

**Biogeographic variations and
response of epifauna to varying levels of
herbivory intensity over the seagrass
*Cymodocea nodosa***

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TITLE

Biogeographic variations and response of epifauna to varying levels of herbivory intensity over the seagrass *Cymodocea nodosa*.

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ABSTRACT (key words)

Seagrasses worldwide constitute a key habitat on shallow soft bottoms, but they are experiencing a rapid decline affecting biodiversity. In order to determine how habitat structure may influence processes that shape faunal communities, we experimentally assessed the effects of different levels of herbivory intensity over the seagrass *C. nodosa* on the composition and abundance of associated epifauna. We tested these effects at two regions throughout the distribution range of *C. nodosa*: the Canary Islands (Gran Canaria) and the Balearic Islands (Mallorca). High herbivory was simulated by clipping seagrass leaves in 5 plots with respect to 5 control plots. Epifaunal abundances and taxon richness differed between regions and treatment. These effects varied amongst epifaunal taxonomic groups, as well as when considering their abundances per area or per biomass of seagrass. For several groups, epifaunal responses to seagrass clipping were the opposite at the two regions. These results highlight the importance of carrying out experimental comparisons at multiple sites to obtain a more complete understanding of ecological patterns and processes governing marine benthic communities.

Key words: Seagrass, *Cymodocea nodosa*, herbivory, epifauna, Mallorca-Gran Canaria.

INTRODUCTION

Seagrass meadows perform essential ecosystem functions and provide services at a global scale; they produce large quantities of organic carbon and support food webs (Duarte et al. 2008; Hemminga and Duarte 2000), provide shelter for associated fauna (Pollard 1984, Espino et al. 2011), produce O₂ (Peduzzi and Vukovic 1990), and stabilize sediments and protect coastlines from turbulence (Hemminga and Nieuwenhuize 1990, Cabaço et al. 2010). Therefore, seagrasses worldwide constitute a key habitat on shallow soft bottoms, where they profoundly influence the physical, chemical, and biological environments - acting as 'ecological engineers' (Wright and Jones 2006). However, seagrass meadows are experiencing a rapid decline worldwide (Orth et al. 2006). Waycott et al. (2009) estimated a global loss of 29% of their coverage between 1879 and 2006, with an increasing trend of annual loss of 7% since 1990. Studies on different topics of seagrass beds have increased in the last decades, stimulated by their high ecological importance for coastal systems and their importance as habitat-formers or ecological engineers. The fact that they are among the most endangered coastal habitat, with a regression worldwide affecting biodiversity on both local and global scales, highlights that their conservation is a primary issue for preserving healthy marine environments (Jones et al. 1994, Short and Neckles 1999; Hemminga and Duarte 2000; Duarte 2002).

A major goal of marine ecology is to determine how habitat structure may influence processes that shape faunal communities (Sirota and Hovel 2006). Studies on the associated fauna, their spatial and temporal variation, as well as their trophic interactions are of special interest to evaluate the importance of seagrass beds for local biodiversity and to improve the management of local fisheries resources (Marina et al. 2012). These meadows serve as a refuge for many species because the structural complexity of emergent shoots (measured as shoot density, leaf biomass, or leaf surface area) inhibits the detection and capture of prey by predators (Orth 1992). This complexity can modify the interaction between predation and competition (Hixon and Menge, 1991). In addition to providing shelter, they also provide food for diverse invertebrates and fish assemblages (Tuya et al. 2006, Espino et al. 2011, Tuya et al. 2014a), including a wide range of animals that live closely associated to seagrass blades, e.g. epifaunal organisms (Gartner et al. 2013). Mobile invertebrates are not randomly distributed in seagrass habitats; the abundances of most species are correlated with macrophytic biomass (Brook, 1978; Heck and Orth, 1980; Stoner, 1980; Lewis & Stoner, 1983). Studies have suggested that epiphytic algae on seagrass meadows positively influence the abundance and/or diversity of motile seagrass epifauna by increasing habitat complexity and food abundance (Nagle, 1968; Novak, 1982; Lewis and Hollingworth, 1982; Bell et al., 1984; Virnstein and Curran, 1986).

Herbivory is probably one of the most ecologically significant interactions in the biosphere. Beyond its role on plant performance and trophic fluxes, herbivory has profound effects on vegetation structure, composition and productivity, and has probably been a strong evolutionary driver since the dawn of life (McNaughton 2001). A great variety of species, ranging in size from small amphipods to dugongs (*Dugong dugong*) feed, at least in part, on live seagrass tissues (Moran et al. 2005). Recent evidence has proved that grazing in seagrasses is by far more important than previously thought (Heck and Valentine 2006, Valentine and Duffy 2006), affecting their population dynamics, composition, distribution and production (Valentine and Heck 1999, Tomas et al. 2005, Moran and Bjorndal 2005).

Several groups of macro and mega grazers (marine mammals, fishes, echinoids) remove relevant amounts of above-ground seagrass material and associated flora. In turn, herbivory is now considered as a disturbance that, at certain places and times, may significantly affect the functioning and resilience of seagrasses (e.g. Rivers and Short 2007, Ruiz et al. 2009, Fourqurean et al 2010). Since seagrasses constitute the habitat for associated epifauna (providing food and refuge), periodical removal of seagrass leaves through grazing could concurrently affect the composition and abundance of epifaunal organisms. At present, however, the implications of seagrass leaf consumption on associated epifauna remains elusive. This is particularly relevant at

temperate latitudes, due to the spread of tropical herbivorous fishes into temperate areas as a result of global warming, which is increasing the intensity of herbivory on recipient habitats (Vergés et al. 2014a,b, 2016).

Cymodocea nodosa (Ucria) Ascherson is a seagrass distributed across the Mediterranean Sea and adjacent eastern Atlantic coasts, including the Macaronesian archipelagos of Madeira and the Canaries, all the way down to Senegal in the western African coast (Alberto et al. 2006, Cunha and Araújo 2009, Mascaró et al. 2009, Tuya et al. 2014a). Together with *P. oceanica*, it represents one of the most important and abundant seagrasses in Mediterranean coastal systems (Scipione and Zupo 2010). It has been treated as a pioneer species, capable of colonizing soft bottoms under a wide range of conditions and generally growing faster than other seagrass species in the Mediterranean Sea (Marbà et al. 2004). This seagrass originated, from an evolutionary point of view, in the eastern Mediterranean by divergence from its close relative in the Indian Ocean/Red Sea and colonized the western Mediterranean and Atlantic by spreading westwards (Masucci et al. 2012). Several studies have demonstrated that herbivory on *C. nodosa* can have strong effects on plant attributes and performance, particularly under certain environmental conditions, e.g. fertilization events (Cebrián et al. 1996, Fernández et al. 2012, Tuya et al. 2013, Sanmartí et al. 2014). Yet, the effects of increased herbivory on associated epifauna remain largely unexplored.

