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Low Diversity of Intertidal Canopy-Forming Macroalgae at Urbanized Areas along the North Portuguese Coast

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Abstract: Canopy-forming macroalgae are the main component in some of the most diverse and productive coastal habitats around the world. However, canopy-forming macroalgae are very sensitive to anthropogenic disturbances. In coastal urban areas, intertidal organisms are exposed to the interactive effect of several anthropogenic disturbances that can modify the community's structure and diversity. Along the North-East Atlantic shores, many studies explored the effect of anthropogenic disturbances on canopy-forming macroalgae, but mainly focused on kelps and fucoids. However, along the intertidal rocky shores of the Atlantic coast of the Iberian Peninsula, the most abundant and frequent canopy-forming macroalgae belong to the family Sargassaceae. To explore the effect of urbanization on these intertidal canopy-forming species the diversity and assemblage structure of canopy species were compared between four urban and four non-urban shores in the north of Portugal. Intertidal canopy assemblages on urban shores were dominated by the non-indigenous *Sargassum muticum* that was the only canopy-forming species on three of the four studied urban shores. Canopy assemblages on all non-urban shores were more diverse. Moreover, stands of canopy-forming species on urban shores were always monospecific, while at non-urban shores multi-specific stands were common. Therefore, results suggest that urbanization reduces canopy's biodiversity.

Keywords: sargassaceae; urbanization; rocky shores; North Portugal; Atlantic Ocean

1. Introduction

Canopy-forming macroalgae play a key role in the functioning of coastal ecosystems providing habitat and food for other organisms, sequestering carbon and nitrogen from the environment, ameliorating the effect of waves and creating very diverse and productive coastal habitats [1]. Moreover, large perennial canopy-forming species are considered as good indicators of the ecological status of coastal habitats, because most of them are very sensitive to anthropogenic disturbances [2]. Unfortunately, coastal ecosystems are nowadays threatened by the interaction of many stressors [2,3] resulting in a loss of high diversity habitats that are replaced by low diversity habitats [2,4,5] that in many cases prevent their colonization by canopy-forming species [6]. One of the main stressors in coastal areas is urbanization that increases the industrial and domestic pollution, modifies natural habitats due to proliferation of artificial structures and increases the exploitation of living and non-living resources [7].

Recently many efforts have focused on restoring the shores where canopy-forming species have been locally extinct [8]. However, results of canopy-forming restoration are still very limited and suggest that nowadays efforts on mitigation of stressors and preservation will be the most efficient strategy to preserve these habitats [8]. However, mitigation and restoration strategies need baseline information with an in-depth knowledge of the species' ecology to be successfully restored. Along the North-East Atlantic shores, many studies have explored the effect of different anthropogenic disturbances on canopy-forming macroalgae, but these have mainly focused on kelps and fucoids [5,9]. However, along the intertidal rocky shores of the Atlantic coast of the Iberian Peninsula, the most abundant and frequent intertidal canopy forming macroalgae belongs to the family Sargassaceae (i.e., genus *Bifurcaria*, *Carpodesmia*, *Cystoseira*, *Sargassum* and *Treptacantha*). While in the Mediterranean Sea, many studies explored the effect of anthropogenic disturbances such as eutrophication [10], metal pollution [11] or urbanization [12] on canopy-forming species of *Cystoseira*(*sensulato*) or *Sargassum*, the response to disturbance of the Sargassaceae intertidal canopy-forming species along the Atlantic shores remains unexplored. The main aim of this study was to compare the diversity and assemblage structure of intertidal canopy-forming species between two areas characterized by different levels of urbanization along the north coast of Portugal.

2. Materials and Methods

2.1. Study Area

This study was done at eight different rocky shores along the North Portuguese coast, between latitudes $41^{\circ}50'20.93''$ and $41^{\circ}02'43.22''$ N covering about 90 km (Figure 1 and Table S1), during March and April 2019.

