

# Herbivory intensity over two coexisting macrophytes on soft bottoms of Gran Canaria Island

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## ABSTRACT

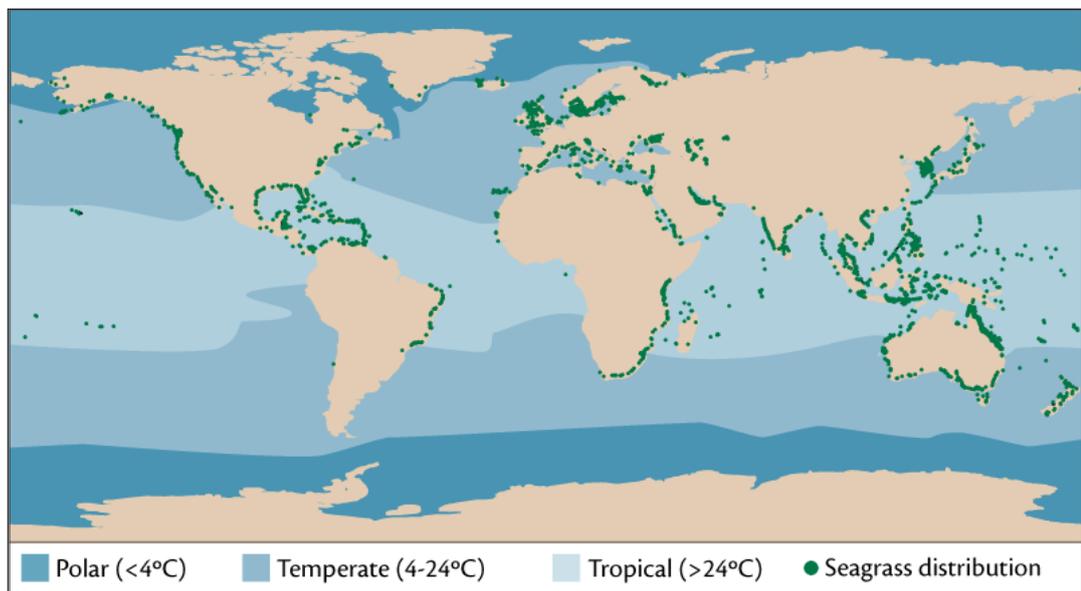
Seagrass meadows are highly productive and complex ecosystems delivering key ecosystem goods and services. Typically, seagrasses are found on shallow-water soft bottoms interspersed with other macrophytes, e.g. green macroalgae. Herbivory over seagrasses has a larger influence than previously thought and can contribute significantly to seagrass dynamics

In this study, we aimed to assess whether the magnitude of herbivory differed between two coexisting macrophytes, the seagrass *Cymodocea nodosa* and the green seaweed *Caulerpa prolifera* on Gran Canaria Island. We focused on the impact of macrograzers over these two macrophytes during two seasons, and we combined outdoor experimentation (through direct and indirect approaches that estimated differences in the intensity of herbivory between both macrophytes) and indoor experimentation. Our results showed a significantly larger consumption of *C. prolifera* than *C. nodosa*. This fact notoriously contrasts with the classic idea that the genus *Caulerpa* contains chemical compounds, as caulerpenyne that deter herbivores. Fish abundance predicted the intensity of herbivory over *C. nodosa* leaves and *C. prolifera* fronds. Moreover, our results revealed that a physical feature of macrophytes (e.g. toughness) is a major driver of macro-herbivore feeding choices.

## INTRODUCTION

Seagrasses are located in coastal areas through the world (Fig. 1). Seagrass meadows cover about 0.1-0.2% of the global oceans, and support highly productive ecosystems which fulfil a key role in the coastal realm (Duarte, 2002). They are the most important structural habitat on sandy bottoms, delivering major contributions to coastal primary production and nutrient dynamics.

Seagrass meadows are critical ecosystems for associated species, including their paramount “nursery” role (Hemminga et al. 1991, 2000; Boström et al. 2006; Gera et al. 2013), offering foraging areas for many adult fishes and birds (Valentine et al. 1991) and providing food and shelter for diverse invertebrate and fish assemblages (Hemminga et al. 2000, Duarte, 2002; Heck et al. 2003; Tuya et al. 2006; Espino et al. 2011a, 2011b). At the same time, seagrass meadows enhance particle sedimentation and protect the coastline from erosion (Ginsberg et al. 1958; Harlin et al. 1982). Seagrasses also provide an enormous source of carbon to the detrital pool, some of which is exported to the deep sea, where it provides a critical supply of organic matter in an extremely food-limited environment (Suchanek et al. 1985).



**Figure 1** Distribution of seagrasses around the world (Source: [www.teachoceanicscience.net](http://www.teachoceanicscience.net))

Many organisms are associated with seagrasses, below the sediment linked to the rhizomes, upon the leaves and stems, and over the seagrass canopy (supra- and epibenthic organisms) that can move along the meadow and constitute the main consumers of seagrass and associated vegetated material. This three-dimensional ecosystem typically harbors large and complicated food webs; the specific role of grazers in structuring seagrass assemblages is sometimes hard to understand (Heck et al. 2006). Seagrasses are plants evolutionarily adapted to herbivory. As some terrestrial plants, seagrasses share certain common structural elements, including a complex physiology through integration of ramets that generate a clonal functional structure.

Traditionally, it has been postulated that a small fraction of seagrass production is directly consumed by marine herbivores (Cebrián, 1998, 2002). The low consumption rates of seagrass by grazers have been explained by their poor nutritional quality, their high C:N ratios (Duarte, 1990), the high content in cellulose, which act as a structural deterrent, as many organisms found difficult to digest. Recent studies, however, has pointed out that herbivory over seagrasses has a larger influence than previously thought (Tomas et al. 2005; Heck et al. 2006; Prado et al. 2007; Vergés et al. 2011; Poore et al. 2012). One of these first studies that directly estimated the proportion of seagrass production consumed by herbivorous fishes (Kirsch et al. 2002) reported that, on average, fishes consumed up to 80% of the net above ground production of turtlegrass (*T. testudinum*) by the parrotfish, *Sparisoma radians*, in the Florida Keys. Tomas et al. (2005) estimated that up to 70% of the production of the seagrasses *Posidonia oceanica* in Mediterranean Sea was consumed by herbivorous fish (*Sarpa salpa*). Prado et al. (2007) also found evidence of a substantial grazing of *Posidonia oceanica* in Mediterranean Sea, reaching a gross annual estimate of 57% of annual leaf production. Chiu et al. (2013) demonstrated that tropical intertidal seagrass leaves are also important food sources for herbivores, which may take up to 20% of seagrass leaf production

Seagrasses offer grazers two potential food sources: epiphytes on seagrass leaves and the seagrass itself (Wressning, 2007). Epiphytes are considered as a key element in the relationship between herbivores and seagrasses. Some studies suggest that the epiphytes production may be elevated to exceed even that of seagrasses (Morgan et al. 1984; Moncreiff et al. 1992; Chiu et al. 2013). Temperate fishes grazing on seagrass material are thought to select seagrass leaves and parts of leaves with abundant epiphytic loads, whereas mesograzers usually feed on algae attached to seagrass leaves. These temperate fishes, such as certain *Sparids*, have locally intense impacts on seagrass. Normally, herbivorous fishes and large-sized invertebrates, e.g. sea urchins, graze seagrasses directly, while smaller invertebrates feed mainly on algae/epiphytes attached to seagrass leaves (Valentine et al. 1999, 2006; Goecker et al. 2005; Chiu et al. 2013).

