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The effects of the invasive seaweed *Asparagopsis armata* on native rock pool communities: evidences from experimental exclusion

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Abstract

Biological invasions represent a threat to ecosystems, through competition and habitat destruction, which may result in significant changes of the invaded community. *Asparagopsis armata* is a red macroalgae (Rodophyta) globally recognized as an invasive species. It is found from the intertidal to shallow subtidal areas, on rock or epiphytic, forming natural vegetation belts on exposed coasts. This study evaluated the variations on native intertidal seaweed and macroinvertebrate assemblages inhabiting rock pools with and without the presence of the invasive macroalgae *A. armata*. To achieve this, manipulation experiments on Atlantic (Portugal) rock pools were done. Three rock pools were maintained without *A. armata* by manual removal of macroalgae, and three others were not experimentally manipulated during the study period and *A. armata* was freely present. In this study the variations between different rock pools were assessed. Results showed different patterns in the macroalgae composition of assemblages but not for the macrobenthic communities. *Ellisolandia elongata* was the main algal species affected by the invasion of *A. armata*. Invaded pools tended to show less species richness, showing a more constant and conservative structure, with lower variation of its taxonomic composition than the pools not containing *A. armata*, where the variability between samples was always higher. Despite the importance of the achieved results, further data based on observation of long-term series are needed, in order to further understand more severe effects of the invader *A. armata* on native macroalgal assemblage.

Keywords: *Asparagopsis armata*, Biodiversity, Intertidal assemblages, Invasive exotics, Marine invasion, Non-indigenous species (NIS)

1. Introduction

The extent and significance of biological invasions in coastal marine ecosystems has become increasingly evident in recent years and has been recognized as one of the main causes of biodiversity loss and changes for native populations, community dynamics, and major ecosystem processes [1]. Contrarily to the presence of an non-indigenous species (NIS) (also exotic species, introduced species, alien species, foreign species, or non-native species), which may be present in a community for long time without significantly affecting it, the increasing rate of biological invasions (by invasive exotics) is affecting ecosystems with strong ecological impacts on resident assemblages [2, 3, 4].

Displacement of native flora by invasive exotic macroalgal species has been largely reported as provoking changes of species' composition and their trophic food webs [3, 4, 5]. Together with climate change, NIS macroalgae are becoming one of the most important threats to marine biodiversity [6]. The impacts associated with these introductions are typically expressed as community dominance through the monopolization of space and changing competitive relationships in native assemblage [7].

Invasive exotics have been shown to alter benthic habitats and biotic communities, resulting in potential ecosystem impacts in a wide geographical range. Important examples are: *Sargassum muticum* in the Galician coast (northwestern Spain) [2, 7], *Asparagopsis armata* Harvey 1855 in western Atlantic coast of the Iberian Peninsula [9], *Codium fragile* and *Grateloupia turuturu* in New England, USA [10], to name a few.

The rhodophyta seaweed *Asparagopsis armata* is native from Southern Australia and New Zealand (Horridge, 1951). In Europe, this macroalgae was introduced in the Atlantic and Mediterranean in the 1920s. The species is now widely distributed from the British Isles to Senegal, including the Azores, Canary, and Madeira Islands [11], where it is considered an invasive exotic species. This seaweed is regarded as invasive because it spreads avidly in receiving habitats in short time, colonizing a wide area, displacing native species and producing a significant change in terms of community composition [12, 13]. *Asparagopsis armata* presents lateral basal branches with retrorse spines (harpoon-like branches), which become entangled among other marine organisms thus permitting thalli to sprawl loosely over large areas [14]. It is found from the intertidal to shallow subtidal areas, on rock or epiphytic, forming compact vegetation belts on exposed coasts.

In this study, the main aim was to evaluate the effect of *A. armata* on intertidal seaweed and macroinvertebrate assemblages using a removal experiment in which the presence of this exotic species was manipulated. It is predicted that with the presence of *A. armata* in certain pools, the remaining assemblages would be distinct, in terms of composition and structure, from those found in rockpools where *A. armata* was experimentally removed.

2. Materials and methods

2.1 . Study area

The study was carried out from February 2018 to December 2018 in Portinho da Areia Norte (WGS84: 39.369587, -9.377899) at the south part of the Peniche peninsula, central western coast of Portugal. It belongs to an exposed coast to high wave action, the national coastal water type A5, but very near of the transition southward to coastal water type A6 [15]. In January 2018, 6 tide pools were randomly selected in the rocky intertidal area, with relatively similar size and invaded by *A. armata*. All pools were located at approximately the same tide level, which allow them to be isolated from the sea about the same time during a tidal cycle. The tidal rock pools position (semi-exposed sites) allowed seawater renewal every tidal cycle. Four hours was the duration time of rockpools, from formation to its complete disappearance. Two hours before and 2 hours after the low tide moment was the full period to the complete exposure.