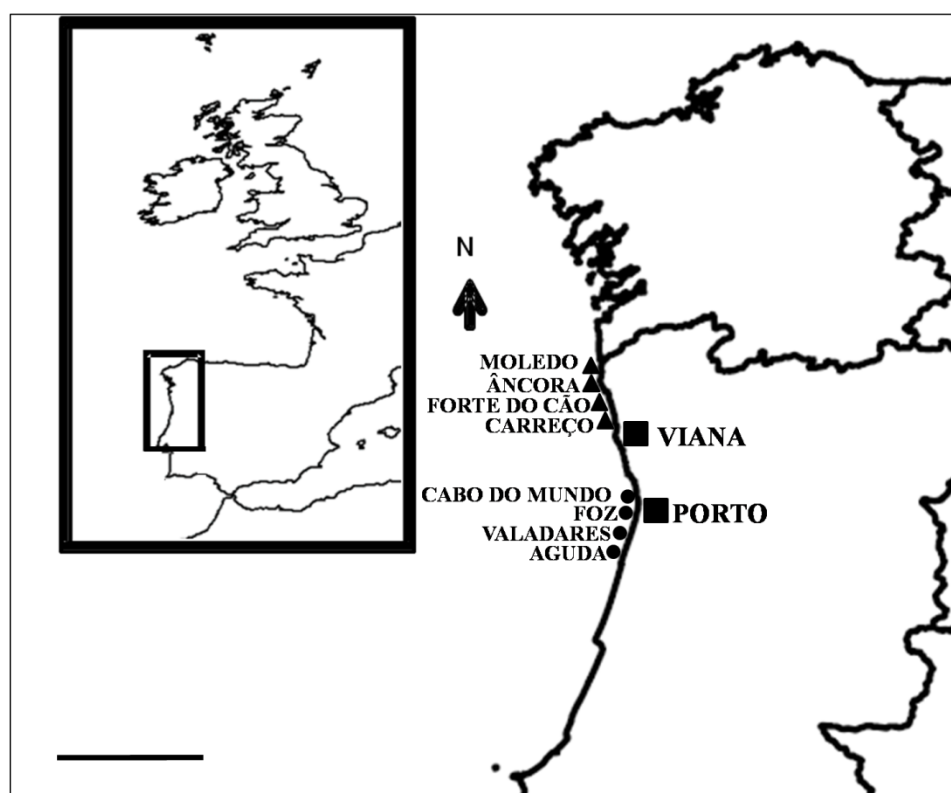


Figure 1. Situation of the study area showing the location of sampled shores (triangles) and the harbor of Porto and Viana (squares). Scale bar 50 km.

The shoreline on the studied area (less than 100 Km) is largely straight and shows very homogenous oceanographic conditions. The shore is exposed to wave action, with the dominant swell directions being W and NW and the common wave height ranging between 1.5–2 m, with maximum values about 7 m during winter. The coastal landscape is fragmented by the presence of estuaries, varying from soft to hard substrata, resulting in many cases in a patched mixture of both substrates. The tidal regime is semidiurnal, with the largest spring tides of 3.5–4.0 m. Moreover, the studied area is subjected to a seasonal upwelling during spring and summer months that provides nutrient supply for primary producers.

Considering the population density as a proxy of the degree of urbanization, we can define two different regions in our study area. One highly urbanized region in the south, encompassing the metropolitan area of Porto with more than 1500 residents per square kilometer. A second region in the north had a low degree of urbanization and less than 300 residents per square kilometer. Another good proxy of urbanization can be the maritime traffic. In the highly urbanized region, the main harbor (Leixões in Porto) moved during 2019 a total of 19,556,008 tons of commodities by 2600 vessels. The main harbor on the region with a low degree of urbanization is Viana, which moved during 2019 a total of 380,196 tons of commodities by 200 vessels. Additionally, in urbanized regions a higher concentration of pollutants on the water and marine organism can be expected than on regions with a low degree of urbanization. On our area of study, previous works found higher concentrations of heavy metals and nutrients in the urbanized region than in the non-urbanized one ([13] and references therein).

2.2. Sampling and Sample Processing

At each one of these rocky shores one transect about 1 km long and parallel to the coastline was defined. Along this transect, 20 random stands of canopy-forming macroalgae of the Sargassaceae family were selected. The abundance of each canopy-forming macroalga species within each stand was visually estimated with a quadrat (50 × 50 cm). Percentage cover estimates were obtained by dividing each quadrat into 25 sub-quadrats of 10 × 10 cm, assigning to each taxon a score from 0 (absence of that taxon) to 4 (a whole sub-quadrat covered by that taxon) and adding up the 25 estimates [14]. Moreover, all the individuals of the most frequent species (i.e., species present on all the studied shores) were removed from 10 quadrats to calculate their biomass. These specimens were stored in labelled bags, transported to the laboratory and dried for 48 h at 70 °C. Their dry weight, as proxy of their biomass, was determined using a precision balance.

