

Effects of simulated herbivore pressure at different biogeographic levels  
on epifaunal communities associated with *Cymodocea nodosa*.

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Resum – Les praderies de fanerògames marines constitueixen un complex hàbitat costaner i juguen un paper clau en els ecosistemes marins a causa de la gran diversitat d'organismes que estan lligats a aquests ecosistemes. Actualment, molts d'aquests ecosistemes es troben en declivi i, en conseqüència, la biodiversitat que engloben. Les comunitats d'epifauna vàgils que viuen associats a les fulles de les praderies tenen un paper clau per transmetre la producció portada a terme per les herbes marines a nivells tròfics superiors. En aquest estudi, s'ha proposat examinar les variacions que poden arribar a partir les comunitats d'epifauna associades a les praderies de *Cymodocea nodosa* a causa de la pressió d'herbivoria simulada. Aquest experiment es va realitzar a diferents escales per obtenir diferències biogeogràfiques. Les localitzacions van ser Arinaga i Castillo de Romeral (a la regió de Gran Canària), Aucanada y Es Barcares (a la regió de Mallorca). El tractament d'herbivoria simulada va causar variacions en l'abundància tota de l'epifauna, la diversitat de l'epifauna i en l'abundància d'alguns grups taxonòmics. A més, les direccions de respostes dels grups taxonòmics no van ser uniformes entre localitats. Les biomasses de *Cymodocea nodosa* i els epífits semblen estar directament relacionades amb les comunitats d'epifauna.

**Resumen** - Las praderas de fanerógamas marinas constituyen un complejo hábitat costero y juegan un papel clave en los ecosistemas marinos debido a la gran diversidad de organismos que están ligados a estos ecosistemas. Actualmente, muchos de estos ecosistemas se encuentran en declive y, en consecuencia, la biodiversidad que abarcan. Las comunidades epifaunales vágiles que viven asociados a las hojas de las praderas tienen un papel crucial para transmitir la producción llevada a cabo por las hierbas marinas a niveles tróficos superiores. En este estudio, se ha propuesto examinar las variaciones que pueden llegar a sufrir las comunidades epifaunales asociadas a praderas de *Cymodocea nodosa* a causa de la presión de herbivoría simulada. Este experimento se realizó a diferentes escalas para obtener diferencias biogeográficas. Las localizaciones fueron Arinaga y Castillo de Romeral (en la región de Gran Canaria), Aucanada y Es Barcares (en la región de Mallorca). El tratamiento de herbivoría simulada causó variaciones en la abundancia total de la epifauna, la diversidad de la epifauna y en la abundancia de algunos grupos taxonómicos. Además, las direcciones de respuestas de los grupos taxonómicos no fueron uniformes entre localidades. Las biomásas de *Cymodocea nodosa* y epifitos parecen estar directamente relacionadas con las comunidades de epifauna.

**Abstract** – Seagrass meadows constitute a complex coastal habitat and play a key role in marine ecosystems due to the high diversity of organisms that is linked to these ecosystems. Currently, many of these ecosystems are in decline and, consequently, their biodiversity is decreasing. Vague epifaunal communities that live associated with leaves of seagrass meadows have a crucial role in transmitting the production carried out by seagrasses to higher trophic levels. In this work, it has been proposed to study the variations that may occur to epifaunal communities associated with *Cymodocea nodosa* due to the pressure of simulated herbivorism. This experiment was carried out at different scales to obtain biogeographical differences. The locations were Arinaga and Castillo de Romeral (in the Gran Canaria region), Aucanada and Es Barcares (in the Mallorca region). The treatment of simulated herbivorism caused variations in the total abundance of epifauna, the diversity of epifauna and in the abundance of some taxonomic groups. In addition, the directions of responses of the taxonomic groups were not uniform among localities. Moreover, the biomasses of *Cymodocea nodosa* and epiphytes appear to be directly related to the epifauna communities.

## 1. Introduction

Human impacts have increased in recent times and, as a consequence, ecosystems have been affected. Marine ecosystems located close to the coast as coral reefs, mangrove forests, salt marshes and seagrass meadows support high impacts due to their geolocation and proximity to the impact sources (Balmford & Bond 2005, Orth et al. 2006, Beaumont et al. 2007). The impacts that these ecosystems receive are numerous such as nutrients and organic inputs, overfishing or susceptibility to climate change (seawater temperature rise or increased CO<sub>2</sub> concentration) (Jackson et al. 2007, Duarte 2002, Reynolds et al. 2018, Khan et al. 2016, Olsen et al. 2012). Mechanical damages (e.g. push nets or anchoring), aquaculture, eutrophication and salinity changes could produce changes in the meadow structures, erosion and finally a loss of the meadows (Duarte, 2002).

It is a well-known fact that seagrass meadows play a key role in marine ecosystems, due to the large number of ecological functions that they perform and services that they provide. In fact, seagrasses are considered “ecological engineers”, as a result of physical, chemical and biological actions that seagrasses perform (Wright and Jones, 2006). Seagrasses influence physical factors such as the capture and stabilization of sediments and the attenuation of waves protecting coastal areas from turbulence (Nieuwenhuize & Hemminga 1990, Cabaço et al. 2010). The presence of seagrass meadows has an effect on the chemical factors in these ecosystems because they improve the quality of the water producing O<sub>2</sub> and incorporating nutrients into the water since they are responsible for the production of large amounts of organic carbon (Peduzzi & Vukovic, 2007). Furthermore, seagrass beds harbor a diverse community of small invertebrates, mainly formed by amphipods, copepods, annelids, gastropods, among other groups (Barberá-Cebrián et al. 2002, Tuya et al. 2006; Espino et al., 2011; Tuya et al. 2014b; Png-gonzalez, et al., 2014; Mateo Ramírez & García Raso, 2012). In some studies such as the one carried out by Lewis Allan W et al., (1983), a greater presence of macrofauna was observed in areas with seagrass meadows than areas without them. In fact, several studies claim that seagrass beds are a source of refuge for many organisms, some of them in danger of extinction (Duarte et al., 2008) and others with important economic value (Espino et al., 2011). Furthermore, some studies have shown that some fish

species have adapted their life cycle to the structural complex of seagrasses (Espino et al., 2011; Heck & Orth 2013).

The presence of epiphytic algae in the seagrass leaves is typically correlated with greater abundance and/or richness of species of the motile epifauna due to an increase in habitat complexity and a greater abundance of food (Nagle, 1968; Lewis and Hillingworth, 1982; Bell et al., 1984; Carr et al., 2011). Indeed, some organisms are strongly dependent on the epiphytes as their main food source (e.g. Tomas et al. 2006; Buza Jacobucci & Pereira Leite, 2014). In addition, it has been demonstrated the relevant role of mesograzers on seagrass meadows due to the crucial regulation that they perform as a result of their epiphytic consumption and, consequently, they control the competition for light and nutrients between seagrass and epiphytes (Neckles, et al., 1993). Epifaunal organisms are prey to a wide variety of consumers. In this way, they act as intermediate steps between primary and secondary producers transmitting the production carried out by seagrass meadow to higher levels in the trophic web. (Heck & Valentine 2006, Fernandez et al.; 2012). Moreover, seagrass leaves are also an important food source for macrograzers such as fish or sea urchins as well as megagrazers such as turtles, waterfowl and dugongs (*Dugong dugong*) (Moran & Bjorndal, 2005; Fourqurean et al., 2010).

Herbivory has fluctuations over time and space in different scales (Tomas et al., 2005; Prado et al. 2007,2008), but, even so, it has been shown that herbivory has important effects in seagrass meadows ecosystems. For instance, herbivory strongly influences the structure, composition and production of vegetation (McNaughton, 2001). Due to the alterations that herbivory can produce in these ecosystems, recent studies affirm that the effect of herbivory is a control factor in the fluctuations of dynamics population, composition, distribution and production, which can be stronger than temperature or salinity (Valentine & Heck 1999; Tomas et al., 2004; Gartner et al., 2013; Duffy et al. 2015; Carr et al. 2011). Therefore, herbivory effects can change the food and shelter resources that seagrass meadows offer to specific organisms that live associated with them and, consequently, alter epifauna abundances (McGlathery, 1995; Lal et al., 2010) as they.

Alter competition and predation, since habitat modification regulates the availability of food and shelter (Hixon & Menge 1991, Sirota & Hovel 2006, Gartner et al., 2013).

*Cymodocea nodosa* (Ucria) Ascherson is a dioecious seagrass distributed throughout the Mediterranean (including Balearic Islands), and adjacent eastern Atlantic coast but it is limited by southwestern Iberia, northwestern Africa and the Canary Islands and Maderia (Alberto et al., 2006; Mascaró et al., 2009; Tuya et al. 2014a). This species is considered a pioneer species colonizing soft bottoms under a wide range of environmental conditions. *C. nodosa* is the second species of seagrasses in terms of abundance in the Mediterranean Sea (being *Posidonia oceanica* the first one; Scipione & Zupo, 2010) and the most important macrophyte in the Canary Island. (Reyes et al., 1995; Barbera et al. 2005). The evolutionary origin of this seagrass was the Indian Ocean (Red Sea), by divergence, because of its proximity, colonized the eastern Mediterranean, and spread across the Atlantic to the west, thus constituting the central and peripheral part of this seagrass (Masucci & Arnaud-haond 2012, Alberto et al. 2006). As previously mentioned, many studies have shown that herbivory has effects linked to the performance of seagrass and its ecology, as it also happens in the case of *C. nodosa* (Cebrián et al. 1996, Fernandez et al. 2012, Tuya et al., 2013). Even so, the effects of herbivory on the epifauna associated with *C. nodosa* have not been studied yet and remain unknown.

The aim of this study is to evaluate the effects of simulated herbivory and biogeographic location on epifaunal organisms associated with the seagrass *C. nodosa*. To examine these effects, meadows locations have been selected in two distinct regions within the distribution area of *C. nodosa*.



## 2. Methods

### 2.1 Study sites

Four *C. nodosa* meadows from two regions (Canarias and Mallorca) were selected in order to study the effects of simulated herbivore pressure at different biogeographical scales. The meadows from Canary Island belong to the peripheral zone of distribution of *C. nodosa*, whereas the meadows located in Baleares are within the central part of the distribution range of *C. nodosa* (Alberto et al., 2006; Masucci & Arnaud-haond, 2012).

In Canary Island, the two meadows selected were located in Gran Canaria Island. The first sampling site was Arinaga (27°51'N 15°24'W), where there is a meadow of *C. nodosa* with an area of 1.5ha, located at 10m depth and with a shoot density of  $424 \pm 83 \text{ m}^2$ . The second sampling sites was Castillo del Romeral (27°47'N 15°29'W), where a meadow with an area of 25ha, located at 5m depth and with a shoot density of  $787 \pm 156 \text{ m}^2$  is found. These two location are situated in the southeast region of Canary Island (Figure 1, Canary Island map). The other two meadows are located in the northern region of Balearic Island.

