

Article

# Non-Indigenous Species on Artificial Coastal Environments: Experimental Comparison between Aquaculture Farms and Recreational Marinas

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**Abstract:** Globally, there is growing concern regarding the effects of the increasing anthropogenic pressures in marine communities. Artificial structures such as marinas and aquaculture facilities serve as invasion hotspots; hence, monitoring fouling communities on these structures can be valuable for detecting new invasions. In the current study, 24 settlement PVC plates were deployed for three months to compare the recruitment ability of these two artificial environments along the south coast of the offshore island of Madeira (NE Atlantic). The results showed higher variations in the species richness between regions (SW vs. SE) than between artificial habitats (sea-cages vs. marinas), although the community composition differed. Cnidaria and Bryozoa were the most representative groups in the aquaculture systems, while Bryozoa and Chordata were in the marinas. A sum of 18 NIS was recorded for the study, accounting for between 21.88% and 54.84% of the total number of species in the aquaculture facilities and marinas, respectively. The higher NIS percentage from the marinas was even more explicit in the SE coast, where *Cradoscrupocellaria bertholletii*, *Parasmittina alba*, and *Botrylloides niger* distinctly dominated fouling populations. The results suggest that at least some particular NIS previously reported in the studied marinas successfully colonized sea-cages. Future assessments need to address the potential role of aquaculture facilities as drivers for the secondary spread of NIS. Additionally, two new records are considered for Madeira: *Eudendrium capillare* and *Erichthonius punctatus*.

**Keywords:** biofouling; artificial substrate; sea-cages; shipping; Madeira; Macaronesia



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## 1. Introduction

Aquaculture is considered a growing activity worldwide and the outermost regions such as Macaronesia, where the physical and oceanographic conditions contribute to fish farming activities in open waters [1], are not an exception. As part of the Macaronesian region, the Madeira Archipelago presents stable temperatures throughout the year (17–24 °C) and a narrow continental shelf, rapidly increasing the depth near the coastline [2,3]. Therefore, high-energy environments are easily found close to the coast (0.3–1.0 km), supporting the deployment of floating fish cages as the main aquaculture method in the region and increasing the production from 386 Tn in 2015 to 1234.6 Tn in 2020 [4].

Marine fish aquaculture operations consist of various floating and submerged structures (i.e., platforms, circular plastic rings, buoys, ropes, and nets), mainly classified as artificial polymer materials, which also serve as surfaces for biofouling colonization and settlement [5]. Biofouling on these artificial habitats is one of the main barriers to efficient and sustainable production [6,7], involving negative impacts (e.g., increasing drag forces on fish cages and restricting the water exchange through nets that could compromise the fish behavior and health) [8,9] with additional economic costs to the industry [10]. Moreover, fouling species in aquaculture cages may serve as a supplementary food source for the cultured fish and farm-associated species, stimulating the inspection and bites on the net that can lead on to more significant damages, such as escapes [11,12].

Due to the ongoing increase of aquaculture and trade, offshore aquaculture activities are also critical for facilitating the local dispersion of non-indigenous species (NIS) [13,14], which are well-known serious environmental threats [15] and considered among the primary threats to global biodiversity and ecosystem function [16,17]. These artificial substrates may serve as stepping stones, offering novel niches for opportunistic colonizers, including NIS, favoring their dispersal [18] and supplying the substrate to establish other NIS [19], with potential ecological impacts [20–22]. Consequently, the study of fouling communities on aquaculture structures may likewise contribute to detecting NIS arrivals and updating their distribution patterns [23].

A wide range of literature has characterized the fouling communities for many regions of the world on different aquaculture production systems and associated structures, such as buoys [24], ropes [5], or fish-cage nets [25]. Depending upon the study's aims, the choice of the method to quantify and/or identify the biofouling assemblages differs, but so far, the studies dealing with the characterization of biofouling assemblages, including the detection and richness of NIS on these artificial substrates remain scarce, especially in the North eastern Atlantic region.

Besides aquaculture substrates, several studies have been carried out in other anthropogenic structures, namely ports and recreational marinas, to assess the distribution and diversity of NIS [26–28], as these structures play an important role as main hubs for the introduction of NIS via the maritime transport [29–31]. Recently, Giangrande et al. [32] analyzed and compared the fouling communities found on several artificial substrates, including pontoons, quays, and ropes of a fish aquaculture facility of an enclosed part of the Mar Grande of Taranto (Ionian Sea). The authors concluded that the assemblages differed according to the age and size of the substrate, but also to their distance and depth from the fish aquaculture facility, as a proxy of organic enrichment.

In recent years, the Madeira Archipelago has been the site of a comprehensive monitoring survey of marine NIS using PVC plates in marinas [33–37]. This methodology, also used by several other authors [28,38–40], has turned out to be a sensitive and reliable option to measure NIS across different habitats.