*Cymodocea nodosa* is a seagrass distributed across the entire Mediterranean and the adjacent Atlantic coasts, from the southern Iberian Peninsula to Senegal, including the Macaronesian archipelagos of Madeira and the Canary Islands (Tuya et al. 2014). Meadows constituted by *C. nodosa* are the dominant vegetated communities on shallow soft substrates throughout Gran Canaria Island; where it may form mixed meadows with green rhizophytic seaweeds of the genera *Caulerpa*, such as *Caulerpa prolifera* (Fig. 2). As a result of environmental deterioration, frondose *C. nodosa* meadows can turn into bottoms dominated by *Caulerpa prolifera*; this has been reported from the Mediterranean and the southern Iberian Peninsula (Ceccherelli et al. 1997; Lloret et al. 2005), as well as from the Canary Islands, e.g. Gran Canaria Island (Tuya et al. 2013).



**Figure 2** Mixed meadow of *Cymodocea nodosa* and *Caulerpa prolifera*.

*Cymodocea nodosa* may be an important food source for macro-herbivores (Cebrián et al. 1996). In addition, leaves of this seagrass are extensively colonized by complex vegetated epiphytic assemblages that may provide food and habitat for associated invertebrates (Vinzini et al. 2002, Tuya et al. 2013). Macrophytes, e.g. green seaweeds, associated with seagrasses can also represent an additional food source for herbivores. However, certain seaweeds associated with seagrasses have developed several mechanisms to minimize herbivory damages (Duffy et al. 1990), including high levels of secondary metabolites that, in turn, have influenced the evolution of plant-herbivore interactions (Hay et al. 1988). Seaweeds may also deter herbivores by association with other plants that interfere with herbivore foraging or feeding.

In the particular case of the genera *Caulerpa*, it has been largely hypothesized that the presence of repulsive (toxic) secondary metabolites, e.g. caulerpenyne, may deter herbivory (Paul et al. 1992; Erickson et al. 2006; Box et al. 2010). Preference for vegetated material among herbivores is not exclusively related to chemical attributes, but also to the

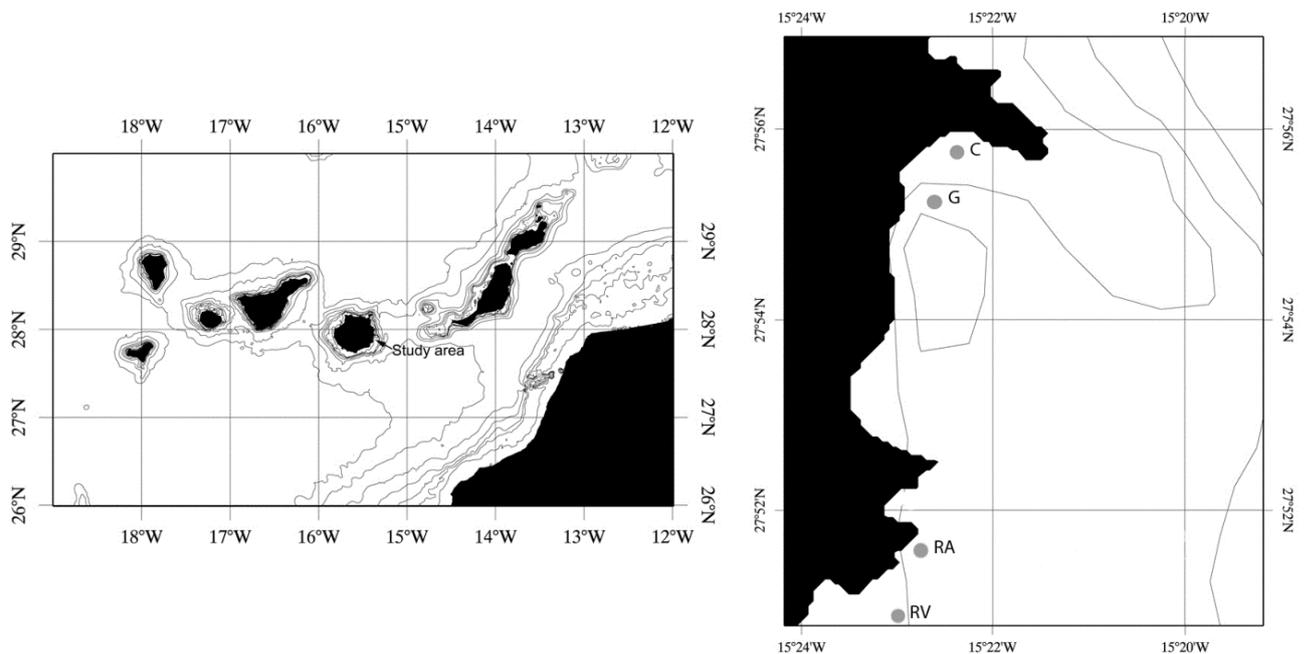
physical structure and configuration of macrophytes, e.g. their toughness (Duffy et al. 1990; Hay et al. 1994; Goecker et al. 2005; Prado et al. 2011).

In mixed meadows (i.e. seagrasses and green seaweeds), macro-herbivores have several choices of food preference, what may generate different patterns of vegetated material consumption. The aim of this work was to assess the magnitude of herbivory at mixed meadows of *C. nodosa* and *C. prolifera* at Gran Canaria Island. The intensity of herbivory was estimated by combining outdoor assays, that assessed indirect (bite marks) and direct (rates of consumption of fresh material) measures of herbivory, and an indoor experiment that quantified rates of consumption of fresh material under controlled laboratory conditions. More specifically, we set out these procedures to test whether the intensity of herbivory differed between *C. nodosa* and *C. prolifera*.

## MATERIALS AND METHODS

### Study sites

Four study sites were selected in meadows dominated by the seagrass *Cymodocea nodosa* and some accompanying green algae, particularly the rhizophytic seaweed *Caulerpa prolifera* at Gran Canaria Island (Fig. 3; Table 1).



**Figure 3** Study area. Grey dots indicate locations where sampling and experimentation took place; C (Caballo), G (Gando), RA (Roque de Arinaga) and RV (Risco Verde).

**Table 1** Localization and depths of sampled meadows.

Meadow	UTM	Depth (m)
Caballo (C)	27°56'54.41" N 15°22'32.41"W	8
Gando (G)	27°56'54.41" N 15°22'32.41"W	10
Roque Arinaga (RA)	27°51'41.50" N 15°22'54.24"W	12
Risco Verde (RV)	27°51'29.37" N 15°23'07.78"W	15.6

### *Outdoor sampling and experimentation*

We developed different types of assays to evaluate the magnitude of grazing on *C. nodosa* seagrass leaves and fronds of the green alga *C. prolifera* inhabiting Gran Canaria meadows. Firstly, we conducted an indirect approach by estimating grazing pressure as the number of bite marks left by herbivores on both *C. nodosa* leaves and *C. prolifera* fronds. The study was carried out at two different seasons; autumn (October 2013) and spring (May 2014) to test for the effect of seasonality on responses. At each of the 4 meadows, two sites were randomly selected. Within each site, 6 replicated shoots of *C. nodosa* and 6 fronds of *C. prolifera* were randomly collected by scuba divers. All material was placed in separated plastic bags and properly labeled. Samples were quickly transported to the laboratory and preserved in ice until analysis. At the same time of collection, fish communities were assessed at each meadow through standard underwater visual surveys (Tuya et al. 2006). Visual censuses were conducted following 25 x 4 m transects (100 m<sup>2</sup> of observation per census, n=4), so the abundance and size of each fish species was annotated. Once in laboratory, samples were placed in trays to firstly measure the length of *Cymodocea nodosa* leaves (from the ligule to the upper tip of the leaf) and *Caulerpa prolifera* fronds (from the base of the stipe to the upper tip of the frond). At the same time, bite marks were recorded for each leaf/frond; all material was then preserved in silica gel. Some bite marks are clearly crescent-shaped (Fig.4) a doubtless indication of grazing by herbivorous fishes (Hay, 1984; Kirsch et al. 2002; White et al.2011). In these cases, we recorded the size (in cm). The cover of epiphytic material was also determined through visual estimation by using a qualitative scale (Table 2).