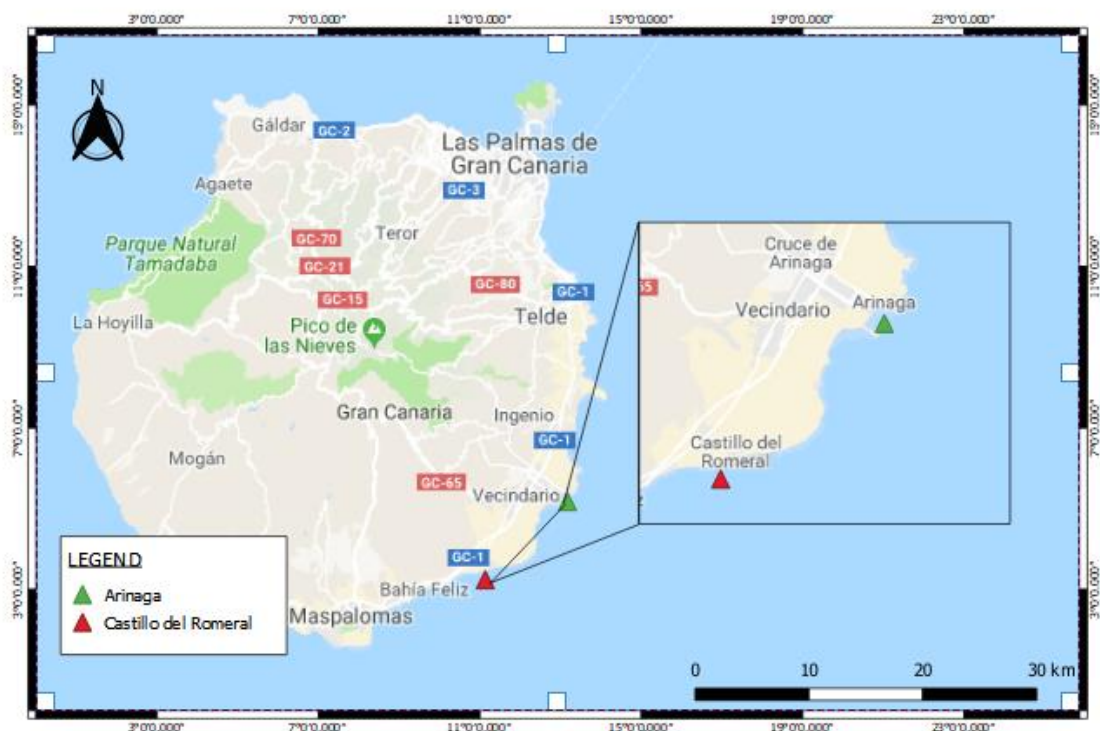


Figure 1. Location the *C. nodosa* meadows in Gran Canaria: Arinaga (green) and Castido del

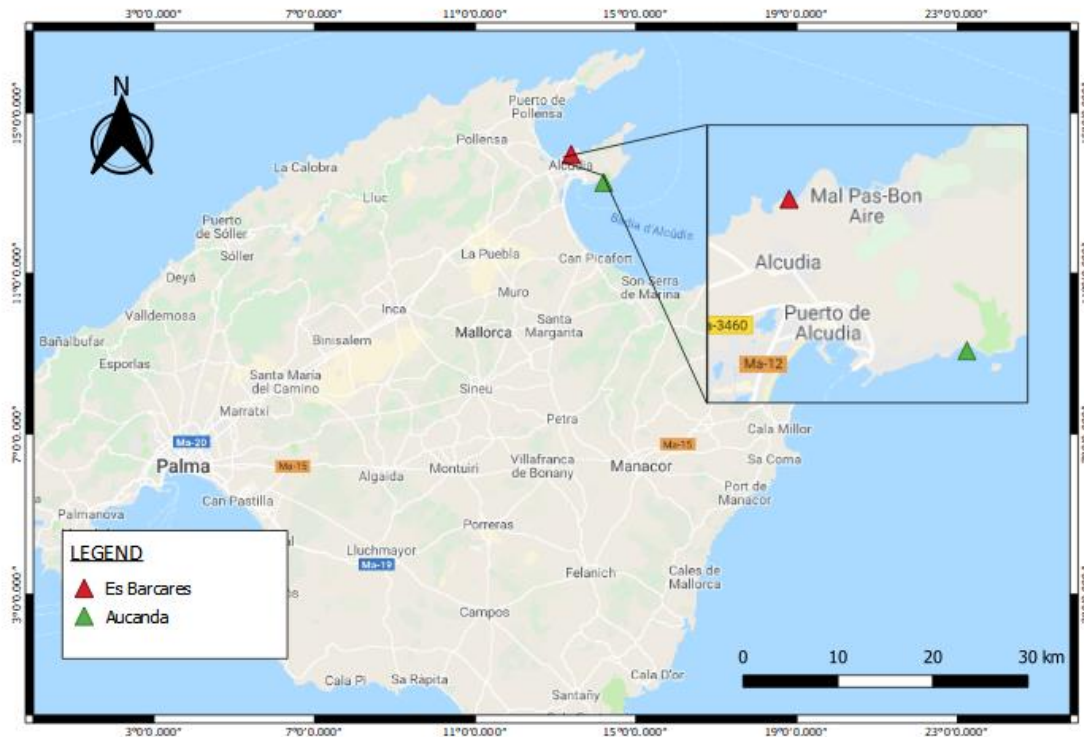


Figure 2. Location the *C. nodosa* meadows in of Mallorca: Es Barcares (red) and Aucanda (green).

One of them was located in Aucanada ( $39^{\circ}50'N$   $3^{\circ}10'E$ ), where the meadow present an area of 2.2ha, located at 2.7m and with a shoot density of  $1183 \pm 204 \text{ m}^2$ . The last study meadows was located in Es Barcares ( $39^{\circ}51'N$   $3^{\circ}07'E$ ), where the meadow has an area of 1.6ha, located at 3.5m and with a shoot density of  $756 \pm 188 \text{ m}^2$  is found (Tuya et al., 2018) (Figure 2, Balearic Island map).

## 2.2 Field sampling methods

Different levels of herbivory were simulated in each *C. nodosa* meadows though a manipulate experiment. This experiment and the subsequent sampling were performed by SCUBA diving. First of all, 10 plots (1 x 1 m) were put in the seabed haphazard, separated from each other by 2 meters. Then, high herbivory level was set up in 5 plots cutting the seagrass leaves at 80%, whereas the rest of plots were established as control (i.e. natural low herbivory level). The experiment was carried out during the summer season, from June to September of 2016, coinciding with highest activity of fish grazing (Tomas et al., 2005) and leaves were cut once a month to maintain treatment levels. At

the end of the experimental period, a 0.04m<sup>2</sup> quadrat with a cotton bag mesh (size 250µm) was placed in the center of each plot (avoiding any border effects) to collect the epifauna. Associated fauna and aboveground biomass were caught inside the mesh bag by carefully, cutting the seagrass leaves just above the sediment surface with scissors (Figure 3). Collected samples were kept in individual plastic bags filled with seawater and transported to the laboratory in coolers for further sorting.



Figure 3. Sampling of epifauna after having applied the simulated herbivorous treatment.

### 2.3 Laboratory analyses

All plastic bags with the corresponding samples were stored in the freezer until further manipulation in the laboratory. The content of each bag including leaves of *C. nodosa* were rinsed with freshwater using a three-rinse cycle separating epifauna from leaves (Carr et al., 2011). Then, samples were filtered through a 50 and 125 micrometers mesh sieve to separate epifauna by size. The different samples resulting from the separation by size were preserved in 70% ethanol. Carefully, epiphytes were scrapped off the leaves by a glass slide and were placed in a previously weighed filter. Epiphytes and leaf biomass were dried in an oven for 48 hours at 60°C, and subsequently weighted (Dry Weight, DW). Epifauna specimens with a size of 500 micrometers or larger were sorted into taxonomic units and their abundance was quantified using a binocular microscope (Tuya et al., 2013)(Figure 4).



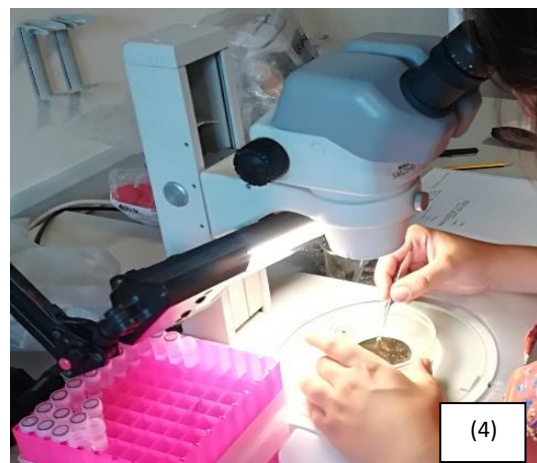
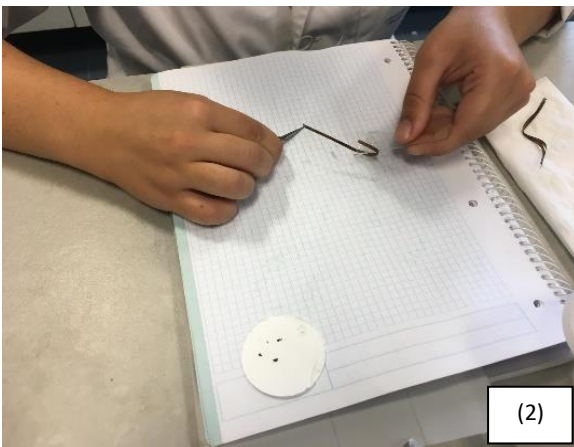


Figure 4. Laboratory analysis process. Rinse the leaves of *C. nodosa* with fresh water causing an osmotic shock and the subsequent separation of the epifauna from the leaves (1). Separation of epiphytes and leaves (2). Filtration through a tapestry of 500 micrometers of the epifauna (3). Classification of taxonomic groups by binocular microscope (4).

## 2.4 Statistical analyses

In order to examine the existence of significant differences between biomass of *C. nodosa* (gDW/100cm<sup>2</sup>) and biomass of epiphytes (gDW/100cm<sup>2</sup>) across the factors region, location and treatment, three-way multivariate permutational ANOVA (PERMANOVA) was carried out. The correlation between the biomass of epiphytes, the biomass of *C. nodosa*, the total abundance of individuals (ind/100cm<sup>2</sup>) as well as the taxonomic richness (species/100cm<sup>2</sup>) was analyzed by the Spearman correlation test. Parametric analyses were used when normality (Shapiro Wilk's and Q-Q norm quantiles based on residuals (Annex S1; Annex S3; Annex S5)) and homoscedasticity (Levenne test) of the data were detected. Then, a three-way Analysis of Variance (ANOVA) was performed to compare the differences in total abundance of individuals (ind/100cm<sup>2</sup>), taxa richness (species/100cm<sup>2</sup>), and different diversity measures such as Pielow evenness (*J*) (Feldman, 2006) and Shannon Wiener diversity index (*H'*) (Shannon and Weaver, 1963) across the factors of Region (fixed factor; 2 levels), Locations (nested within region; 2 levels) and clipping treatment (fixed factor; 2 levels). Fourth root transformation data were used to generate a similarity matrix based on Bray Curtis index. Subsequently, multidimensional scaling (MDS) representation and three-way multivariate permutational ANOVA (PERMANOVA) were performed to examine the differences in the structure of the epifauna community associated with the different *C. nodosa* meadows. The design was the same than ANOVA analysis. To conclude, a similitude test (SIMPER routine) was used to determine which epifauna groups contributed to a greater differences per regions and between them. Two-way Analysis of Variance (ANOVA) was performed to compare the differences in the taxonomic groups that produce a greater differences between region (fixed factor, 2 levels) and clipping treatment (fixed factor, 2 levels). When the criteria of normality and homoscedasticity were not respected Kruskal-Wallis test was used.

Univariate statistical analyses, homogeneity test and homoscedasticity test were performed using Rstudio software package (Version 1.1.463). Graphics and maps were plotted with RStudio, Excel and QGIS 3.6. Diversity index, multivariate methods (PERMANOVA, MDS), and SIMPER analyses, were performed with the PRIMER 7 software package.

### 3. Results

#### 3.1 *Cymodocea nodosa* and epiphytes biomass

Biomass of *Cymodocea nodosa* presents significant differences between Region, Location and Treatment factors (Table 1). Higher abundance of seagrass is found in Gran Canaria, is also the region where the treatment factor affects with greater magnitude (Figure 5). Epiphyte biomass presents differences between region, location and treatment, according to the statistical results made with three-way multivariate permutational ANOVA (PERMANOVA) (Figure 6, Table 1).

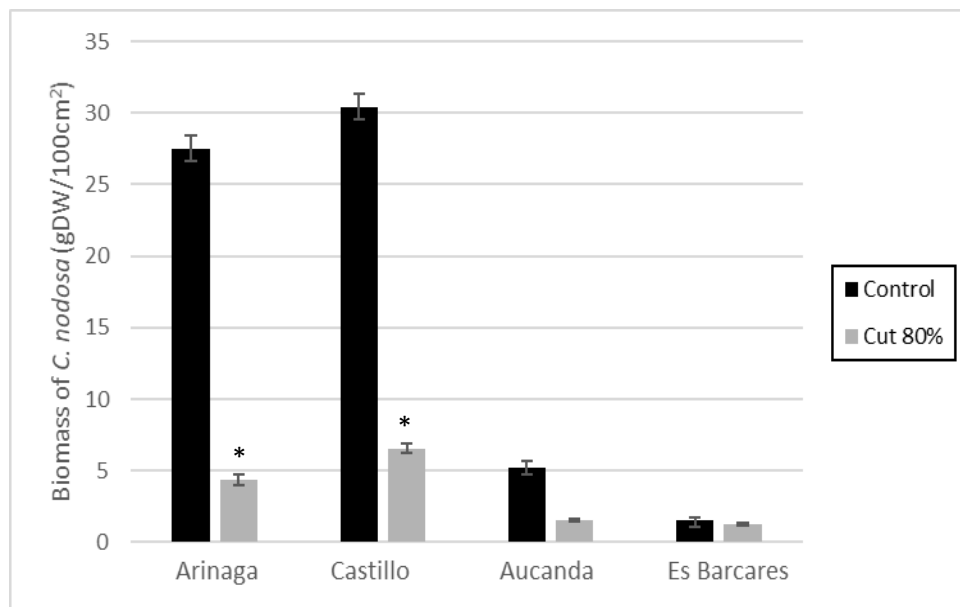


Figure 5. Biomass of *Cymodocea nodosa* (g\*100cm<sup>-2</sup>) (mean and SE) at the four location Arinaga, Castillo, Aucanada i Es Barcares and across clipping treatments (Control and Cut (80%)). Data (n= 18 plots per treatment). Significant values of Wilcoxon rank sum test (\*).