In this study, we experimentally assessed the effects of different levels of herbivory intensity over the seagrass *C. nodosa* on the composition and abundance of associated epifauna. To encompass a wide range of environmental conditions, we tested these effects at two regions throughout the distribution range of *C. nodosa* the Canary Islands (Gran Canaria) and the Balearic Islands (Mallorca).

MATERIAL AND METHODS

Study region and landscape characteristics

A ‘comparative-experimental approach’ (Wernberg et al. 2010) was adopted where the same experimental manipulations and procedures were performed at the two meadows. One study site was located in the southern region of Gran Canaria (27°44.92’N, 015°33.86’W; Canary Islands) and the other one on the northern part of the island of Mallorca (39°55.37’N, 003°08.24’E; Balearic Islands). The Gran Canaria meadow is approximately 35 ha and located at 7–11 m depth with a density of 900-1200 shoots m⁻², and the meadow in Mallorca is approximately 3125 m² and located at 2–4 m depth with a density of 450-875 shoots m⁻². *C. nodosa* complexity varies seasonally in both regions. Shoot density and biomass peak in summer when growth is robust but are lower and more variable in winter (Drew 1978, Perez and Romero 1992).

Clipping and sample collection procedures

A manipulative experiment was set at the two meadows to simulate different levels of herbivory. Using metallic bars, 10 plots (1 x 1 m), which were at least 2 meters apart were set up at each meadow. High herbivory (80% of leaf canopy removed) was simulated by clipping the seagrass leaves using scissors in 5 plots (Fig. 1), and the other 5 plots were used as controls (natural low levels). The experiment was set up in the summer season and lasted 4 months, with clipping being conducted periodically every 4 weeks (June to September of 2016), when fish grazing activity is highest (Tomas et al. 2005).

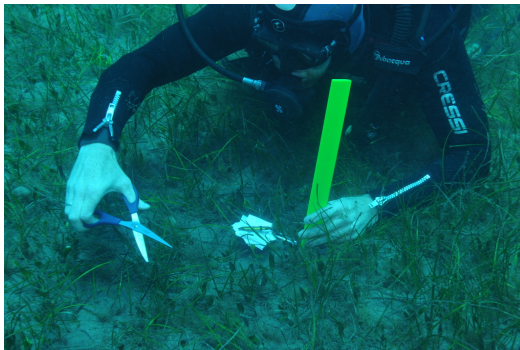


Figure 1: Plots of the experimental simulation of herbivory



Figure 2: Sampling epifauna

At the end of the experimental period, a 25x25cm quadrat was deployed in the centre of each plot (avoiding the limits of the plot), and all aboveground material and associated fauna were covered with a calico bag. At the tip of the bag a small buoy was clipped in order to maintain the bag in a vertical position, which facilitated the retrieval of the samples (Fig. 2). Leaves were removed by cutting their base with scissors and collected inside the bag, and bags were transported to the laboratory for further processing.

Laboratory procedures

All bags were preserved in ice until sorting in the laboratory. Each sample was rinsed with freshwater using a three-rinse cycle as in Carr et al. (2011). During each rinse, seagrass was agitated to remove the epifauna and rinsed through a 500 micrometer mesh sieve and preserved in 70% ethanol after rinsing. The leaf biomass was separated from the rest of the sample. It was dried in an oven at 60°C during 48 hours approximately, and subsequently weighted to the nearest mg. Macrofaunal (i.e. 0,05 – 3cm) specimens were sorted into broad taxonomic units to class/order level using a binocular microscope and their abundances were quantified (Tuya et al., 2013). The remaining fraction that was smaller than 500 microns was preserved in 70% ethanol. During sampling procedures, one sample was lost.

Statistical analysis

I used Analysis of Variance (ANOVA) to test whether region and herbivory treatment affected the abundance of seagrass as well as the composition and abundance of epifaunal organisms associated to *C. nodosa*. Region and herbivory treatment were treated as fixed and crossed factors. Epifauna abundances were considered both in terms of number of individuals per 100cm² as well as individuals per 100 g of seagrass dry weight. Prior to performing ANOVAs, data were examined for normality using the Shapiro-Wilk's test, and for homogeneity of variances using Bartlett's test. When necessary, data were square-root transformed to avoid heterogeneous variances. Because one sample was lost during processing, for that experimental treatment, the average of the other four replicates was used to replace the missing value. When assumptions for ANOVA were not met even after transformation, data were analyzed using the non-transformed data and alpha was set to 0.01 instead of 0.05 to reduce Type I Error (Underwood 1997). All graphics were plotted using Sigmaplot 8.0.2 package and statistical analyses were performed using [R].

RESULTS

Seagrass biomass differed across the two regions, being ca. 8 times higher at the Gran Canaria site than in Mallorca. The abundance of seagrass was also affected by clipping, although these effects differed between Mallorca and Gran Canaria (significant interaction term; Table 1), with the magnitude of the effect being much larger in Gran Canaria (Figure 3).

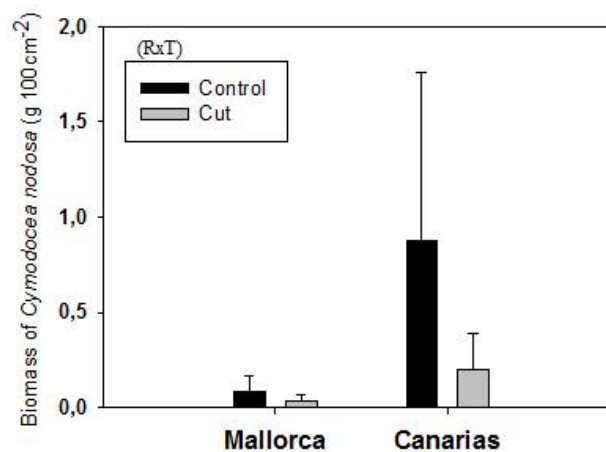


Figure 3: Biomass of *Cymodocea nodosa* (g 100cm⁻²) across regions and experimental treatments. Statistically significant effects are indicated with letters: R=region; T= treatment

A total of 944 epifaunal individuals, belonging to 48 taxa (Table Appendix 1), were found including: ostracoda (52), gammaridae (194), caprellidae (29), copepoda (139), decapoda (37) isopoda (17), tanaidacea (65), cumacea (17), prosobranchia (330), opisthobranchia (4), nudibranchia (1), ophiuroidea (3) and annelida (56).

Table 1: Results of the ANOVA test comparing biomass of *Cymodocea nodosa* g100cm⁻². P-value in bold = significant. Data were square-root transformed

	Biomass of <i>Cymodocea nodosa</i> (g100cm ⁻²)				
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	1.013	1.013	1	173.971	<0.01
Treatment	0.464	0.464	1	79.570	<0.01
RxT	0.190	0.190	1	32.577	<0.01

The abundance and taxon richness of epifauna were influenced by region and the clipping treatment, but responses differed whether considering organism per surface area or per biomass of seagrass (compare figures 4 and 5). While there was no significant effect of either region or treatment when considering total number of organisms per 100cm², (Figures 4o, Table 2), we did observe effects regarding taxon richness. Indeed, the number of taxa were significantly higher in Mallorca than in Gran Canaria, and at both sites plots that underwent clipping harboured a higher number of taxa (Figure 4p, 5p, Table 2, 3).

