf/Ci) - Hf], where Hi and Hf were initial and final wet weights of tissue exposed to herbivores, and Ci and Cf were initial and final weights in controls (Cronin & Hay 1996; Parker & Hay 2005; Tomas et al. 2011a, b). Macrophytes consumption was expressed as mg of wet weight consumed per day.

**Food choice experiments**

Given the high consumption of *Caulerpa* spp by *S. salpa* shown in previous studies (Tomas et al. 2011b, Marco-Méndez et al. 2015), and the reported importance that *C. nodosa* and *P. oceanica* can have in its diet (Prado et al. 2007a; Marco-Méndez et al. 2015), we conducted paired feeding experiments to examine the relative palatability of *C. prolifera*, *C. cylindracea*, *C. nodosa* and *P. oceanica*. In addition, since epiphytes can have a large influence on herbivore feeding choices (Marco-Méndez et al. 2012), paired experiments were
carried out with epiphytized and non-epiphytized seagrass leaves (*Caulerpa* species were not epiphytized). These experiments did not only test fish selectivity for seagrass or *Caulerpa* species but also helped to elucidate whether the manifested selectivity is consistent in presence and absence of epiphytes in seagrass leaves. Despite seagrass are naturally epiphytized, these experiments will help to understand whether *S. salpa* consumption rates and feeding behavior observed in the field could be related to food preferences and epiphytes presence. Food choice experiments were conducted in summer, when macroalgae and epiphyte biomasses have maximum values and they undergo the highest pressure from *S. salpa* (Alcoverro et al. 1997a). Experiments were deployed in large sandy patches (ca. 2-4 m depth; at least 5 m away from rocks, seagrasses or macroalgae) to ensure that no other macrophytes could interfere with fish feeding choices and that invertebrate herbivores did not have access to experimental setups. A total of 9 paired floating tethering experiments were carried out with the following paired combinations: 1) *C. nodosa* epiphytized vs. *C. prolifera*; 2) *C. nodosa* epiphytized vs. *C. cylindracea*; 3) *P. oceanica* epiphytized vs *C. prolifera*; 4) *P. oceanica* epiphytized vs *C. cylindracea*; 5) *C. nodosa* non-epiphytized vs. *C. prolifera*; 6) *C. nodosa* non-epiphytized vs. *C. cylindracea*; 7) *P. oceanica* non-epiphytized vs. *C. cylindracea* and 9) *C. prolifera* vs. *C. cylindracea*. For each experiment, similar amounts of freshly collected algal and seagrass biomass were offered in pairs (ca. 3 g wet weight). Replicate pairs (n = 18) and their respective controls (individually protected from herbivores by 0.5 cm² mesh cages) were deployed at least 1 m apart and collected after four days. Consumption was estimated as for tethering experiments and expressed as mg wet weight lost by *S. salpa* bite marks. Despite the amount of epiphytes was not quantified before and after experiments, we used control leaves to make some calculation in order to estimate the amount of epiphytes potentially growing in *C. nodosa* and *P. oceanica* leaves offered in food choice experiments. Estimates of epiphytic
loads growing on *P. oceanica* leaves offered with *C. prolifera* were 219.02 ± 8.22 mg DW per shoot and with *C. cylindracea* was 279.51 ± 8.17 mg DW per shoot. For *C. nodosa* leaves offered with *C. prolifera* estimated loads were 100.46 ± 3.46 mg DW per shoot and with *C. cylindracea* 138.36 ± 5.57 mg DW per shoot. Differences in the amount initially offered with leaves possibly respond to natural differences between shoots collected. We considered that the most important changes in the epiphytic community occurring during four days would be in terms of biomass, which is also related with epiphytic coverage, so using control leaves for weight correction would resolve this issue. Despite we did not analyzed the epiphytic community before and after the experiments, we think that giving the short duration of the experiments, changes in the community composition in terms of taxa composition would possibly be small.

**Gut contents, stable isotope analyses and nutrient contents**

A total of 26 individuals of *S. salpa* (average length: 23.12 ± 0.62 cm) were haphazardly collected within the area for a dietary study. Since individuals were caught at two different moments, we studied them separately (n = 13 individuals per group or school). We used all individuals for gut content analyses and 10 individuals (n = 5 from each school) for nutrient content and stable isotope analyses (SIA). In the laboratory, fish muscle was isolated for SIA and nutrient content analysis. Gut contents were extracted and food items separated under the microscope (eg., *P. oceanica* leaves, *C. prolifera*, *C. cylindracea* and epiphytic macroalgae). Each fraction was dried to constant weight at 60º C.