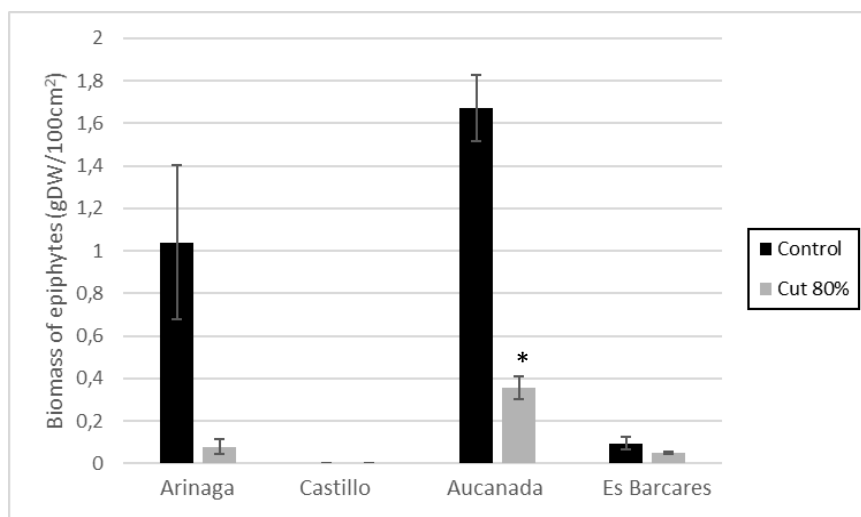


Figure 6. Biomass of epiphytes ( $\text{g} \cdot 100\text{cm}^{-2}$ ) (mean and SE) at the four location Arinaga, Castillo, Aucanada i Es Barcares and across clipping treatments (Control and Cut (80%)). Data ( $n= 18$  plots per treatment). Significant values of Wilcoxon rank sum test (\*).

Table 1. Results of three-way multivariate permutational ANOVA (PERMANOVA) examining differences in biomass of *C. nodosa* ( $\text{gDW}/100\text{cm}^2$ ) and biomass of epiphytes ( $\text{gDW}/100\text{cm}^2$ ) between Region (Re), locations between regions (Loc(Re)), treatment (Tr) and their interactions. Both variables have need a pre-treatment (square root). Pseudo-F = pseudo-F values, df = degrees of freedom, MS = mean squares, unique perms = numbers of permutations. Significant p-values are highlighted in bold.

Source of variation	df	MS	Pseudo-F	P (perm)	Unique perms
<i>Biomass of C. nodosa</i>					
Re	1	0.5033	239.85	<b>0.0001</b>	9840
Tr	1	0.2297	109.49	<b>0.0001</b>	9850
Loc(Re)	2	0.0171	8.1255	<b>0.0017</b>	9951
RexTr	1	0.0636	30.298	<b>0.0001</b>	9848
Loc(Re)xTr	2	0.0018	0.8672	0.4205	9954
Residuals	28	0.0021			
Total	35				
<i>Biomass of epiphytes</i>					
Re	1	0.31423	9.7281	<b>0.0042</b>	9831
Tr	1	0.13875	4.2956	<b>0.0347</b>	9849
Loc(Re)	2	0.27206	8.4224	<b>0.0015</b>	9963
RexTr	1	0.00807	0.2499	0.6589	9874
Loc(Re)xTr	2	0.05287	1.6369	0.21	9956
Residuals	28	0.03230			
Total	35				

The results obtained from the Spearman correlation test determine that the abundance of individuals (N) and the taxonomic richness (T) are positively correlated with epiphytes biomass, whereas, the results between the abundance of epiphytes and the abundance of the seagrass show a significant negative correlation (Table 2, Figure 7, Figure 8)

Table 2. Results of the Spearman correlation test on the relationship between biomass of epiphytes (gDW/100cm<sup>2</sup>) and biomass of *Cymodocea nodosa* (g/100cm<sup>2</sup>), total abundance of individuals (N; Ind/100cm<sup>2</sup>) and Taxon Richness (T; species/100cm<sup>2</sup>). S = Spearman correlation rank, rho = Spearman coefficient rank. P-value in bold indicates positive correlation.

Source of variation	S	p-value	rho
<i>Epiphytes</i>			
Leaf	10509	<b>0.035</b>	-0.352472
N	4406.7	<b>0.008367</b>	0.4328629
T	2817.6	<b>0.00002909</b>	0.6373765

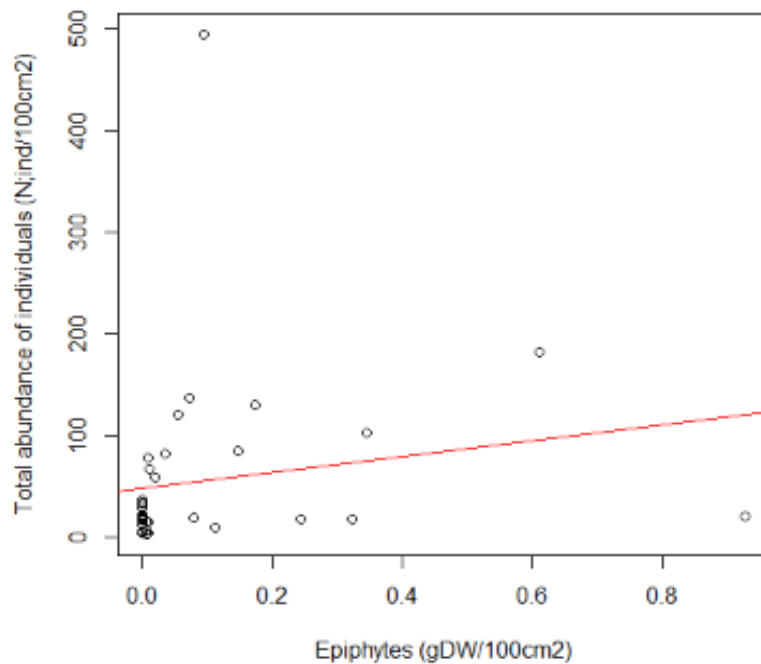


Figure 7. Relationship between Total abundance of individual (N; ind/100cm<sup>2</sup>) and Epiphytes biomass (gDW/100cm<sup>-2</sup>). R<sup>2</sup>=0.0299



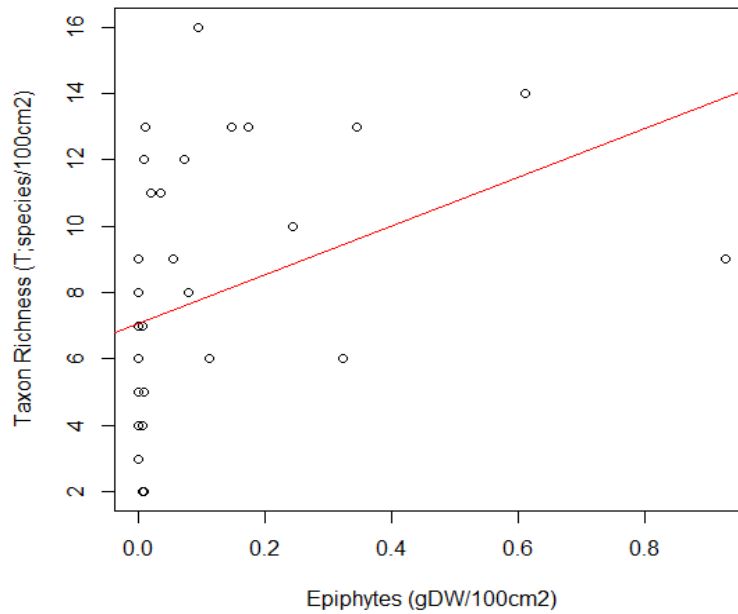


Figure 8. Relationship between Taxon Richness (T; species/100cm<sup>2</sup>) Epiphytes biomass (gDW/100cm<sup>-2</sup>). R<sup>2</sup>=0.1424

### 3.2 Epifaunal groups

A total of 1952 individuals were counted and classified in taxonomic groups resulting in 543 Nematoda, 305 Polychaeta, 250 Copepoda, 220 Prosobranchia, 195 Gammaridea, 152 Caprellidea, 70 Cumacea, 55 Tanaidacea, 41 Isopoda, 31 Bivalvia, 22 Ostracoda, 13 Pycnogonida, 13 Arachnida, 11 Echinoidea, 8 Sipunculidea, 6 Sagittoidea, 5 Anomura, 5 Caridea, 3 Mysidacea, 3 Brachyura, 1 Clitellata (Hirudinea).

The abundance of individuals (N; ind/100cm<sup>2</sup>) presents significant differences between region and location. In addition, the effects of the treatment factor (clipping) were different between regions (i.e. nearly significant interaction treatment x region). Whereas abundance of individuals tended to decrease under clipping, in location of Aucanda, in Mallorca, the treatment cut 80% exhibited higher abundance of individuals than the control (Table 3; Figure 9 Post Hoc; p>0.05 Annex S4).

Table 3. Results of univariate Three-way nested ANOVAs comparing differences in total abundance of individuals (N; ind/100cm<sup>2</sup>), taxa richness (T; species/100cm<sup>2</sup>), Pielow and Shannon Wiener (*H'*) per region (Re), location (Loc) and treatment (Tr) and their interactions. Region factor (Re) is nested within location (Loc(Re)). df = degrees of freedom, SS = sum of squares. Significant p-values are highlighted in bold.

Source of variation	n	df	SS	F	p-value
<i>Abundance (N, ind/100 cm<sup>2</sup>)</i>	36				
Re		1	1.667	9.330	<b>0.00491</b>
Loc(Re)		2	1.992	5.574	<b>0.00917</b>
Tr		1	0.128	0.716	0.40453
Re:Tr		1	0.069	0.388	0.53859
Loc (Re):Tr		2	1.140	3.190	<b>0.05647</b>
Residuals		28	5.004		
<i>Taxon Richness (T)</i>	36				
Re		1	124.69	13.020	<b>0.00119</b>
Loc(Re)		2	81.61	4.261	<b>0.02424</b>
Tr		1	0.40	0.042	0.83927
Re:Tr		1	2.81	0.294	0.59216
Loc (Re):Tr		2	15.08	0.787	0.46487
Residuals		28	268.15		
<i>Pielou's evenness (J)</i>	36				
Re		1	0.00901	2.567	0.12034
Loc(Re)		2	0.04148	5.912	<b>0.00721</b>
Tr		1	0.00055	0.156	0.69610
Re:Tr		1	0.02063	5.879	<b>0.02202</b>
Loc (Re):Tr		2	0.03391	4.834	<b>0.01573</b>
Residuals		28	0.09823		
<i>Shannon Wiener (H')</i>	36				
Re		1	0.335	2.256	0.1443
Loc(Re)		2	0.303	1.022	0.3730
Tr		1	0.088	0.590	0.4490
Re:Tr		1	0.791	5.332	<b>0.0285</b>
Loc (Re):Tr		2	0.057	0.193	0.8256
Residuals		28	4.156		

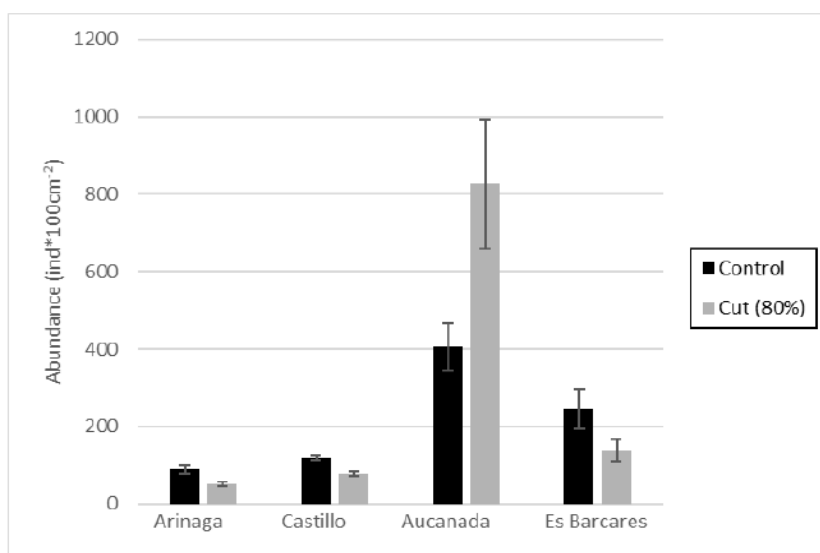


Figure 9. Total abundance of individuals (N; ind\*100cm<sup>-2</sup>) (mean and SE) at the four Location and across clipping treatments (Control and Cut (80%)). Data (n= 5 plots per treatment).