Effects of region and treatment differed amongst specific groups as well as when considering their abundances per area or per biomass of seagrass. When considering abundance data normalized by area (i.e. individuals per 100 cm²), several groups exhibited regional differences (Table 2). For instance, while cumacea and the non-polychaeta annelids were more abundant in Mallorca (Fig 4h,m), decapods were approximately two to eight times more abundant in Gran Canaria (Fig. 4e) than in Mallorca. Isopods were also more abundant in Gran Canaria and were positively affected by clipping (Fig. 4f, Table 2). Interestingly, several groups that responded to the clipping treatment exhibited contrasting responses at the two different regions (Table 2). Indeed, gammarids (Fig. 4b), tanaidacea (Fig. 4g) and polychaeta (Fig. 4n) underwent an increase in their abundance under clipping in Gran Canaria while they exhibited the opposite pattern (i.e. a decrease in abundance under clipping) at the Mallorca site. Copepods and caprellids exhibited a similar pattern (Fig. 4c,d), although the trends were not statistically significant (Table 2). Finally, some groups with relatively low abundances in Gran Canaria (i.e. ostracoda, opisthobranchs, nudibranchs, and ophiurioids; Figure 4a,d,j,k,l) were largely absent in the Mallorca samples, although the ANOVA did not detect a significant effect of region (Table 2).

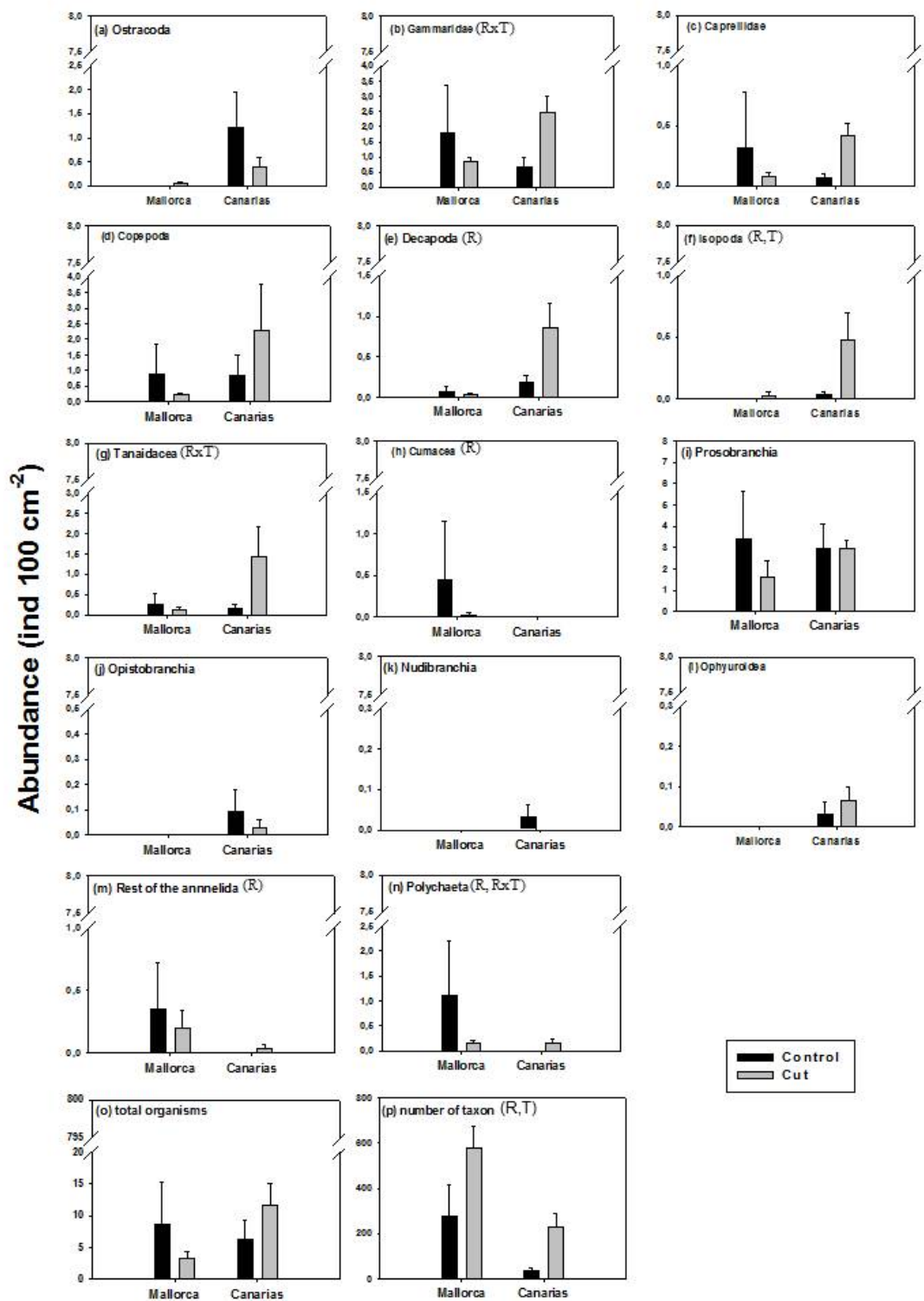


Figure 4: Abundance (individuals 100cm⁻²) of the different epifaunal groups found in *Cymodocea nodosa* across regions and experimental treatments. Statistically significant effects are indicated with letters: R=region; T= treatment.

When considering abundance data normalized by biomass of seagrass (i.e. individuals per 100 g⁻¹ DW), several groups exhibited regional differences (Table 3). For instance, while cumacea were only present in Mallorca (Fig 5h), polychaeta and the non-polychaeta annelids were present in both sites but more abundant in Mallorca (Fig 5m, n) than in Gran Canaria. Gammaridae showed a significant effect of region and treatment, that is the abundance in Mallorca was higher than in Gran Canaria and in both regions there was an increase of abundance in clipped plots (Fig. 5b, Table 3). Numerous groups appeared to be positively affected by clipping (gammaridae, decapoda, isopoda, tanaidacea, prosobranchia, opistobranchia, ophyuroidea and annelida non-polychaeta; Fig. 5, b, e, f, g, i, j, l, m) but there was no statistical evidence, except in gammaridae and isopoda (Table 3). As observed for measures of individuals per area, some groups that responded to the clipping treatment exhibited contrasting responses at the two different regions (Table 3). Indeed, cumacea (Fig. 5h) and polychaeta (Fig. 5n) underwent an increase in their abundance under clipping in Gran Canaria while their abundances decreased with clipping of the leaves at the Mallorca site. Caprellidae (Fig. 5c) and copepoda (Fig. 5d), exhibited a similar pattern of differential responses to treatment with site, although these trends were not statistically significant (Table 3). Opistobranchia, nudibranchia and ophyuroidea were only present in Gran Canaria (Fig. 5j, k, l), although the ANOVA did not detect a significant effect of region (Table 3).