**Figure 4** Crescent-shaped bite mark on a *C. nodosa* leaf.

**Table 2** Qualitative scale of epiphytic coverage.

SCALE	0	1	2	3	4	5
COVERTURE	< 1%	1-10%	10-20%	20-40%	40-60%	>60%

When the apical part of either seagrass leaves or *C. prolifera* fronds was damaged, we omitted to record these marks as bite marks, due to the difficult to ascertain if these marks result from grazing or other type of damage. Finally, we took measurements of the leaf/frond toughness as a way to assess the physical resistance to breakage of both macrophytes; these measurements were assessed with a dynamometer. Each of (n=30) leaves/fronds was attached to the pin of the dynamometer; the force (Newtons) necessary to break up the leaf/frond was annotated.

In an *in situ* experiment, we offered herbivores fresh *C. nodosa* leaves and *C. prolifera* fronds alternatively attached with clothespins to plastic mesh frames (Fig.5). This was a way to directly estimate consumption rates over both *C. nodosa* and *C. prolifera*.



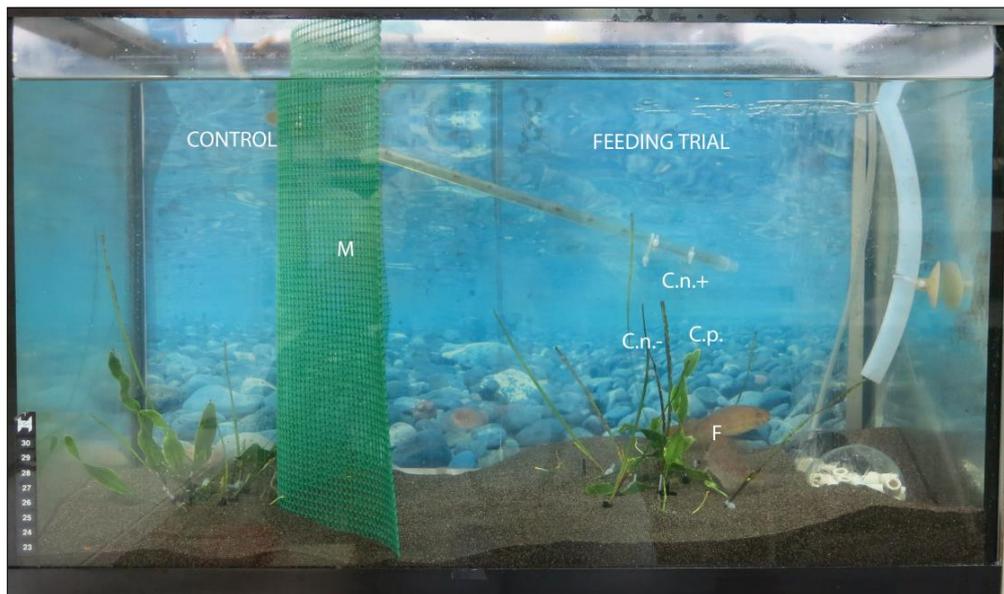
**Figure 5** Mesh frame attached to the ocean floor with *C. nodosa* leaves and *C. prolifera* fronds.

Fresh material was initially collected by scuba divers minutes before to set up the experiment, which was located at G (Fig.3). A total of 14 plots (mesh frames) were directly attached to the ocean floor with metal bars and cover with sand to minimize any visual impact. Plots were placed at two randomly-selected areas within this meadow. The distance between adjacent plots was approximately 0.5 meters. All leaves were of similar length; we only used leaves with no previous herbivore marks and with no signs of necrosis (Vergés et al. 2011). Furthermore, in order to offer herbivores material under similar conditions, epiphytes were scraped off the leaves using a thin blade (Wressning et al. 2007). The length of all *C. nodosa* leaves were standardized to 15 cm and *C. prolifera* fronds to 7 cm; this was accomplished by removing the excess length by cutting the apical part with scissors before the experiment was initiated. Three leaves of *C. nodosa* and 3

fronds of *C. prolifera* were used per each plot. After 7 days, all material was collected and stored in labelled plastic bags. Once in the laboratory, the number of bites marks per leave/frond was recorded, as in the previous assay.

### *Indoor experimentation*

We tested for differences in feeding preferences by the main grazer fish in seagrass meadows of the study region (Tuya et al. 2013), the parrotfish *Sparisoma cretense*, on *C. nodosa* seagrass leaves with or without epiphytes and fronds of *C. prolifera* under controlled laboratory conditions. Fresh material of both macrophytes (seagrass leaves with epiphytes, seagrass leaves without epiphytes and *C. prolifera* fronds) was offered to 3 *Sparisoma cretense* per aquaria, which were collected in Gando meadow through a seine net. Fish were allowed to acclimatize to indoor conditions for 7 days, with a lack of food provision prior to experimentation (Prado et al. 2011; Tuya et al. 2012). All fish specimens were small-sized (juveniles), with lengths ranging from 7 to 18 cm. This experiment was replicated in a total of 4 aquaria (84 l) with constant aeration. Only seagrass leaves/algae fronds without grazing scars were selected. Each aquaria had two compartments separated by a mesh net (Fig. 6); one for the feeding trial including the fish, while the other compartment lacked fish and so acted as a control to correct for possible autogenic changes in macrophytes biomass not directly caused by grazing (Wressing et al. 2007; Prado et al. 2011). A total of 5 seagrass leaves (with and without epiphytes) and 5 fronds of *C. prolifera* were placed into each experimental compartment per tank. All vegetated material was distributed randomly and secured to the bottom with wire stakes (Fig. 6).



**Figure 6** Aquaria with two separated compartments by a mesh (M), including a CONTROL without fish, and a FEEDING TRIAL with fish (F). The three treatments included: C.n. +, *C. nodosa* leaves with epiphytes, C. n. -, *C. nodosa* leaves without epiphytes, and C. p. *C. prolifera* fronds.

Prior to experimentation, the wet weigh of the 15 vegetative fragments per aquaria was obtained. We the conducted the feeding trial over a 24 h period; after that the remaining plants were removed from each tank, blot-dried, weighed, measured and the numbers of bite marks counted.

