










Article

# Monitoring Non-Indigenous Species with Passive Sampling Methods in an Oceanic Island

Anna Diem <sup>1,\*</sup> , Patrício Ramalhosa <sup>1,2</sup>, Eva Cacabelos <sup>1,3</sup>, Jasmine Ferrario <sup>4</sup> , Nuno Castro <sup>1,5</sup> ,  
Filipe Henriques <sup>1</sup>, João Gama Monteiro <sup>1,6</sup> , Paula Chainho <sup>5</sup> , Christopher Kim Pham <sup>7</sup> ,  
João Canning-Clode <sup>1,8</sup> , José Paula <sup>5</sup>  and Ignacio Gestoso <sup>1,8,9,\*</sup> 

- <sup>1</sup> MARE—Marine and Environmental Sciences Centre/ARNET—Aquatic Research Network, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), 9020-105 Funchal, Portugal
  - <sup>2</sup> OOM—Oceanic Observatory of Madeira, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, 9020-105 Funchal, Portugal
  - <sup>3</sup> Hydrosphere—Environmental Laboratory for the Study of Aquatic Ecosystems, Isla de Toralla s/n, 36331 Vigo, Spain
  - <sup>4</sup> Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy
  - <sup>5</sup> MARE—Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal
  - <sup>6</sup> Faculty of Life Sciences, Universidade da Madeira, 9020-105 Funchal, Portugal
  - <sup>7</sup> IMAR/Oceanos, Department of Oceanography and Fisheries, University of the Azores, 9900-140 Horta, Portugal
  - <sup>8</sup> Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA
  - <sup>9</sup> Department of Biology, Faculty of Marine and Environmental Sciences, University of Cádiz, 11510 Puerto Real, Spain
- \* Correspondence: anna.diem@mare-centre.pt (A.D.); ignacio.gestoso@uca.es (I.G.)

**Abstract:** The synergistic effects of biological invasions have long been considered significant causes of biodiversity loss worldwide. Therefore, early detection monitoring is crucial in mitigating non-indigenous species (NIS) threats. In the marine environment, settlement plates were used as monitoring devices in ports, but this method was mainly applied to assess the sessile benthic community and is less efficient in collecting the mobile biota and accessing its diversity. Moreover, as the potential expansion of NIS to the surrounding coastal environment is still poorly understood, a pilot study was conducted focusing on two aspects: (i) improving the feasibility of the settling method under different environmental contexts and (ii) enhancing the capacity of the developed prototypes to collect more representative samples (i.e., sessile and mobile biota). Three different prototypes were designed: a box prototype consisting of PVC plates encapsulated by a plastic bottle, a CD prototype with CDs surrounded by a net, and a PVC prototype with uncovered PVC plates. The prototypes were deployed inside a marina and in an outside area on Madeira Island, Portugal. Results indicate that the PVC prototype was the most efficient regarding monitoring the sessile community, whereas the box prototype showed the highest abundance of the mobile fauna. The location influenced both the sessile community composition and the number of mobile taxa. Our findings suggest combining features from prototypes to encompass the whole benthic community better.

**Keywords:** biological invasions; monitoring; coastal ecosystems; biofouling



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## 1. Introduction

Anthropogenic pressures and their resulting impacts on the environment are increasing at a global scale [1,2]. Among the most significant impacts, climate change, widespread pollution, and habitat destruction have severe consequences to the marine environment [3].

Human population rise and development increase the demand for goods and products transported via commercial ships [4]. Furthermore, with the expected rise in global tourism [5], the number of cruise ships and recreational boats is predicted to increase accordingly [6]. The global shipping industry has some negative impacts on the environment,

such as habitat fragmentation and coastal habitat destruction for the construction of marinas and harbors [7], and is, among other drivers, responsible for most of the translocation and introduction of nonindigenous species (NIS) into new areas [8].

Ports provide suitable environments for recruiting potential incoming species, that is, by creating new free available substrates [7] in sheltered areas, and are considered critical habitats for the NIS' first successful establishment [7,9]. The translocated species settle mostly on hard artificial substrates [10], but also on natural substrates [11,12]. From these primary hubs, the introduced species can then spread into neighboring natural areas [13], where they can affect the performance of native species and the functioning of resident communities [14–16]. Furthermore, some NIS can outcompete native species and are difficult to eradicate once they are successfully established in new areas [11]. Additionally, they show a comparably rapid colonization and can impact the local community structure, food web, and nutrient cycling [14,17]. Occasionally, these NIS can also disperse from harbors and port infrastructures to other artificial substrates, for example, to neighboring aquaculture facilities [18] or ecosystems [19] via recreational shipping [9,19–21]. As the impacts of NIS can go beyond initial introduction sites, their expansion may have unpredictable consequences for the receiving ecosystems and areas [14,17]; therefore, early detection of NIS is needed to prevent their establishment and dispersal [22].