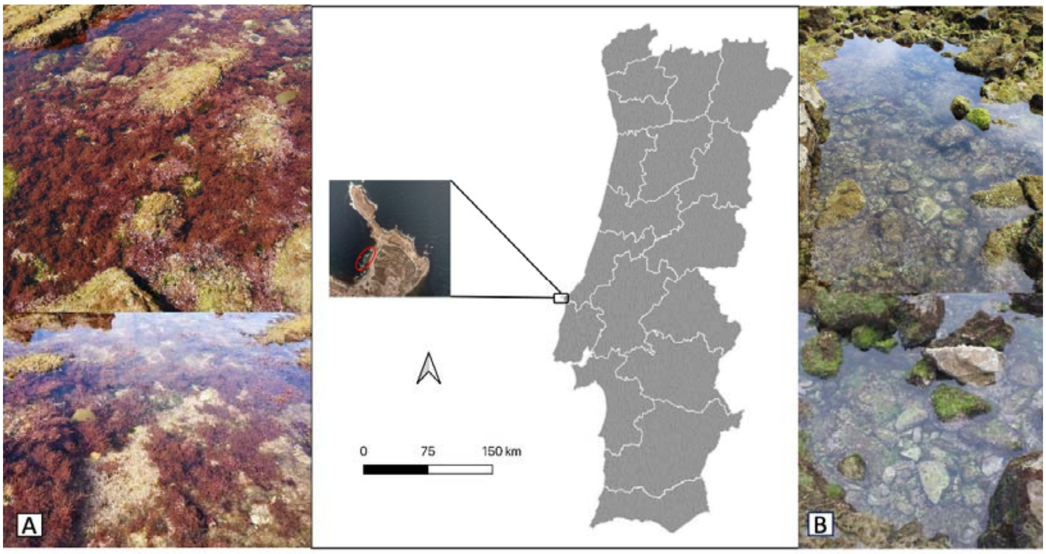

Figure 1 - Map of Peniche peninsula, western coast of Portugal, showing the location and photos of the sampling areas: **A** – Pools (A) with *A. armata*; **B** – Pools (C) without *A. armata*

2.2 . Field sampling and laboratory procedures

Sampling was performed during low-tide. Six intertidal rock pools (exposed during low tide) were used on experimental manipulations. Three rock pools were maintained without *A.*

armata (C pools) by regular manual removal of macroalgae so that the effect of its absence could be assessed in the community. Three other rock pools were not experimentally manipulated during the study period and *A. armata* was freely present (A pools). The manipulation period lasted for 10 months and community samples were collected twice per season. The manipulation was maintained over the course of the experiment by periodically removing new *Asparagopsis* recruits, every 2-3 weeks.

When rockpools were formed, physical and chemical parameters were measured for Initial water pool conditions. The water temperature (°C), conductivity (µS cm^{-1}), oxidation-reduction potential (ORP) (mV), salinity, dissolved oxygen (DO) (%), and pH parameters were registered in situ (YSI Professional Plus handheld multiparameter probe). This procedure was repeated before the rockpools disappear, in all rockpools, for the assessment of final conditions. While the rockpools were formed, biological samples were collected inside each selected pool by removing the inner material, using a paint scraper, from a 0.1 × 0.1 m sampling square, randomly placed on the rocky surface, and immediately sorted for taxonomic identification and abundance quantification of biological material.

In all cases, sampling consisted of complete removal of all algae and macroinvertebrates present in randomly chosen plots. In the laboratory, each sample was washed in tap water. Water was then sieved (mesh size: 0.5 mm) to retain the macrofaunal invertebrates and macroalgae were separated. Macroalgae were identified to species, dried to constant weight (60°C, 48h), and weighed to the nearest 0.01g. Macroalgae biomass was determined as dry weight (DW). Macroinvertebrates were preserved in formalin (4%) and inked with rose Bengal for later counting and identification, to the lowest possible taxonomic level (usually species).

2.3 Statistical analyses

2.3.1 Physical-chemical parameters analysis

The environmental parameters (temperature, conductivity, oxidation-reduction potential, salinity, dissolved oxygen and pH) were used to ordinate the sampling pools by performing principal coordinate (PCO) analyses. Euclidean similarity measure was used in the calculation of similarity matrices, after square root transformation, followed by normalization. Then, to see differences between samples, average for factor “pool*season” was selected.

2.3.1 Macroalgae data analysis

Macroalgae biomass was converted to dry-weight per unit (g DW m^{-2}). Bray Curtis similarity measure was used in the calculation of similarity matrices, after fourth root transformation of data.

The statistical significance of variance was tested using 9999 permutations of residuals under a reduced model, with a significance level of α-level of 0.05. PERMANOVA was applied including two fixed factors, ‘Pools’ (two levels: A - with *A. armata* and C - without *A. armata*) and ‘Season’ (nested in four levels: Wi (winter), Sp (spring), Su (summer) and Au (autumn)). *Asparagopsis armata* was not included in this analysis in order to identify effects on native diversity. To test whether differences of assemblages between pools were due to different multivariate dispersion between groups rather than in the location of centroids, the PERMDISP procedure was done.

To identify the taxa which contribute mostly to the communities’ structural variation between sites, Similarity Percentage Analysis (SIMPER) was applied. Dissimilarities between groups were assessed using two-way crossed designs with factors ‘Pools’ and ‘Season’ (as for PERMANOVA), with a 95% cut off for macroalgae. Principal Coordinate Analysis (PCO) was used as an ordination method to visualize patterns in data. Vectors based on Pearson correlations (greater than 0.5 to target variables with high correlations) were used.

2.3.2 Macrofauna data analysis

Abundance data of invertebrates was converted to density (ind. m^{-2}). The Bray-Curtis similarity measure was used in the calculation of similarity matrices, after the fourth root transformation of data (to reduce natural species dominance). PERMANOVA was performed to test differences between pools and stations, followed by pair-wise tests. The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model, with an a priori chosen significance level of $\alpha = 0.05$. The Similarity Percentages- species contributions (SIMPER) analysis was used to determine which macrofauna species contributed most for the dissimilarity between pools and stations. Dissimilarities between groups were assessed using two-way crossed designs with factors 'Pools' and 'Season' (as for PERMANOVA), with an 85% cut off for macroinvertebrate. Principal Coordinate Analysis (PCO) was used as an ordination method to visualize patterns in data. Vectors based on Pearson correlations (greater than 0.5 to target variables with high correlations) were used.