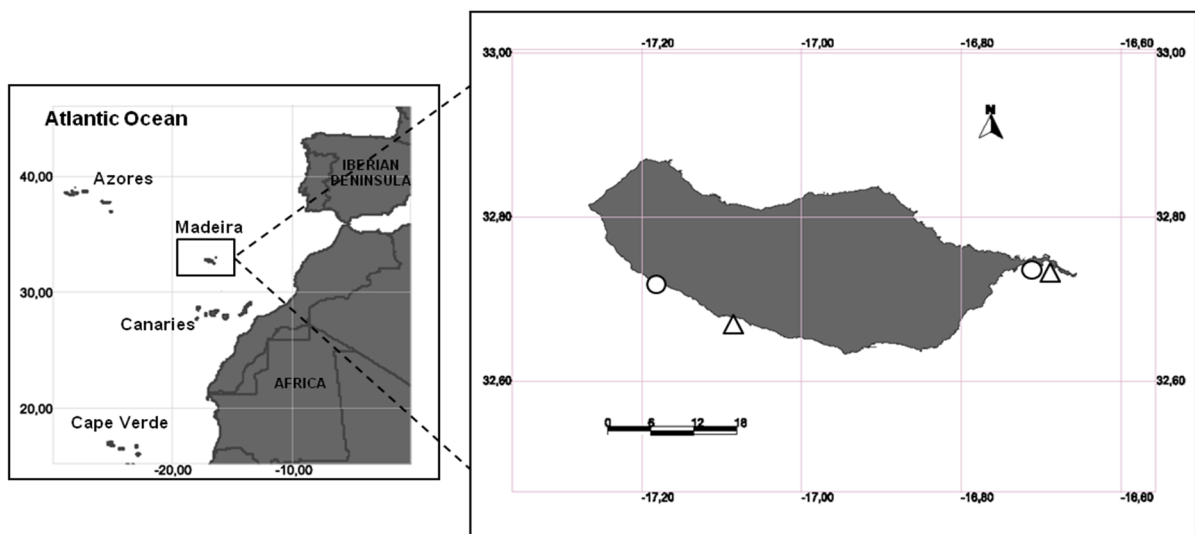
The aims of the present study were, for the first time, (i) to describe and analyze the fouling community settled on experimental PVC plates in two aquaculture facilities located along the south coast of Madeira island and (ii) to compare the fouling communities with the ones found in recreational marinas. By standardizing the substrate and time of exposure in these two different but very closely situated artificial environments, we aimed to compare the variability in the macrofouling's recruitment process, including the community composition and to detect the occurrence of NIS.

## 2. Materials and Methods

### 2.1. Study Area and Sampling Design

The study was carried out in two regions along the south coast (SW and SE) of Madeira Island (Portugal, NE Atlantic; Figure 1). In each region, the experiment was performed in two different artificial environments: (a) an aquaculture facility devoted to the growing of gilthead sea bream (*Sparus aurata*) and (b) one recreational marina located in the closest

bay. The distance between the SE marina and the respective aquaculture operation was approximately 1 km, whereas the distance reached nearly 4 km in the SW region.



**Figure 1.** Study regions in Madeira along the southern coast. Triangles, aquaculture facilities; circles, marinas.

Based on the design employed by Canning-Clode et al. [41] and Ramalhosa et al. [42], a total of 24 PVC plates ( $14 \times 14 \times 0.3$  cm) were horizontally attached to a brick and faced downwards to favor the recruitment of macroinvertebrates, rather than macroalgae (Figure S1). All replicates ( $n = 6$  per study region) were submerged at approximately 1-m depth from the external float of the sea-cages (Figure S2) and marina's pontoons (Figure S3), with a minimum distance of 5 m between replicates. After three months (August–November 2018), experimental plates were retrieved to collect representative samples of mature communities and plates were photographed (out of the water with bottom face up) using an Olympus TG-5 camera (Figure S4).

In the laboratory, benthic species settled on the plates were identified and confirmed using a stereomicroscope (Leica S8APO) to account for total species richness. All sessile macroinvertebrates and algae were identified to the lowest possible taxonomic level and later assigned to four biogeographic categories: native, NIS, cryptogenic (i.e., unspecified origin; [43]), or unresolved (based on an inability to identify to species level). In all data analyses, the NIS status was attributed only to those species with a confirmed non-indigenous category and verified by specific scientific literature [33,36,44,45].

The biogeographical status (NIS, cryptogenic, unresolved, and native) of the fouling communities was compared between marinas and sea-cages. To evaluate the NIS settlement success on experimental plates, a more conservative approach was followed [35,36], and those species categorized as cryptogenic and unresolved were included within the native species for the statistical analyses.

Previous photographs taken from the plates were used to determine species richness and total percent cover by using the image analysis software CPCe [46]. Consequently, each image was sub-divided into  $3 \times 3$  grids of 9 cells, with 11 random points per cell, resulting in 99 points analyzed per picture. This stratified random sampling method ensured that points were sampled in each region of the image [46], as it has been successfully developed in recent sampling analyses [36,41,44].

## 2.2. Statistical Analyses

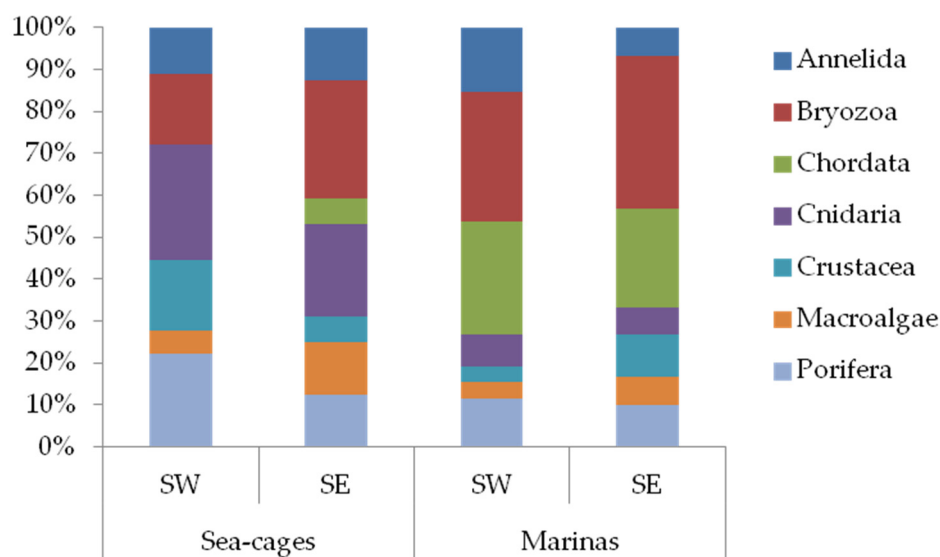
Univariate analyses were performed considering the total percent cover and species richness of the overall species (i.e., total, NIS, and native species) between regions and environments. A 2-way permutational ANOVA was used and included the orthogonal factors: 'Region' (random factor with 2 levels: SW and SE), and 'Environment' (fixed factor