DISCUSSION AND CONCLUSIONS

Using a comparative-experimental approach we observed that there are differences in the abundance of epifaunal communities (individuals per cm² and individuals per g⁻¹DW) inhabiting *Cymodocea nodosa* meadows between both regions, with higher abundances in Mallorca than in Gran Canaria (Fig. 5o, table 3). Furthermore, not only were there differences between regions, but epifauna abundances also changed in response to clipping. Interestingly, we observed both positive and negative responses of epifauna abundance with the reduction of the leaf canopy. Furthermore, in several instances there were opposite responses between regions to the clipping treatment, with epifauna increasing with clipping in Gran Canaria and decreasing in Mallorca.

In Gran Canaria, *Cymodocea nodosa* meadows are generally located along the eastern and southern coasts of the islands, sheltered from the dominant oceanic swells from the north and north-west, forming extensive, but often fragmented, subtidal meadows (Tuya et al. 2014c). Despite of the location of Gran Canaria's meadow (south-east) hydrodynamic effects on this meadow are likely more relevant than in the Mallorca meadow, especially in winter (Tuya et al. 2014c), since the Mallorca meadow is inside a very protected cove.

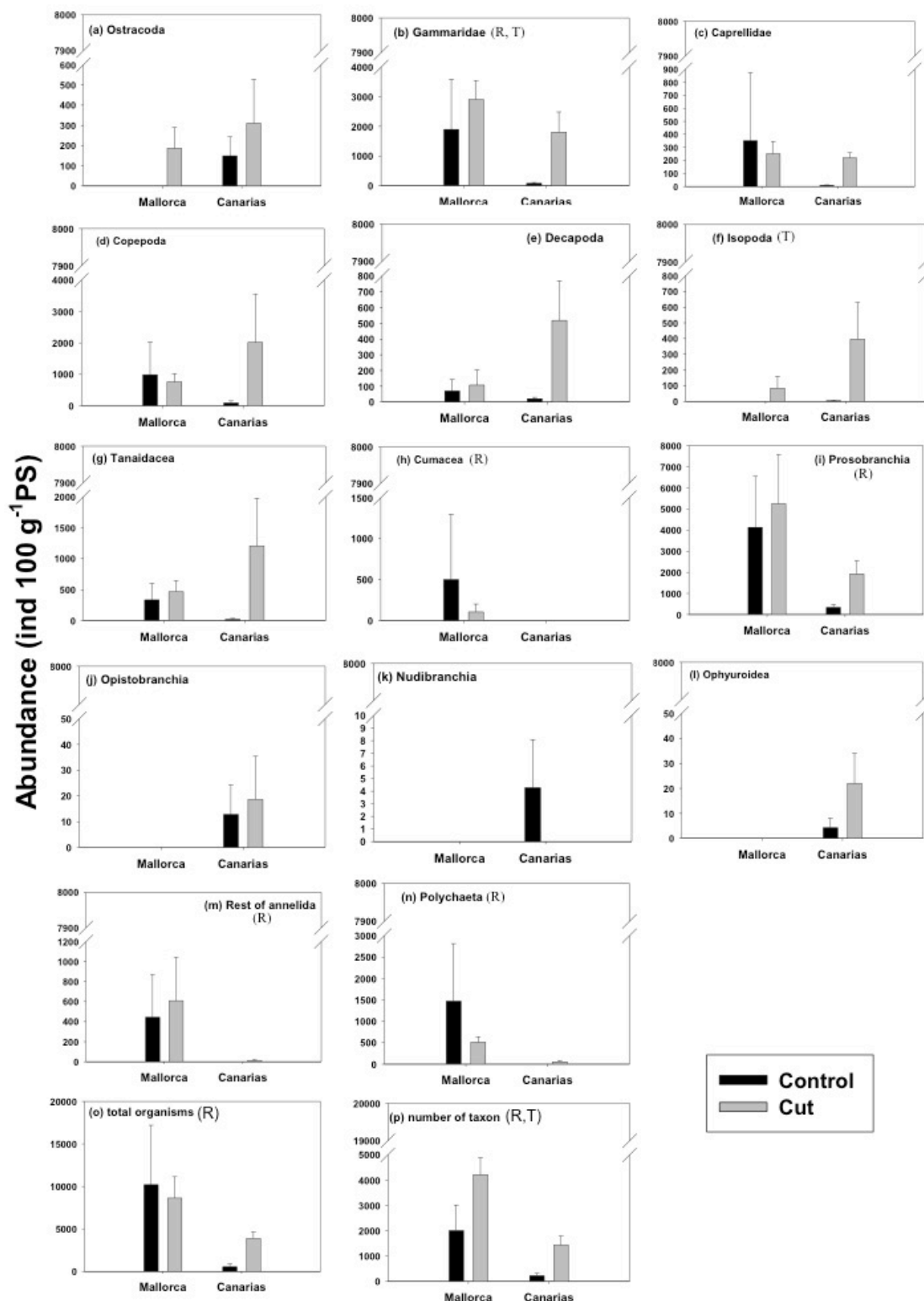


Figure 5: Abundance (individuals 100g⁻¹DW) of the different epifaunal groups found in *Cymodocea nodosa* across regions and experimental treatments. Statistically significant effects are indicated with letters: R=region; T=treatment.

Turner et al. (1999) analysed patterns of variation in epifauna community composition in relation to seagrass patch (patch size, seagrass % cover, seagrass biomass) landscape characteristics (fractal geometry, patch isolation), and wave exposure variables (mean wind velocity). They found that differences in faunal abundance were largely explained by landscape and wave exposure variables. Higher exposure to swell and hydrodynamic conditions in Gran Canaria may explain why some epifauna groups might be more abundant in Mallorca than in Gran Canaria.

The groups for which there were more individuals in Mallorca were gammaridae (96), copepoda (40), type 5 prosobranchia (56) and polychaeta (38). Indeed, looking at table 3, gammaridae, prosobranchia and polychaeta were significantly more abundant per seagrass biomass in Mallorca than in Gran Canaria. In addition to higher abundance of epifauna, the Mallorca site also exhibited higher taxon richness than the Gran Canaria bed. The group that mainly contributed to this taxon richness in Mallorca were crustaceans, which encompassed eight different subgroups. The differences in total abundance and diversity observed between the two regions might be potentially explained because shallow vegetated areas on the Mediterranean coast often include a matrix of vegetated habitats (e.g. presence of *Posidonia oceanica*), and such habitat diversification supports a higher abundance and species richness in comparison with more homogeneous habitats (Barberá-Cebrian 2002) such as the seagrass bed studied in Gran Canaria. Interestingly, crustaceans, which are typically a common group inhabiting *Cymodocea nodosa* beds of the Canary Islands (Png-Gonzalez 2013) and the Mediterranean (Scipione et al. 1996) were more abundant in Gran Canaria (360 individuals counted) than in Mallorca (190 individuals counted), but their taxa richness within the groups was lower (7). Groups such as ophiuroids, nudichanchs and opisthobranchs were only present in Gran Canaria whereas cumacea were only found in Mallorca.