2.3. Data Analyses

In order to explore the potential effect of urbanization on the diversity and assemblage structure of the intertidal canopy-forming Sargassaceae along the north coast of Portugal, different univariate and multivariate analyses were done. Differences between urban and non-urban regions on the number of taxa (S) and Shannon index (H') of canopy-forming macroalgae were examined by a two-way nested analysis of variance (ANOVA). The design of these analyses considered two factors: Region (2 levels), fixed and orthogonal, and Shore (4 levels), random and nested in Region, considering 20 replicates. Cochran's C test was employed to assess homogeneity of variances prior to the analysis. When necessary, data were transformed. The most stringent criterion of $p < 0.01$ was used to reject null hypotheses when variances were heterogeneous [15].

Differences between urban and non-urban regions on the structure of the canopy-forming species assemblages were explored with PERMANOVA analyses based on a Bray–Curtis similarity matrix built from the abundance data of each species. PERMANOVA analysis was based on the same design described previously for the univariate analyses. The statistical significance of multivariate components of variance was tested using a maximum of 999 permutations under a reduced model with significance level set, a priori, at $p < 0.05$. When the number of unique permutations was less than 30, the statistical significance of multivariate components of variance was tested using Monte Carlo p -values [16]. To test whether differences of assemblages between regions were due to different multivariate dispersion

between groups rather than in the location of centroids, the PERMDISP procedure was done [17]. Multivariate patterns were illustrated by non-metric multidimensional scaling (nMDS) ordination of sampled quadrats for each shore.

The SIMPER procedure [18] was used to determine the percentage contribution ($\delta i\%$) of each taxon to the Bray-Curtis dissimilarity between assemblages sampled in urban and non-urban shores (δi). The ratio $\delta i/SD(\delta i)$ was used to quantify the consistency of the contribution of a particular taxon to the average dissimilarity in all pair-wise comparisons of samples between urban and non-urban. Values ≥ 1 indicated a high degree of consistency.

Data on the abundance of relevant (according to the SIMPER results) individual taxa and biomass of the most frequent taxa were analyzed with analysis of variance (ANOVA), using the same design described above.

3. Results

3.1. Canopy-Forming Diversity

We found a total of five different species of canopy-forming macroalgae of the family Sagassaceae: *Bifurcaria bifurcata* R. Ross, *Treptacantha baccata* (S.G. Gmelin) S. Orellana and M. Sansón, *Cystoseira humilis* Schousboe ex Kützing, *Carpodesmia tamariscifolia* (Hudson) S. Orellana and M. Sansón and *Sargassum muticum* (Yendo) Fensholt. However, the distribution of the different species among the study shores was very variable. At Valadares and Foz only *S. muticum* was found, at Aguda only *S. muticum* and *T. baccata* were present, at Cabo do Mundo, Forte do Cão and Vila Praia de Âncora *B. bifurcata*, *S. muticum* and *T. baccata* were found, at Carreço *B. bifurcata*, *S. muticum*, *T. baccata* and *C. tamariscifolia* were found and finally, at Moledo *B. bifurcata*, *S. muticum*, *T. baccata* and *C. humilis* were found. Values of S and H (Figure 2) were significantly different between urban and non-urban regions (Table 1).

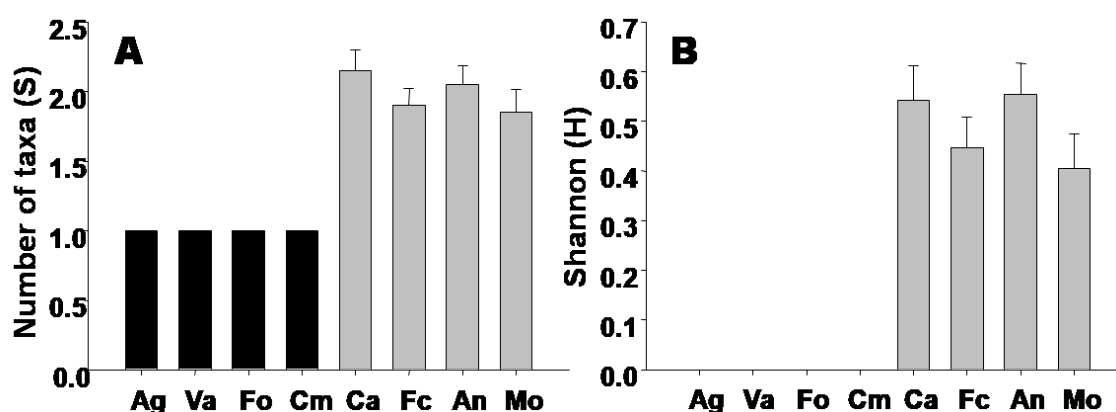


Figure 2. Values (+SE) of (A) number of taxa and (B) Shannon index for all the studied rocky shores: Aguda (Ag), Valadares (Va), Foz (Fo), Cabo do Mundo (Cm), Carreço (Ca), Forte do Cão (Fc), Âncora (An) and Moledo (Mo).