Samples of *Caulerpa* and seagrass species were haphazardly collected from the study area for SIA and nutrient content analyses. These samples included: *C. prolifera*, *C. cylindracea*, *P. oceanica* and *C. nodosa* epiphytized leaves with and without epiphytes (n = 5 of each type), as well as epiphytes themselves (n = 5 of each type). The latter included both
epifauna (heterotrophic metazoans) and epiflora (macroalgae). Samples from schools and food resources were dried to constant weight at 60°C and ground to fine powder for determination of nutrient contents (C:N) and isotopic signatures ($\delta^{15}$N and $\delta^{13}$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, Spain). 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 $R_{\text{sample}}$ is the $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N ratio in the sample; $R_{\text{standard}}$ is the $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N ratio for the reference material (i.e. CaCO$_3$ from belemnite (PBD) for $\delta^{13}$C and atmospheric nitrogen for $\delta^{15}$N measurements), calibrated against an internal standard (i.e. atropine, IAEA and/or UGS).

**Epiphytic community**

The epiphytic community of both *P. oceanica* and *C. nodosa* was investigated in shoots (n = 10) collected at the two study times (summer and autumn 2012). For each shoot the oldest leaf was selected as representative of the epiphyte community during the entire life span of the shoot (Prado et al. 2008a). Epiphytic cover (%) on the 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 weight at 60°C for biomass determination (mg DW·cm$^{-2}$).

**Data analyses**

The significance of differences in the cover of each macrophyte species (percentage of bottom covered and rocky substrate covered) between “Time” (fixed factor with two levels)
and “Location” (random factor with two levels) was investigated using the PERMANOVA+ β20 software package (Anderson and Gorley, 2007).

Differences in consumption rates by *S. salpa* among macrophyte species, times (T1: summer; T2: autumn) and locations (A and B) were investigated with a three-way ANOVA design with two fixed factors (“Macrophyte” and “Time”) and a random orthogonal factor (“Location”). The factor “Macrophyte” had four levels (*C. prolifera*, *C. cylindracea*, *C. nodosa*, *P. oceanica*). Factors “Time” and “Location” both had two levels in all analyses.

Differences in the abundances of *S. salpa* during the study were analyzed with a two-way ANOVA with “Time” and “Location” as fixed and random factors, respectively.

A two-way ANOVA was also used to analyze differences in the number of epiphyte taxa, cover and biomass between *P. oceanica* and *C. nodosa* leaves during the two times of study. Factors “Macrophytes” and “Time” were both fixed and had two levels.

Differences in isotopic signatures (δ15N and δ13C) and nutrient content (C: N molar ratio) among food resources were tested through a one-way ANOVA with 8 levels (*C. prolifera*, *C. cylindracea*, *P. oceanica* epiphytized, *P. oceanica* non-epiphytized, *C. nodosa* epiphytized, *C. nodosa* non-epiphytized, epiphytes of *C. nodosa* and epiphytes of *P. oceanica*). Differences in the mean isotopic signatures between the two different schools of fish were subjected to standard t-tests. The IsoSource (Phillips & Gregg 2003) isotope mixing model was used to identify the contributions of each food source to the diets of school 1 and 2 separately. Since results by Prado et al. (2012) concluded that there is a strong dietary effect on fractionation (i.e. seagrass, macroalgae, and omnivorous diet fractionations were different) and both schools of *S. salpa* were collected where all those diets were available, the model was run with the means of the fractionation values found for seagrass and macroalgae diets (0.63 ± 0.29 ‰ for δ15N and 2.49 ± 0.25 ‰ for δ13C, means ± SE). Since seagrasses have very low digestibility, those mean values were considered more accurate than assuming the
theoretical 3.4‰ enrichment between trophic levels. The input parameters for the model were
the isotopic values of the consumer and trophic resources (measured in this study) and the
overall fractionation rates (Mean ± SE). Since no significant differences were found between
epiphytes of *C. nodosa* and epiphytes of *P. oceanica* and this prevents the detection of
differences by the model (Phillips & Gregg 2003), $\delta^{15}$N and $\delta^{13}$C values were averaged to run
the model. We also used averaged $\delta^{15}$N and $\delta^{13}$C values for *C. cylindracea* and for *C.
prolifera*, since no significant differences were found between their $\delta^{15}$N values, despite $\delta^{13}$C
values differed.

ANOVA assumptions of normality and homogeneity of variance were assessed with
the Kolmogorov-Smirnov and Cochran’s C- tests, respectively. When necessary, an
appropriate transformation was performed before further analysis. When assumptions were
not met, the level of significance was set at 0.01 to reduce the possibility of committing Type
I errors (Underwood 1997). Student-Newman-Keuls post-hoc tests were used to single out
significant groupings. The statistical tests were done using PASW software and GMAV 5
software (University of Sydney, Australia).

The n-MDS ordination (Bray-Curtis similarity index), ANOSIM and SIMPER
(available in the PRIMER-E v.6 software package, Clarke & Warwick 1994) were applied to
stomach contents (percentage) and epiphytic assemblages (presence-absence transformation).