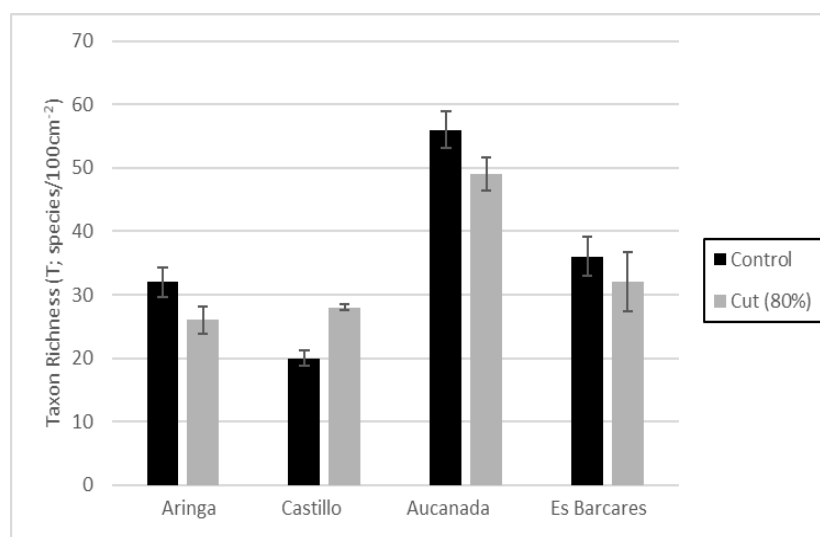


Figure 10. Taxon Richness (T; species/100cm<sup>2</sup>) (mean and SE) at the four Location and across clipping treatments (Control and Cut (80%)). Data (n= 5 plots per treatment).

The number of taxa (T/species/100cm<sup>2</sup>) showed significant differences between region, with Mallorca presenting higher taxon richness values than Gran Canaria (Table 3; Figure 10; Post Hoc;  $p > 0.05$  Annex S4). In the case of Shannon Wiener index the location of Aucanada shows the highest value of this index. The location of Castillo de Romeral shows a smaller value of Shannon Wiener index and it was the unique location where

the effect of the treatment cut 80% showed higher values than control (Table 3; Figure 11; Post Hoc;  $p > 0.05$  Annex S4). The effects of the 80% clipping treatment in Pielow's evenness index varies by Region (Table 3; Figure 12; Post Hoc;  $p > 0.05$  Annex S4), where there were also significant differences between regions.

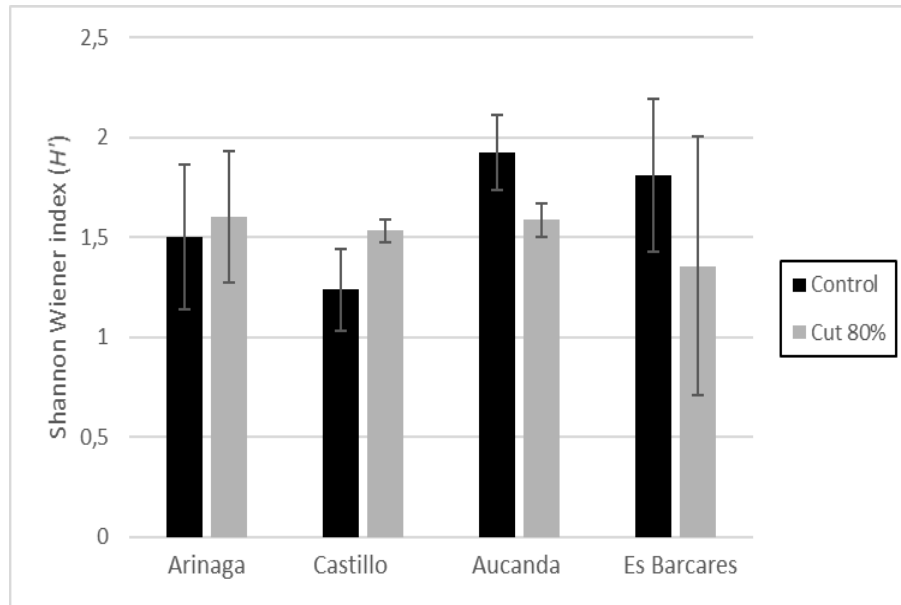


Figure 11. Shannon Wiener index ( $H'$ ) (mean and SE) at the four Location and across clipping treatments (Control and Cut (80%)). Data ( $n = 18$  plots per treatment).

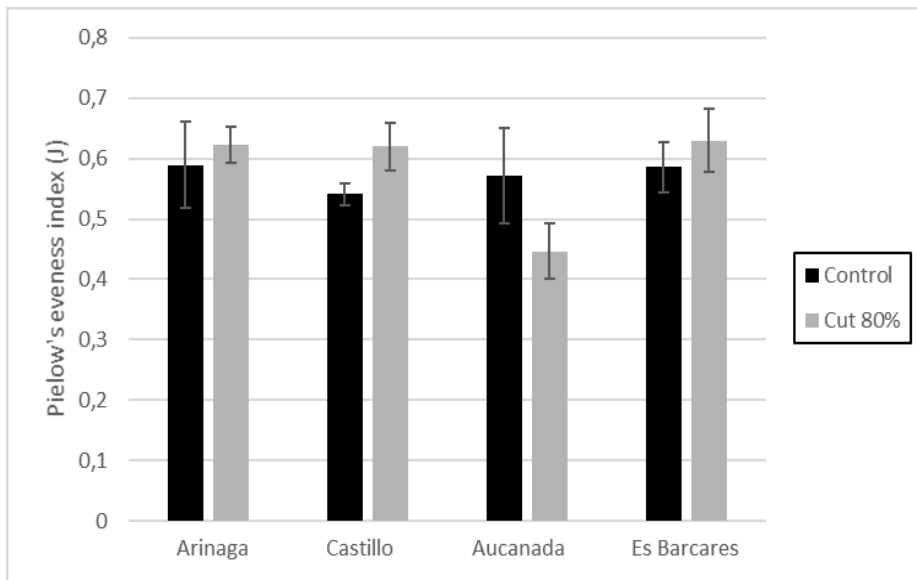


Figure 12. Pielow's evenness index ( $J$ ) (mean and SE) at the four Location and across clipping treatments (Control and Cut (80%)). Data ( $n = 18$  plots per treatment).

Pielow's evenness index ( $J$ ) showed significant values in terms of the locality factor between Aucanda and Es Barcares, and the interaction between locality (region factor nested within) and treatment between Aucanda and Es Barcares after 80% clipping treatment (Significant results of the post hoc analysis of Tukey HSD test). The values of Pielow's evenness are greater in the treatment of 80% clipping with the exception of Aucanada where Pielow's evenness index is greater in the control treatment.

The structure of the community displayed significant differences between regions but not between treatment levels (Table 4). The MDS show a coherent grouping arrangement according to the regions (Figure 13).

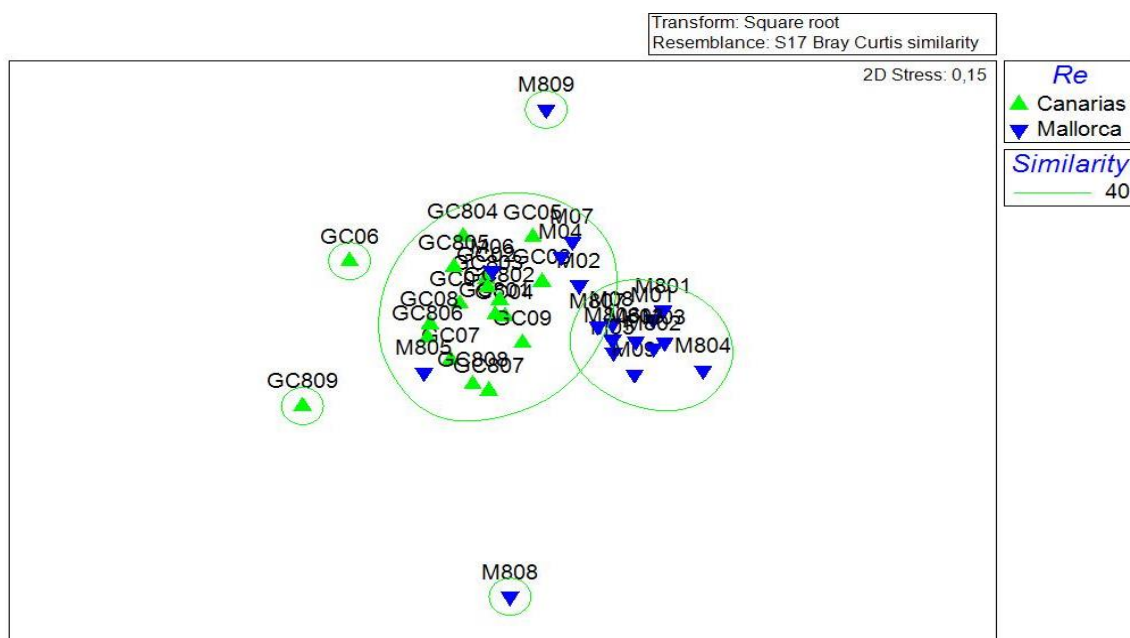


Figure 13. Multidimensional scaling plot from the transformation of the data to the fourth root and the generation of the similarity matrix generated by an index of similarity of Bray Curtis considering the abundance of the different groups of epifauna at each site according to the region (green=Canarias, blue=Mallorca) and treatment factors (Control, Cut 80%). Circles group samples under with a 40% similarity array. Data (n= 5 plots per treatment).

Table 4. Results of three-way multivariate permutational ANOVA (PERMANOVA) examining differences in the abundance of each taxon (ind/100cm<sup>2</sup>) between Region (Re), locations between regions (Loc(Re)), treatment (Tr) and their interactions. Pseudo-F = pseudo-F values, df = degrees of freedom, MS = mean squares, unique perms = numbers of permutations. Significant p-values are highlighted in bold.

Source of variance	df	MS	Pseudo-F	P(perm)	Unique perms
Abundance of each taxon (ind/100cm <sup>2</sup> )					
Re	1	14373	10.614	<b>0.0001</b>	9947
Loc(Re)	2	4836.1	3.5715	<b>0.0001</b>	9909
Tr	1	1880.1	1.3885	0.2017	9941
Re:Tr	1	833.07	0.61523	0.7762	9939
Loc(Re):Tr	2	1185.7	0.87561	0.6037	9922
Residual	28	1354.1			
Total	35	66631			

Nematoda, Caprellidea, Polychaeta, Copepoda, Prosobranchia, Gammaridea, Cumacea and Tanaidacea were responsible of the 70% differences between Gran Canaria and Mallorca. Therefore, the differences found between regions depend mainly on a group of determined taxa (Figure 5). Furthermore, the results of three-way multivariate permutational ANOVA (PERMANOVA) that there is no effect of clipping on the community structure overall, although, there were some specific taxonomic groups in which the treatment factor has effects on their abundances (Figure 14).

For instance, the Nematoda taxon presents significant differences at the level of both the region and treatment factors (Table 6; Figure 14; Post Hoc analysis p-value<0.05 Annex S6).

Table 5. Results of SIMPER test for similarity between regions: Gran Canarias (GC) and Mallorca (M). Av. Abund = abundance of each unit taxon, Av. Diss = average dissimilarity, Diss/SD = quotient of dissimilarity and standard deviation, Contrib. % = contribution of each taxon to the overall dissimilarity between high and low groups, Cum% = cumulative contribution.