The development of monitoring programs to assess NIS presence and early detection of new arrivals are necessary for the cost-effective eradication of pioneering populations of invasive species [23]. Moreover, establishing standardized monitoring methods could contribute to the management of biological invasions, for example, to Marine Strategy Framework Directive (MSFD) Descriptor 2, which requires EU member states to consider NIS in their marine management strategies [24]. Additionally, a standardized monitoring method could contribute to EU Regulation 1143/2014 on Invasive Alien Species, which is a list of invasive alien species of Union concern, and to the European Alien Species Information Network, EASIN [25]. Standard continuous monitoring could also be beneficial to contribute to the IUCN Global Invasive Species Database, GISD [26]. However, a European standardized monitoring method still needs to be established [27] for both sessile and mobile taxa, as fouling organisms cover a broad taxonomic range: up to now, sessile organisms, such as sponges, hydroids, tube worms, barnacles, bryozoans, mussels, ascidians, and algae, have been extensively reported to be introduced to harbors or marinas [19,28], whereas literature about mobile species is relatively scarce (but see [29] or [30]). In fact, according to Ruiz et al. [31], nonindigenous mobile species also require attention, as they have been responsible for around one-half of the marine biological invasions on North American coasts.

Several active and passive sampling methods have been implemented to monitor species, such as traps, collectors, nylon bath sponges, or settling plates [32,33]. Settling plates are commonly and widely used by researchers and managers across the globe due to their high cost efficiency and easy application [33–35]. This method, usually carried out with Polyvinyl chloride (PVC) plates deployed at specific sites (such as ports and docks), allows for monitoring the settlement of benthic marine biota and is accepted worldwide as a common technique, especially efficient in collecting NIS [36,37]. However, in a study by Outinen et al. [32], several passive sampling methods were compared regarding their fouling efficiencies. They concluded that settling plates are only appropriate for monitoring sessile fauna when combined with baited traps [32].

In other studies, such as Obst et al. [38], settling plates were assembled as autonomous reef monitoring structures (ARMS), consisting of several PVC plates parallelly fixed horizontally via a backbone. These ARMS can be deployed in different environments and are commonly used to monitor hard-bottom communities, including mobile species, efficiently. However, the construction and application of ARMS are relatively challenging, as each structure should contain at least nine plates, which is time-consuming to assemble and results in a lot of material to sample and analyze. Therefore, a simplified version that can be

easier assembled and applied might be more suitable for long-term monitoring in different habitats [39,40].

Madeira is a Portuguese subtropical island in the Macaronesian region, located in the Northeast Atlantic [41]. The port of Funchal, Madeira's capital, has a long history of maritime activities. In the past decades, the increase in the recreational and cruise tourism sector has contributed to the growing intensity of the marine traffic influx in the island, facilitating the dispersal and establishment of NIS [42]. In fact, the number of NIS has been positively correlated to vessel traffic [23,43]. During the last decade, a monitoring program for ports was implemented in Madeira to assess the settlement and the diversity of fouling organisms, including early NIS detections [23,43–47]. However, data obtained by this monitoring program were mainly based on downward-facing PVC plates and focused on sessile species only (e.g., [23,48]). Mobile fauna was detected just through opportunistic sampling (e.g., dry dock inspections [45]).

Despite the success of the monitoring method with PVC plates in Madeira harbors, studies about the potential application of a standard translatable method to monitor the arrival of NIS also in the open coasts are scarce. Some studies have documented the presence of NIS in the natural coastal environment of the archipelago (e.g., [43,49]), but very few examples of the potential spread and the underlying mechanisms of introduced species have been performed outside harbor areas [50]. In this context, the present study was designed to test different passive sampling methods for marine NIS detection on Madeira Island. Specifically, the study wanted to cover two objectives:

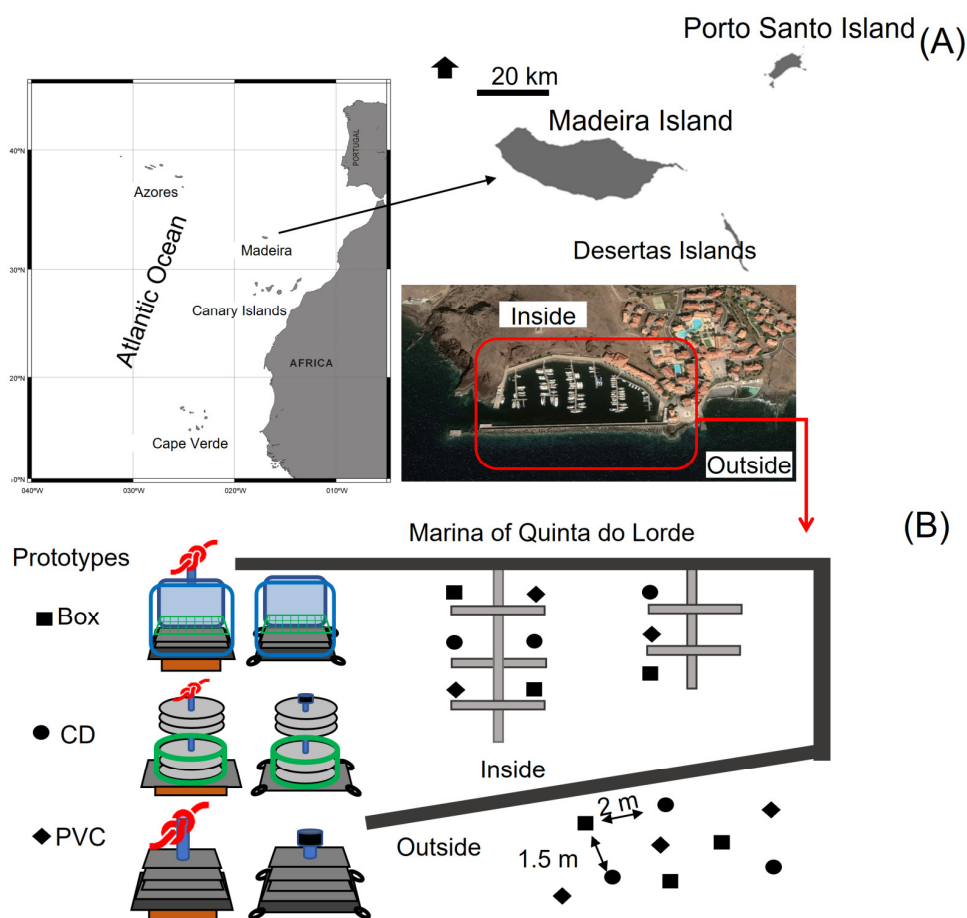
(a) To evaluate the capacity of three different prototypes to collect more representative samples of hard-bottom benthic communities. The aim was to collect both sessile organisms and mobile fauna.

(b) To assess the possible spread of NIS from the recreational marina to neighboring areas. Furthermore, the study was finalized by investigating the influence of environmental conditions in determining species composition, especially regarding the settlement behavior or spread of NIS.

## 2. Materials and Methods

### 2.1. Study Site

This study was conducted at the marina of Quinta do Lorde, on the SE coast of Madeira Island (Caniçal, 32.741667 N, −16.713333 W, Figure 1A), in two environments: inside the marina along pontoons and outside on the open coast (relatively close to the marina environment, Figure 1B). The marina of Quinta do Lorde was established in 2002. Recent studies showed that the number of vessels arriving at Quinta do Lorde Marina has increased since 2006, from around 300 to more than 400 arriving vessels annually [23]. Ships arriving at Quinta do Lorde's marina usually come from Porto Santo, Portugal's mainland, the Canary Islands, the Mediterranean, or Northern Europe [23,43], and are primarily recreational boats.

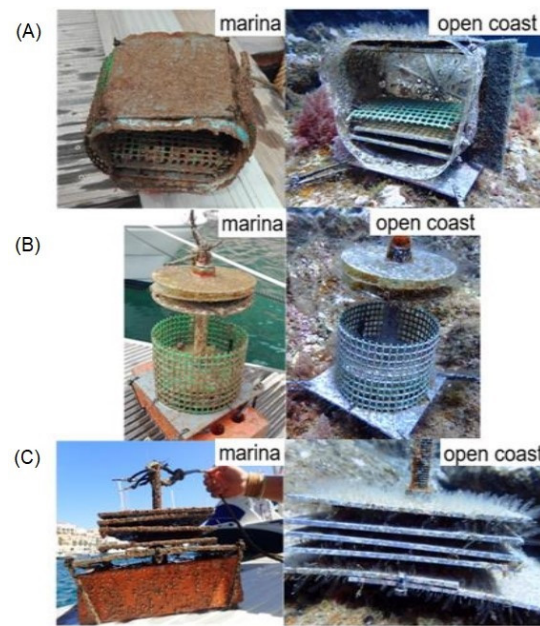


**Figure 1.** (A) Madeira Archipelago with the location of Quinta do Lorde Marina at the south coast of Madeira Island, where the present study was carried, and (B) a diagram showing the experimental setup of the three different prototypes, of which three replicates each were deployed on both locations (inside and outside the marina).