The diversity of macrobenthic fauna was assessed by different ecological indices: 1) Margalef richness index (d) [16]; 2) Shannon-Wiener diversity index ($\log_e$); 3) Pielou evenness index (J') [17]; and 4) Simpson domination index ($1-\lambda$) [18], using the following algorithms:

(1) $d=(S-1)/\log(N)$;

(2) $H'=\Sigma pi\log(pi)$;

(3) $J'=H'/\log(S)$

(4) $1-\lambda=1-\Sigma(Ni*(Ni-1)/(N*(N-1))$

Where S is the number of species, N is the total number of individuals, pi is the proportion of abundance of species.

All multivariate analyses were carried out with PERMANOVA+ for PRIMER software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.).

Two-way analysis of variance (ANOVA) was applied to ecological indices**.** Considered factors were: 1) Pools, with two levels; and 2) Season with four levels, using SigmaPlot software for Windows, version 12.0.

3. Results

3.1 . Environmental data

The physico-chemical parameters exhibited no significant differences between the initial and final rockpools conditions. Although, at the end of emersion period, the environmental variables were markedly different between seasons (*P*(perm)=0.0001) (Table I).

Table I - Summary of PERMANOVA of physico-chemical parameters from the sampling pools after it was created (Initial) and before it was flooded (Final).

	Initial	Final

Source of variation	df	MS	Pseudo-F	P(perm)	df	MS	Pseudo-F	P(perm)
Pools	1	3.891	0.667	0.582	1	1.058	0.234	0.9636
Seasons	3	9.480	1.626	0.090	3	28.037	6.213	**0.0001**
Pools x Seasons	3	4.977	0.853	0.544	3	2.303	0.510	0.9496

The PCO analysis indicated that the first two axes explained a total of 80.3% and 82.2% variation for the initial pool conditions and final pool conditions, respectively Figure 2 A and B). In the Initial conditions, the parameters strongly correlated with the first axis (PCO1; r > ± 0.80) were temperature (r = 0.80) and conductivity (r = - 0.91). And DO (%) (r = 0.95) and ORP (mV) (r = -0.86), with PCO2. In the Final conditions, pH showed the strongest correlations (r = 0.97) with PCO1 and conductivity (r = 0.83) with PCO2. Before the emersion (A), there is no clear separations between seasons. After being isolated from the sea during low tide, the parameters showed more consistency, and different pools "A" and "C" showed similar pattern per season.

The selected rockpools presented different volumes and depth, which could affect assemblage composition and bias the final results, but no physico-chemical variability existed between pool A and C initial conditions.

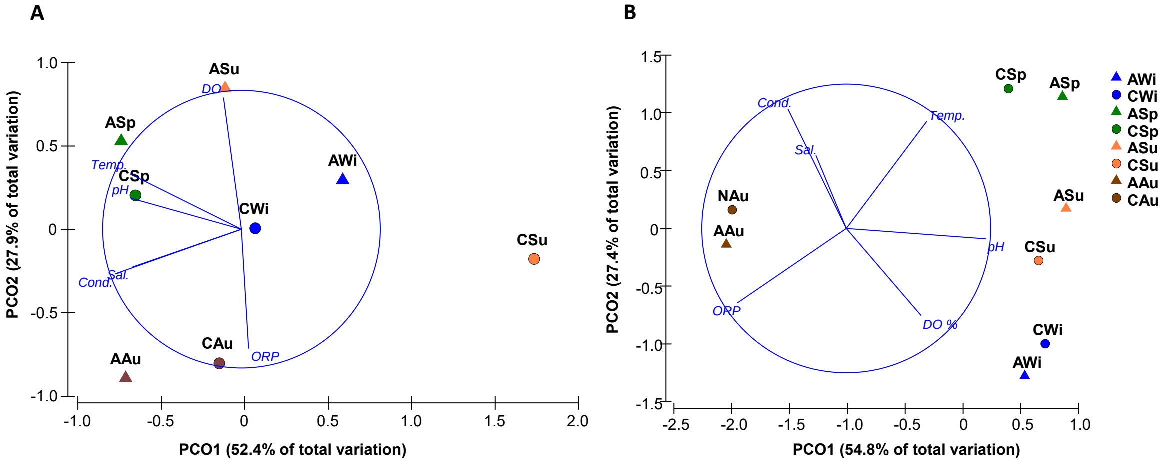

Figure 2 - Principal Coordinates Ordination (PCO) of the physico-chemical parameters: (**A**) Initial pool conditions; (**B**) Final pool conditions. Vectors are the raw Pearson correlations of variables with the PCO axes. Key: upward triangles = Pool "A"; circles = Pool "C"; Wi = winter; Sp = spring; Su = summer; Au = autumn.

3.2 . Assemblage composition

In the studied assemblages, 50 macroalgal taxa were identified plus *Asparagopsis armata* and its tetrasphorophyte phase (*Falkenbergia rufolanosa*); in total: 37 Rhodophyta, 7 Chlorophyta and 8 Ochrophyta (Appendix A). In both pool A and pool C there were 48 and 44 macroalgae taxa, respectively. The group Rhodophyta showed higher species richness (34 and 30 taxa for pool A and pool C, respectively). Chlorophyta and Phaeophyceae had the same number of taxa (7) in pool C and pool A. Pools C and A from summer season obtained the highest mean number of species (15.4±0.66 and 15.4±0.93, respectively), whereas pool C in winter obtained the lowest number (6.25±0.74) (Fig.1, A). Mean biomass highest value was found for pool C in winter (9.13±2.84 g DW m^{-2}), when *Asparagopsis armata* mean biomass was 1.95±0.65 g DW m^{-2} while the lowest value (0.17±0.07 g DW m^{-2}) was found for pool A in summer, with 0.70±0.33 g DW m^{-2} of *A. armata* biomass (Fig. 3).