Group Gran Canarias & Mallorca Average of dissimilarity = 64.93						
Species	Group GC Av. Abund	Group M Av. Abund	Av. Diss	Diss/SD	Contrib. %	Cum. %
<i>Nematoda</i>	0.06	3.93	9.72	1.38	14.98	14.98
<i>Caprellidea</i>	0.44	2.39	6.66	1.55	10.25	25.23
<i>Polychaeta</i>	0.39	2.67	6.64	1.13	10.23	35.46
<i>Copepoda</i>	1.32	2.70	6.25	1.34	9.62	45.08
<i>Prosobranchia</i>	2.12	2.19	5.90	0.95	9.09	54.17
<i>Gammaridea</i>	2.25	1.89	5.28	0.89	8.13	62.30
<i>Cumacea</i>	0.00	1.47	3.86	1.11	5.94	68.24
<i>Tanaidacea</i>	0.40	1.28	3.81	1.21	5.87	74.11
<i>Bivalvia</i>	0.00	1.01	3.01	1.07	4.63	78.74
<i>Isopoda</i>	0.65	0.90	3.00	0.96	4.61	83.35
<i>Echinoidea</i>	0.47	0.00	1.93	0.62	2.98	86.33
<i>Ostracoda</i>	0.22	0.61	1.83	0.85	2.82	89.15
<i>Arachnida</i>	0.32	0.30	1.51	0.69	2.32	91.48
<i>Pycnogonida</i>	0.08	0.39	1.18	0.60	1.81	93.29
<i>Caridea</i>	0.25	0.00	1.13	0.42	1.73	95.02
<i>Sipunculidea</i>	0.00	0.34	0.80	0.60	1.23	96.25
<i>Mysidacea</i>	0.06	0.11	0.60	0.31	0.93	97.18
<i>Sagittidea</i>	0.00	0.27	0.59	0.50	0.91	98.09
<i>Anomura</i>	0.13	0.08	0.57	0.35	0.87	98.97
<i>Brachyura</i>	0.06	0.11	0.53	0.39	0.82	99.79
<i>Hirudinea</i>	0.00	0.06	0.14	0.24	0.22	100.00

In addition, the taxonomic group Prosobranchia displayed significant differences only at the level of treatment factor, being less abundant under clipping (Table 7; Figure 14; Post Hoc analysis p-value<0.05 Annex S7).

On the other hand, the taxonomic group Gammaridea did not show significant differences in any factor involved. On the other hand, the majority of taxonomic groups such as Caprellidea, Cumacea, Copepoda and Tanidacea presented significant differences at the level of region factor, generally being more abundant in Mallorca than in Gran Canaria (Table 6; Table 7).

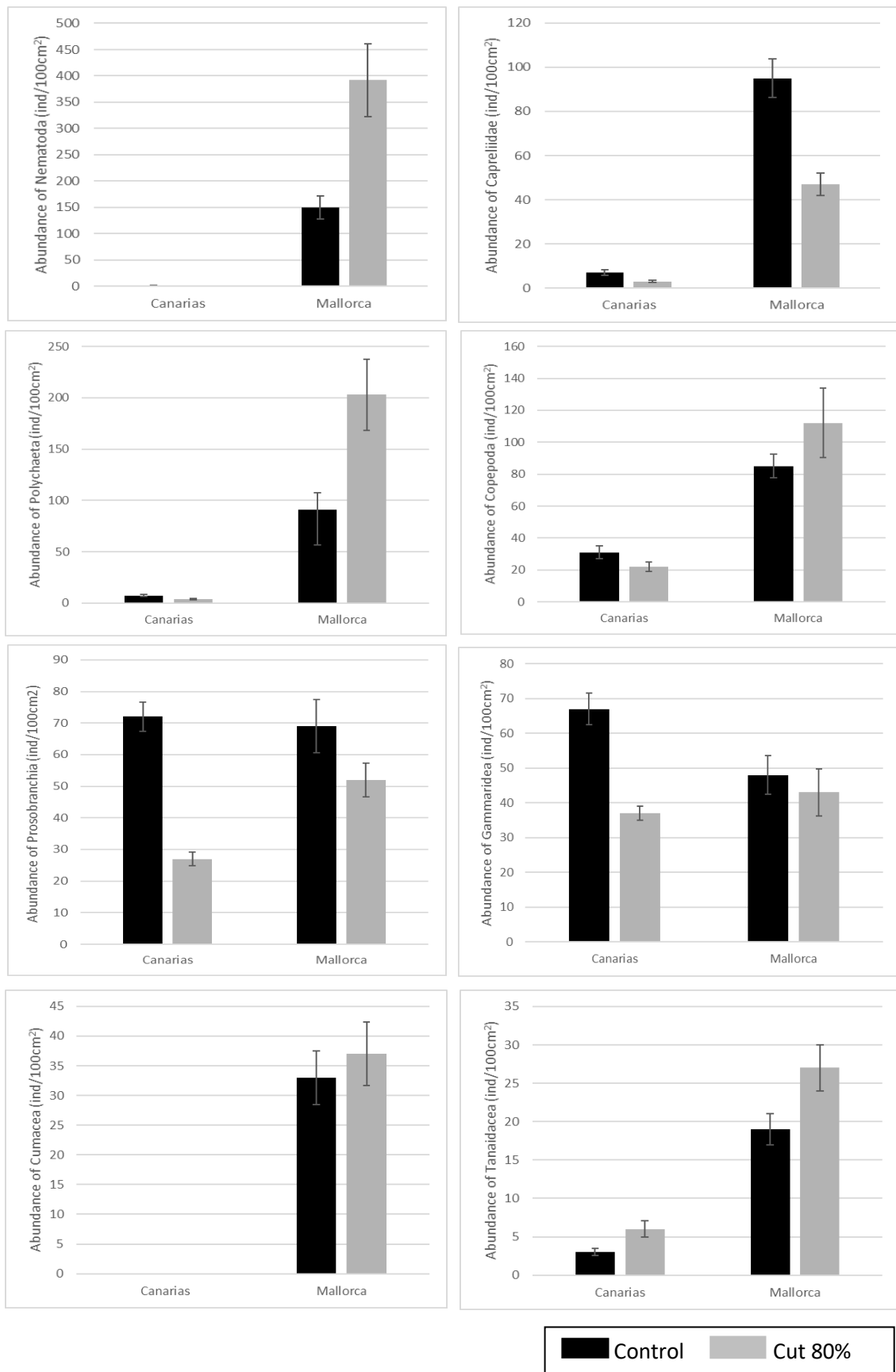


Figure 14. Results of Abundance of individuals (ind/100 cm<sup>2</sup>) of the Nematoda, Caprelliidae, Polychaeta, Gammaridea, Copepoda, Prosobranchia, Gammaridea, Cumacea, Tanaidacea taxon groups found in *Cymodocea nodosa* across regions and experimental treatments.



Table 6. Results of the non-parametric Kruskal-Wallis  $H$  test on the Nematoda, Polychaeta, Caprellidea, Cumacea and Tanaidacea abundance (ind/100cm<sup>2</sup>) across the fixed factors region (Re) and clipping treatment (Tr). df = degrees of freedom,  $\chi^2$  =Kruskal-Wallis Chi-squared. Significant p-values are highlighted in bold.

Source of variation		df	$\chi^2$	p-value
<i>Nematoda</i>	Re	1	19.202	<b>0.000011176</b>
	Tr	1	3.8467	<b>0.04984</b>
<i>Polychaeta</i>	Re	1	7.3547	<b>0.006689</b>
	Tr	1	0.04979	0.8234
<i>Caprellidea</i>	Re	1	16.032	<b>0.00006227</b>
	Tr	1	1.6669	0.1967
<i>Cumacea</i>	Re	1	16.594	<b>0.00004629</b>
	Tr	1	0.00035567	0.985
<i>Tanaidacea</i>	Re	1	8.2606	<b>0.004052</b>
	Tr	1	0.36612	0.5451

Table 7. Results of univariate Three-way nested ANOVAs comparing differences in Prosobranchia (pre-treatment Ln+1), Gammaridea (pre-treatment Ln+1) and Copepoda (pre-treatment Ln+1) abundance (ind/100cm<sup>2</sup>) per region (Re), location (Loc) and treatment (Tr) and their interactions. Region factor (Re) is nested within location (Loc(Re)). df = degrees of freedom, SS = sum of squares. Significant p-values are highlighted in bold.

Source of variation	n	df	SS	F	p-value
<i>Prosobranchia</i>	36				
Re		1	0.000	0.001	0.9795
Loc(Re)		2	0.896	0.669	0.5201
Tr		1	3.097	4.628	<b>0.0402</b>
Re:Tr		1	0.783	1.170	0.2887
Loc (Re):Tr		2	2.788	2.084	0.1434
Residuals		28	18.736		
<i>Gammaridea</i>	36				
Re		1	0.869	1.505	0.230
Loc(Re)		2	1.224	1.060	0.360
Tr		1	0.920	1.593	0.217
Re:Tr		1	0.074	0.129	0.722
Loc (Re):Tr		2	1.825	1.580	0.224
Residuals		28	16.170		
<i>Copepoda</i>	36				
Re		1	7.292	12.889	<b>0.001246</b>
Loc(Re)		2	14.484	12.801	<b>0.000113</b>
Tr		1	0.625	1.104	0.302306
Re:Tr		1	0.061	0.109	0.744303
Loc (Re):Tr		2	1.659	1.466	0.247952
Residuals		28	15.841		

#### 4. Discussion

The results obtained in this study suggest that the factor that regulates the structure and composition of the epifauna communities is the different biogeographical regions. Moreover, our results also suggest that the clipping treatment mimicking the herbivory pressure modifies the total abundance and diversity index of epifauna. Unexpectedly, the effect of the clipping treatment has had opposite outcomes depending on the location (Table 3). For instance, the abundance of the Prosobranchia taxonomic group decreased upon clipping treatment whereas, the abundance of Nematoda taxonomic group increased (Table 7). These data support the idea that epifauna is very sensitive to environmental variations as it was previously shown in different studies, such as the study carried out by Tanner et al. (2006). This study unveiled that the variation of the epifauna composition is affected by the patch of meadows, landscape characteristics and wave exposure variable. Our study suggests a new factor to the epifauna community regulation: the herbivory pressure.

However, the differences found in this study could be caused by the location of the analyzed regions. Firstly, because these regions are located in archipelagoes but with the difference that Mallorca is located very close to the Iberian Peninsula. The study carried out by Benedetti-Cecchi et al. (2003) showed that both the algae and the set of invertebrates differed from the islands to the continental shallow waters, since isolated islands provide fundamentally different habitat patterns of those of the continental shallow waters.

Secondly, these locations are exposed to different hydrodynamism. For instance, the open sea location of the *Cymodocea nodosa* meadow in Gran Canaria (southeast region) causes the fragmentation of seagrass probably due to hydrodynamic effects caused by waves and tides. These damaging hydrodynamic effects may be stronger in Gran Canaria than in Mallorca because *Cymodocea nodosa* meadows located in Mallorca are in areas more protected from hydrodynamism such as caves and bays (Tuya et al., 2014b). This weaker hydrodynamism may explain the higher abundance of individuals in Mallorca compared to Gran Canaria (Figure 9, Table 3).

Our data also suggest a positive effect of biomass of *C. nodosa* and epiphytes in the abundance and taxa richness of epifaunal communities (Figure 7, Table 2). Indeed, several studies have previously shown that changes in the habitat structure (epiphytic biomass and leaf biomass) affects the diversity and abundance of the epifauna community (Gartner et al. 2013; Edgar and Robertson, 1992; Heck and Wetstone, 1977; Stoner, 1980; Attrill et al. 2000). Specifically, some studies link epifaunal composition to the biomass of epiphytes instead of leaf biomass because epiphytes provide an increased complexity to habitat that promotes epifauna diversity (increase in shelter and food) (Nagle, 1968; Novak, 1982; Lewis & Hollingworth, 1982; Bell et al., 1984). Interestingly, our data show differences between the leaf biomass and epiphytes biomass between regions and locations (Table 1). These results suggest that there are latitudinal differences in the growth of alga as have been previously demonstrated in others studies (Heck & Valentine, 2006; Gartner et al. 2013).

The abundance of epifaunal individuals was significantly influenced by the clipping treatment, but the responses differed amongst localities (Table 3). Whereas clipping tended to decrease the total abundance of epifauna, in Aucanada (in the Mallorca region), total abundance was not only the highest of all localities, but was also where clipping actually increased total abundance. In fact, the clipped plots of Aucanada exhibited the highest overall abundance recorded. Similarly to abundance, response of epifaunal diversity patterns to the clipping treatment also differed between regions. The Shannon Wiener index ( $H'$ ) before the treatment tended to be higher in the Mallorca sites compared to Gran Canaria (although they were still relatively low specific biodiversity values;  $H' < 2$ ). In these Mallorca locations, the effects of clipping tended to decrease diversity. However, the Shannon Wiener index tended to increase in the clipping 80% treatment in the localities of Gran Canaria (Table 3). This unexpected increase in the Shannon Wiener index could be explain by the structural complexity of macroalgal beds, since the excessive vegetative development of algae could prevent the access of new individuals due to the high compression of algal ramifications. This increase in the Shannon Wiener index is not observed in Mallorca due to the initial conditions of lower biomass and lack of excessive development of *C. nodosa*. The increase in epifauna diversity caused by the lower algal development was also observed

in cold months when the structural complexity of macroalgal beds could facilitate the increase of the number of individuals (Mateo-Ramírez et al., 2018).