### *Statistical analyses*

Differences in the number of bite marks between the two macrophytes was tested using a 3-way ANCOVA, which incorporated the factors: “Time” (fixed factor with 2 levels: Time 1, October 2013 and Time 2 May 2014), “Meadow” (random factor with 4 levels: Caballo, Gando, Roque Arinaga and Risco Verde) and “Species” (fixed factor with two levels: *Cymodocea nodosa* and *Caulerpa prolifera*); in addition, “Leave length” and “Epiphytism” were included as covariates to remove their potential influence. Data were square root transformed prior to analyses, and analyses based on Euclidean distances. Differences in consumption between both macrophytes in the indoor experiment was tested by a 3-way ANOVA, that included a similar design relative to the previous analysis, but the factor “Meadow” was replaced by “Mesh”. A t-test tested for significant differences in toughness between *C. nodosa* and *C. prolifera* at the range of sizes they typically occur.

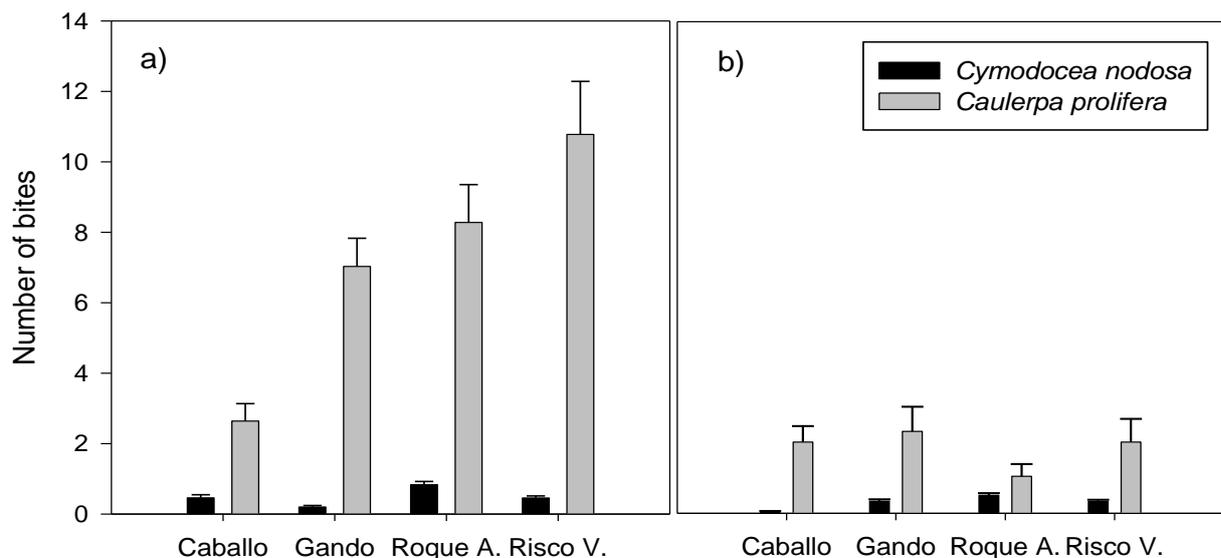
To test for differences in consumption between aquaria and the type of vegetation in the indoor trial, a 2-way ANOVA was used; “Aquaria” (random factor with 4 levels: aquaria 1, aquaria 2, aquaria 3 and aquaria 4) and “Treatment” (fixed with 3 levels: *C. nodosa* with epiphytes, *C. nodosa* without epiphytes and *C. prolifera*). When appropriate, pair-wise comparisons were performed.

ANOVAs and ANCOVAs were carried out using the PERMANOVA 1.6 statistical package (courtesy of M. J. Anderson).

## RESULTS

### Outdoor experimentation

The number of bites differed between *Cymodocea nodosa* and *Caulerpa prolifera* (Fig.7; Table 3, “C.n. Vs.C.p.” P=0.008) consistently through times and meadows (Table 3, “TimexC.n Vs. C.p.” and “MeadowxC.n Vs. C.p.” P>0.05). The number of bites at Time 1 (October, 2013) was larger than at Time 2 (May, 2014) (Fig.7; Table 3 “Time” P=0.03) irrespectively of the meadow (Table 1, “TimexMeadow” P>0.05).

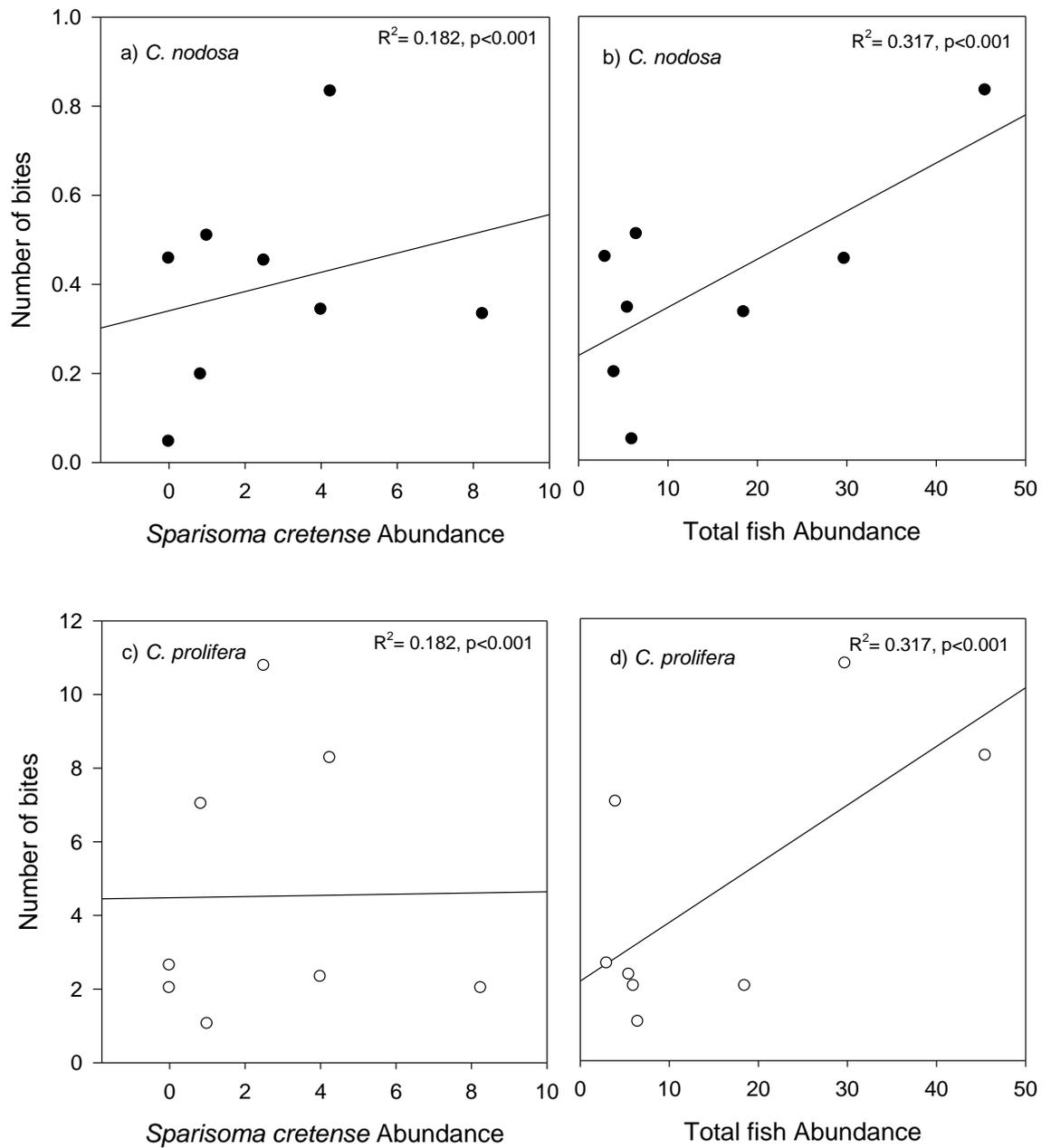


**Figure 7** Grazing pressure over *C. nodosa* leaves and *C. prolifera* fronds, estimated as the number of grazing marks per leaf/frond, at each seagrass meadow, at a) October 2013 and b) May 2014. Error bars are +SE of means.