with 2 levels: sea-cages and marinas;  $n = 6$ ). A PERMDISP test was used to assess the data for heterogeneity of dispersions, and transformations were applied when necessary. Analyses were based on Euclidean distances, using an approach similar to parametric ANOVA [47].  $p$ -values for the pseudo- $F$  ratios were calculated by permutation of raw data through 9999 permutations. Significant effects ( $p < 0.05$ ) were further investigated through pairwise comparisons between treatments. Pooling procedures were used where appropriate ( $>0.25$ ) to improve the power of tests concerning terms of interest [48].

Differences in the multivariate structure of the fouling community (composition and abundance) were visualized through a Non-metric Multidimensional Scaling (nMDS) plot to evaluate the community assembling responses to different artificial environments (i.e., sea-cages vs. marinas). The significance of multivariate differences was tested by a two-way PERMANOVA, following the same design outlined above, and analyses were based on Bray–Curtis similarities. Finally, to explain the community changes, taxa that contributed most to similarity within and dissimilarity among groups were identified using SIMPER analysis [49]. All statistical analyses were performed using the software PRIMER v6 [50,51] with the PERMANOVA+ for PRIMER (PRIMER-E Ltd., Plymouth, UK).

### 3. Results

A total of 54 species was found on the experimental plates (Table 1), belonging to 7 different phyla: Bryozoa (18), Chordata (10), Cnidaria (8), Crustacea (5), Porifera (5), Annelida (4), and Macroalgae (4). Of these taxa, 10 were categorized as native (16.7%), 18 as NIS (33.3%), 15 as cryptogenic (29.6%), and 11 were considered as unresolved (20.4%). The average species richness within each artificial environment was  $25 \pm 10$  species for the aquaculture sea-cages and  $29 \pm 4$  species for the marinas. In the aquaculture installations, Cnidaria was the most representative group ( $R = 5$ ) in the SW location, followed by Porifera ( $R = 4$ ), while Bryozoa ( $R = 9$ ) and Cnidaria ( $R = 7$ ) were in the SE location (Table 1; Figure 2). On the other hand, Bryozoa ( $R = 8$  in the SW and  $R = 11$  in the SE) and Chordata ( $R = 7$  in each region; Table 1; Figure 2) were the most representative phyla of both recreational marinas.



**Figure 2.** Taxa contributions for similarity among plates (SIMPER, 100% stacked column,  $n = 6$ ) in 3-month-old experimental plates for each type of artificial environment (sea-cages vs. marinas) and region (SW vs. SE).

**Table 1.** List of recorded species found in sea-cages and marinas located on the south coast of Madeira. Taxa were categorized as native (N), non-indigenous species (NIS), cryptogenic (C), or unresolved (U). Filled symbols, present; open symbols, absent.