Regarding Pielow's evenness ( $J$ ), higher values were observed under the 80% clipping treatment except in the case of Aucanada. This result suggest that the removal of leaf biomass produces a modification on habitat structure. This restructuring effect affects negatively the abundance of dominant taxa such as Prosobranchia (Table 3). This decrease in Prosobranchia abundance after the 80% clipping treatment would allow the increase in the abundance of other taxonomic groups that have lower values before clipping treatment, such as Isopoda, Copepoda, Tanaidacea and Caprellidae. Similarly to Prosobranchia, other dominant taxa, such as Caprellidae or Gammaridea, showed a decreased abundance after the 80% clipping treatment. However, significant differences were only observed in the Prosobranchia taxon. On the other hand, as expected, the several taxa, such as Nematoda, Polychaeta, Copepoda, Cumacea and Tanaidacea showed higher abundance values after the 80% clipping treatment compared to the control. Therefore, these changes in the abundances of the different taxa would explain the increase in the value of Pielow's evenness index after clipping treatment, since after clipping treatment the different taxa populations tend to equal.

There are different reasons to explain the population changes of the different taxa after clipping treatment. For instance, Prosobranchia population decreases because this taxon population is highly linked to the abundance of seagrass (Urra et al., 2013a; Marina et al., 2012; Urra et al., 2013b). On the other hand, Nematoda population increases probably due to the decrease of predatory pressure on this taxon by fishes and other predators that require high abundance of seagrass in their habitat to subsist (Mateo-Ramírez et al., 2018).

Unexpectedly, the Pielow's evenness index in Aucanada location decreases after clipping treatment. This result is apparently contradictory to the rest of the data obtained in other locations. However, Aucanada is different from the rest of locations before the clipping treatment, since Aucanada shows a higher biomass of epiphytes and a higher abundance of epifauna than the rest, even with a lower seagrass biomass. The Aucanada data obtained before the clipping treatment suggest that Aucanada is a specially rich habitat probably due to the high deposition of organic material of a river

that flows into the bay less than 500 meters away from the study area. The clipping treatment would facilitate the access to this organic material to the epifauna. Therefore, and in contrast to the rest of locations, the clipping treatment in Aucanada increases the abundance of almost all the taxa studied. This unexpected increase in the abundance of epifauna after clipping treatment would explain the decrease in the Pielow's evenness index in Aucanada.

The structure and complexity of seagrass habitat is defined by two main group of factors: the natural factors, such as surface area, number of leaves per seagrass shoot, patch mosaic, leaf growth taxa, leaf biomass, latitude or temperature (Sirota & Hovel, 2006; Larkum et al., 2006; Tanner, 2006); and the anthropic factors, such as organic matter or pollution (Duarte, 2002). The results obtained during this study suggest that the effect of herbivorism is a factor involved in a broader and more important way in the seagrass habitat complexity regulation. The data of this study suggests that the herbivorism could be regulating the abundance and composition of the epifauna community in the seagrass meadow. However, the degree of influence that the herbivorism has on abundance of epifauna, taxonomic richness or structure of the community remains still unclear. Further investigations should clarify the role of herbivorism in the regulation of the epifauna community that live on the leaves of seagrass. The importance of the epifauna in the proper regulation of the seagrass ecosystem is widely known and that is why it is important to continue studying the different factors that affect epifauna in order to assure the conservation of these important marine habitats.

## 5. Conclusions

With the results obtained in this study, it has been possible to demonstrate that the pressure of herbivory can cause impacts on the mobile fauna that lives above the leaves of *C. nodosa* at a regional level and, specifically, only in some taxonomic groups. The results have shown differences in the total abundance and taxa richness between the regions of Mallorca and Gran Canaria. In addition, the abundance of species and Pielow's evenness index has presented significant differences after clipping treatment in the localities of Mallorca (Aucanda and Es Barcares). A large number of factors and

phenomena can alter the structure of the epifauna community and, therefore, its resilience. Due to this complexity, it is difficult to know the role of herbivory pressure in the regulation of epifauna community. Even so, replications made at different geographical scales have shown that herbivory pressure is present in the variations that this type of ecosystem can suffer. It should be pointed out that the changes in the epifauna community due to the herbivory pressure differs depending on the location. For this reason, it is important to perform more studies in different locations to be sure that representative patterns are obtained. Altogether, this work emphasize the importance of taking into account the herbivory pressure as factor to investigate in subsequent studies that are carried in *C. nodosa* meadows.

## 6. Ethics and morality of the project

In the daily activity, carried put in the laboratory of Instituto Mediterráneo de Estudios Avanzados (IMEDEA) located in Esporlas (Mallorca), the safety regulations have been complied with. In addition, personnel have been informed of the management of both chemical and daily waste. In this laboratory, waste reduction has been carried out using recycled materials, when have been possible.

In the fieldwork, precautions have been taken to avoid any damage during the dives. In addition, maintenance has been carried out on the material used during, before and after the dives.

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## 8. References

- Alberto, F., Arnaud-haond, S., Duarte, C. M., & Serrão, E. A. (2006). Genetic diversity of a clonal angiosperm near its range limit the case of *Cymodocea nodosa* at the Canary Islands Mar Ecol-P, 309, 117–129. <https://doi.org/10.1074/mcp.M110.004457>
- Attrill, Martin J., James A.S, & Ashley A.R. (2000). Are macroinvertebrate communities influenced by seagrass structural complexity? Ecography 23.1, 114-121. doi: 10.1111/j.1600-0587.2000
- Balmford, A., & Bond, W. (2005). Trends in the state of nature and their implications for human well-being. Ecology Letters, 8(11), 1218–1234. <https://doi.org/10.1111/j.1461-0248.2005.00814.x>
- Barberá-Cebrián, C., Sánchez-Jerez, P., & Ramos-Esplá, A. A. (2002). Fragmented seagrass habitats on the Mediterranean coast, and distribution and abundance of mysid assemblages. Marine Biology, 141(3), 405–413. <https://doi.org/10.1007/s00227-002-0852-3>
- Barbera, C., Tuya, F., Boyra, A., Sanchez-Jerez, P., Blanch, I., & Haroun, R. J. (2005). Spatial variation in the structural parameters of *Cymodocea nodosa* seagrass meadows in the Canary Islands: A multiscaled approach. Botanica Marina, 48(2), 122–126. <https://doi.org/10.1515/BOT.2005.021>
- Beaumont, N. J., Austen, M. C., Atkins, J. P., Burdon, D., Degraer, S., Dentinho, T. P., Zarzycki, T. (2007). Identification, definition and quantification of goods and services provided by marine biodiversity: Implications for the ecosystem

approach. Marine Pollution Bulletin, 54(3), 253–265.  
<https://doi.org/10.1016/j.marpolbul.2006.12.003>

Bell, S. S., Walters, K., & Kern, J. C. (1984). Meiofauna from seagrass habitats: A review and prospectus for future research. *Estuaries*, 7(4), 331–338. <https://doi.org/10.2307/1351617>

Benedetti-Cecchi, L., Maggi, E., Bertocci, I., Vaselli, S., Micheli, F., Osio, G., & Cinelli, F. (2003). Variation in rocky shore assemblages in the northwestern Mediterranean: Contrasts between islands and the mainland. *Journal of Experimental Marine Biology and Ecology* (Vol. 293). [https://doi.org/10.1016/S0022-0981\(03\)00220-X](https://doi.org/10.1016/S0022-0981(03)00220-X)

Buza Jacobucci, G., & Pereira Leite, F. P. (2014). The role of epiphytic algae and different species of *Sargassum* in the distribution and feeding of herbivorous amphipods. *Latin American Journal of Aquatic Research*, 42(2), 353–363. <https://doi.org/10.3856/vol42-issue2-fulltext-6>

Cabaço, S., Ferreira, Ó., & Santos, R. (2010). Population dynamics of the seagrass *Cymodocea nodosa* in Ria Formosa lagoon following inlet artificial relocation. *Estuarine, Coastal and Shelf Science*, 87(4), 510–516. <https://doi.org/10.1016/j.ecss.2010.02.002>

Carr, L. A., Boyer, K. E., & Brooks, A. J. (2011). Spatial patterns of epifaunal communities in San Francisco Bay eelgrass (*Zostera marina*) beds. *Marine Ecology*, 32(1), 88–103. <https://doi.org/10.1111/j.1439-0485.2010.00411.x>

Cebrián, J., Duarte, C. M., & Marbà, N. (1996). Herbivory on the seagrass *Cymodocea nodosa* (Ucria) Ascherson in contrasting Spanish Mediterranean habitats. *Journal of Experimental Marine Biology and Ecology*, 204(1–2), 103–111. [https://doi.org/10.1016/0022-0981\(96\)02574-9](https://doi.org/10.1016/0022-0981(96)02574-9)

Duarte, C. M. (2002). The future of seagrass meadows. *Environmental Conservation*, 29(2), 192–206. <https://doi.org/10.1017/S0376892902000127>

Duarte, C. M., Dennison, W. C., Orth, R. J. W., & Carruthers, T. J. B. (2008). The charisma of coastal ecosystems: Addressing the imbalance. *Estuaries and Coasts*, 31(2), 233–238. <https://doi.org/10.1007/s12237-008-9038-7>



- Duffy, J. E., Reynolds, P. L., Boström, C., Coyer, J. A., Cusson, M., Donadi, S., Stachowicz, J. J. (2015). Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach. *Ecology Letters*, 18(7), 696–705. <https://doi.org/10.1111/ele.12448>
- Edgar, G. J., & Robertson, A. I. (1992). The influence of seagrass structure on the distribution and abundance of mobile epifauna: pattern and process in a Western Australian *Amphibolis* bed. *Journal of Experimental Marine Biology and Ecology*, 160(1), 13–31. [https://doi.org/10.1016/0022-0981\(92\)90107-L](https://doi.org/10.1016/0022-0981(92)90107-L)
- Espino, F., Tuya, F., Brito, A., & Haroun, R. J. (2011). Ictiofauna asociada a las praderas de *cymodocea nodosa* en las islas canarias (Atlántico centro oriental): Estructura de la comunidad y función de “guardería.” *Ciencias Marinas*, 37(2), 157–174. <https://doi.org/10.7773/cm.v37i2.1720>
- Feldman, M. W. (2006). *An Introduction to Mathematical Ecology*. E. C. Pielou. Wiley-Interscience, New York, 1969. x + 294 pp., illus. \$14.95. *Science*, 169(3940), 43–44. <https://doi.org/10.1126/science.169.3940.43-a>
- Fernandez, C., Ferrat, L., Pergent, G., & Pasqualini, V. (2012). Sea urchin-seagrasses interactions: Trophic links in a benthic ecosystem from a coastal lagoon. *Hydrobiologia*, 699(1), 21–33. <https://doi.org/10.1007/s10750-012-1151-8>
- Fourqurean, J. W., Manuel, S., Coates, K. A., Kenworthy, W. J., & Smith, S. R. (2010). Effects of excluding sea turtle herbivores from a seagrass bed: Overgrazing may have led to loss of seagrass meadows in Bermuda. *Marine Ecology Progress Series*, 419, 223–232. <https://doi.org/10.3354/meps08853>
- Gartner, A., Tuya, F., Lavery, P. S., & McMahon, K. (2013). Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. *Journal of Experimental Marine Biology and Ecology*, 439, 143–151. <https://doi.org/10.1016/j.jembe.2012.11.009>
- Heck, K. L., & Orth, R. J. (2013). *Seagrass Habitats: the Roles of Habitat Complexity, Competition and Predation in Structuring Associated Fish and Motile*

- Macroinvertebrate Assemblages. *Estuarine Perspectives*, (November), 449–464.  
<https://doi.org/10.1016/b978-0-12-404060-1.50043-5>
- Heck, K. L., & Valentine, J. F. (2006). Plant-herbivore interactions in seagrass meadows. *Journal of Experimental Marine Biology and Ecology*, 330(1), 420–436.  
<https://doi.org/10.1016/j.jembe.2005.12.044>
- Heck, K., & Wetstone, G. (1977). Habitat Complexity and Invertebrate Species Richness and Abundance in Tropical Seagrass Meadows. *Journal of Biogeography*, 4(2), 135–142. doi:10.2307/3038158
- Hixon, M. A., & Menge, B. A. (1991). Species diversity: Prey refuges modify the interactive effects of predation and competition. *Theoretical Population Biology*, 39(2), 178–200. [https://doi.org/10.1016/0040-5809\(91\)90035-E](https://doi.org/10.1016/0040-5809(91)90035-E)
- Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Peterson, C. H. (2007). Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science*, 629(2001), 1–17. <https://doi.org/10.1126/science.1059199>
- Khan, F., Alexandre, A., Ullah, H., & Santos, R. (2016). Effects of elevated CO<sub>2</sub> and nutrients on the community metabolism of a *Cymodocea nodosa* bed. *Turkish Journal of Botany*, 40(3), 250–257. <https://doi.org/10.3906/bot-1404-119>
- Lal, A., Arthur, R., Marbà, N., Lill, A. W. T., & Alcoverro, T. (2010). Implications of conserving an ecosystem modifier: Increasing green turtle (*Chelonia mydas*) densities substantially alters seagrass meadows. *Biological Conservation*, 143(11), 2730–2738. <https://doi.org/10.1016/j.biocon.2010.07.020>
- Larkum, A. W. D., Orth, R. J., & Duarte, C. M. (2006). Seagrasses: Biology, ecology and conservation. *Seagrasses: Biology, Ecology and Conservation*. <https://doi.org/10.1007/978-1-4020-2983-7>
- Lewis Allan W., F. Graham. and Stoner. (1983). Distribution of macrofauna within seagrass beds: an explanation for patterns of abundance. *Bulletin of Marine Science*, 33(2): 296-304., (April 1983).