**Table 3** Results of 3-way ANCOVA testing for differences in the number of grazing marks between times, meadows and macrophytes (*C. n.* = *Cymodocea nodosa* and *C. p.* = *Caulerpa prolifera*). \*Significant differences at P<0.05.

	df	MS	F-ratio	P-valor
<b>Length = Covariate1</b>	1	1	0.10	0.752
<b>Degree of Epiphytism = Covariate 2</b>	1	225	18.46	0.000*
<b>Time</b>	1	994	15.61	0.030*
<b>Meadow</b>	3	126	2.74	0.690
<b>C.n. Vs. C.p.</b>	1	1644	27.75	0.008*
<b>Time x Meadow</b>	3	61	0.58	0.669
<b>Time x C.n. Vs. C.p.</b>	1	1014	9.36	0.055
<b>Meadow x C.n. Vs. C.p.</b>	3	92	0.84	0.555
<b>Time x Meadow. x Cn. Vs. C.p.</b>	3	110	8.98	0.000*

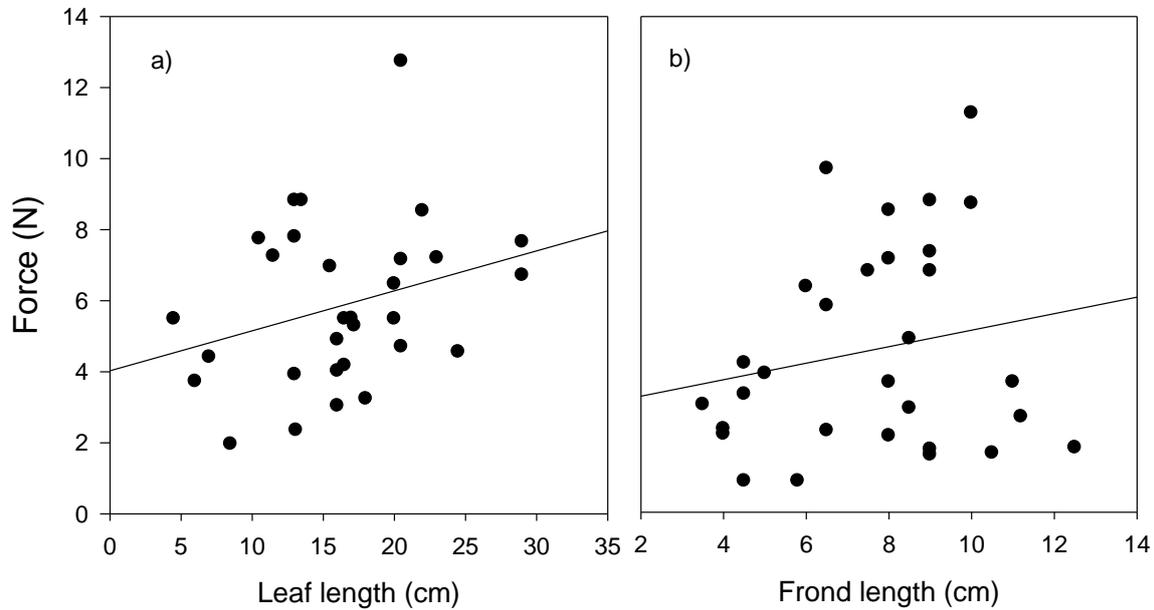
In general, the total abundance of herbivorous fishes (Appendix 2) and the abundance of the most conspicuous herbivorous fish, the parrotfish *Sparisoma cretense*, significantly predicted differences in herbivory intensity between meadows (Fig.8).



**Figure 8** Mean number of bites on (a and b) *C. nodosa* leaves and (c and d) *C. prolifera* fronds according to the abundance of the parrotfish, *Sparisoma cretense*, and the total grazers fish abundance. Data were pooled for the two sampling times.

### Differences in toughness

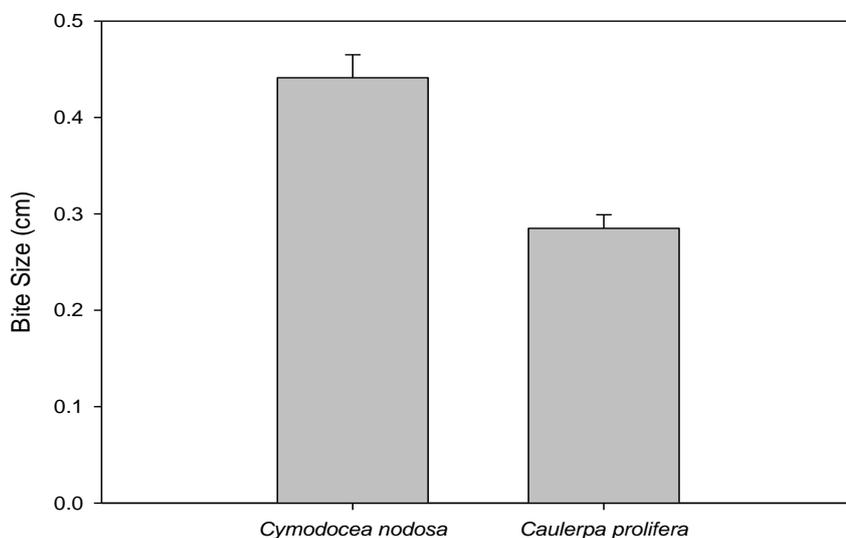
Overall, *C. nodosa* leaves had a larger toughness than fronds of *C. prolifera*, when considering the range of sizes that both macrophytes typically reach in situ (Fig. 9;  $t$ -student = 1.9047,  $P$ = 0.061).



**Figure 9** Differences in leaf toughness between (a) *Cymodocea nodosa* leaves and (b) *Caulerpa prolifera* fronds (measurements were carried out with a dynamometer and force is expressed in Newton).

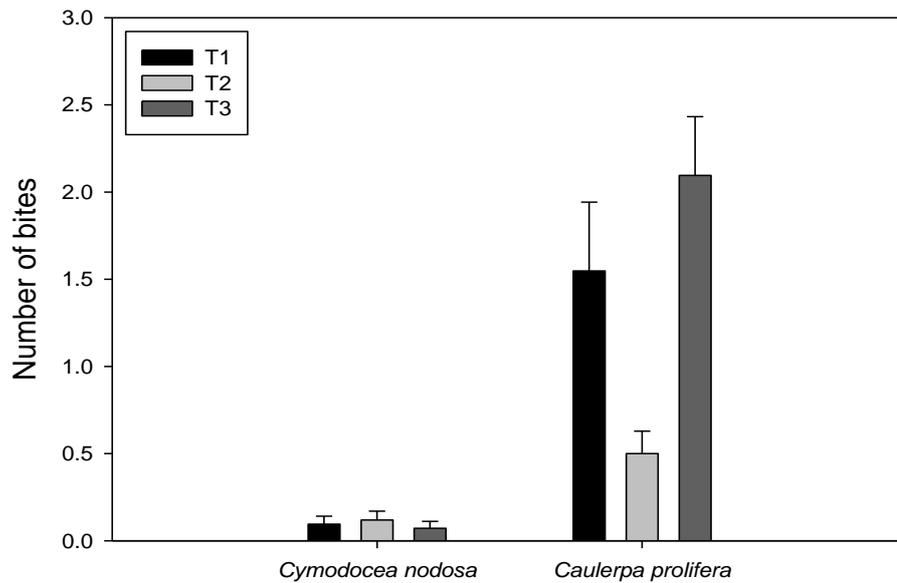
### Bite size

The size of grazing marks (bites) on *C. nodosa* leaves were larger than those of *C. prolifera* fronds (Fig. 10;  $t$ -test=6.0134,  $P$ <0.00001).