Wilcoxon signed-ranks paired test was applied to food-choice experiments, due to lack
of normality and homoscedasticity of data.

Pearson’s product-moment correlation coefficient (r) was used to test how fish
densities correlate with feeding rates and weather this two variables correlate negatively with
macrophytes cover.

RESULTS
Bottom characterization

No significant differences were found in the shoot density of *Posidonia oceanica* and *Cymodocea nodosa* either between times or between locations (Summer, *C. nodosa*: 1029.2 ± 217.4 shoots·m⁻²; *P. oceanica*: 478.1 ± 66.6 shoots·m⁻²; Autumn, *C. nodosa*: 875.0 ± 219.7 shoots·m⁻²; *P. oceanica*: 367.7 ± 45.1 shoots·m⁻²).

PERMANOVA analyses for bottom coverage did not show significant effects between “Times” (P = 0.5296) or “Location” (P = 0.317). The highest percentages of cover were recorded for *P. oceanica* (Summer: 46.2 ± 6.6 %; Autumn: 34.6 ± 8.4 %; average of A and B) and *Caulerpa prolifera* (T1: 19.7 ± 6.8 %; T2: 27.5 ± 5.3 %; average of A and B). On rocky substrates, no significant effects were found between “Times” (P = 0.992) or “Location” (P = 0.988). The highest percentage of rocky substrate covered was recorded in summer for *C. prolifera* (48 ± 22.2 %). *Caulerpa cylindracea* and *P. oceanica* recorded similar percentages during the study, with values slightly higher in summer (8.65 ± 1.74 % and 8.98 ± 5.28 % respectively) than in autumn (5.95 ± 3.54 % and 4.6 ± 4.6 %). The remaining percentages corresponded to other macroalgae species identified (*Cystoseira compressa; Dylophus sp; Enteromorpha compressa; Jania rubens, Padina pavonica* and *Halopteris scoparia*).

Herbivore densities and feeding observations

There was a significant “Time x Location” interaction in the abundance of *S. salpa*. The highest abundances reported during the study were those recorded in summer and location A (0.56 ± 0.15 ind·m⁻²) (Two way ANOVA; p < 0.01; Fig. 2A).

Feeding observations showed that individuals fed on a variety of species during summer (*P. oceanica, C. nodosa, C. prolifera,* and other algae) but mainly on *C. prolifera* and
C. nodosa during autumn (Fig. 2B). The average fish sizes recorded for the schools observed during the study were in summer: 15.72 ± 1.14 cm in location A and 17.78 ± 1.22 cm in location B; while in autumn: 13.05 ± 2.25 cm in location A and 12.5 ± 2.58 cm in location B. These sizes were considered small-medium size individuals (small < 17cm and medium 17-29 cm), based on Francour (1997).

**Tethering experiments**

There were significant differences for the interaction “Macrophyte x Time” (Fig. 2C; Table 1). Consumption rates of C. nodosa were only significantly higher than the consumption recorded for the other macrophyte species in summer. In addition, consumption rates of C. nodosa in summer were also significantly higher than in autumn (0.51 ± 0.13 % of wet plant biomass per day). Despite SNK analyses did not detect further significant differences between the rest of macrophyte species or times, consumption of C. prolifera tended to be higher in summer; P. oceanica displayed low but consistent consumption rates, and C. cylindracea showed no herbivory (Fig. 2C; Table 1).

**Correlations between bottom coverage, herbivore abundances and consumption rates**

No significant correlations were found between any of the variables analyzed. Bottom coverage of C. nodosa and P. oceanica displayed a tendency to correlate negatively with fish abundances (r = -0.70; -0.550 respectively) and C. prolifera and P. oceanica with consumption rates (r = -0.350; -0.068 respectively). Consumption of C. nodosa showed a tendency to correlate positively with fish abundances (r = 0.070).

**Food choice experiments**
Sarpa salpa displayed higher consumption rates of C. prolifera relative to C. nodosa non-epiphytized (NE) (Fig. 3A) but not of C. cylindracea vs. C. nodosa non-epiphytized (NE) (Fig. 3B). Regarding P. oceanica, a significant higher consumption of C. prolifera was recorded vs. P. oceanica non-epiphytized (NE) (Fig. 3C) but no consumption of C. cylindracea or P. oceanica (NE) was detected (Fig. 3D). In presence of epiphytes, no significant differences were found either in the consumption of C. nodosa (E) vs. C. prolifera or in that of C. nodosa vs. C. cylindracea (Fig. 3E, F). Similarly, no significant differences were found either in the consumption of P. oceanica epiphytized (E) vs. C. prolifera or in P. oceanica (E) vs. C. cylindracea (Fig. 3G, H). Finally, the consumption of C. prolifera was significantly higher than C. cylindracea (Fig. 3I). The highest consumption rates for C. prolifera were observed vs. C. nodosa non-epiphytized (2.58 ± 0.91 mg WW·d\(^{-1}\)), followed by those observed vs. C. cylindracea and P. oceanica non-epiphytized (1.07 ± 0.49 mg WW·d\(^{-1}\); 0.34 ± 0.17 mg WW·d\(^{-1}\) respectively).