Moreover, the mean values of S and H showed a different distribution of species between the two studied regions. On the urbanized region, all the studied stands were monospecific independently of the total number of species recorded on each shore, while in the non-urbanized shores most of the studied stands harbored two, three or even four different species.

Table 1. Results of ANOVAs testing for differences in the total number of taxa (S) and Shannon's diversity index (H') on canopy assemblages from urban and non-urban shores. Significant effects are indicated in bold. s: significant.

Source of Variation	df	S			H		
		MS	F	p	MS	F	p
Urbanization = Ur	1	39.0	205.7	0.000	9.48	175.6	0.000
Shore(Ur) = Sh(Ur)	6	0.18	0.9	0.49	0.05	1.23	0.29
Residual	152	0.21			0.04		
Total	156						
Transform		none			none		
Cochran's Test		C = 0.3312			C = 0.2832		
				s			s

3.2. Canopy-Forming Assemblage Structure

PERMANOVA analysis showed significant differences between the assemblage structure of urbanized and non-urbanized regions (Table 2).

Table 2. Permutational multivariate analysis of variance (PERMANOVA) on canopy assemblages from urban and non-urban shores. Significant effects are indicated in bold.

Source of Variation	df	MS	Pseudo-F	p	Unique Permutations
Urbanization = Ur	1	64289	2.73	0.04	35
Shore(Ur) = Sh(St)	6	23527	14.03	0.001	999
Residual	152	1677			
Total	159				

The multivariate pattern was visualized as a clear separation between urban and non-urban regions in the nMDS ordination (Figure 3).



Figure 3. Plots of sampled shores from urbanized (black) and non-urbanized (gray symbols) regions. Different symbols represent different shores.

PERMDISP analysis indicated that the dispersion of samples did not contribute to the significant differences detected by the PERMANOVA analysis ($F = 1.2$, $p > 0.05$). SIMPER analysis identified three taxa as the main ones responsible for differences between urbanized and non-urbanized regions. Collectively, these taxa contributed more than 98% (Table 3). The contribution to percentage of dissimilarity of *B. bifurcata*, *T. baccata* and *S. muticum* was consistent among pair-wise comparisons of samples between the two groups (Table 3).

Table 3. Contribution (δi) of individual canopy species to the average Bray-Curtis dissimilarity between urban and non-urban shores.

Species	Average Abundance		δi	$\delta i\%$	$\delta i/SD(\delta i)$
	Urban	Non-Urban			
<i>Bifurcariabifurcata</i>	2.49	26.41	33.01	42.48	1.26
<i>Sargassummuticum</i>	22.10	13.10	29.85	38.41	1.33
<i>Treptacanthabaccata</i>	0.49	7.11	13.66	7.77	17.57

3.3. Abundance and Biomass of Most Relevant Species

Individual ANOVAs were done on the abundance of relevant taxa identified through SIMPER (Figure 4).