**Figure 10** Difference in bite size between *Cymodocea nodosa* leaves and *Caulerpa prolifera* fronds.

Consumption of *Caulerpa prolifera* fronds greatly exceeded consumption on seagrass leaves (*Cymodocea nodosa*) (Fig. 11; Table 4, "C.n. Vs. C.p." P=0.003), particularly on time 3 (June, 2014) (Fig. 11; Table 4, "Time x C.n. Vs. C.p." P=0.008).



**Figure 11** Mean number of bites over *Cymodocea nodosa* and *Caulerpa prolifera* leaves and *Caulerpa prolifera* fronds after 1 week at each replicated assay (T1= October 2013, T2= May 2014 and T3= June 2014). Error bars are +SE of means.

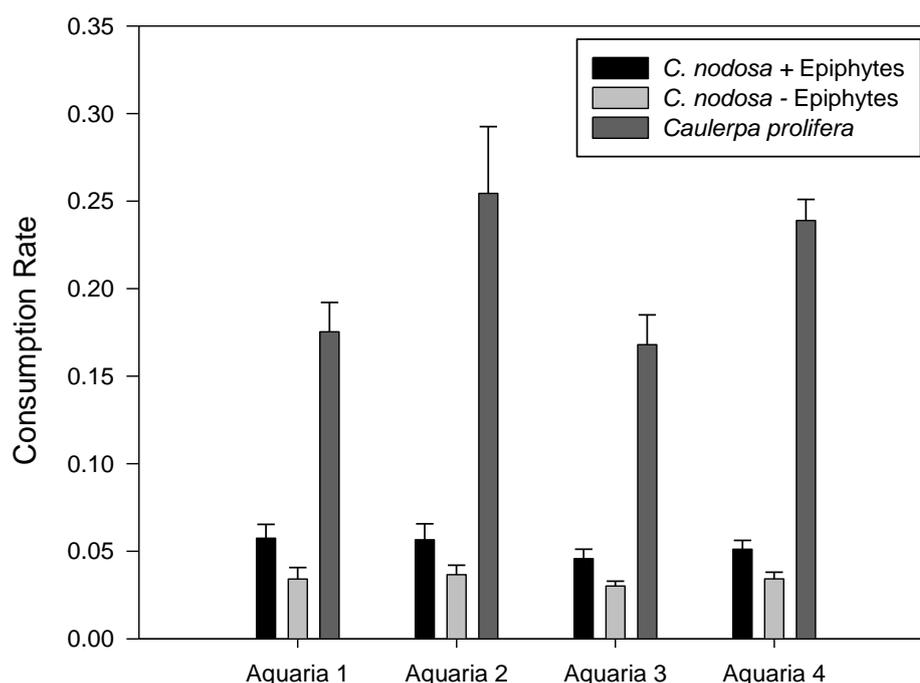
**Table 4** Results of 3-way ANOVA testing for differences in grazing marks between times, meadows and macrophytes (C. n. =*Cymodocea nodosa* and C. p. = *Caulerpa prolifera*).

\* Significant differences at P<0.05 \*\* Significant differences at P<0.01

	df	SS	MS	F	P
<b>Time</b>	1	1.3216	1.326	8.21778	0.0150*
<b>Mesh</b>	13	5.4303	0.4177	0.9650	0.5000
<b>C.n. Vs. C.p.</b>	1	9.2099	9.2099	22.7752	0.0030**
<b>Time x Mesh</b>	13	2.1009	0.1616	0.3733	0.9770
<b>Time x C.n. Vs. C.p.</b>	1	1.7002	1.7002	11.4539	0.0080**
<b>Mesh x C.n. Vs. C.p.</b>	13	5.2570	0.4044	0.9342	0.5360
<b>Time x Mesh x C.n. Vs. C.p.</b>	13	1.9297	0.1484	0.3429	0.9870
<b>Residual</b>	112	48.4829	0.4329		
<b>Total</b>	167	75.4325			

### Indoor assay

Overall, consumption of *Caulerpa prolifera* fronds was larger than consumption on either *Cymodocea nodosa* leaves with or without epiphytes (Fig. 12; Table 5, “Treatment” P=0.03). However, the magnitude consumption among treatments varies from aquaria to aquaria (Fig. 12; Table 5, “Aquaria x Treatment” P=0.01).



**Figure 12** Consumption rates of vegetated material (g wet weight ind<sup>-1</sup>d<sup>-1</sup>) by the parrotfish *S. cretense*. Error bars are +SE of means.

**Table 5** Results of 3-way ANOVA testing for differences in consumption rates between aquariums and treatments (*C. nodosa* leaves with epiphytes, *C. nodosa* leaves without epiphytes and *Caulerpa prolifera* fronds).

\* Significant differences at P<0.05    \*\*Significant differences at P<0.01

	df	SS	MS	F	P
<b>Aquaria</b>	3	70.0214	23.3405	17.2912	0.0010**
<b>Treatment</b>	2	54.2843	27.1422	6.0696	0.0380*
<b>Aquaria x Treatment</b>	6	26.8309	4.4718	3.3128	0.0100*
<b>Residual</b>	48	64.7925	1.3498		
<b>Total</b>	59	215.9291			

## DISCUSSION

Our study has demonstrated that herbivory may remove substantial amounts of vegetated material in mixed seagrass meadows of *C. nodosa* and *C. prolifera* in Gran Canaria Island. *Caulerpa prolifera* had higher rates of herbivory than *Cymodocea nodosa*, both in the field through direct and indirect measures and in aquaria (indoors) conditions. Average feeding rates of *Sparisoma cretense* were estimated at  $0.209 \text{ ind}^{-1}\text{d}^{-1}$  for *C. prolifera* and  $0.086 \text{ g wet weight ind}^{-1}\text{d}^{-1}$  for *C. nodosa* under laboratory conditions. These results are in accordance with previous studies (Appendix 3), which reported significant amounts of consumption by herbivores over seagrasses and algae.

Fish abundance recorded at the studied meadows significantly predicted the intensity of herbivory over *C. nodosa* leaves and *C. prolifera* fronds, existing a positive correlation between the number of bite marks on *C. nodosa* leaves and the total fish abundance. Herbivory intensity was strongly seasonal, reaching maximum values in the late summer (October 2013) and minimum values in spring (May 2014). There is a clear correlation between the annual vitality cycle of *C. nodosa* and the richness and abundance of associated fish assemblages in the study region (Tuya et al. 2006 and Polifrone et al. 2006), with maximum values in spring-summer and minimum in autumn-winter (Espino et al. 2011). Our results are, moreover, in accordance with seasonal trends for herbivory in shallow seagrass meadows from the Mediterranean, where maximum rates of seagrass material occur in summer (Tomas et al. 2005; Prado et al. 2007, 2010). Chiu et al. (2013) also demonstrated that leaf grazing rates were significantly greater in summer and autumn than in winter and spring. Seasonal differences in herbivory pressure can be related to migratory or seasonal feeding behavior of herbivore species (Prado et al. 2007).