Gut contents

Gut contents of S. salpa individuals from school 1 comprised epiphytes (6.7 %), P. oceanica (39.7 %) and C. prolifera (53.5 %), while school 2 samples showed a diet of P. oceanica (0.5 %), C. prolifera (31.8 %) and C. cylindracea (67.7 %).

n-MDS ordination of the gut items showed different groupings between individuals from schools 1 and 2. ANOSIM results confirmed that gut contents of these two schools were significantly different (Global R: 0.48; p = 0.001). The average similarity among school 1 gut contents was 42.87 % and school 2 was 59.85 %. The average dissimilarity between the two schools of fish was 80.81 %, mostly due to C. cylindracea (41.89 %), C. prolifera (29.44 %) and P. oceanica (24.52 %).
Stable isotope analyses

Both $\delta^{13}$C and $\delta^{15}$N signatures showed significant differences among food items (one-way ANOVA, $p < 0.001$; Fig. 4A; Table 2). The highest $\delta^{15}$N values were recorded for *C. prolifera* and *C. cylindracea* (7.25 ± 0.27 ‰ and 7.59 ± 0.07 ‰ respectively) and the lowest for *P. oceanica* epiphytized and non-epiphytized (4.49 ± 0.07 ‰ and 4.36 ± 0.09 ‰ respectively). For $\delta^{13}$C, the highest values were recorded for *C. nodosa* non-epiphytized (-9.58 ± 0.01 ‰) and the lowest for *C. cylindracea* (-16.67 ± 0.11 ‰), and the epiphytes from *P. oceanica* and *C. nodosa* leaves (-17.06 ± 0.17 ‰; -15.93 ± 0.23 ‰ respectively).

Regarding consumers, significant differences were found in the $\delta^{15}$N values between the two *S. salpa* schools (t = 5.004; df = 7.527; $p = 0.001$) and these values (school 1: 12.85 ± 0.46 ‰; school 2: 9.95 ± 0.35 ‰) were closer to *C. cylindracea* and *C. prolifera* values. In contrast, no significant differences were found for $\delta^{13}$C signals between the two schools (t = 2.620; df = 7.824; $p = 0.31$) and values (school 1: -16.23 ± 0.42 ‰; school 2: -17.90 ± 0.35 ‰) lay much closer to *C. cylindracea*, *C. prolifera* and epiphytes (including both ‘macroalgae’ and metazoans), than to seagrass values (Fig. 4A; Table 2).

Results from the IsoSource model indicated that, in the long term, the diet of both schools of *S. salpa* consisted of *Caulerpa* spp, epiphytes and seagrasses (school 1, *P. oceanica*: 20%; *C. nodosa*: 12%; epiphytes: 28%; *Caulerpa* spp: 32%; school 2, *P. oceanica*: 10%; *C. nodosa*: 16%; epiphytes: 38%; *Caulerpa* spp: 30%; both at the percentile 50%).

Nutrient contents in seagrass leaves and epiphytes

There were significant differences among C:N molar ratios of food items, with the highest values found for *P. oceanica* non-epiphytized and epiphytized (34.06 ± 0.45; 29.93 ± 1.48 respectively) and the lowest for both types of epiphytes (epiphytes of *C. nodosa*: 14.66 ±
epiphytes of \(P.\) oceanica: 10.06 ± 0.98; one-way ANOVA, \(p < 0.001\); Fig. 4B; Table 2).

For \(S.\) salpa, no differences were found in the C:N molar ratios between the two schools (3.47 ± 0.01 for both; \(t = -0.188\); df = 7.957; \(p = 0.855\)).

### Epiphytic community

Significant differences were found in the epiphytic biomass due to the interaction “Macrophyte x Time” (Two way ANOVA; Table 3). \(C.\) nodosa supported the highest epiphytic biomass in autumn (6.233 ± 0.284 mg DW·cm\(^{-2}\); Table 3) but no differences were detected between the epiphytic biomass of \(C.\) nodosa in summer and the recorded in \(P.\) oceanica leaves, which epiphytic biomass was similar between the two seasons (two-way ANOVA; Table 3). We found significant differences in leaf epiphytic cover with respect to “Macrophyte” and “Time” (two-way ANOVA; Table 3). During the study, the recorded values were consistently higher for \(C.\) nodosa vs. \(P.\) oceanica leaves and in autumn vs. summer (Table 3). Concerning the number of epiphytic taxa, significant differences were found for the interaction “Macrophytes x Time” (two-way ANOVA, \(p < 0.01\); Table 3). The highest number of epiphytic taxa was found on \(C.\) nodosa leaves at both times of study and the lowest was recorded on \(P.\) oceanica leaves in autumn.