Epiphytes are considered important in determining abundance and diversity of epifauna as they play two important ecological roles: providing refuge from predation as well as being a food source (Gartner et al. 2013). If there had been more epiphytes in Mallorca, epiphyte abundance could have been a factor contributing to the patterns observed. However, in our study we did not quantify the amount of epiphytes because they were present in very low abundances. Yet, these small differences may also contribute to the different patterns. On the other hand, the low abundance of epiphytes observed in our samples may be resulting from the high abundance of grazers such as gammarids and caprellid amphipods as well as some prosobranchia that consume algae. Indeed, mesograzers such as these have been identified as major epiphyte eaters, potentially providing a positive feedback to the seagrass by eliminating algal competitors (e.g. Duffy et al. 2015).

Several groups (individuals 100cm⁻²), including some of the most abundant ones, such as gammarids, tanaidacea, polychaeta, copepods and caprellids, tended to be less abundant in control plots than in the clipping ones in Gran Canaria while they exhibited the opposite patterns in Mallorca (Fig. 4). Sanmartí et al. 2014 found that simulated herbivory in *Cymodocea nodosa* causes several responses to compensate for defoliation by the addition of new modules (leaves and shoots), while leaf growth is not affected. Increased complexity is likely to provide additional refuge value, either as protection from predation (Heck and Orth, 2005) or specialist habitat niche (Edgar and Robertson, 1992), and live algal epiphytes are likely to provide trophic resources (Gartner et al 2013). We did not measure shoot density shoot at the end of experiment in the different treatments (these data should be compared with Nerea Royo's TFG, which is not published yet), but if the Gran Canaria plots responded to clipping by increasing shoot density, this could contribute to explain the positive response of epifauna abundance to this treatment. Gran Canaria clipping plots may have experienced an epifaunal migration from adjacent areas due to increasing complexity.

Discerning between the responses observed by each group to the treatment is difficult because within groups we may have species that could differ in multiple aspects such as type of life cycle (e.g. opportunist or not), food preference and availability (epiphytes, other epifauna, seagrass, etc.), habitat that they typically use (in the epiphytes or not), susceptibility to predation, competition (for food, for habitat), etc. For example, a large scale study examining predation pressure on mesograzers in *Zostera marina* communities found that amphipods were much more susceptible to predators than gastropods (Reynolds et al. in review). We did observe a lower abundance of gammarids in the clipped plots in Mallorca, but prosobranchia did not appear to be positively affected by that. In addition, the communities of predators may differ between regions and thus could be feeding on different groups of epifauna. It is likely a combination of multiple mechanisms which is driving the different patterns observed.

Surface area, epiphyte growth, leaves per seagrass shoot, patch age and patch size all contribute to seagrass habitat structure (Sirota and Hovel 2006), and different species and life stages may respond differently to these changes in habitat structure. Right now we cannot discern which of these may have had the strongest influence on epifaunal abundance and community structure in our study. Only until very recently studies have started comparing different locations (e.g. Wernberg et al. 2010, Duffy et al. 2015) to examine ecological processes. The work performed here is following this multiple site approach and precisely highlights the importance of incorporating different areas. Indeed, the fact that the results are very different (responses to the clipping treatment are opposite in the two regions for multiple groups) emphasizes the importance of doing

these comparative studies in several places. If I had only examined the epifauna data from the study in Mallorca, I would have obtained a totally different conclusion than if I had done the same experiment in GC. In conclusion, there is a high variability of epifaunal response to clipping treatment between regions. This makes it difficult to get to firm conclusions about the effect of the disturbance when considering only two meadows. Biogeography (spatial variability on a large scale) is as, or more important, than the disturbance effect we manipulated at small spatial scales. For this reason, studies addressing ecological responses to environmental stressors should encompass large spatial gradients across the range of distribution of the founding species being analyzed. Therefore, more studies in different places and replicated in time are needed to develop a good understanding of ecological patterns of seagrass epifaunal communities.

Table 2: Results of ANOVA tests for abundance (individuals 100cm⁻²) of epifaunal groups. P-value in bold = significant. *Does not meet conditions of normality and homoscedasticity. **Data were square-root transformed.

Total organisms					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	42.430	42.430	1	0.953	0.344
Treatment	0	0	1	0	0.996
RxT	136.960	136.960	1	3.039	0.100
Number of taxa**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	455.38	455.38	1	170.492	<0.01
Treatment	332.49	332.49	1	124.483	<0.01
RxT	7.1	7.1	1	0.266	0.613
Ostracoda*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	2.983	2.983	1	3.382	0.085
Treatment	0.755	0.755	1	0.855	0.369
RxT	0.755	0.755	1	0.115	0.307
Gammaridae**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.015	0.015	1	0.061	0.808
Treatment	0.407	0.407	1	1.604	0.224
RxT	1.602	1.602	1	6.309	0.023
Caprellidae**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.034	0.034	1	0.435	0.519
Treatment	0.106	0.106	1	0.134	0.263
RxT	0.506	0.506	1	0.643	0.022

Copepoda					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	5.103	5.103	1	1.166	0.296
Treatment	0.672	0.672	1	0.154	0.7
RxT	5.423	5.423	1	1.239	0.282
Decapoda**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.913	0.913	1	8.12	0.012
Treatment	0.144	0.144	1	1.282	0.274
RxT	0.44	0.44	1	3.91	0.065
Isopoda**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.428	0.428	1	7.137	0.017
Treatment	0.411	0.411	1	6.858	0.019
RxT	0.226	0.226	1	3.768	0.07
Tanaidacea**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.322	0.322	1	1.919	0.185
Treatment	0.444	0.444	1	2.644	0.124
RxT	1.288	1.288	1	7.664	0.014
Cumacea**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.364	0.364	1	4.594	0.048
Treatment	0.191	0.191	1	2.413	0.139
RxT	0.191	0.191	1	2.413	0.139
Prosobranchia					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	1.049	1.049	1	0.224	0.643
Treatment	3.945	3.945	1	0.841	0.373
RxT	3.945	3.945	1	0.841	0.373
Opisthobranchia*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.02	0.02	1	1.6	0.224
Treatment	0.005	0.005	1	0.4	0.536
RxT	0.005	0.005	1	0.4	0.536
Nudibranchia*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.001	0.001	1	1	0.332
Treatment	0.001	0.001	1	1	0.332
RxT	0.001	0.001	1	1	0.332
Ophyuroidea*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.012	0.012	1	3.6	0.076
Treatment	0.001	0.001	1	0.4	0.536
RxT	0.001	0.001	1	0.4	0.536

Rest of the annelida **					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	0.559	0.559	1	7.041	0.017
Treatment	0.031	0.031	1	0.387	0.542
RxT	1.255	1.255	1	1.58	0.228
Polychaeta**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	1.238	1.238	1	8.888	0.009
Treatment	0.097	0.097	1	0.696	0.416
RxT	0.764	0.764	1	5.486	0.032

Table 3: test ANOVA abundance (individuals 100g⁻¹DW) for every single group. P-value in bold = significant. *=Does not meet conditions of normality and homoscedasticity. **Data were square-root transformed.