- Lewis, J. B., & Hollingworth, C. E. (1982). Leaf epifauna of the seagrass *Thalassia testudinum*. *Marine Biology*, 71(1), 41–49. <https://doi.org/10.1007/BF00396991>
- Marina, P., Urra, J., Rueda, J. L., & Salas, C. (2012). Composition and structure of the molluscan assemblage associated with a *Cymodocea nodosa* bed in south-eastern Spain: Seasonal and diel variation. *Helgoland Marine Research*, 66(4), 585–599. <https://doi.org/10.1007/s10152-012-0294-3>
- Mascaró, O., Oliva, S., Pérez, M., & Romero, J. (2009). Spatial variability in ecological attributes of the seagrass *Cymodocea nodosa*. *Botanica Marina*, 52(5), 429–438. <https://doi.org/10.1515/BOT.2009.055>
- Masucci, P., & Arnaud-haond, S. (2012). Genetic flow directionality and geographical segregation in a *Cymodocea nodosa* genetic diversity network arXiv : 1206 . 5453v3 [ q-bio . PE ] 3 Oct 2012, 1–14.
- Mateo Ramírez, Á., & García Raso, J. E. (2012). Temporal changes in the structure of the crustacean decapod assemblages associated with *Cymodocea nodosa* meadows from the Alboran Sea (Western Mediterranean Sea). *Marine Ecology*, 33(3), 302–316. <https://doi.org/10.1111/j.1439-0485.2011.00496.x>
- Mateo-Ramírez, Urra, J., Rueda, J. L., Marina, P., & García Raso, J. E. (2018). Decapod assemblages associated with shallow macroalgal communities in the northwestern Alboran Sea: Microhabitat use and temporal variability. *Journal of Sea Research*, 135(February), 84–94. <https://doi.org/10.1016/j.seares.2018.02.009>
- McGlathery, K. J. (1995). Nutrient and grazing influences on a subtropical seagrass community. *Marine Ecology Progress Series*, 122(1–3), 239–252. <https://doi.org/10.3354/meps122239>
- McNaughton, J.S. (2001). Herbivory and Trophic Interactions. *Terrestrial Global Productivity*. <https://doi.org/10.1016/B978-012505290-0/50007-7>
- Moran, K. L., & Bjorndal, K. A. (2005). Simulated green turtle grazing affects structure and productivity of seagrass pastures. *Marine Ecology Progress Series*, 305, 235–247. <https://doi.org/10.3354/meps305235>

- Nagle, J. (1968). Distribution of the epibiota of macroepibenthic plants. [Port Arkansas Texas]. Retrieved from <https://www.worldcat.org/title/distribution-of-the-epibiota-of-macroepibenthic-plants/oclc/17001403#.XOUf4eWnA84.mendeley>
- Neckles, H. A., Wetzel, R. L., & Orth, R. J. (1993). Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia*, 93(2), 285–295. <https://doi.org/10.1007/BF00317683>
- Nieuwenhuize, J., & Hemminga, M. A. (1990). Seagrass Wrack-induced Dune Formation on a Tropical Coast. *Estuarine, Coastal and Shelf Science*, 31, 499–502.
- Novak, R. (1982). Spatial and seasonal distribution of the meiofauna in the seagrass *Posidonia oceanica*. *Netherlands Journal of Sea Research*.
- Olsen, Y. S., Sánchez-Camacho, M., Marbà, N., & Duarte, C. M. (2012). Mediterranean Seagrass Growth and Demography Responses to Experimental Warming. *Estuaries and Coasts*, 35(5), 1205–1213. <https://doi.org/10.1007/s12237-012-9521-z>
- Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Hech, K. L., Williams, S. L. (2006). A Global Crisis for Seagrass Ecosystems. *BioScience*, 56(12), 987. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:agcfse\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2)
- Peduzzi, P., & Vukovic, A. (2007). Primary production of *Cymodocea nodosa* in the Gulf of Trieste (Northern Adriatic Sea): a comparison of methods. *Marine Ecology Progress Series*, 64(Stevenson 1988), 197–207. <https://doi.org/10.3354/meps064197>
- Prado, P., Alcoverro, T., Martínez-Crego, B., Vergés, A., Pérez, M., & Romero, J. (2007). Macrograzers strongly influence patterns of epiphytic assemblages in seagrass meadows. *Journal of Experimental Marine Biology and Ecology*, 350(1–2), 130–143. <https://doi.org/10.1016/j.jembe.2007.05.033>
- Prado, P., Farina, S., Tomas, F., Romero, J., & Alcoverro, T. (2008). Marine protection and meadow size alter fish herbivory in seagrass ecosystems. *Marine Ecology Progress Series*, 371(November), 11–21. <https://doi.org/10.3354/meps07662>

- Png-gonzalez, L., Tuya, F., Ambiental, G., Ciencias, F. De, & C, U. D. L. P. G. (2014). Diferencia en la comunidad de epifauna asociada a praderas de *Cymodocea nodosa* y *Caulerpa prolifera*, (October). <https://doi.org/10.13140/2.1.2014.2080>
- Reyes, J., Sansón, M., & Afonso-Carrillo, J. (1995). Distribution and reproductive phenology of the seagrass *Cymodocea nodosa* (Ucria) Ascherson in the Canary Islands. *Aquatic Botany*, 50(2), 171–180. [https://doi.org/10.1016/0304-3770\(95\)00451-5](https://doi.org/10.1016/0304-3770(95)00451-5)
- Reynolds, P. L., Stachowicz, J. J., Hovel, K., Boström, C., Boyer, K., Cusson, M., Duffy, J. E. (2018). Latitude, temperature, and habitat complexity predict predation pressure in eelgrass beds across the Northern Hemisphere. *Ecology*, 99(1), 29–35. <https://doi.org/10.1002/ecy.2064>
- Scipione, M. B., & Zupo, V. (2010). Crustacean amphipods from the seagrasses *Zostera marina*, *Cymodocea nodosa* and *Posidonia oceanica* in the Adriatic Sea ( Italy ): a first comparison Anfípodos crustáceos asociados a las fanerógamas *Zostera marina* ,. *Zool. Baetica*, 21(February 2015), 15–32.
- Sirota, L., & Hovel, K. A. (2006). Simulated eelgrass *Zostera marina* structural complexity: Effects of shoot length, shoot density, and surface area on the epifaunal community of San Diego Bay, California, USA. *Marine Ecology Progress Series*, 326(Moksnes 2002), 115–131. <https://doi.org/10.3354/meps326115>
- Stoner, A. W. (1980). The role of seagrass biomass in the organization of benthic macrofaunal assemblages. *Bulletin of Marine Science*, 30(3): 537-551, 1 Figure, 7 Tables., 30(3), 537–551.
- Tanner, J. E. (2006). Landscape ecology of interactions between seagrass and mobile epifauna: The matrix matters. *Estuarine, Coastal and Shelf Science*, 68(3–4), 404–412. <https://doi.org/10.1016/j.ecss.2006.01.029>
- Tomas, F., Álvarez-Cascos, D., Turon, X., & Romero, J. (2006). Differential element assimilation by sea urchins *Paracentrotus lividus* in seagrass beds: Implications for trophic interactions. *Marine Ecology Progress Series*, 306(June 2014), 125–131. <https://doi.org/10.3354/meps306125>

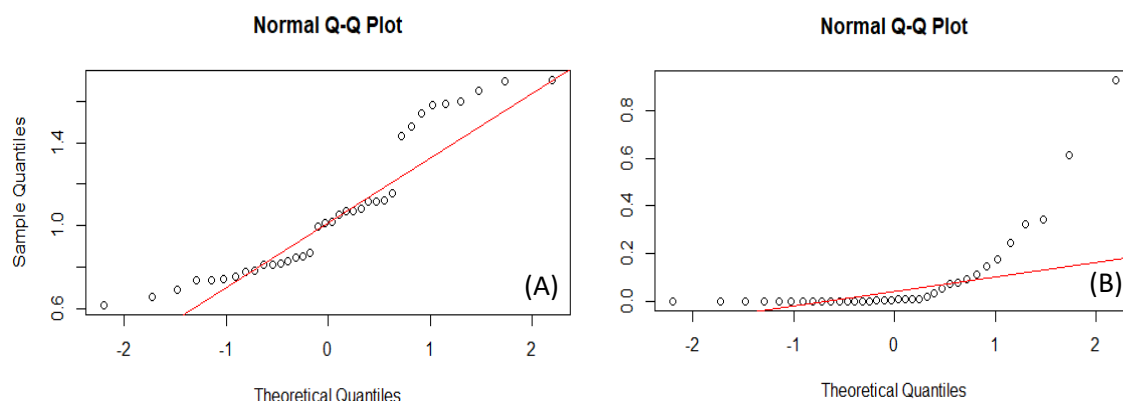
- Tomas, F., Romero, J., & Turon, X. (2004). Settlement and recruitment of the sea urchin. *Marine Ecology Progress Series*, 282, 173–184. <https://doi.org/10.3354/meps282173>
- Tomas, F., Turon, X., & Romero, J. (2005). Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Marine Ecology Progress Series*, 301(May 2014), 95–107. <https://doi.org/10.3354/meps301095>
- Tuya, F., Martín, J. A., & Luque, A. (2006). Ciclo estacional de una pradera marina de *Cymodocea nodosa* y la ictiofauna asociada en Playa Dorada (Lanzarote, Islas Canarias, Atlántico oriental). *Ciencias Marinas*, 32(4), 695–704. <https://doi.org/10.7773/cm.v32i4.1158>
- Tuya, F., Png-Gonzalez, L., Riera, R., Haroun, R., & Espino, F. (2014b). Ecological structure and function differs between habitats dominated by seagrasses and green seaweeds. *Marine Environmental Research*, 98, 1–13. <https://doi.org/10.1016/j.marenvres.2014.03.015>
- Tuya, F., Ribeiro-Leite, L., Arto-Cuesta, N., Coca, J., Haroun, R., & Espino, F. (2014a). Decadal changes in the structure of *Cymodocea nodosa* seagrass meadows: Natural vs. human influences. *Estuarine, Coastal and Shelf Science*, 137(1), 41–49. <https://doi.org/10.1016/j.ecss.2013.11.026>
- Tuya, F., Viera-Rodríguez, M. A., Guedes, R., Espino, F., Haroun, R., & Terrados, J. (2013). Seagrass responses to nutrient enrichment depend on clonal integration, but not flow-on effects on associated biota. *Marine Ecology Progress Series*, 490, 23–35. <https://doi.org/10.3354/meps10448>
- Urra, J., Rueda, J. L., Mateo Ramírez, Á., Marina, P., Tirado, C., Salas, C., & Gofas, S. (2013a). Seasonal variation of molluscan assemblages in different strata of photophilous algae in the Alboran Sea (western Mediterranean). *Journal of Sea Research*, 83, 83–93. <https://doi.org/10.1016/j.seares.2013.05.016>
- Urra, Javier, Mateo Ramírez, Á., Marina, P., Salas, C., Gofas, S., & Rueda, J. L. (2013b). Highly diverse molluscan assemblages of *Posidonia oceanica* meadows in northwestern Alboran Sea (W Mediterranean): Seasonal dynamics and

environmental drivers. *Estuarine, Coastal and Shelf Science*, 117, 136–147.  
<https://doi.org/10.1016/j.ecss.2012.11.005>