Herbivorous fishes inhabiting seagrass meadows are typically small-sized, i.e. juveniles. In the study region, for example, 98.68% of fishes were juveniles (Espino et al. 2011). The most abundant herbivorous fish in the Canary Islands, the parrotfish *Sparisoma cretense*, is majorly found as small-sized individuals (7-18 cm). Our data has demonstrated that the varying intensity of herbivory between *C. nodosa* and *C. prolifera* was size-dependent, as grazing marks were significantly larger on *C. nodosa* leaves than in *C. prolifera* fronds. The mean bite size on *C. nodosa* leaves normally exceeded 0.4 mm, which likely corresponds with adult and sub-adult fishes (e.g. *Sparisoma cretense*). In contrast, bite sizes on *C. prolifera* fronds ranged from 0.1 to 0.5 mm, which likely correspond with juvenile fish stages (Fig. 10). In other words, small-sized herbivorous fishes preferably consume *C. prolifera*; once a certain size is reached, herbivorous fish can consume both macrophytes, and so *C. nodosa* leaves. Adult fishes are big enough to move across the meadows without necessary to hide of predators and so the entire canopy is available to

them, while juveniles hide from predators within the dense canopy provided by seagrass leaves. As a result, small-sized fishes have a direct access to the shorter fronds of *C. prolifera*, which on other hand are softer to feed in. This hypothesis is consistent with the assumption that adult fishes have a different trophic niche in comparison with juvenile transients (Livingston, 1982; Barry et al. 1996; Vizzini et al. 2002).

Many herbivores find difficulties to consume tough plant material (Steneck et al. 1982; Watson et al. 1985). Calcification and toughness usually correlate with low feeding preference (Litter et al. 1983; Hay, 1984; Paul et al. 1986). *C. prolifera* shows a higher total internal N content than *C. nodosa* and hence a lower C:N ratio (García-Sánchez et al. 2012). *C. nodosa* is a seagrass with high fibre content (large amounts of cellulose in their cell walls), with a C:N ratio of 14:4 (Goldenberg et al. 2014). In turn, a high fraction of its internal C is used for the synthesis of structural carbohydrates that forms cell walls and fibre bundles, which means that *C. nodosa* has a high mechanical resistance, exhibiting a low leaf nutritional value (Lucas et al. 2000; De Los Santos et al. 2012). *C. nodosa* is tougher than foliose unicellular seaweeds such as *C. prolifera* (our data supported this idea). Fast-growing species (such as *C. prolifera*) do not invest resources in leaf/frond toughening as much as large, long-lived seagrass species (De Los Santos et al. 2012). Fibre content influences herbivore feeding selectivity, since fibrous tissues are difficult to break down mechanically and digest (Klumpp et al. 1983; Lanyon et al. 2006). This fact supports the hypothesis that plant physical features (as C:N ratio, fibre content and leaf-fracture properties) have ecological consequences (Read et al. 2006), influencing consumer preferences and the relationship between physical features of fishes (e.g. the degree of development of the jaws) and their diets (Vergés et al. 2007, 2011). Probably, the jaws of *Sparisoma cretense* juveniles do not have enough force to rip off pieces of *C. nodosa* leaves; this idea is consistent by the presence of unsuccessful bite marks of seagrass blades (Fig. 13).



**Figure 13** “Unsatisfactory” bite on *Cymodocea nodosa* leaf.

Other studies support this fact, Jernakoff et al. (1997, 1998) described that grazers did not eat *Posidonia* leaves, but consume their epiphytes and periphyton. Wressing et al. (2007) measured direct consumption by monacanthid fish over seagrass leaves and they recorded a preference toward seagrass leaves with high epiphyte loads (old leaves) whereas young, soft, nutrient-rich seagrass blades were consumed less than mature blades; Doropoulos et al. (2009) reported results of preference towards kelp and periphyton and red algae and avoidance of seagrass consumption. Prado et al. (2011) considered that plant physical attributes do not significantly explain food preferences by macrograzers (fish and sea urchins, in particular); selection of seagrass material was primarily based on nutritional characteristics. In their study, however, they only included adult fishes, so the resistance of seagrass to breakage is relatively low as a result of well-developed jaws. Small-sized fishes (juveniles) and small-sized invertebrates, which may constitute a large quantity of consumers within meadows. It is likely that these small-sized assemblages have more difficulties to feed on seagrass leaves than macrograzers.

The production and utilization of secondary metabolites has been indicated to justify that plant chemistry is the central factor determining herbivore feeding choices (Ehrlich et al. 1988; Schultz, 1988). However, the relative importance of these factors probably varies from system to system depending upon the identity of herbivores (Pennings et al. 1992). Numerous studies reported that caulerpenyne, the main secondary metabolites from genus *Caulerpa*, actively deter herbivores (e.g. gastropods and fishes), which is toxic to larval and adult stages of many marine invertebrates and vertebrates (Paul et al. 1986; Hay et al. 1988; Paul et al. 1992; Lemee et al. 1993; Pedrotti et al. 1996; Nelson et al. 2003). Raniello et al. (2007) also reported phytotoxicity of caulerpenyne on the leaf tissue of *C. nodosa*. Sea urchins (McConnell et al. 1982) and a few reef fish (Targett et al. 1986; Paul et al. 1987) have been deterred by caulerpenyne, despite, most reef fish were not deterred by *Caulerpa* extracts containing caulerpenyne (Paul et al. 1987; Wylie et al. 1988; Paul et al. 1990, 1992). Caulerpenyne plays a major role in the macroalgae chemical defence against epiphytes and herbivores (Erickson et al. 2006). Macroalgal fronds have often higher concentrations of caulerpenyne than stolons and this concentration may even change during the year (Box et al. 2010). Variation in herbivory may relate to caulerpenyne concentration, which varies within and among species. In any case, the classic paradigm that *Caulerpa* species deter herbivores through the presence of secondary metabolites seems to be overrated by our data. We demonstrated a larger consumption over *C. prolifera* fronds than *C. nodosa* leaves. The convergence of results from indirect and direct in situ measurements and indoor assays reinforce this idea. In all cases, herbivory pressure was significantly higher over *C. prolifera* despite their secondary metabolite. With these results, we hypothesized that caulerpenyne may have a higher effect on small organisms (like epibiota or invertebrates) than in vertebrates. Probably, macrograzers that inhabiting mixed seagrass meadows may be adapted to allelochemicals produced in the system (like related Reigosa et al. 1999; Prado et al. 2011 and Goldenberg et al. 2014).

Seagrass leaves, *C. nodosa* in particular, are extensively colonised by a complex epiphytic community (cyanobacteria, diatoms, crustose and ephemeral algae, invertebrates...), which provide food and habitat for invertebrates and so increases the spatial complexity of the habitat (Mazzella et al. 1992; Vizzini et al. 2002). Our results showed that that *C. nodosa* leaves with epiphytes are preferred by herbivores over leaves devoid of epiphytes despite the assumption that epiphytes are a negligible food source for herbivores due to their low biomass (Tomas et al. 2005). This is consistent with previous findings which confirm that fishes grazing on seagrass prefer epiphytes growing on the seagrass leaves (Conacher et al. 1979; Cebrián et al. 1996; Wressing et al. 2007).