Taxa	Status	Sources	Sea-Cages		Marinas		
			SW	SE	SW	SE	
<b>Annelida (4)</b>							
<i>Protula tubularia</i> (Montagu, 1803)	C	[36,52]	○	●	●	○	
<i>Salmacina dysteri</i> (Huxley, 1855)	N	[33,52,53]	●	●	●	●	
<i>Spirobranchus triqueter</i> (Linnaeus, 1758)	N	[36,44]	●	●	●	●	
<i>Spirorbis</i> sp.	U		○	●	●	●	
<b>Bryozoa (18)</b>							
<i>Aetea sica</i> (Couch, 1844)	C	[54]	○	●	○	○	
<i>Amathia verticillata</i> (delle Chiaje, 1822)	NIS	[36,46,55]	○	○	●	○	
<i>Bugula</i> sp. Oken, 1815	U		●	○	○	○	
<i>Bugula neritina</i> (Linnaeus, 1758)	NIS	[36,54,56]	○	○	●	●	
<i>Bugulina simplex</i> (Hincks, 1886)	NIS	[36,56]	○	○	●	●	
<i>Celleporaria inaudita</i> Tilbrook, Hayward and Gordon, 2001	NIS	[33,36,57]	○	○	○	●	
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	NIS	[33,36,46]	○	●	●	●	
<i>Crisia</i> sp. Lamouroux, 1812	U		●	●	●	●	
<i>Fenestrulina</i> sp. Jullien, 1888	U		○	○	○	●	
<i>Nolella gigantea</i> (Busk, 1856)	C	[36]	○	●	○	○	
<i>Parasmittina alba</i> Ramalho, Muricy and Taylor, 2011	NIS	[36,57]	○	○	●	●	
<i>Schizoporella errata</i> (Waters, 1878)	C	[36]	○	●	●	●	
<i>Scruparia</i> sp. Oken, 1815	U		○	●	○	○	
<i>Scrupocaberea maderensis</i> (Busk, 1860)	N	[54,58]	○	○	○	●	
<i>Scrupocellaria</i> sp. Van Beneden, 1845	U		●	○	○	○	
<i>Smittoidea</i> sp. Osburn, 1952	U		○	●	○	●	
<i>Tricellaria</i> sp. Fleming, 1828	U		○	●	○	○	
<i>Watersipora subtorquata</i> (d'Orbigny, 1852)	NIS	[33,36,46]	○	●	●	●	
<b>Chordata (10)</b>							
<i>Aplidium glabrum</i> (Verrill, 1871)	NIS	[36,37]	○	○	●	○	
<i>Botrylloides niger</i> Herdman, 1886	NIS	[36,37]	○	○	●	●	
<i>Botryllus schlosseri</i> (Pallas, 1766)	NIS	[33,36,37]	○	●	●	○	
<i>Diplosoma listerianum</i> (Milne Edwards, 1841)	C	[36,37,44]	○	●	○	●	
<i>Distaplia corolla</i> Monniot F., 1974	NIS	[33,36,37,44]	○	○	●	●	
<i>Ecteinascidia</i> sp. Herdman, 1880	U	[37]	○	○	○	●	
<i>Perophora listeri</i> Wiegman, 1835	C	[36,37]	○	○	●	●	
<i>Symplegma rubra</i> Monniot C., 1972	C	[36,37]	○	○	●	○	
<i>Symplegma brakenhielmi</i> (Michaelsen, 1904)	C	[37,44]	○	○	○	●	
<i>Trididemnum cereum</i> (Giard, 1872)	C	[33,36,37]	○	○	●	●	
<b>Cnidaria (8)</b>							
<i>Aiptasia diaphana</i> (Rapp, 1829)	NIS	[33,37]	○	●	○	●	
<i>Clytia hemisphaerica</i> (Linnaeus, 1767)	C	[59]	○	●	○	○	
<i>Ectopleura crocea</i> (Agassiz, 1862)	NIS	[46,59,60]	●	●	○	○	
<i>Eudendrium capillare</i> Alder, 1856	C	This study	●	○	○	○	
<i>Kirchenpaueria halecioides</i> (Alder, 1859)	C	[36,59]	●	●	●	●	
<i>Obelia dichotoma</i> (Linnaeus, 1758)	C	[36,59]	●	●	○	○	
<i>Pennaria disticha</i> Goldfuss, 1820	C	[36,59]	●	●	○	○	
<i>Sertularella ellisii</i> (Deshayes and Milne Edwards, 1836)	C	[59,61]	○	●	●	○	

Table 1. Cont.

Taxa	Status	Sources	Sea-Cages		Marinas	
			SW	SE	SW	SE
Crustacea (5)						
<i>Caprella scaura</i> Templeton, 1836	NIS	[34,36]	○	○	○	●
<i>Chthamalus stellatus</i> (Poli, 1791)	N	[62]	●	○	○	○
<i>Erichthonius punctatus</i> (Spence Bate, 1857)	C	This study	●	●	●	●
<i>Megabalanus azoricus</i> (Pilsbry, 1916)	N	[63]	●	●	○	○
<i>Paracerceis sculpta</i> (Holmes, 1904)	NIS	[64]	○	○	○	●
Macroalgae (4)						
<i>Bryopsis</i> sp. J.V. Lamouroux, 1809	U		○	●	○	○
<i>Champiaceae</i> sp. Kützing, 1843	U		○	●	○	○
<i>Lithophyllum incrustans</i> Philippi, 1837	N	[33,36,65]	●	●	●	●
<i>Neosiphonia sertularioides</i> (Grateloup) K.W. Nam and P.J. Kang, 2012	N	[33,36,66]	○	●	○	●
Porifera (5)						
<i>Clathrina clathrus</i> (Schmidt, 1864)	N	[36]	●	●	○	○
<i>Mycale (Carmia) senegalensis</i> Lévi, 1952	NIS	[33,36,44]	●	●	●	●
<i>Paraleucilla magna</i> Klautau, Monteiro and Borojevic, 2004	NIS	[33,36,44]	●	●	○	●
<i>Prosuberites longispinus</i> Topsent, 1893	NIS	[36]	○	○	●	○
<i>Sycon ciliatum</i> (Fabricius, 1780)	N	[33,36,67]	●	●	●	●
Total Species Richness	54	Total species per region	18	32	26	31
		Percentage (%)	33.3	59.3	48.1	57.4

Only eight species were found in common between the study regions (Table 1): *Salmacina dysteri*, *Spirobranchus triqueter* (Annelida); *Crisia* sp. (Bryozoa); *Kirchenpaueria halecioides* (Cnidaria); *Erichthonius punctatus* (Crustacea); *Lithophyllum incrustans* (Macroalgae); *Mycale (Carmia) senegalensis*, and *Sycon ciliatum* (Porifera). It should be mentioned that, besides the sessile fauna, some vagile species were found and therefore considered, given their cryptogenic and NIS status (i.e., *Caprella scaura*, *E. punctatus*, and *Paracerceis sculpta*; Table 1). However, these species were not representative of the whole epifaunal community, which was underestimated with the following methodology. Additionally, two new records were considered for Madeira with *Eudendrium capillare* and *E. punctatus*, which were then deposited at the Natural History Museum of Funchal (MMF), Madeira (under voucher number MMF48340 and MMF47931/MMF47932, respectively).