Total organisms**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	9822.5	9822.5	1	6.489	0.022
Treatment	5333.7	5333.7	1	3.524	0.079
RxT	3534.4	3534.4	1	2.335	0.146
Number of taxa					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	25969413	25969413	1	14.098	0.001
Treatment	14668312	14668312	1	7.963	0.012
RxT	1236743	1236743	1	0.6714	0.292
Ostracoda*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	93076	93076	1	0.901	0.357
Treatment	151324	151324	1	14.649	0.274
RxT	842	842	1	0.008	0.929
Gammaridae**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	2586.5	2586.5	1	8.729	0.009
Treatment	2363.2	2363.2	1	7.976	0.012
RxT	458.7	458.7	1	1.548	0.231
Caprellidae*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	178454	178454	1	2.093	0.167
Treatment	14531	14531	1	0.171	0.685
RxT	124051	124051	1	1.455	0.245
Copepoda*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	151599	151599	1	0.037	0.85
Treatment	3545661	3545661	1	0.87	0.365
RxT	5880064	5880064	1	14.435	0.247

Decapoda*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	160620	160620	1	1.405	0.253
Treatment	352675	352675	1	3.085	0.098
RxT	267049	267049	1	2.336	0.146
Isopoda**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	192.45	192.45	1	2.759	0.116
Treatment	439.03	439.03	1	6.296	0.023
RxT	139.36	139.36	1	1.099	0.176
Tanaidacea*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	228403	228403	1	0.232	0.637
Treatment	2186837	2186837	1	2.219	0.156
RxT	1384333	1384333	1	1.405	0.253
Cumacea**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	508.13	508.13	1	4.619	0.047
Treatment	148.23	148.23	1	1.348	0.263
RxT	148.23	148.23	1	1.348	0.263
Prosobranchia**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	4370.6	4370.6	1	4.925	0.041
Treatment	972.8	972.8	1	1.096	0.311
RxT	677.6	677.6	1	0.764	0.395
Opisthobranchia*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	1241.3	1241.3	1	1.933	0.184
Treatment	43.1	43.1	1	0.067	0.799
RxT	43.1	43.1	1	0.067	0.799
Nudibranchia*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	1.068	1.068	1	1	0.332
Treatment	1.068	1.068	1	1	0.332
RxT	1.068	1.068	1	1	0.332
Ophyuroidea*					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	863.6	863.6	1	3.448	0.082
Treatment	393.3	393.3	1	1.57	0.228
RxT	393.3	393.3	1	1.57	0.228
Rest of the annelida**					
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	1183.49	1183.49	1	7.141	0.017
Treatment	2.65	2.65	1	0.016	0.901
RxT	25.08	25.08	1	0.151	0.702

	Polychaeta**				
	Sum of square	Mean square	Degrees of freedom	F value	p-value
Region	2813.42	2813.42	1	16.226	<0.01
Treatment	66.26	66.26	1	0.382	0.545
RxT	344.31	344.31	1	1.986	0.178

DETAILED DESCRIPTION OF THE ACTIVITIES CARRIED OUT DURING THE IMPLEMENTATION OF THE TFT

Experimental in situ examination of light limitation

At three *Cymodocea nodosa* meadows in Mallorca an experiment was set up to study the effects of light limitation on seagrass and the associated community. Using metallic bars, 15 plots (1 x 1 m), which were at least 2 meters apart, were set up at each meadow. Three different experimental treatments (low, medium and high light reduction) were set up using tarpaulin mesh of different densities (Figure 6).



Figure 6: Tarpaulin mesh on a plot

Measures of seagrass abundance

The abundance of *Cymodocea nodosa* was quantified at each site through different measurements. Shoot density is defined as the number of plant shoots per unit area and it is determined using a square of 20x20 cm. This quadrat is made of pvc tubes and is thrown randomly throughout the meadow. The number of shoots that are included within the quadrat is counted and the density of shoots per 1m² of meadow is estimated from the mean of 20 quadrats.

Percent cover

To estimate abundance using cover, a 25x25 cm quadrat which was subdivided into 10 subquadrats of 5 x 5 cm was used. Cover is estimated by counting the number of subquadrats in which the species of interest is present and divided by the total number of subquadrats. In a similar fashion as for shoot density estimates, twenty quadrats were haphazardly tossed along the area of study. This approach allows to obtain a measure of abundance without destroying the plant and it is particularly useful for macrophytes (such as many macroalgae) for which counting independent individuals is hard or impossible.

Epifauna sampling and laboratory procedures

See the above materials and methods section in the TFG manuscript (pages 6 and 7) for details on these techniques.

Estimation of rhizome growth

In order to quantify the growth rate of *C. nodosa* rhizomes an in situ marking of twenty different plants is conducted. An apical shoot that presents a rhizome in which there are not many nodes and internodes are relatively far from each other (rhizomes presenting numerous and close internodes are probably older plants that are unlikely to exhibit significant growth during the growing season) is chosen. Once an appropriate rhizome is found, it is marked. A cable tie is placed in a space between two nodes (ensuring that there are roots or leaves to hold it in place) and the distance between nodes to the apex is measured to the nearest mm. All this is written on a blackboard in situ. Next to the marked rhizome a peg with a tag number is placed in order to identify it and to mark the area for future monitoring. Later (after 6 months) the spaces between knots will be measured again and the growth will be quantified.

Seed quantification and collection

To quantify seed abundance in a *Cymodocea nodosa* meadows, 50 corers of 10cm in diameter are collected haphazardly throughout the meadow. The corer is pushed into the sediment approximately 15 cm deep. Once the corer is removed, the sample is sieved in situ using a colander to remove the sediment particles and the seeds are collected and kept in a zip-lock bag.

Seed collection is also performed for further seed culture in mesocosms. In this case, rather than conducting corers, divers collect the seeds by removing the sediment that is covering the rhizomes with their hands and collect the seeds that are buried below.

Sampling of abiotic variables

Sediment samples were collected for future characterization (grain size and organic matter content). Sediment of the surface layers surrounding the seagrass was collected in 50 ml falcon tubes and frozen for further analysis.

To obtain in situ measurements of water temperature and irradiance, Hobo® temperature and light recorders were deployed in the different meadows of study by attaching them to a pvc bar that was anchored to the sediment.

Mapping the presence of a new invasive alga.

A new invasive alga, *Halimeda incrassata*, has been recently detected in Mallorca (Alós et al. 2016), and it was very recently discovered in Cabrera National Park. An initial mapping and estimate of abundance of this species was performed in a shallow sandy area where it was found. An initial map of its general distribution was drawn in situ and cover estimates were obtained using quadrats as explained above.

TRAINING RECEIVED

During the performance of this internship I have received training on experimental design, experimental setup and sample collection in situ, processing of epifauna and seagrass samples in the laboratory, organization of a scientific project and task coordination, coexistence during a sampling field trip, performing SCUBA diving from a boat, awareness of diving accidents, solving the problems that one can find once in the field and during the collection of samples, tracking an invasive seaweed and mapping an algae in an area. I have also been trained in data entering, statistical analysis of data using [R], and graphic representation of data using Sigmaplot.