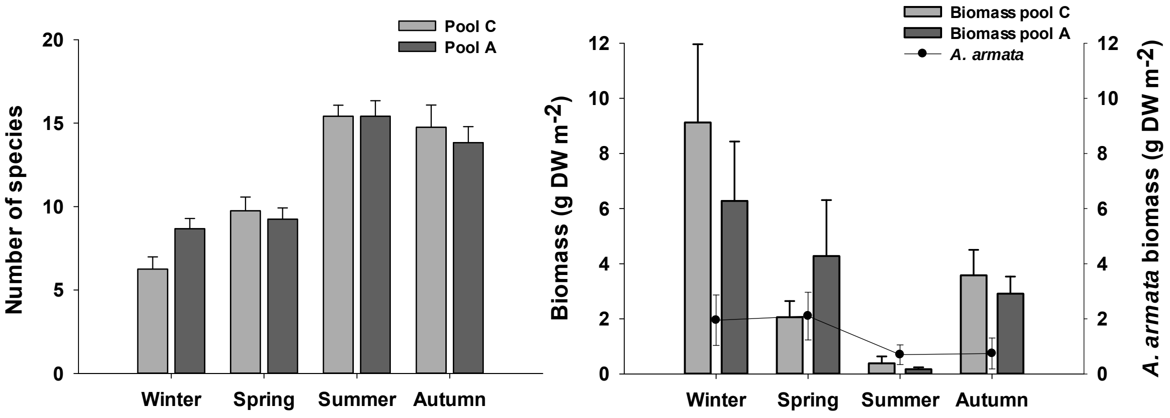

Figure 3 – Mean number of species (± SE) (A) and macroalgae mean biomass (g DW m^{-2} ± SE) with *Asparagopsis armata biomass* mean (SE) (B) per rockpool for all seasons.

PERMANOVA (Table II) revealed statistically significant differences in macroalgae biomass between pools (*P*(perm)=0.0192), between seasons (*P*(perm)=0.0001) and also the interaction Pools*Seasons (*P* (perm)=0.0046. But such patterns were not consistent in time. The pairwise on the “pools” revealed only significant differences between spring and summer season (*P*(perm)=0.0025 and 0.0257), respectively. The lack of significant differences on the PERMDISP analysis indicated that the dispersion of samples did not provide a significant contribution (F = 1.4, $p = 0.3$) to the differences detected by PERMANOVA, indicating that there is only a location effect.

Table II - Summary of PERMANOVA analyses

Source	**df**	**MS**	**Pseudo-F**	**P(perm)**
Stations	1	3636	2.5185	**0.0192**
Seasons	3	14861	10.294	**0.0001**
Stations x Seasons	3	2967.9	2.0558	**0.0046**
Groups		**t**		**P(perm)**
Winter A ≠ C		1.198		0.2208
Spring A ≠ C		1.9789		**0.0025**
Summer A ≠ C		1.4354		**0.0257**
Autumn A ≠ C		0.9784		0.4438

SIMPER analysis identified taxa that contributed for the differentiation between invaded (Pools A) and non-invaded (Pools C) rockpools, with 60.2% dissimilarity between groups. The top three species at the *A. armata* invaded sites (Pool A) that most contributed to this dissimilarity percentage (Table III) were *Vertebrata thuyoides/Vertebrata fruticulosa* (5.91%), *Ulva* spp. (5.86%) and *Lithophyllum incrustans* (5.09%). At the pools without the invasive macroalgae (Pool C), the species that most contributed to this dissimilarity were *Ellisolandia elongata* (21.73%), *Mesophyllum lichenoides* (4.12%), and *Jania rubens* (3.41%). Some species were equally abundant along all sampling areas (as *Gastrocolonium ovatum* and *Amphiroa* spp.)

Table III - Contribution of individual taxa to the average Bray-Curtis dissimilarity in macroalgae assemblage (Pool C, without *Asparagopsis armata*; and Pool A, with *A. armata*).

Taxa	**Pool C**	**Pool A**	**Average Dissimilarity**	**Contribution %**
	Average abundance			
Ellisolandia elongata	**1.79**	1.26	13.08	21.73
Vertebrata thuyoides/Vertebrata fruticulosa	0.39	**0.42**	3.56	5.91
Ulva spp.	0.32	**0.54**	3.53	5.86

Lithophyllum incrustans	0.23	**0.24**	3.06	5.09
Mesophyllum lichenoides	**0.22**	0.18	2.48	4.12
Chondracanthus acicularis	0.19	**0.30**	2.27	3.77
Jania rubens	**0.19**	0.14	2.05	3.41
Ulva clathrata	0.16	**0.17**	1.91	3.18
Ceramium spp./*Gayliella* spp.	0.25	**0.30**	1.69	2.80
Champia parvula	0.18	**0.21**	1.63	2.71
Osmundea pinnatifida/Laurencia pyramidalis	0.12	**0.15**	1.51	2.52
Crouania attenuata	0.11	**0.14**	1.47	2.45
Cladophora spp.	**0.11**	0.10	1.39	2.30
Polysiphonia spp. *or Other Rhodomelaceae*	0.10	**0.11**	1.32	2.19
Peyssonelia spp.	**0.11**	0.06	1.27	2.11
Caulacanthus ustulatus	**0.10**	0.08	1.25	2.07
Gastrocolonium ovatum	0.08	0.08	1.11	1.84
Amphiroa spp.	0.07	0.07	1.05	1.74

Regarding the macroinvertebrates, in both pool types, five groups contributed to about 90% of the total density. They were Gastropoda, Malacostraca, Bivalvia, Polyplacophora, and Polychaeta, with the higher species richness belonging to Gastropoda (17 and 12 taxa for pool C and A, respectively) (Appendix B). The macroinvertebrates highest mean number of species was registered in pool C in autumn (13.83±1.23) and the lowest values were recorded in pool C in winter (6.73±0.93) (Fig. 4, A). Regarding the mean density, the highest value was found for pool C in autumn (1743.3 ± 285.3 ind m^{-2}), when *A. armata* biomass was 0.74 ± 0.40 g DW m^{-2} (nearly the lowest), while lower values were found for pool C in winter (251.8 ± 37.8) when *A. armata* biomass was registered with values of 1.95 ± 0.65 g DW m^{-2} (nearly the highest) (Fig. 4, B).