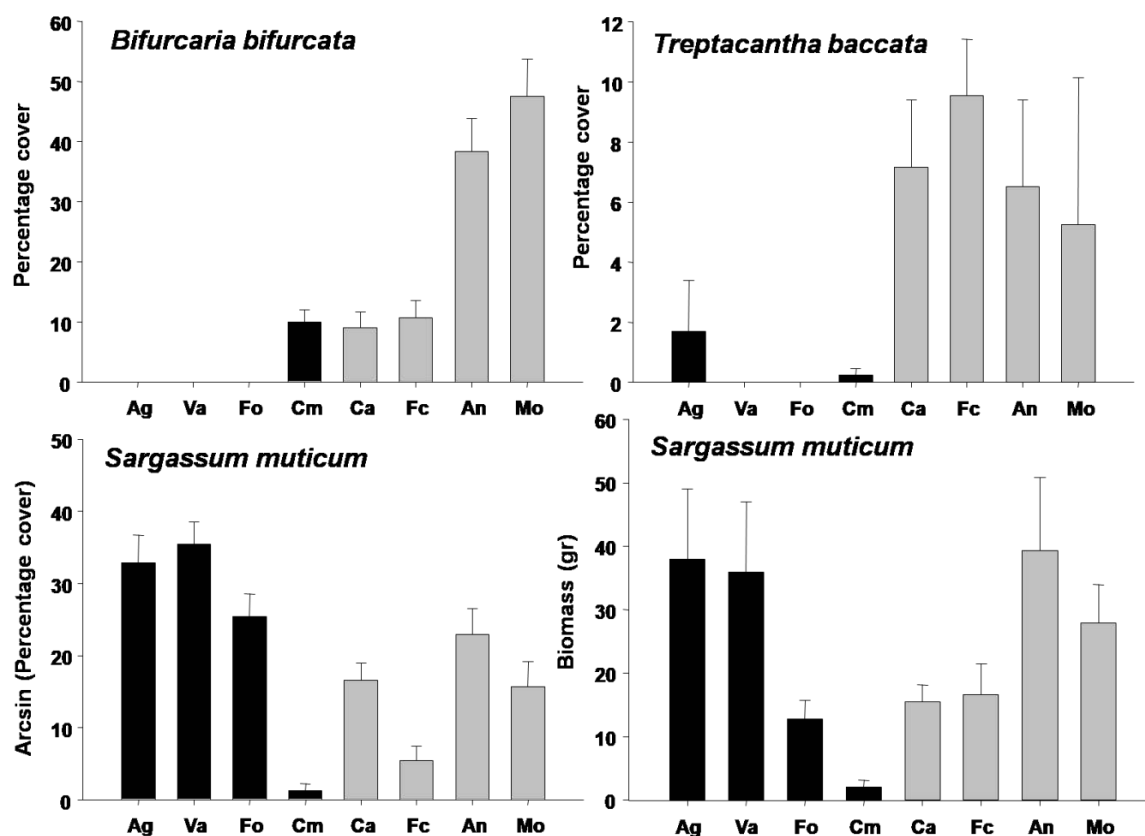


Figure 4. (+SE) percentage cover of individual taxa and biomass of *S. muticum*. Aguda (Ag), Valadares (Va), Foz (Fo), Cabo do Mundo (Cm), Carreço (Ca), Forte do Cão (Fc), Âncora (An) and Moledo (Mo).

Significant differences in the abundance of *B. bifurcata* and *T. baccata* were found between urban and non-urban regions (Table 4).

Table 4. Results of ANOVAs testing for differences on main canopy species from urban and non-urban shores. Significant effects are indicated in bold. ns: not significant; s: significant.

Source of Variation	df	<i>S. muticum</i>			<i>B. bifurcata</i>			<i>T. baccata</i>		
		MS	F	p	MS	F	p	MS	F	p
Urbanization = Ur	1	2979.8	1.01	0.35	22,393.4	7.46	0.03	1755.6	44.68	0.001
Shore(Ur) = Sh(Ur)	6	2952.8	17.16	0.000	3000.4	20.14	0.000	39.3	0.36	0.9
Residual	152	172.0			149			109.4		
Total	156									
Transform		ArcSin (%)			none			none		
Cochran's Test		C = 0.2075			C = 0.2487			C = 0.5454		
			n.s.			n.s.			s.	

In contrast, no significant differences between these two studied regions were found for abundance of *S. muticum* (Table 4). Differences in biomass were only explored for *S. muticum* because it was the only species present in all the studied shores. ANOVA did not find significant difference in the biomass of *S. muticum* between urbanized and non-urbanized regions (Table 5).

Table 5. ANOVAs testing for differences on the biomass of *S. muticum* from urban and non-urban shores. Significant effects are indicated in bold. ns: not significant.

Source of Variation	df	<i>S. muticum</i> (Biomass)		
		MS	F	p
Urbanization = Ur	1	141.0	0.06	0.81
Shore(Ur) = Sh(Ur)	6	2177.2	3.82	0.002
Residual	72	569.9		
Total	79			
Transform		none		
Cochran's Test		C = 0.2919		
				ns

4. Discussion

Most of the studies that explore the effects of different anthropogenic disturbances on canopy-forming species of the family Sargassaceae are focused on the Mediterranean Sea due to the high diversity and ecological relevance of the species of this family at that area [8,10–12]. Despite the fact that species of the family Sargassaceae (genus *Carpodesmia*, *Cystoseira*, *Sargassum* and *Treptacantha*) are also very abundant and with ecological relevance along the Atlantic shores of the Iberian Peninsula, there is a lack of information about the effect of anthropogenic disturbances on their diversity, distribution or functioning.