Finally, in this section I would like to highlight not only the professional training that I have received but also how enriching this experience has been at the personal level. It allowed me to have a first contact with the world of research, also showed me how to deal with different situations and has enabled me to learn from other professionals, in order to improve.

LEVEL OF INTEGRATION AND INVOLVEMENT WITHIN THE DEPARTMENT AND RELATIONSHIPS WITH STAFF

First of all, in this section I would like to express my deepest thanks to Fiona Tomas Nash, my tutor and principal investigator of the RESIGRASS project in Mallorca, Fernando Tuya Cortes, main project PI for the Canary Island and my advisor at ULPGC, the research technicians Laura Royo Marí and Inés Castejón, Jorge Terrados, principal investigator of the project and director of IMEDEA, as well as IMEDEA. I thank them all for giving me the opportunity to develop my subject of external practices and my TFG, always willing to solve my doubts and for guiding me in this experience.

From the beginning, I felt their support and they provided everything I needed, so that my first contact with the world of research was as enriching and satisfying as possible, showing me a new perspective of learning. For me it is a privilege to have been able to access IMEDEA to carry out the practices, where I have always been treated like one more of the group, an even as a friend. I always had the availability and support of all the aforementioned, which gave me security, and also allowed me to learn about my strengths and weaknesses, in order to improve. From the beginning, I decided to make the most of the time spent at IMEDEA facilities and in the field sampling. At all times they made me feel like one more of the team and they allowed me to see myself with the capacity to assume the responsibility of being involved in this project that has been developed for years, jointly with the ULPGC, IMEDEA and the University of Alicante .

MOST SIGNIFICANT POSITIVE AND NEGATIVE ASPECTS RELATED TO TFG DEVELOPMENT

Firstly, in the positive aspects I must emphasize the fantastic treatment received by all my colleagues. I have always been given all the necessary facilities to carry out my work and the stay in IMEDEA was as rewarding as possible, to the point of feeling like one more and even as a friend. Specifically, my tutor was willing to answer the different questions I posed and provided me with all the materials needed to develop my work. I would also like to point out the gratitude I feel for giving me the opportunity to collaborate on a project of this magnitude, with which a great number of scientific research could be developed, and thanks to which I have learned a lot. In addition, it has given me a new vision of what it is to work in a united group and the advantages of having support in one another and being able to count on each and every one of them at any time even in the most difficult ones. I would also like to highlight the effort that all my colleagues have made to help me look for information and opportunities outside of IMEDEA.

On the other hand, if I had to comment on some negative aspect would be the little funding that the projects receive. Specifically, RESIGRASS is barely funded to pay a part-time person for a year. That is not enough, considering that the project lasts 4 years.

PERSONAL EVALUATION OF THE LEARNING EXPERIENCE OBTAINED THROUGHOUT THE TFG

This internship has been one of the most enriching experiences of the degree. All the professional and personal knowledge and information obtained throughout my degree have really helped me develop my skills while working at IMEDEA. This experience has showed me a real environment in which I could develop my profession. For all the above, it has been a very beneficial opportunity that gives greater meaning to everything learned during these four years. Although I have many things to learn and many experiences to live through my professional development, I have found a great opportunity that helps to strengthen knowledge, improve as a person and learn from other workers, as well as their experience.

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APPENDIX 1

Table 1: Data base

Region	Treatment	g PS Cymo	Surface cm	CRUSTACEA							
				Ostracoda	Gammaridae	Caprellidae	Copepoda	Decapoda	Isopoda	Tanaidacea	Cumacea
Mallorca	control	0,3602	726	0	1	0	1	0	0	1	0
Mallorca	control	0,7883	726	0	22	0	8	1	0	1	1
Mallorca	control	0,6634	726	0	2	1	0	0	0	1	0
Mallorca	control	0,639	726	0	27	8	17	1	0	5	12
Mallorca	control	0,612725	726	0	13	2,25	6,5	0,5	0	2	3,25
Mallorca	80%	0,239	726	0	5	0	3	0	1	2	0
Mallorca	80%	0,1861	726	0	9	0	3	1	0	1	1
Mallorca	80%	0,2071	726	1	7	1	1	0	0	2	0
Mallorca	80%	0,2213	726	1	8	1	1	0	0	0	0
Mallorca	80%	0,3153	726	0	2	1	0	0	0	0	0
Canarias	control	6,1063	625	13	11	1	23	3	0	1	0
Canarias	control	5,28	625	0	0	0	0	0	0	0	0
Canarias	control	4,68	625	25	7	1	4	2	1	4	0
Canarias	control	7,29	625	0	1	0	0	1	0	0	0
Canarias	control	4,14	625	0	2	0	0	0	0	0	0
Canarias	80%	0,63	625	8	28	1	56	10	9	29	0
Canarias	80%	0,81	625	1	21	3	4	0	2	6	0
Canarias	80%	1,07	625	0	11	2	3	2	2	4	0
Canarias	80%	1,89	625	3	8	5	6	10	0	5	0
Canarias	80%	1,75	625	0	9	2	2	5	2	1	0

Total per group	52	194	29	139	37	17	65	17
Total Crustacea	550							

Region	Treatment	g PS <i>C. nodosa</i>	Surface cm	PROSOBRANCHIA							
				<i>type1</i>	<i>type2</i>	<i>type3</i>	<i>type4</i>	<i>type5</i>	<i>type6</i>	<i>type7</i>	<i>type8</i>
Mallorca	control	0,3602	726	0	0	0	0	12	0	0	0
Mallorca	control	0,7883	726	0	0	0	0	31	0	0	0
Mallorca	control	0,6634	726	0	0	0	0	15	0	0	0
Mallorca	control	0,639	726	0	0	0	0	0	0	0	0
Mallorca	control	0,612725	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,239	726	0	0	1	0	20	0	0	0
Mallorca	80%	0,1861	726	0	0	0	0	2	0	0	0
Mallorca	80%	0,2071	726	0	0	0	0	6	0	0	0
Mallorca	80%	0,2213	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,3153	726	0	0	0	0	0	0	0	0
Canarias	control	6,1063	625	2	1	1	0	2	6	5	1
Canarias	control	5,28	625	3	0	0	0	0	3	0	0
Canarias	control	4,68	625	0	0	1	1	7	13	1	2
Canarias	control	7,29	625	0	0	0	0	1	5	0	0
Canarias	control	4,14	625	0	0	0	0	0	1	0	0
Canarias	80%	0,63	625	1	1	1	0	6	6	0	0
Canarias	80%	0,81	625	0	1	0	0	5	2	2	0
Canarias	80%	1,07	625	1	0	0	0	2	3	0	0
Canarias	80%	1,89	625	2	1	4	0	1	4	0	0
Canarias	80%	1,75	625	2	1	5	0	5	4	0	0