n-MDS ordination of epiphytic taxa displayed four distinctive groupings considering times (T1: summer; T2: autumn) and seagrass species (\(C.\) nodosa; \(P.\) oceanica) (one-way ANOSIM, four levels: Global \(R = 0.749, p = 0.001\)). SIMPER analyses indicated that the epiphytic community on \(C.\) nodosa leaves displayed an average similarity of 70.19 % at time and 82.97 % at time 2. The epiphytes on \(P.\) oceanica had an average similarity of 63.14 % at time 1 and 65.33 % at time 2. The average dissimilarity between \(C.\) nodosa and \(P.\) oceanica was 64.50 % at time 1 (\(R = 0.85; p = 0.001\)) and 62.75 % in time 2 (\(R = 0.99; p = 0.001\)), mainly due to \(Myrionema\) magnusii, \(Ceramium\) sp, \(Lyngbya\) sp and \(Sphacelaria\) cirrhosa. The
epiphytic community on *C. nodosa* leaves showed an average of dissimilarity between time 1 and time 2 of 53.79% mainly due to *Sphacelaria cirrhosa*, *Myriactula gracilis* and *Cladophora* sp. (*R* = 0.90; *p* = 0.001) while *P. oceanica* epiphytic community did not display significant dissimilarity between times (32.55%; *R* = -0.092; *p* = 0.99). The average dissimilarity between T1-Sum-CE and T2-Aut-PE was 63.91% and between T2-Aut-CE and T1-Sum-PE was 63.54%, due in both cases mainly to *Myrionema magnusii* (*R* = 0.85; *p* = 0.002 and *R* = 0.92; *p* = 0.001 respectively).
DISCUSSION

This study points to seagrass *Cymodocea nodosa* and green alga *Caulerpa prolifera* as the “most consumed” and the “most preferred” food species, respectively, by the Mediterranean fish *Sarpa salpa*. In summer, *C. nodosa* recorded the highest consumption rates in the mixed meadow, which seems to be related to higher fish abundances. *C. prolifera* was the most preferred macrophytes in food choice experiments but different nutritional content and epiphyte presence likely explains why the preference of *S. salpa* for *C. prolifera* was not sustained vs. epiphytized leaves and therefore did not deflect herbivory pressure on the most epiphytized and nutritious seagrass *C. nodosa*, the “most consumed” macrophyte in the mixed meadow. Our results highlight the possible mediating role of epiphytes and nutrient contents in *S. salpa* selectivity. However, results also show the high variability in *S. salpa* diet and herbivory pattern as a consequence of the multiple factors potentially involved.

Tethering experiments showed that *C. nodosa* was the most consumed macrophyte, recording in summer consumption rates significantly higher than the reported for the other macrophytes species during the whole study. Despite analyses did not detect further significant differences among the rest of the species, the consumption of *C. prolifera* in summer tended to be higher than in autumn (~2.5 times), while consumption of *P. oceanica* was consistently low and no consumption of *C. cylindracea* was detected during the study. Our results evidence that herbivory on *C. nodosa* can even exceed some previous estimates on *P. oceanica* (ca. 2 times higher in our study than in Prado et al. 2007). The high variability observed during the study concurs with the high temporal and spatial variability in the previous estimates of *S. salpa* herbivory on *P. oceanica* (Prado et al. 2007, Tomas et al. 2005a). In addition, results also suggest that *C. prolifera* and *C. nodosa* bottom coverage tend to decrease with higher consumption and fish abundances, reinforcing the idea that herbivory on these macrophytes species can also be important (see also Marco-Méndez et al. 2015,
Tomas et al. 2011b) and should be considered when studying herbivory in Mediterranean seagrass meadows.