Univariate analyses showed that the total percentage cover of fouling communities was affected by the region and the type of artificial environment (significant interaction: 'Reg × Env'; Table 2). The total cover was significantly higher in the marinas than in the sea-cages, but only for the SW coast (Figure 3a). The type of artificial environment (sea-cages vs. marinas) significantly altered both the native and NIS cover, promoting a higher cover of native communities in both aquaculture facilities, while the NIS cover was significantly higher in the marinas (Table 2; Figure 3a). The total and native species richness were more affected by spatial differences than between the types of artificial environments (Table 2), as fouling assemblages maintained significantly higher values for both response variables in the SE coast (Figure 3b). By contrast, NIS richness was significantly affected by the artificial environment, presenting higher richness in both marinas (Table 2; Figure 3b), with the highest number of NIS found in the SE region.

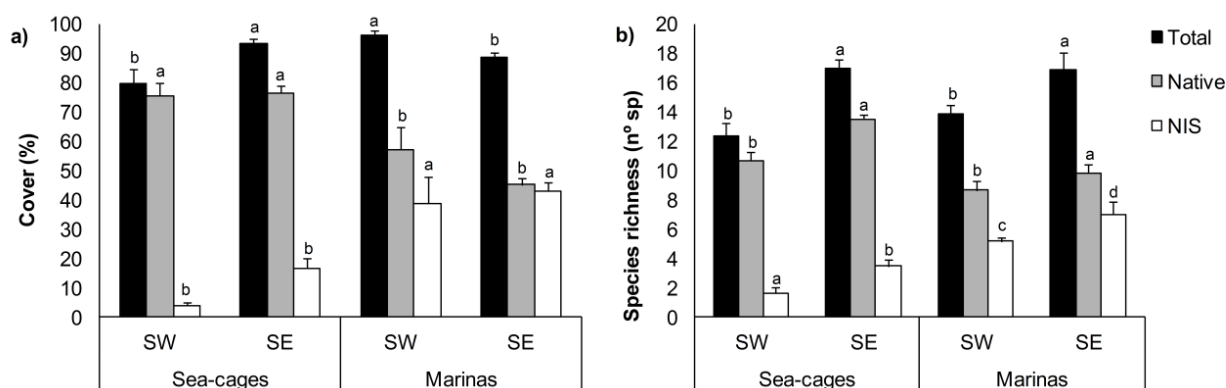
**Table 2.** Results of 2-way PERMANOVA testing for variability in fouling community composition, and 2-way permutational ANOVA examining changes in percent cover and species richness of total community, native species, and non-indigenous species (NIS;  $n = 6$ ).

Source	df	Full Community					
		Composition		Cover (%)		Species Richness	
		MS	Pseudo-F	MS	Pseudo-F	MS	Pseudo-F
Region (Reg)	1	12380	<b>11.45 ***</b>	58.16	1.05	100.04	<b>20.313 ***</b>
Environment (Env)	1	16861	2.23	214.32	0.32	5.04	0.72
Reg × Env	1	7568.3	<b>7.00 ***</b>	664.23	<b>12.04 **</b>	7.04	1.43
Residual	20	1081.2		55.17		4.93	
Total	23						

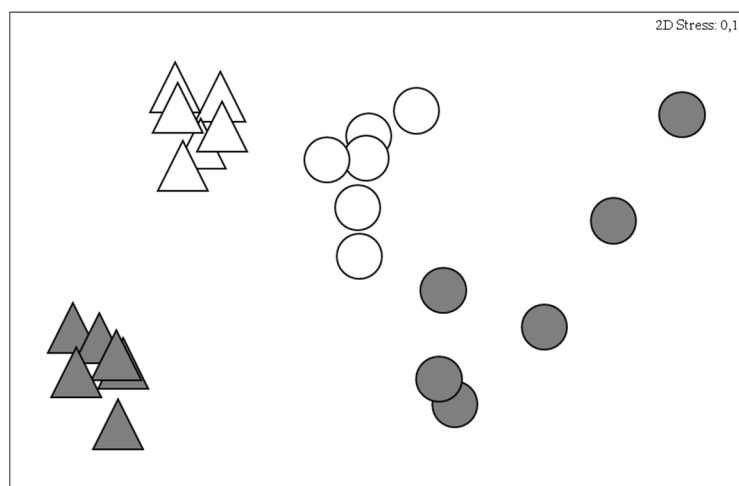
Source	df	Native Species				NIS			
		Cover (%)		Species Richness		Cover (%)		Species Richness	
		MS	Pseudo-F	MS	Pseudo-F	MS	Pseudo-F	MS	Pseudo-F
Region (Reg)	1	138.1	0.90	30.38	<b>16.2 ***</b>	383.52	2.16	20.17	<b>10.86 **</b>
Environment (Env)	1	3075.6	<b>19.96 ***</b>	35.04	4.98	4942.7	<b>27.86 ***</b>	66.67	<b>35.90 ***</b>
Reg × Env	1	154.09	pooled	7.04	3.76	177.38	pooled	1.86	pooled
Residual	20			1.88					
Total	23								

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Significant  $p$ -values are indicated in bold.



**Figure 3.** (a) Mean cover (% + SE) and (b) species richness (mean + SE) of total, native, and non-indigenous species (NIS) across artificial environments (sea-cages vs. marinas) and regions (SW vs. SE). Significances of *a posteriori* pairwise tests between regions within environments are indicated with different letters ( $p < 0.05$ ).