Total per type	11	5	13	1	115	47	8	3
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Region	Treatment	g PSC. <i>nodosa</i>	Surface cm	PROSOBRANCHIA							
				<i>type9</i>	<i>type10</i>	<i>type11</i>	<i>type12</i>	<i>type13</i>	<i>type14</i>	<i>type15</i>	<i>type16</i>
Mallorca	control	0,3602	726	0	0	0	0	0	0	0	0
Mallorca	control	0,7883	726	0	0	0	0	0	0	0	0
Mallorca	control	0,6634	726	0	0	0	0	0	0	0	0
Mallorca	control	0,639	726	0	0	0	0	0	0	0	0
Mallorca	control	0,612725	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,239	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,1861	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,2071	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,2213	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,3153	726	0	0	0	0	0	0	0	0
Canarias	control	6,1063	625	1	0	1	1	0	1	1	3
Canarias	control	5,28	625	0	0	0	0	0	0	0	0
Canarias	control	4,68	625	5	1	1	2	1	0	0	0
Canarias	control	7,29	625	0	0	0	0	0	0	0	0
Canarias	control	4,14	625	0	0	0	0	0	0	0	0
Canarias	80%	0,63	625	6	0	0	0	0	0	0	1
Canarias	80%	0,81	625	5	0	0	0	0	0	0	0
Canarias	80%	1,07	625	0	0	0	0	0	0	0	2
Canarias	80%	1,89	625	3	0	0	0	0	0	0	0
Canarias	80%	1,75	625	1	0	0	0	0	0	0	0

Total per type	21	1	2	3	1	1	1	6
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Region	Treatment	g PSC. <i>nodosa</i>	Surface cm	PROSOBRANCHIA							
				<i>type17</i>	<i>type18</i>	<i>type19</i>	<i>type20</i>	<i>type21</i>	<i>type22</i>	<i>type23</i>	<i>type24</i>
Mallorca	control	0,3602	726	0	0	2	0	4	0	0	0
Mallorca	control	0,7883	726	0	1	0	0	0	0	0	0
Mallorca	control	0,6634	726	0	2	1	0	2	0	0	0
Mallorca	control	0,639	726	0	0	0	0	0	0	0	0
Mallorca	control	0,612725	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,239	726	0	8	1	1	2	1	1	0
Mallorca	80%	0,1861	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,2071	726	0	1	0	0	2	0	0	0
Mallorca	80%	0,2213	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,3153	726	0	0	0	0	0	0	0	0
Canarias	control	6,1063	625	3	0	0	0	1	0	0	4
Canarias	control	5,28	625	0	0	0	0	0	0	0	0
Canarias	control	4,68	625	0	0	0	0	0	0	0	0
Canarias	control	7,29	625	0	0	0	0	0	0	0	1
Canarias	control	4,14	625	0	0	0	0	0	0	0	0
Canarias	80%	0,63	625	1	0	0	0	0	0	0	0
Canarias	80%	0,81	625	0	0	0	0	0	0	0	0
Canarias	80%	1,07	625	0	0	0	0	0	0	0	0
Canarias	80%	1,89	625	0	0	0	0	0	0	0	0
Canarias	80%	1,75	625	0	0	0	0	0	0	0	0

Total per type	4	12	4	1	11	1	1	5
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Region	Treatment	g PSC. <i>nodosa</i>	Surface cm	PROSOBRANCHIA							
				<i>type25</i>	<i>type26</i>	<i>type27</i>	<i>type28</i>	<i>type29</i>	<i>type30</i>	<i>type31</i>	<i>type32</i>
Mallorca	control	0,3602	726	0	0	1	2	0	0	0	0
Mallorca	control	0,7883	726	0	0	0	2	1	10	0	0
Mallorca	control	0,6634	726	1	0	0	3	5	3	0	0
Mallorca	control	0,639	726	0	0	0	0	0	0	0	0
Mallorca	control	0,612725	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,239	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,1861	726	0	0	0	1	0	7	1	0
Mallorca	80%	0,2071	726	0	0	0	1	0	0	0	0
Mallorca	80%	0,2213	726	0	0	0	2	0	0	0	0
Mallorca	80%	0,3153	726	0	0	0	0	0	0	0	0
Canarias	control	6,1063	625	2	3	0	0	0	0	0	0
Canarias	control	5,28	625	0	0	0	0	0	0	0	0
Canarias	control	4,68	625	0	0	0	0	0	0	0	0
Canarias	control	7,29	625	0	0	0	0	0	0	0	0
Canarias	control	4,14	625	0	0	0	0	0	0	0	0
Canarias	80%	0,63	625	0	2	0	0	0	0	0	1
Canarias	80%	0,81	625	0	0	0	0	0	0	0	0
Canarias	80%	1,07	625	0	1	0	0	0	0	0	0
Canarias	80%	1,89	625	0	0	0	0	0	0	0	0
Canarias	80%	1,75	625	0	0	0	0	0	0	0	0

Total per type	3	6	1	11	6	20	1	1
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Biogeographic variations and response of epifauna to varying levels of herbivory intensity over the seagrass *Cymodocea nodosa*.

Laura Antich Homar

Region	Treatment	g PSC. <i>nodosa</i>	Surface cm	PROSOBRANCHIA			OPISTOBRANCHIA	NUDIBRANCHIA	OPHYUROIDEA	ANNELIDA	
				type33	type34	type35				Rest of annelida	polychaeta
Mallorca	control	0,3602	726	0	0	0	0	0	0	2	8
Mallorca	control	0,7883	726	0	0	0	0	0	0	1	3
Mallorca	control	0,6634	726	0	0	0	0	0	0	7	21
Mallorca	control	0,639	726	0	0	0	0	0	0	0	0
Mallorca	control	0,612725	726	0	0	0	0	0	0	0	0
Mallorca	80%	0,239	726	0	0	0	0	0	0	6	0
Mallorca	80%	0,1861	726	0	0	0	0	0	0	1	1
Mallorca	80%	0,2071	726	0	0	0	0	0	0	0	1
Mallorca	80%	0,2213	726	0	0	0	0	0	0	0	2
Mallorca	80%	0,3153	726	0	0	0	0	0	0	0	2
Canarias	control	6,1063	625	0	0	0	0	0	0	0	0
Canarias	control	5,28	625	0	0	0	0	0	0	0	0
Canarias	control	4,68	625	0	0	0	3	1	1	0	0
Canarias	control	7,29	625	0	0	0	0	0	0	0	0
Canarias	control	4,14	625	0	0	0	0	0	0	0	0
Canarias	80%	0,63	625	0	0	0	0	0	0	0	0
Canarias	80%	0,81	625	0	0	0	0	0	0	0	0
Canarias	80%	1,07	625	1	0	0	1	0	0	0	0
Canarias	80%	1,89	625	0	1	0	0	0	1	0	0
Canarias	80%	1,75	625	0	0	1	0	0	1	1	0

Total per type or group	1	1	1	4			4	1	3	18	38
	Total prosobranchia 330			Total opistobranchia 4			Total nudibranchia 1	Total ophyuroidea 3	Total annelida 56		