In this study, five canopy-forming macroalgae of the family Sargassaceae of the seven species previously reported in the intensive checklist [19] were found. The lack of the two remaining species: *Halidrys siliquosa* (Linnaeus) Lyngbye and *Treptacantha nodicaulis* (Withering) S. Orellana and M. Sansón can be explained because they are more frequent in subtidal habitats than in intertidal habitats. Moreover, in the particular case of *H. siliquosa* the north of Portugal is the southern boundary of its distribution range and thus the records of this species are very rare in the studied area [19].

It is remarkable that in our study the only species present in all the studied rocky shores and the only one found in two of the urban ones (i.e. Valadares and Foz) was the non-indigenous *S. muticum*. This species was observed for first time in the north of Portugal in 1989 [20] and nowadays it is the most frequent and locally more abundant intertidal canopy, as reported in our study. This high abundance of *S. muticum* is very relevant because although *S. muticum* is a complex canopy-forming species its ability to provide habitat to different invertebrates is different to the ability of native species [21,22]. Resulting from these changes on faunal species composition, other ecosystem functions such as primary production or food web connectivity may be significantly modified [23]. Moreover, results of this study showed that diversity of canopy-forming species on urbanized areas was lower than at non-urbanized

areas. Many studies showed that anthropogenic disturbances related to urbanization can negatively affect canopy-forming species of the genus *Cystoseira*(*sensulato*) [10–12] or *Sargassum* [24] while the abundance and diversity of these species is high at areas with low influence of urbanization such as marine protected areas [25]. However, results of our study also showed that the assemblage structure of canopy-forming stands was significantly different between urbanized (only monospecific stands) and non-urbanized (mainly multi-specific stands). Most of the studies about the effects of urbanization on canopy-forming macroalgae were focused on a single species that forms mainly monospecific stands [10–12]. However, [26] found that *Cystoseira*(*sensulato*) stands within harbors showed lower diversity (two species) than mixed stands out of harbors (six species) in the northern Adriatic. Results by [26] are in agreement with our study, suggesting a negative effect of urbanization on the diversity of canopy-forming macroalgae. Additionally, significant differences on the relative abundance of shared species between urbanized and non-urbanized areas were detected in our study and similar results were found by [12] for different species of *Cystoseira*(*sensu lato*) at the Mediterranean Sea. Curiously, neither the abundance nor biomass of the non-indigenous species *S. muticum* was affected by urbanization. This lack of significant differences on the abundance and biomass of *S. muticum* between urbanized and non-urbanized regions is in agreement with the study by [27] in Galicia (Northwest Spain). Therefore, it seems that the non-indigenous *S. muticum* is more tolerant to the associated disturbances with urbanization, but it is not able to increase its abundance after the elimination of other native canopy-forming species. Despite the lack of significant differences between urbanized and non-urbanized regions, the high variability of abundance and biomass of *S. muticum* among rocky shores is remarkable. Similar results were found by [27] and this suggests that ecological drivers acting at shore scale should be more relevant in the invasion of *S. muticum* than urbanization. These results contrast with many studies that suggest a positive effect of urbanization on the invasion success of other marine species [28].

Finally, many studies found that key anthropogenic disturbances like nutrient enrichment or urbanization can eliminate canopy-forming macroalgae that are replaced by turf-forming macroalgae [2,4,5]. In the studied area, canopy-forming species were present in all urban shores suggesting a moderate degree of disturbance due to urbanization. However, a more general study exploring the effect of anthropogenic disturbances along the same studied area [13] found a reduction in the abundance of canopy-forming species (i.e., *B. bifurcata* and *S. muticum*) and an increase in turf-forming species (i.e., *Gelidium pulchellum* (Turner) Kützinger) in rocky shores of the Porto region in comparison with non-urban shores in the north.

We can conclude that results suggest that urbanization has significantly reduced the diversity and the structure of canopy-forming assemblages in the urban region of north Portugal. Moreover, the non-indigenous species *S. muticum* seems to be the more tolerant canopy species and nowadays is the more frequent canopy along intertidal rocky shores in north Portugal.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/12/6/211/s1>, Table S1: Coordinates of each of the studied shores.

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