The high variance in herbivory has been partially attributed to changes in herbivore abundance and distribution, which can be a consequence of the interaction among recruitment rates (Camp et al. 1973), predation effects (McClanahan et al. 1994) or fishing pressure (Klumpp et al. 1993; Prado et al. 2008). In addition, the fish *S. salpa* displays seasonal mobility patterns according to nutritional and life cycle needs. This accounts for massive schools of fish feeding actively in summer on seagrass meadows in order to accumulate reserves for the winter period, when fish eat less, migrate to greater depths and prepare for reproduction (Peirano et al. 2001). This seasonal migration explains the high temporal variability in the abundances of *S. salpa* individuals detected in our study, with the highest fish densities during summer (up to 0.56 ± 0.15 ind·m⁻² in location A) but decreasing during autumn (Tomas et al. 2005a, Prado et al. 2007). Since the significantly higher fish abundance in location A was recorded in summer, it was probably related to variability in the mobility pattern within the home range of the species (ca. 4.3 ha; Jadot et al. 2002, 2006), rather than to spatial differences in recruitment rates, predation or overfishing. Accordingly, temporal variability in fish abundance strongly influenced the more intense herbivory in summer, especially on *C. nodosa*, and the low consumption rates of all macrophyte species during the autumn (Ruitton et al. 2006, Tomas et al. 2011b). In fact, our results suggest that *C. nodosa* consumption tend to increase and its coverage to decrease with higher fish densities. Nevertheless, these results contrast with a previous study carried out in a differently located mixed meadow (Marco-Méndez et al. 2015), where herbivory by *S. salpa* on *C. nodosa* in late summer was not detected despite similar fish densities and habitat features. Furthermore, the lack of consumption of *C. cylindracea* detected by the tethering experiment, feeding observations, and food choice trials contrasts with its presence in gut contents and with the
findings of Tomas et al. (2011b), where *S. salpa* consumed large quantities of that invasive alga. This apparent contradiction between studies reinforces the idea that herbivory varies strongly both spatially and temporally (Tomas et al. 2005a, Prado et al. 2008b). It is not only influenced by temporal changes in fish abundances but probably also by their home-range size, habitat selection or variability in individual behavior (Jadot et al. 2002, 2006). Plant availability and accessibility or feeding preferences for some macrophyte species could also be mediating herbivory on Mediterranean seagrasses meadows (Prado et al. 2008b, 2009, 2010).

Food choice experiments recorded the highest consumption on *C. prolifera* vs. *C. nodosa* non-epiphytized leaves (2.58 ± 0.92 mg WW·shoot<sup>−1</sup>·d<sup>−1</sup>) and showed that *S. salpa* individuals only preferred to feed on *C. prolifera* vs. *P. oceanica* and *C. nodosa* when epiphytes were removed, pointing to the mediating role of epiphytes in herbivore selectivity (Tomas et al. 2005b, Marco-Méndez et al. 2012, 2015). In addition, their consistent preference for *C. prolifera* vs. *C. cylindracea* suggests other factors inherent to macrophyte features could also be involved. Even though some experiments showed no preferences for *C. cylindracea* vs. seagrasses (epiphytized or non-epiphytized leaves), the strong preference for *C. prolifera* and the lack of consumption detected by tethers suggest that *S. salpa* may prefer feeding on native species. In fact, a large number of studies have evidenced that preferences and feeding rates of marine herbivores may respond to a combination of high nitrogen and protein content, enhanced epibiotic loads, or with low amounts of chemical and structural components (Cebrián & Duarte 1998, Mariani & Alcoverro, 1999, Verges et al. 2007).

In our study, differences in C:N ratios among *Caulerpa* species, seagrass species and epiphytes are likely to have influenced the observed patterns of herbivory and selectivity. However, although both *Caulerpa* species recorded lower C:N ratios than seagrasses, preferences were only manifested for *C. prolifera*. On the one hand, lower C:N ratio values...
are consistent with *S. salpa*'s preference for *C. prolifera* vs. *C. cylindracea*. On the other, the preference for *C. prolifera* vs. seagrasses, which was dissipated in the presence of epiphytes suggest that epiphytes and macroalgae sustain a comparatively higher herbivore pressure than seagrass leaves (Duarte & Cebrián 1994), due to their typically lower C:N ratios (Duarte 1992). In fact, C:N ratios values were ca. 2 times higher in non-epiphytized leaves of both seagrasses than in *C. prolifera*. These nutritional differences were slightly reduced when seagrasses were epiphytized. Together with the significantly lower C:N ratios and higher nutritional content of epiphytes compared to *C. prolifera* (% N was ca. 3 times higher and % C ca. 4 times higher), such differences could explain why preference for *C. prolifera* vs. seagrasses is dissipated in the presence of epiphytes and also the higher herbivory on *C. nodosa* recorded in the mixed meadow (i.e. tethering results). It also confirms that epiphytes and their higher nutritional value (e.g. Alcoverro et al. 1997a, 2000) can mediate herbivore preferences and consumption rates (Marco-Méndez et al. 2012). Furthermore, it seems plausible that higher consumption of *C. nodosa* compared to *P. oceanica* in mixed meadows (Marco-Méndez et al. 2015) is explained by the lower C:N ratio of its leaves, plus the increased nutritional value resulting from the presence of epiphytes.