Overall, our results along with the compilation of published reports presented show herbivory on seagrasses as a very variable process which depends of season, the level of the complexity that we search (include epiphytes, micrograzers, macrograzers, “stage of age” of the specie, relationship-competition between grazed species...) the latitudinal zone, the physical features of the meadow.

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## APPENDIX

**Appendix 1** Average percentage of daily change in length of *C. nodosa* leaves and *C. prolifera* fronds as a result of autogenic processes.

	<i>C. nodosa</i> with epiphytes	<i>C. nodosa</i> without epiphytes	<i>Caulerpa</i> <i>prolifera</i>
<b>Aquaria 1</b>	-0.377	2.432	0.069
<b>Aquaria 2</b>	1.877	0.860	1.848
<b>Aquaria 3</b>	0.631	1.263	1.322
<b>Aquaria 4</b>	0.068	-0.886	2.387
<b>TOTAL</b>	2.199	3.670	5.628

**Appendix 2** Fish abundances at each studied meadow (mean number of fish 100m<sup>-2</sup> ±SE); their species trophic classification are also included: CAR: Carnivorous, OMN: Omnivorous, HER: Herbivorous (\*: only juveniles) at time 1 (T1, October 2013) and time 2 (May 2014).

Specie	Trophic class	Caballo		Gando		Roque Arinaga		Risco Verde	
		T1	T2	T1	T2	T1	T2	T1	T2
<i>Boop boops</i>	OMN					17.5 ± 17.5		25 ± 25	
<i>Bothus podas maderensis</i>	CAR			0.67 ± 0.33					0.25 ± 0.25
<i>Canthigaster capistrata</i>	OMN	1.25 ± 0.75	1.5 ± 0.65	1.5 ± 0.22	1.25 ± 0.25	3.5 ± 0.28	3.5 ± 1.26	0.75 ± 0.47	3.75 ± 0.85
<i>Dasyatis pastinaca</i>	CAR					0.5 ± 0.5			
<i>Diplodus annularis</i>	HER*			0.16 ± 0.16					
<i>Mullus surmuletus</i>	CAR					10 ± 5.7		0.25 ± 0.25	
<i>Pagrus auriga</i>	CAR						0.25 ± 0.25		0.25 ± 0.25
<i>Pagrus pagrus</i>	CAR			0.16 ± 0.16		0.25 ± 0.25			
<i>Sparisoma cretense</i>	HER			0.83 ± 0.54	3 ± 2.35	4.25 ± 0.47	0.25 ± 0.25	2.5 ± 0.86	8.25 ± 3.97
<i>Spondyllosoma cantharus</i>	OMN					7.5 ± 4.41			1 ± 1
<i>Sphyræna viridensis</i>	CAR								

<i>Spherooides marmoratus</i>	OMN	1.75 ± 0.63	3 ± 0.41	0.5 ± 0.22	1 ± 0.41	1.5 ± 0.65	1.5 ± 0.87	1 ± 0.7	2.25 ± 1.03
<i>Stephanolepis hispidus</i>	CAR		1.5 ± 0.86						
<i>Synodus sp.</i>	CAR			0.16 ± 0.16		0.25 ± 0.25			
<i>Xyrichtys novacula</i>	CAR					0.25 ± 0.25	1 ± 1	0.25 ± 0.25	1.75 ± 1.03
<i>Trachinus draco</i>	CAR								1 ± 0.71
<i>Thalassoma pavo</i>					0.25 ± 0.25				

**Appendix 3** Literature review on herbivory rates over seagrasses and algae (\*Seagrass defoliation; \*\*Leaf consumption; \*\*\*Feeding rate).

Reference	Consumer	Seagrass	Herbivory rates
Cebrián et al. 1996	-	<i>Cymodocea nodosa</i>	17.6 ± 7.3 (mg DW shoot <sup>-1</sup> y <sup>-1</sup> )
Cebrián et al. 1998	-	<i>Thalassodendron ciliatum</i>	36.2 (mg DW m <sup>-2</sup> d <sup>-1</sup> ) areal leaf removal
		<i>Enhalus acoroides</i>	3 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Cymodocea serrulata</i>	70.5 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Thalassia hemprichii</i>	319 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>T. testudinum</i>	376± 300 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Zostera marina</i>	130±50.2 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Z. noltii</i>	40±8.6 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Cymodocea nodosa</i>	87.6±38.9 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Posidonia oceanica</i>	56.5±7.7 (mg DW m <sup>-2</sup> d <sup>-1</sup> )
Goecker et al. 2005	-	<i>Thalassia testudinum</i> high [N]	> 15% (Percent/2h)

		<i>Thalassia testudinum</i> low [N]	<15% (Percent/2h)
Tomas et al. 2005	<i>Sarpa salpa</i>	<i>Posidonia oceanica</i>	16.65 (gDW m <sup>-2</sup> d <sup>-1</sup> )
	<i>Paracentrotus lividus</i>	<i>Posidonia oceanica</i>	0.29 (gDW m <sup>-2</sup> d <sup>-1</sup> )
Unsworth et al. 2007	<i>Leptoscarus vaigiensis</i>	<i>Thalassia hemprichii</i>	> 0.1 (gDW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Enhalus acoroides</i>	0.05(gDW m <sup>-2</sup> d <sup>-1</sup> )
		<i>Cymodocea rotundata</i>	> 0.05(gDW m <sup>-2</sup> d <sup>-1</sup> )
Prado et al. 2007	<i>Sarpa salpa</i>	<i>Posidonia oceanica</i>	0.365 ± 0.08 (cm <sup>2</sup> shoot <sup>-1</sup> d <sup>-1</sup> ) *
	<i>Paracentrotus lividus</i>	<i>Posidonia oceanica</i>	0.158 ± 0.04 (cm <sup>2</sup> shoot <sup>-1</sup> d <sup>-1</sup> ) *
Prado et al. 2011	<i>Stephanolepis hispidus</i>	<i>Halodule wrightii</i>	0.04 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
		<i>Thalassia testudinum</i>	< 0.02 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
		<i>Syringodium filiforme</i>	> 0.06 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
	<i>Lagodon rhomboides</i>	<i>Halodule wrightii</i>	> 0.002 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
		<i>Thalassia testudinum</i>	0 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
		<i>Syringodium filiforme</i>	> 0.004 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
	<i>Nicholstina usta</i>	<i>Halodule wrightii</i>	> 0.4 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
		<i>Thalassia testudinum</i>	0.2 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
		<i>Syringodium filiforme</i>	> 0.6 (g WW ind <sup>-1</sup> d <sup>-1</sup> ) **
Chiu et al. 2013	<i>Siganus fuscescens</i> <i>Tripneustes gratilla</i>	<i>Thalassia hemprichii</i>	0.77 ± 0.13 (g DW m <sup>-2</sup> d <sup>-1</sup> )
Goldenberg et al. 2014	<i>Sarpa salpa</i>	<i>Cymodocea nodosa</i>	32 ± 9 g seagrass (kg fish) <sup>-1</sup> d <sup>-1</sup> ***
		<i>Zostera marina</i>	21 ± 11 g seagrass (kg fish) <sup>-1</sup> d <sup>-1</sup> ***
		<i>Zostera noltii</i>	40 ± 11 g seagrass (kg fish) <sup>-1</sup> d <sup>-1</sup> ***