Multivariability associated with the two regions induced significant differences in the type of fouling communities recruited on the plates deployed in the sea-cages compared to those deployed in the marinas (significant interaction: ‘Reg × Env’; Table 2). Clear segregation from sea-cages communities vs. marinas communities can be appreciated in the nMDS: fouling assemblages associated with aquaculture sea-cages are on the left-hand side of the ordination space, while assemblages inhabiting recreational marinas are on the right-hand side of the plot (Figure 4). Posteriori pairwise comparisons confirmed significant differences between communities from sea-cages and marinas in both regions, but the effect of the marina environment was stronger in the SE than in the SW (similarities marina group: 53.3 vs. 37.8; Table 2; Figure 4).



**Figure 4.** Two-dimensional n-MDS plot showing similarities of fouling communities between artificial environments and regions ( $n = 6$ ). Triangles, sea-cages; circles, marinas. Filled symbols, SW; open symbols, SE.

Finally, SIMPER analysis revealed the most critical taxa contributing to the communities' dissimilarity and similarity between each type of artificial environment (i.e., contribution  $\geq 2\%$ ; Table 3). The bryozoans *Scrupocellaria* sp. and *Crisia* sp. were the most important taxa in shaping differences between the recruited communities in the SW coast and only *Botrylloides niger*, particularly abundant in the marina, was remarkable among the NIS recruited (Table 3a). By contrast in the SE region, and though the most important species in separating communities was the cryptogenic amphipod *E. punctatus*, the contribution of the NIS *Parasmittina alba* and *B. niger* was particularly relevant, summing together more than 18% of the total dissimilarity (Table 3a). SIMPER analysis highlighted the taxa *Scrupocellaria* sp. and *E. punctatus* as those mostly contributing to similarity within sea-cages in the SW and SE regions, respectively (Table 3b). Similarity results also revealed the bryozoan *Crisia* sp. as the second most important species in the sea-cages, and in this case, affecting the communities from both regions (25.42% in the SW and 15.19% in the SE). Communities in the marina environments showed important abundances of NIS, namely in the SE coast (41.46%) compared to the SW (22.18%), where *Cradoscrupocellaria bertholletii*, *Botrylloides niger*, and *Parasmittina alba* distinctly dominated fouling populations. However, in the SW region, none of the three taxa contributing higher to the similarity of communities from the respective marina was catalogued as NIS (43.40% summed by *S. disteri*, *Spirorbis* sp. and *Schizoporella errata*). Sea-cages presented some contribution of NIS, especially in the SE region (14.04% vs. 6.27% in the SW), but to a much lower extent than the closest marina in both regions (Table 3b).



**Table 3.** Results from SIMPER analysis showing the contribution of taxa to the average Bray–Curtis dissimilarity (a) and similarity (b) between fouling communities fixed on the experimental plates at sea-cages and marinas from two regions on the south coast of Madeira (SW vs. SE). The individual taxa cut-off level was  $\geq 2\%$  for dissimilarity and similarity analyses. Non-indigenous species are highlighted in bold.

(a)	Area	Taxon	Sea-Cages		Marina		Av.Diss	Diss/SD	Contrib%	Cum%
			Av.Abund	Av.Abund	Av.Abund	Av.Abund				
	SW	<i>Scrupocellaria</i> sp.	20.03	0.00	11.40	5.90	12.80	12.80		
		<i>Crisia</i> sp.	19.36	1.01	10.29	2.56	11.55	24.35		
		<b><i>Botrylloides niger</i></b>	0.00	17.34	9.76	0.77	10.96	35.31		
		<i>Symplegma rubra</i>	0.00	11.28	6.69	0.68	7.50	42.81		
		<i>Eudendrium</i> sp.	11.78	0.00	6.66	3.07	7.48	50.29		
		<i>Schizoporella errata</i>	0.00	10.27	5.85	1.23	6.57	56.86		
		<i>Spirorbis</i> sp.	0.00	9.76	5.64	1.49	6.33	63.19		
		<i>Salmacina dysteri</i>	0.34	8.59	4.79	1.82	5.38	68.57		
		<b><i>Prosuberites longispinus</i></b>	0.00	7.24	4.06	0.73	4.55	73.12		
		<b><i>Parasmittina alba</i></b>	0.00	5.56	3.19	1.00	3.58	76.70		
		<i>Lithophyllum incrustans</i>	8.42	5.72	2.91	1.34	3.26	79.96		
		<b><i>Ectopleura crocea</i></b>	3.70	0.00	2.07	1.44	2.32	82.28		
		<i>Erichthonius punctatus</i>	1.85	4.71	1.84	1.28	2.07	84.35		
		SE	<i>Erichthonius punctatus</i>	24.91	3.87	11.59	3.13	16.93	16.93	
	<b><i>Parasmittina alba</i></b>		0.00	12.46	6.87	0.98	10.03	26.97		
	<b><i>Botrylloides niger</i></b>		0.00	10.77	5.89	1.58	8.61	35.58		
	<i>Spirorbis</i> sp.		0.17	9.60	5.22	1.63	7.62	43.20		
	<i>Crisia</i> sp.		12.29	4.88	4.53	1.79	6.62	49.82		
	<b><i>Cradoscrupocellaria bertholletii</i></b>		10.61	11.62	4.21	1.50	6.15	55.97		
	<i>Scruparia</i> sp.		6.73	0.00	3.74	1.55	5.46	61.43		
	<i>Spirobranchus triqueter</i>		10.27	11.45	3.25	1.39	4.74	66.17		
	<i>Diplosoma listerianum</i>		4.71	3.87	2.87	1.23	4.20	70.37		
	<i>Kirchenpaueria halecioides</i>		4.21	0.17	2.23	2.00	3.26	73.62		
	<b><i>Paraleucilla magna</i></b>		3.87	0.51	1.87	1.65	2.73	76.35		
	<i>Perophora listeri</i>	0.00	3.03	1.66	0.88	2.42	78.77			

Table 3. Cont.