Variability in epiphyte composition has also been reported to influence herbivore consumption and preferences (Marco-Méndez et al. 2012, 2015). In the present study, the epiphytic community structure revealed important differences between seagrass species and times. Such differences were probably influenced by differences in light shading (Carruthers 1994), and the effects of shoot morphology and leaf age on the surface area and timing of epiphytic colonization (Lavery & Vanderklift 2002). *C. nodosa* leaves were found to support the highest epiphytic biomass, cover and taxa during the study, which may account for the undergoing of more intense grazing rates than in *P. oceanica*. 
Although they were not measured in this study, we cannot rule out the influence of other macrophytes features in plant–herbivore interactions and feeding preferences (Orians 2002, Taylor et al. 2002; Vergés et al. 2011). For instance, high levels of structural carbohydrates in seagrass leaves, which make their digestion less effective (Thayer et al. 1984, Cebrián & Duarte 1998), could have also influenced selectivity for *C. prolifera* vs. non-epiphytized seagrass leaves. Despite *Caulerpa* species can synthesize caulerpenyne, a secondary metabolite that plays a major role in their chemical defense (Pohnert & Jung 2003) against epiphytes and herbivores (Erickson et al. 2006), the observed preference of *S. salpa* for *C. prolifera* relative to seagrasses (without epiphytes) suggests that this fish could have evolved some tolerance to this compound. Yet, since lower levels of caulerpenyne have been reported (Jung et al. 2002) for the invasive *C. cylindracea* compared to the non-invasive *C. prolifera* (which was the preferred *Caulerpa* species in this study), chemical deterrence was unlikely to be a factor determining the patterns of *S. salpa* herbivory observed in the mixed meadow. From such evidence, it seems that the more intense herbivory on *C. nodosa* and the selectivity for *C. prolifera* must have been mostly influenced by differences in nutritional content rather than in chemical compounds, which seem not to inhibit *S. salpa* herbivory. Although this could theoretically also trigger higher selectivity for *C. cylindracea* vs. seagrasses, our results evidence that *S. salpa* prefers feeding on native species.

During the whole study period, feeding observations revealed that although *S. salpa* individuals were feeding on a mix of species, feeding activity on mixed patches of *C. prolifera* and *C. nodosa* was reiterative, which concur with tethering results and food choice experiments. For *C. prolifera*, gut content analyses were consistent with previous results, confirming it as a “preferred food item” in the diet of the two schools of fish sampled. In contrast, the absence of *C. nodosa* coupled with the presence of *C. cylindracea* in gut samples did not agree with tethering results. Since coverage of *P. oceanica*, *C. prolifera*, *C.
cylindracea and mixed patches of C. nodosa-C. prolifera were consistently present throughout the study without temporal variation in the mixed meadow, different availability could not explain gut content analyses. We hypothesize that spatial variability may be involved and that S. salpa mobility across other sites with lower abundance of C. nodosa, or higher abundance of C. cylindracea within its home range (ca. 4.3 ha according to Jadot et al. 2002, 2006), could account for the absence or enhanced presence of these species within gut contents, also explaining the dietary differences between the two schools. IsoSource mixing model results showed that both seagrasses as well as Caulerpa species and epiphytes all contribute to the long-term diet of S. salpa, and highlights the importance of Caulerpa species (which seems to be mainly attributed to the high consumption of C. prolifera) and epiphytes in their diet. This analytical contribution ultimately reflects preferences and consumption patterns observed during the study and supports the previously reported importance of epiphytes (Marco-Méndez et al. 2012, 2015) and Caulerpa species in S. salpa herbivory (Ruitton et al. 2006, Tomas et al. 2011b).

In conclusion, our study highlights the importance of C. nodosa and C. prolifera in the diet of S. salpa, and also that herbivory in Mediterranean meadows can be highly variable and mediated by multiple factors. In summer, when densities of S. salpa are higher, C. nodosa was the “most consumed” macrophyte, likely influenced by the higher nutritional quality of its leaves and epiphytes, as well as by differences in the epiphytic community composition (Marco-Méndez et al. 2015). Food choice, feeding observations and gut content analyses pointed to C. prolifera as a food consistently selected by S. salpa. In contrast, preference of S. salpa for C. prolifera was not sustained vs. epiphytized leaves, which suggests that epiphyte presence and nutritional contents explain the herbivory patterns in the mixed meadow. In fact, the IsoSource mixing model confirms the importance of Caulerpa species, which from our results, seems to be mostly attributable to the high consumption of C. prolifera and the role of
epiphytes in the long-term diet of *S. salpa*. Although *C. cylindracea* consumption was not observed, the fact that it was found within stomach contents suggests that they may eventually adapt to feeding on this new resource. Our results may suggest that *S. salpa* selectivity for *C. nodosa* and *C. prolifera* could eventually influence their abundances in the mixed meadows. However, macrophyte–herbivore interactions are complex and final consumption rates and dietary differences are not only determined by food preferences, but also by home-range mobility, as well as by temporal and spatial differences in the availability of food resources. This study confirm the need to carry out a multiple methodological approach for a better understanding of herbivory patterns on heterogeneous habitats subjected to seasonal variation.