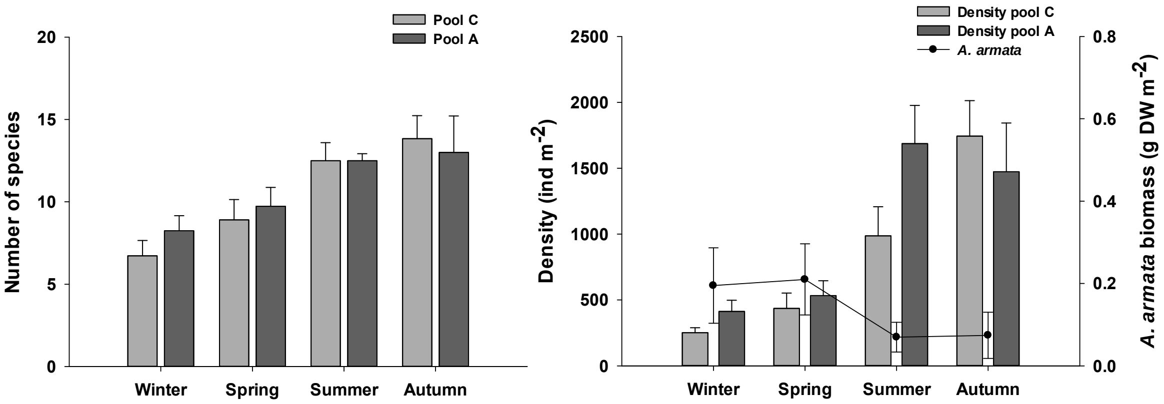

Figure 4 – Mean number of species (SE) (**A**) and macroinvertebrate mean density (ind m^{-2} ± SE) with *Asparagopsis armata biomass* mean (SE) (**B**) per rockpool for all seasons

Univariate analyses did not detect significant differences between the two pools for the diversity of benthic macrofauna or for S, H' or N, except for factor 'season' concerning the number of taxa (S), the total number of individuals (N) and for the Margalef index (Table IV; Fig. 5, A–D).

Table IV- Results of ANOVAs testing for differences in the total number of taxa (S), total number of individuals (N), Shannon's diversity index (H'), Margalef richness (d), Pielou eveness (J) and Simpson domination (1-D). Significant differences are indicated in bold.

Source of variation	df	S			N			H'		
		MS	F	P	MS	F	P	MS	F	P
Pools	1	2.22	0.18	0.68	0.19	1.99	0.16	0.007	0.04	0.85
Season	3	123.35	9.83	**0.001**	2.10	22.53	**0.001**	0.30	1.4	0.24
Pools*Season	3	4.13	0.33	0.804	0.10	1.07	0.37	0.09	0.41	0.75
Residual	61	12.55			0.09			0.21		
	68	16.93			0.18			0.20		
Source of variation	**df**	**d**			**J**			**1-λ**		
		MS	F	P	MS	F	P	MS	F	P
Pools	1	0.005	0.02	0.88	0.001	0.05	0.81	0.005	0.18	0.68
Season	3	1.13	4.84	**0.004**	0.04	2.61	0.06	0.02	0.78	0.51

Pools*Season	3	0.08	0.35	0.79	00.005	0.27	0.85	0.006	0.23	0.88
Residual	61	0.23			0.02			0.03		
	68	0.26			0.02			0.03		

In general, Margalef index (Fig. 5A) presented higher values in Autumn, pool C with 1.72±0.2 and pool A with 1.66±0.3, and lower values in winter, where pool A got higher values (1.22±0.1) this time. Values for Pielou (Fig. 5B) were higher in winter and spring, and Simpson (Fig. 5D) were very similar around all year. Shannon index showed a similar pattern to Simpson index, with values ranging from 1.55 in winter and 1.70 in spring for pool A and to 1.36 in winter and 1.8 in spring for pool C.

For all indices there were no significant differences between pools (*p*>0.05) and the interaction pools*season (*p*>0.05).