(b)	Area	Taxon	Sea-Cages				Taxon	Marinas			
			Av.Abund	Sim/SD	Contrib%	Cum%		Av.Abund	Sim/SD	Contrib%	Cum%
SW		<i>Scrupocellaria</i> sp.	20.03	6.56	31.93	31.93	<i>Salmacina dysteri</i>	8.59	1.44	15.42	15.42
		<i>Crisia</i> sp.	19.36	3.61	25.42	57.34	<i>Spirorbis</i> sp.	9.76	1.07	15.05	30.46
		<i>Eudendrium</i> sp.	11.78	2.92	16.38	73.72	<i>Schizoporella errata</i>	10.27	0.91	12.93	43.39
		<i>Lithophyllum incrustans</i>	8.42	1.37	10.34	84.06	<b><i>Botrylloides niger</i></b>	17.34	0.36	10.23	53.62
		<b><i>Ectopleura crocea</i></b>	3.7	0.96	3.5	87.56	<i>Lithophyllum incrustans</i>	5.72	2.11	10.14	63.76
		<b><i>Paraleucilla magna</i></b>	2.69	1.11	2.77	90.33	<i>Erichthonius punctatus</i>	4.71	1.38	7.91	71.67
		<i>Erichthonius punctatus</i>	1.85	3.15	2.56	92.89	<b><i>Parasmittina alba</i></b>	5.56	0.78	5.88	77.55
		<i>Sycon ciliatum</i>	1.85	3.54	2.19	95.08	<i>Symplegma rubra</i>	11.28	0.26	5.48	83.02
SE							<i>Spirobranchus triqueter</i>	2.69	2.41	4.02	87.05
							<b><i>Prosuberites longispinus</i></b>	7.24	0.36	3.71	90.76
							<b><i>Mycale (Carmia) senegalensis</i></b>	2.02	0.65	2.36	93.12
		<i>Erichthonius punctatus</i>	24.91	2.98	34.99	34.99	<b><i>Cradoscrupocellaria bertholletii</i></b>	11.62	1.4	17.04	17.04
		<i>Crisia</i> sp.	12.29	1.97	15.19	50.18	<i>Spirobranchus triqueter</i>	11.45	1.88	16.8	33.84
		<i>Spirobranchus triqueter</i>	10.27	3.06	12.65	62.83	<b><i>Botrylloides niger</i></b>	10.77	1	14.38	48.21
		<b><i>Cradoscrupocellaria bertholletii</i></b>	10.61	1.54	9.92	72.75	<i>Spirorbis</i> sp.	9.6	1.89	13.59	61.81
		<i>Scruparia</i> sp.	6.73	1.11	6.39	79.15	<b><i>Parasmittina alba</i></b>	12.46	0.68	10.04	71.85
		<i>Kirchenpaueria haleciooides</i>	4.21	1.83	4.72	83.87	<i>Crisia</i> sp.	4.88	1.67	6.08	77.93
		<b><i>Paraleucilla magna</i></b>	3.87	1.93	4.12	87.99	<i>Erichthonius punctatus</i>	3.87	2.08	4.96	82.89
						<i>Diplosoma listerianum</i>	3.87	0.63	3.33	86.22	
						<i>Salmacina dysteri</i>	1.18	37.18	2.3	88.52	

#### 4. Discussion

The increasing number of anthropogenic marine structures, together with aquaculture facilities, will increase submerged artificial structures that inadvertently provide favorable substrates for fouling organisms, including NIS. Theoretically, fouling organisms could be more attracted to aquaculture facilities than the partially enclosed marinas due to the higher organic and nutrient concentration within these facilities [68]. Standard experimental design allowed comparing, for the first time, fouling communities of fish farms and marinas in two regions of Madeira Island (Northeastern Atlantic Region).

A total of 54 species and 18 NIS were identified in the current study, including the new record of the cryptogenic *E. capillare* (Alder, 1856) and *E. punctatus* (Spence Bate, 1857). Comparison of the fouling communities between the two aquaculture farms and recreational marinas showed significant differences among species within each artificial environment in both regions. Sea-cages were mostly characterized by cnidarians, whilst in the marinas, bryozoans and chordates were found in higher percentages. Moreover, the number of NIS significantly differed between the types of artificial environments rather than between regions, with a higher presence of NIS in the marinas.

From the 18 NIS identified, 14 NIS were already reported for the same marinas in previous works [33,36]; nonetheless, *Bugulina simplex*, *C. scaura*, and *P. sculpta* were newly recorded in several marinas of Madeira island [34,36,56,64]. Additionally, *Prosuberites longispinus*, previously reported from the neighboring island of Porto Santo [36], is documented for the first time in Madeira. Most of the NIS observed in the aquaculture sea-cages were also present in the nearby marina, with the exception of *Botryllus schlosseri* and *P. magna*. Interestingly, the hydroid *Ectopleura crocea* was only detected in the sea-cages.