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**Figure 1.** Map of the study area, Cabo de Santa Pola (Spain), showing the two study locations (A and B).

**Figure 2.** A. *S. salpa* census (ind·m⁻²); B. Feeding observations (%): swimming (SW); feeding on mixed *C. prolifera* and *P. oceanica* (; mixed *C. prolifera* and *C. nodosa*; C. *prolifera* and other algae and C. Macrophyte consumption by *S. salpa* (mg WW·d⁻¹) at both locations (A and B) and both times of sampling (T1: summer 2012; T2: autumn 2012). Mean ± SE (in SNK, a and b indicate significant groupings).

**Figure 3.** Consumption by *S. salpa* during paired food preference experiments (mg WW·d⁻¹):

A. *C. nodosa* non-epiphytized (NE) vs. *C. prolifera*; B. *C. nodosa* non-epiphytized (NE) vs. *C. cylindracea*; C. *P. oceanica* non-epiphytized (NE) vs. *C. prolifera*; D. *P. oceanica* non-epiphytized (NE) vs. *C. cylindracea*; E. *C. nodosa* epiphytized (E) vs. *C. prolifera*; F. *C. nodosa* epiphytized (E) vs. *C. cylindracea*; G. *P. oceanica* epiphytized (E) vs. *C. prolifera*; H. *P. oceanica* epiphytized (E) vs. *C. cylindracea* and I. *C. prolifera* vs *C. cylindracea*.

Percentage of the different food items found in the gut contents of *P. lividus* and *S. salpa* (%). Mean ± SE. *p < 0.05; **p < 0.01; ***p < 0.001; NS = non-significant results.

**Figure 4.** A. δ¹⁵N and δ¹³C signatures of *S. salpa* individuals from the two schools and collected food items, including epiphytized and non-epiphytized seagrass leaves and their respective epiphytes (*C. cylindracea*, *C. prolifera*, *C. nodosa* epiphytized (E), *C. nodosa* non-epiphytized (NE), *P. oceanica* epiphytized (E), *P. oceanica* non-epiphytized (NE), epiphytes from *C. nodosa* leaves (Epif-*C. nodosa*) and from *P. oceanica* leaves (Epif-*P. oceanica*); B. C:N molar ratios in consumers and food items. Mean ± SE. *p < 0.05; **p < 0.01; ***p < 0.001; NS = not significant results.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Table 1. Three-way ANOVA showing differences in macrophyte consumption by *S. salpa* (mg WW·d⁻¹) between macrophyte species (CP; CC; CE; PE), times (T1: Summer; T2: Autumn) and locations (A; B). Labels: *C. prolifera* (CP); *C. cylindracea* (CC); *C. nodosa* (CE) and *P. oceanica* (PE). Significant differences are indicated: * p < 0.05, ** p < 0.01, *** p < 0.001, NS: not significant, NT: no transformation was carried out. In SNK, significant differences between investigated groups are indicated.

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Table 2. Differences in $\delta^{15}N$ and $\delta^{13}C$ signatures and nutrient contents (C:N ratios) among food items: *C. cylindracea* (CC); *C. prolifera* (CP); *C. nodosa* epiphytized (CE); *C. nodosa* non-epiphytized (CNE); *P. oceanica* epiphytized (PE); *P. oceanica* non-epiphytized (PNE) and epiphytes of *C. nodosa* (EC) and *P. oceanica* (EP). Significant differences are indicated: * p < 0.05, ** p < 0.01, *** p < 0.001, NS: not significant, NT: no transformation was carried out. In SNK, significant differences between investigated groups are indicated.

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<tr>
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<td>0.1515</td>
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<td>Total</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Transformation</td>
<td></td>
<td>NT</td>
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</table>
Table 3. Differences in biomass (mg DW cm\(^{-2}\)), coverage (%) and number of taxa (taxa cm\(^{-2}\)) between epiphytes of two macrophyte species, *C. nodosa* (EC) and *P. oceanica* (EC) and times (T1: Summer; T2: Autumn). Significant differences are indicated: * p < 0.05, ** p < 0.01, *** p < 0.001, NS: not significant, NT: no transformation was carried out. In SNK, significant differences between investigated groups are indicated.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Biomass (mgWW cm(^{-2}))</th>
<th>Coverage (%)</th>
<th>Taxa cm(^{-2})</th>
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<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>F</td>
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<td>1.901</td>
<td>12.400</td>
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<tr>
<td>Time (Ti)</td>
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<td>1.856</td>
<td>12.110</td>
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<tr>
<td>Total</td>
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<td>SNK</td>
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<tr>
<td>Transformation</td>
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</tr>
</tbody>
</table>

ECAut>ECSum=EPSum=EPAut; Aut>Sum; EC>EP; ECSum=ECAut>EPSum>EPAut

Transformation: Ln(X+1) NT Ln(X)