Valentine, J. F., & Heck, K. L. (1999). Seagrass herbivory: Evidence for the continued grazing of marine grasses. *Marine Ecology Progress Series*, 176, 291–302.  
<https://doi.org/10.3354/meps176291>

Wright, J. P., & Jones, C. G. (2006). The Concept of Organisms as Ecosystem Engineers Ten Years On: Progress, Limitations, and Challenges. *BioScience*, 56(3), 203.  
[https://doi.org/10.1641/0006-3568\(2006\)056\[0203:tcooae\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)056[0203:tcooae]2.0.co;2)

## 9. Appendix

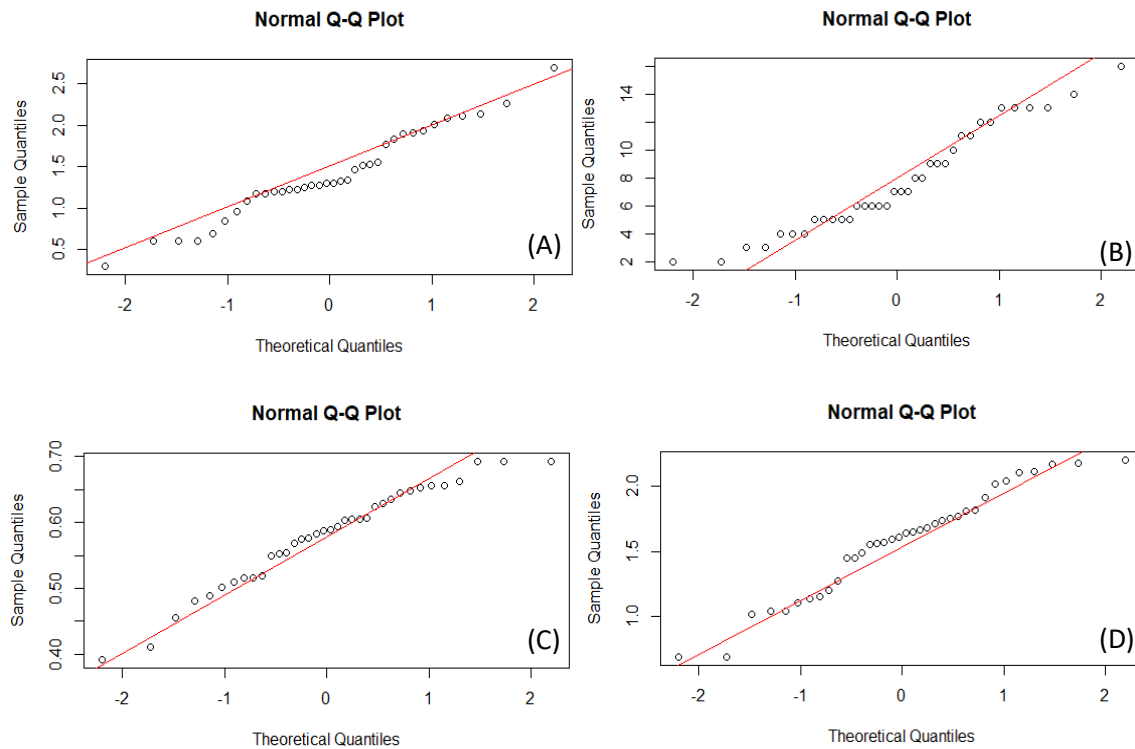


S1. Normal Q-Q plot from biomass of *C. nodosa* (A) and biomass of epiphytes (B)

S2. Wilcoxon sum-rank test (U-Mann Whitney) as a post-hoc analysis according to different region fixed factor, Gran Canaria and Mallorca; and the response biomass of *C. nodosa* (gDW/100cm<sup>2</sup>) and biomass of epiphytes (gDW/100cm<sup>2</sup>). Significant p-values are highlighted in bold.

Source of variation		W	p-value
Biomass of <i>Cymodocea nodosa</i> (gDW/100cm <sup>2</sup> )			
Castillo	Control*Cut80%	20	<b>0.01587</b>
	Arinaga		
	Control*Cut80%	20	<b>0.01587</b>
	Aucanda		
	Control*Cut80%	18	0.06349
Es Barcares	Control*Cut80%	16	0.1905
Biomass of epiphytes (gDW/100cm <sup>2</sup> )			
Castillo	Control*Cut80%	10	NA
	Arinaga		
	Control*Cut80%	12.5	0.5605
	Aucanda		
	Control*Cut80%	19	<b>0.03175</b>
Es Barcares	Control*Cut80%	11	0.9048

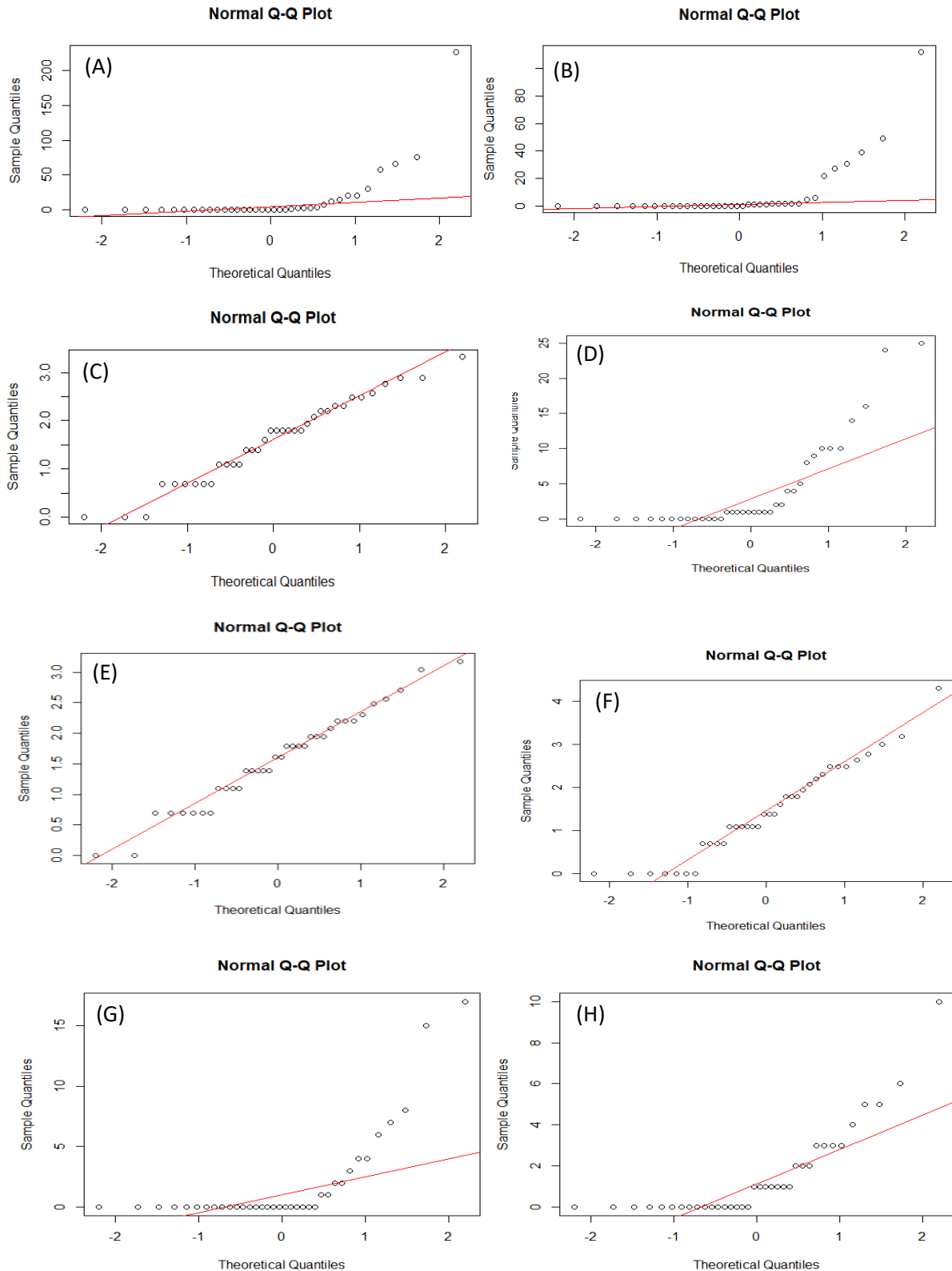




S3. Normal Q-Q plot from abundance of individuals (A), taxa richness (B), Pielow's evenness index (C) and Shannon Wiener index (D).

S4. Tukey HSD test as a post-hoc analysis according to different region fixed factor, Gran Canaria and Mallorca; and the response of abundance of individuals (ind/100cm<sup>2</sup>), taxa richness (species/100cm<sup>2</sup>), Pielow's evenness index (*J'*) and Shannon Wiener index (*H'*).

Source of variation		diff	p-adj
<i>Abundance (ind/100cm<sup>2</sup>)</i>			
	Castillo		
	Control*Cut80%	-0.22964702	0.9911119
	Arinaga		
	Control*Cut80%	-0.01035836	1.0000000
	Aucanada		
	Control*Cut80%	0.37449860	0.8833004
	Es Barcares		
	Control*Cut80%	-0.61450397	0.4001636
<i>Taxa richness (species/100cm<sup>2</sup>)</i>			
	Castillo		
	Control*Cut80%	0.0375000	1.0000000
	Arinaga		
	Control*Cut80%	-0.4625000	0.9999982
	Aucanada		
	Control*Cut80%	1.6125000	0.9930633
	Es Barcares		
	Control*Cut80%	-2.0375000	0.9734540
<i>Pielow's evenness (J)</i>			
	Castillo		
	Control*Cut80%	0.030920401	0.9929853
	Arinaga		
	Control*Cut80%	-0.01524053	0.9999263
	Aucanada		
	Control*Cut80%	-0.07640933	0.5475214
	Es Barcares		
	Control*Cut80%	0.09208919	0.3194721
<i>Shannon Wiener (H')</i>			
	Castillo		
	Control*Cut80%	-0.00247750	1.0000000
	Arinaga		
	Control*Cut80%	-0.19597750	0.9939946
	Aucanada		
	Control*Cut80%	-0.03987250	0.9999999
	Es Barcares		
	Control*Cut80%	-0.15858250	0.9983836



S5. Normal Q-Q plot from abundance of Nematoda (A), Polychaeta (B), Prosobranchia (C), Caprellidea (D), Gammaridea (E), Copepoda (F), Cumacea (G) and Tanaidacea (H).

S6. Wilcoxon sum-rank test (U-Mann Whitney) as a post-hoc analysis according to different region fixed factor, Gran Canaria and Mallorca; and the response of abundance of Nematoda, Caprellidea, Polychaeta, Cumacea and Tanaidacea taxa.

Source of variation			W	p-value
<i>Nematoda</i>				
	Gran Canaria	Control*Cut80%	45	0.3741
	Mallorca	Control*Cut80%	38.5	0.894
<i>Caprellidea</i>				
	Gran Canaria	Control*Cut80%	46.5	0.5715
	Mallorca	Control*Cut80%	56.5	0.1689
<i>Polychaeta</i>				
	Gran Canaria	Control*Cut80%	38.5	0.8667
	Mallorca	Control*Cut80%	37	0.7867
<i>Cumacea</i>				
	Gran Canaria	Control*Cut80%	40.5	NA
	Mallorca	Control*Cut80%	40	1
<i>Tanaidacea</i>				
	Gran Canaria	Control*Cut80%	37.5	0.7911
	Mallorca	Control*Cut80%	34.5	0.6218

S7. Tukey HSD test as a post-hoc analysis according to different region fixed factor, Gran Canaria and Mallorca; and the response of abundance of Prosobranchia and Copepoda taxa.

Source of variation			diff	p-adj
<i>Prosobranchia</i>				
	Gran Canaria	Control*Cut80%	-0.8760326	0.1290929
	Mallorca	Control*Cut80%	-0.2898703	0.8751600
<i>Copepoda</i>				
	Gran Canaria	Control*Cut80%	-0.3439163	0.7674031
	Mallorca	Control*Cut80%	-0.1797644	0.9567442