Fouling organisms related to aquaculture facilities such as the non-indigenous *P. magna* are in agreement with studies developed in the Mediterranean Sea [9,28,69], where it is considered a potential invasive species [70]. The relation of *E. crocea* with aquaculture activities has been also documented in other areas of the globe, namely Australia, where it can produce adverse effects [71]. Along with *Pennaria disticha*, *Ectopleura* spp. have been identified as highly problematic taxon for marine fish farms, also in the Mediterranean Sea [72], due to the asexual reproduction process—hydranth self-detachment (autotomy)—that leads to the release of the tentacled polyp heads in the water column [73]. When liberated, these polyp heads may act as drifting “armed weapons” able to cause harmful injuries on farmed fish skin and gills.

Regarding the new species observed in the current study, the cnidarian *E. capillare* was exclusively found in the sea-cages of the Madeira SW region. It was first described in the North Sea and it is reported to be nearly cosmopolitan. Reliable records based on nematocyst information belong to NE Atlantic, Greenland, the Mediterranean, Bermudas, southern Africa, and Japan [74]; however, a wider distribution can be also attributed to the NW Atlantic, including the Gulf of Mexico, and New Zealand [75]. Although *E. capillare* was already documented in Macaronesia, namely along the Canary Islands [61]; this study provides a new record for Madeira, suggesting maritime transport as the primary introduction vector [76].

The amphipod *E. punctatus* was identified across all surveyed environments and regions of Madeira. Originally described from Wales, the species has a circumglobal distribution in tropical and temperate climates, including the North Atlantic Ocean, the Mediterranean Sea, and the Indian Ocean [75]. In the Mediterranean Sea, *E. punctatus* has been commonly found in fouling communities associated with aquaculture systems [5,77]. By contrast, it is considered an introduced species in the SW Atlantic, where it has successfully colonized Argentinian harbors [78]. In the Macaronesian region, *E. punctatus* was reported from soft bottoms across the Canaries [79] and Azores [80], likely spreading its distribution via maritime transport. In this sense, tube-dweller amphipods such as *E. punctatus* present a high capacity to disperse by attaching the tube to hard substrates, and thus protecting themselves from hostile environmental conditions [81]. This character-

istic explains that *E. punctatus* was highly found on the experimental units and, therefore, accounted as a sessile organism.

Our monitoring showed spatial differences between total and native species richness, as the SE region presented a higher species richness, including a higher cover percentage of NIS. Several factors could contribute to these results. The first factor is that the variation in biofouling is predominantly driven by the light and water flow availability and is often related to the infrastructure's depth and orientation [82–84]. The aquaculture operation and the marina of the SE region are located in an enclosed protected bay with shallow depths (<30 m), mean annual current speeds lower than 0.5 m/s, and stable water temperature throughout the year [85], providing favorable conditions for most NIS [86]. On the other hand, sea-cages of the SW region are deployed in open waters with greater depths (>50 m).

Secondly, the spatial distance between environments might affect the species colonization process [87]. The aquaculture facility of the SE region is located approximately 1 km from the nearby marina, while in the SW region studied, the environments are nearly 4 km away. According to Alharbi and Petrovskii [87], the invasion success depends on the stepping stone size, location, and length of the unfavorable area. Hence, it is reasonable that the largest distance between habitats and the oceanographic features in the SW region significantly interfere with the species distribution.

Finally, it is known that commercial and recreational vessels are responsible for the transport of some of the most widespread non-native species [29–31]. The SE region presents a big port (~4 km) with dense international maritime traffic (e.g., container ships, gas tankers, recreational vessels), opposed to the small shelter port of the SW, used mainly by local fishermen. Moreover, considering that the SE port hosts the aquaculture facility's operational boat, therefore subject to increased fouling, this could support the higher number of NIS found on the SE aquaculture facility than the one located at the SW region. Such connectivity between these two pressurized environments highlights the stepping-stone effect, enhancing opportunities for the spread of fouling species, including NIS. In agreement, Canning-Clode et al. [33] underlined in their conclusions that the NIS occurrence in the Madeira biological system is most likely secondary or tertiary introductions resulting from hull fouling (recreational and cruise vessels). Hence, our results suggest that, at least for some particular NIS, the presence of sea-cages could promote the secondary spread from the hotspots of introduction (i.e., marinas/ports). Future assessments would need to address the potential role of aquaculture facilities as drivers during the introduced NIS invasion process, similarly to studies performed in other basins [9,88,89].

## 5. Conclusions

This study allowed us, for the first time, to assess fouling communities and, more importantly, to detect the NIS richness in aquaculture facilities as a potential result of the stepping-stone effect and spread from marinas on Madeira Island. The methodology employed here was shown to be a valuable tool to characterize and compare sessile assemblages in artificial environments with different anthropogenic pressures. Vagile fauna has been underestimated with this experimental approach, even though relevant species have been found. Furthermore, it has contributed to updating the presence of NIS and biodiversity records for Madeira. Indeed, in the future, if experimental plates are intended to be used in offshore aquaculture environments, they would need to be optimized since these sites are typically exposed to strong currents and storms in Macaronesia, which could affect the integrity of the experimental units. Furthermore, complementary studies aimed at assessing the successional pattern of fouling assemblages in aquaculture systems may be of interest, in order to be compared with similar works performed in other recreational marinas [34–37,57,82].

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/jmse9101121/s1>, Figure S1: PVC plate (experimental unit). Figure S2: (a) Aquaculture operation, (b) Experimental unit deployed at aquaculture sea-cages. Figure S3: (a) Recreational

marina, (b) Experimental unit deployed at marina. Figure S4: Experimental unit with biofouling. Credits: P. Ramalhosa.

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