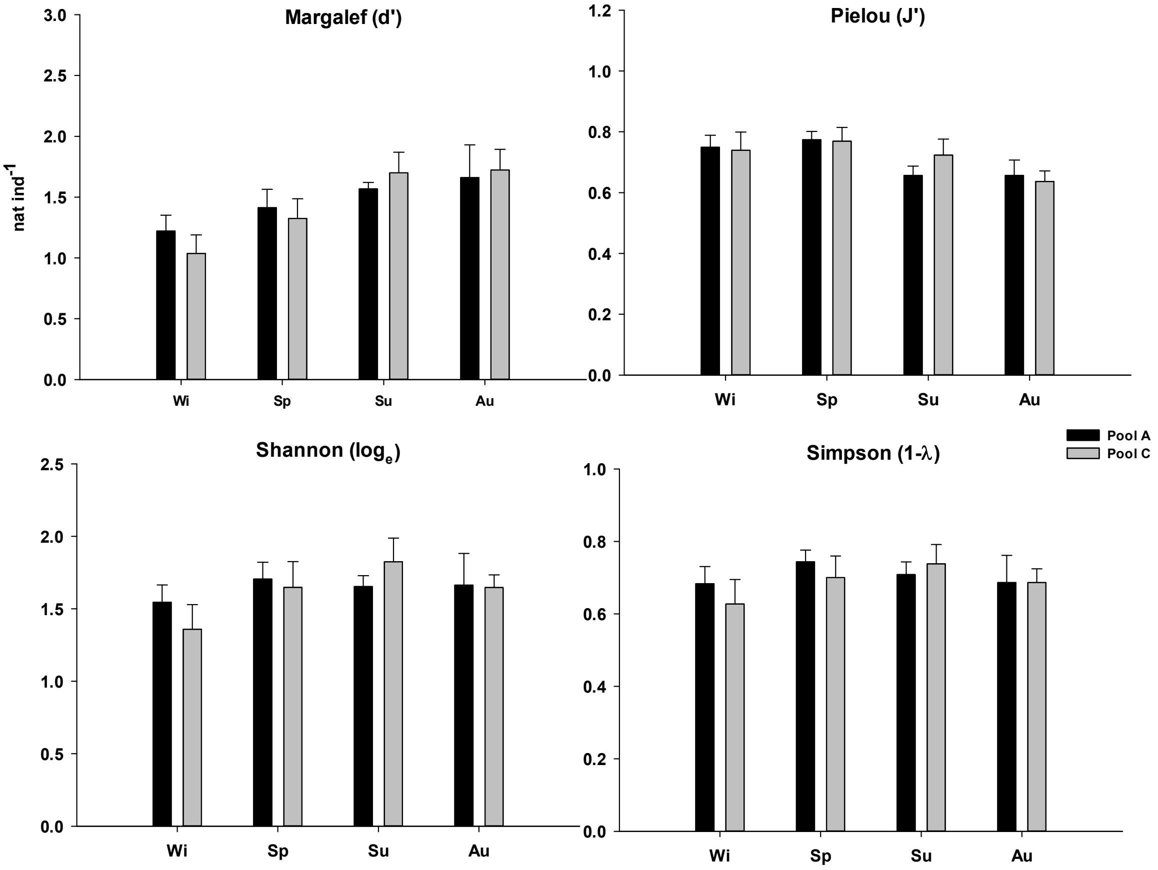

Fig. 5 - Variation of Margalef (**A**), Pielou (**B**), Shannon (**C**), and Simpson (**D**) indices per pool within seasons (Wi, Winter; Sp, Spring; Au, Autumn; Su, Summer).

Concerning macroinvertebrates density, PERMANOVA did not detect any significant differences between control and *A. armata* invaded rock pools (*P*(perm)=0.5095), only a significant temporal variability was confirmed for factor 'Season' (Table V). However, macroinvertebrates density in summer was slightly higher in the invaded pools (A) (1686.7 ind. m^{-2} ± 311.3) than in the non-invaded pools (C). In Autumn, the pools C, without the invasive macroalgae, presented higher macroinvertebrate densities (as already mentioned) than pools A for the same season (1473.3 ind. m^{-2} ± 319.7) (Fig. 4).

Table V - Summary of PERMANOVA analyses

Source	df	MS	Pseudo-F	P(perm)
Pool	1	1331.5	0.93382	0.5095
Season	3	6842.7	4.7988	**0.0001**
Pool x Season	3	12674	0.88883	0.6316

The SIMPER analysis showed 55.5% dissimilarity in mean abundance composition between pools C, without invasive *A. armata*, and pools A, with *A. armata* (Table VI). For the pools A, the top three dominant taxa that most contributed to this dissimilarity percentage were *Skeneopsis planorbis* (8.02%), *Rissoa parva* (6.73%), and *Melarhaphe neritoides* (6.46%) (Table 5). For pools C, where *A. armata* was experimentally removed, the species that most contributed to dissimilarity were *Amphipholis squamata* (6.33%), Polychaeta (5.17%), and *Mytilus sp.* (2.43%). Although most species contributing to differences between pools with and without *A. armata* were mainly present at the former ones, Polyplacophora, Polychaeta, and Ophiuroidea showed higher abundances in pools C, without the invasive species.

Table VI - Contribution of individual taxa to the average Bray-Curtis dissimilarity in macroinvertebrate assemblage (Pool C, without *Asparagopsis armata*; and Pool A, with *A. armata*).

Taxa	**Pool C**	**Pool A**	**Average Dissimilarity**	**Contribution %**
	Average abundance			
Skeneopsis planorbis	2.62	**3.05**	4.45	8.02
Rissoa parva	1.53	**1.87**	3.74	6.73
Melarhaphe neritoides	1.52	**1.68**	3.59	6.46
Amphipholis squamata	**3.18**	3.1	3.51	6.33
Bittium reticulatum	1.65	**1.75**	3.16	5.69
Amphipoda	1.51	**1.88**	2.89	5.2
Polychaeta	**1.92**	1.65	2.87	5.17
Dynamene magnitorata	1.26	**1.62**	2.8	5.04
Cymodoce truncata	1	**1.1**	2.69	4.84
Parvicardium scriptum	0.68	**1.07**	2.44	4.39
Gibbula sp.	0.66	**1.07**	2.36	4.25
Musculus costulatus	0.57	**1**	2.18	3.93
Gibbula pennanti	0.56	**0.59**	2	3.6
Mytilus sp.	**0.54**	0.42	1.35	2.43
Lepidochitona sp.	**0.46**	0.33	1.34	2.42
Gibbula umbilicais	**0.45**	0.31	1.31	2.37

A Principal Coordinates Analysis (PCO) based on the Bray-Curtis resemblance matrix was applied to visualize differences and similarities between samples. The PCO for the macroalgae biomass and macrofauna density, in general, did not show clear differences between the studied pools and seasons. For macroalgae it provided a tenuous distinction between pools and in some cases between seasons of the same pool (pools in spring and in autumn, Fig. 6A). The first two principal component axis explain 32.7% and 19.1% of the samples variability, for macroalgae biomass (A) and invertebrate's density (B) respectively. For both macroalgal assemblages, six taxa showed a moderate to strong correlation with the first two PCO axes (Pearson's coefficient >0.5, Fig. 6A). The macroalgae taxa with higher value of correlation (>0.5) were related to Pool A, in autumn season, while macrofauna taxa with higher correlation are distributed between pools from spring and autumn season. For this assemblages, 5 taxa were the ones influencing more the relative distribution of different pools along seasons (Fig. 6B).

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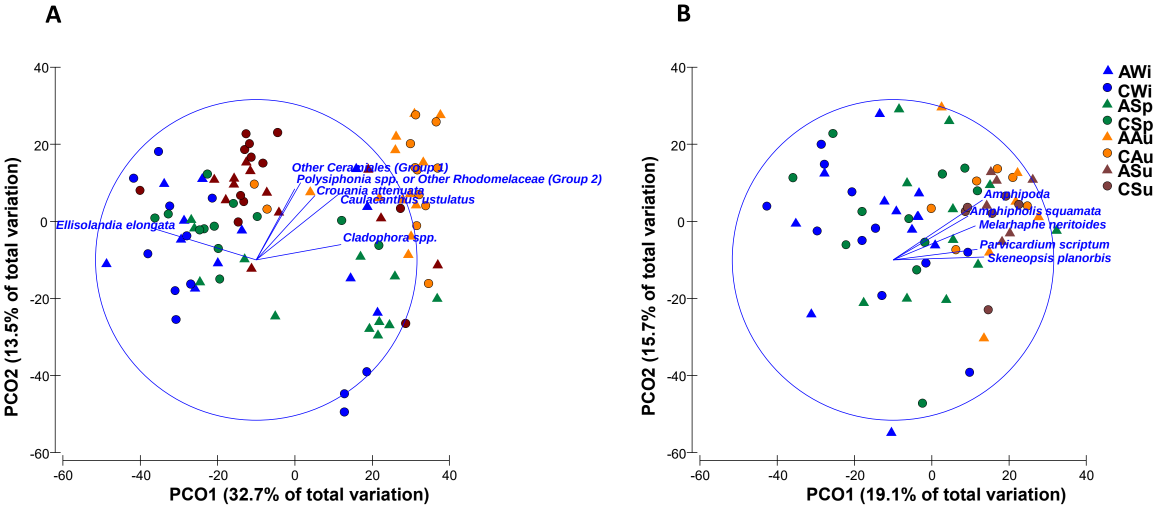

Figure 6 - Principal Coordinate analysis (PCO) plot based on macroalgae biomass (**A**) and macrofauna density (**B**) per pool for all seasons with the representation of the species with vectors longer than 0.5. A and C = pool A and pool C. Wi, Sp, Au and Su =Winter, Spring, August and Summer

4. Discussion

This study aims to evaluate the variation observed on intertidal seaweed and benthic macroinvertebrate assemblages inhabiting rock pools with and without the presence of the invasive macroalgae *A. armata.*

Several studies have been made using invasive exotic species and assessing the induced changes in native macroalgal assemblages [2, 4, 19]. Nevertheless, to the present knowledge, in the literature, there are no studies showing the impact of *A. armata* on macroalgae and macroinvertebrate community assemblages. In the present study, *A. armata* had a significant impact on the native intertidal algal assemblage but not so evident over macroinvertebrate assemblage. Results indicated that physico-chemical parameters, did not have a strong impact on different pools, with most of the variation occurring between seasons. As many invasive macrophytes, *A. armata* exhibits seasonal development patterns having therefore a temporal variation in biomass [20].

In this study, during 2018, *A. armata* reached the peak biomass score in spring, which accumulated and started to decompose in the rock polls. A sharp biomass fall was observed in the summer suggesting spring as an important growing period for the species. Although similar seasonal patterns in biomass of *A. armata* have been recorded in the Azores (NE Atlantic archipelago) [21], this should also be verified in other *A. armata* populations. Also, supporting the growing pattern observed in the study, this algae may disappear from the high intertidal zone during summer, being found mainly in lower abundances in the low intertidal zone or in the shallow subtidal during this period of the year [22]. The capacity of this species, nonetheless, to disperse with water currents, attached to floating objects, suggests that shoreline shape and local currents may play an important role in determining its distribution at the coast [23].

Asparagopsis armata produces very high amounts of secondary metabolites including volatile components as phenolic compounds [24], which toxicity to invertebrates, and seaweeds has often been associated to the invasive process [25, 26]. These phenolic compounds from seaweeds have also shown to vary throughout the year [27], while the release of defensive compounds may also be altered by several abiotic factors such as temperature, light, and nutrient availability [28].

In the present work, the effect of *A. armata* removal on the macroalgal assemblage was detectable in spring and summer seasons. During summer, with lower *A. armata* biomass, a higher algal richness is noted. This result agrees with the concept known as “biotic resistance” proposed by Elton [29] that states that diverse communities should be less susceptible to invasion because of a more complete utilization of resources.

Considering the strong seasonal changes in individual biomass of *A. armata*, faunal shifts are expected to be especially relevant during the growth season (Spring). Episodes of exponential growth can happen followed by sudden drops of abundance like the congeneric species *Asparagopsis taxiformis* [30]. Consequently, macrofauna resilience would be conditioned by their response to high variability in *A. armata* biomass. In spring, where *A. armata* registered higher biomass, in the invaded pools a higher biomass of native macroalgae was registered. In fact, increased light intensity, daylight duration and temperature during the spring and summer stimulates the growth of many native sub-canopy algal [31]. This fact might increase competition for primary resources with *A. armata.* Also, pools with *A. armata* tended to present lower species richness, which may contribute to increased variability within invaded rockpools in the intertidal zone [32]. High densities of *A. armata* inhabiting the tide pools could be responsible for a decrease in the macroalgae abundance through competitive interactions. These hypotheses are not mutually exclusive and experimental evidence from this study and others [5, 33] may explain the observed field patterns. The experimental exclusion of *A. armata* from the studied rockpools, and the difference found on macroalgae assemblages from pools with and without this invasive species may also be an important indication about the recovery potential of these marine ecosystems. Native macroalgal assemblages found in pools C (without *A. armata*), were more diverse then from pools A, foreseeing the importance of management actions on habitat recovery.

Ellisolandia elongata contributed substantially to the total dissimilarity between different pools. This species decreased its biomass when *A. armata* was present. This is sustained by previous studies by [34] that reports *E. elongata* as the main algal species affected by the presence of *A. armata*, while it is also known to dominate algal assemblages by producing massive coverage [35].

Concerning macrofauna, no significant differences were detected by PERMANOVA, showing that both rock pools type did not differ in the abundance of macrofauna species. PCO results (Fig. 6B) did not portray differences among different pools and only the factor season was significant in the PERMANOVA test. Furthermore, the structure of the benthic assemblage tends to be different between the different pools. There was a shift between the season summer to autumn where the higher number of macroinvertebrates started to decrease in the invaded pools and the number of species in pools without *A. armata* started to increase. There is a tendency towards increased variability of assemblage structure and towards decreased species richness in the presence of invasive species. Further work following the trajectory of macroalgal and macrofauna communities over a longer duration would increase information to assess the full community dynamics with and after *A. armata* removal.

However, there is a tendency of Gastropoda, Bivalvia, and Crustacea to preferer invaded pools and Polyplacophora, Polychaeta, and Ophiuroidea to prefer the native macroalgal assemblage pools. Many small and relatively sedentary herbivores such as polychaetes may preferentially feed on defended seaweeds in order to reduce their susceptibility to natural enemies [36]. Crustaceans are the dominant epifaunal group on *A. armata*, representing more than 50% of the total fauna, as also reported by [37].

Due to their nature, grazers play an important role in structuring macroalgal assemblages, but secondary metabolites (terpenes, phenolic compounds, etc.) of *A. armata* ought to affect the palatability, or acceptability to herbivores [25, 26]. Many of the gastropod species are microherbivores that feed on the biofilm established on the macrophyte fronds. In fact, lower densities of bacteria are found on the surface of the alga with halogenated compounds like those found in *A. armata* [38]. For that, a major presence of gastropoda in invaded pools is not expected. Despite Zwerschke et al. [39] stating that NIS can play an important role in maintaining biodiversity in human-altered environments, the stated presence of relatively high levels of phenolic compounds in *A. armata* tend to result in lower grazing losses and organism diversity.

These results are similar to previous studies that have compared faunal assemblages with other invasive species to those associated with other native canopy species. Most of these studies considered that the introduction of invasive macroalgae have not produced substantial modifications in the composition of faunal assemblages (e.g. loss of diversity) suggesting a weak impact in native faunal diversity. Thomsen et al. [40] stated that there was no evidence for severe effects of invasive macroalgae on fauna density and assemblages. Other studies, such as Cacabelos et al. [41], stated that epifaunal assemblages associated with the native *Laminaria ochroleuca* and with the invasive algal species *Sargassum muticum* differed, but only for epifaunal organisms, since the number of taxa and diversity did not clearly differ between the two algae. Viejo [42] also demonstrated that the composition of the epifaunal assemblages associated to the invasive *Sargassum muticum* and the local *Cystoseira nodicaulis* were very similar.

However, Navarro-Barranco et al. [30] showed differences in macrofauna composition between native *Halopteris scoparia* and the invasive species *Asparagopsis taxiformis*, using presence/absence data. Species richness, abundance and diversity of peracarids were significantly lower in invaded assemblages than in *H. scoparia* ones.

The general low abundance of *A. armata* at this intertidal level and its pseudoperennial character (high cover percentages are only recorded during a few months of the year) [43] appear to or may limit competitive processes with native species. Due to the great differences in morphology and size of the two phases of *A. armata* (the diploid tetraesporophyte is known as '*Falkenbergia'*) their ability to harbour mollusc assemblages can differ in macrofauna composition, which further studies would certainly benefit current knowledge.

Nevertheless, macrofauna density in native and invaded rockpools can vary depending on past diet, physiological condition of algae, and other local factors, such as grazing pressure or competition [41]. These factors may influence the upper and/or lower limits of distribution of individual species similarly on rocky shores [44].

The effects of the native and the invasive species on macrofaunal assemblages at larger spatial and temporal scales are yet to be determined, but may include effects on spatial patterns in β-diversity and detrital food webs, and the population dynamics of species associated with macrophytes at some or all stages of their life-history.

Nevertheless, the here seen trend, may point to a progressive replacement of *E. elongata* by *A. armata* that may directly or indirectly have considerable impacts on the ecology of rocky intertidal zone.

5. Conclusions

Results suggest that there was limited effect of *Asparagopsis armata* on community assemblages, due to short time follow-up, but the spreading of this species ought to drive to a homogenisation of communities (lower differences between sampling stations). Nevertheless, the constant increase in the algal biomass, even with seasonal changes, and its presence along the intertidal suggests that the effect will be greater in the future, in an equilibrium situation. Even the protection provided by Marine Protected Areas (MPAs) is very limited to prevent the establishment and spread of the most abundant invasive macroalgae [11, 45]. Predicting the impacts of invasive species on food webs and communities is one of the biggest challenges facing ecologists. Nevertheless, ecological management may have critical importance in the control of biological invasions, specifically by reducing the ecological pressure over native populations. On the other hand, this management may be reinforced when approached in a perspective of "transforming threats into opportunities", using the high *A. armata* biomass as a natural resource with economic values and opening a new window of opportunities for sustainable production.

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