## 2.2. Prototype Construction

To conduct this experimental study, we constructed three different passive sampling methods (prototypes) with various settlement materials (e.g., PVC plates, plastic bottles, CDs, and collector mesh). The material for the construction was selected according to the premises of maintaining the lowest cost and simplicity to assemble. To provide a more extensive availability of surfaces for colonization and to see possible differences in settlements of shadow-preferring organisms, two different orientations (plates facing upwards and downwards) were established for the plates set. In total, we constructed 18 experimental units, corresponding to three replicates of each of the three different prototypes for two different environments. Nine units (three replicates of each prototype) were deployed in each environment (inside the marina and outside on the open coast, Figures 1B and S1).

The prototype box consisted of a 5 L plastic bottle, the upper part removed, serving as a “shelter”. Inside, there were three parallel PVC plates horizontally fixed as a substrate for the settlement of benthic biota. Additionally, an inner plastic net was introduced, protecting the fouling organisms from consumption, and promoting algae growth. Outside of the plastic bottle wall, we attached two additional PVC plates: one served as a base attaching the structure to the bottom of the sea, and the other one was placed at the top of the structure (Figure 2A). This prototype was meant to collect mobile organisms better, but also provide surfaces for sessile biota to grow.



**Figure 2.** The three different prototypes (A–C) placed in the two different environments (marina and open coast).

The prototype CD consisted of a PVC plate as a base and held six CDs, parallelly and horizontally fixed and backboned with a metal rod in the middle. The three lower CD plates were surrounded by a plastic net, protecting the potential recruited fouling communities from consumption. Nuts were used to separate the CD plates equidistantly from each other (Figure 2B). This prototype was supposed to be the most feasible one in terms of financing and assembling.

The prototype PVC was a structure of four horizontal PVC plates fixed to the bottom with a larger PVC plate, backboned with a metal bar in the middle of the plates (Figure 2C). As there was no protective equipment surrounding the plates, this prototype seemed to be the most realistic one to obtain less biased data about the organisms' recruitment. The PVC plates and CDs of all prototypes were previously sanded with sandpaper to provide a better adhesion surface for the settling organisms.

### 2.3. Anchor and Deployment System

All the experimental units were deployed in June 2017 and maintained in the field until October 2017 (4 months). Nine units were placed inside the marina, fixed with a brick that served as ballast to stay at the bottom, and attached to a rope on the pontoon to facilitate the retrieval. The units placed outside the marina were anchored to the rocky bottom with screws (bored into the rocky bottom) tied to and fixed with cable ties. All units were randomly distributed and placed at a 4–6 m water depth. In both locations, the replicates maintained a minimum distance of 1.5–2 m.

### 2.4. Retrieval of the Prototype Units and Laboratory Work

After 4 months, all units were retrieved and transported to the laboratory. In order to collect the mobile fauna, collector mesh bags (size 0.5 mm) were placed over each structure, closed properly, and placed into buckets on land prior to the transport. In the laboratory, all the prototypes were disassembled, and the plates were removed. The plates were inspected under a stereomicroscope (Leica S8APO) on both upward and downward-facing sides. A catalog of all the organisms found was created. The sessile organisms were identified to the species level, if possible, or at least to the genera level, and later classified according to their biogeographic status in Madeira: nonindigenous, native, cryptogenic (unknown origin), or unresolved (species with either insufficient taxonomic resolution or information), based

on scientific literature (e.g., [23,43,46,51–53]). Pictures were taken from each plate (upward and downward) and processed with the image analysis software CPCe (Coral Point Count with Excel extensions [54]) to estimate abundance (species cover in percentage). Each image was divided into  $3 \times 3$  grids of 9 cells and 11 random points, resulting in 99 sampled points per picture (adapted from [46,54]).

Mobile organisms were carefully rinsed off the mesh bags and sieved again to keep only those larger than 0.5 mm. They were preserved in ethanol 96% for their posterior separation and quantification by using light microscopes and dissection, if necessary. In this case, a lower taxonomic resolution was applied to optimize the quantitative analysis of the samples and pragmatism as a strategy to minimize difficulties in identification, as it was considered enough for the study's purpose (i.e., check the capacity of the method to catch also the mobile fraction [55]).

### 2.5. Data Analysis

Data of the sessile biota were analyzed using permutational analysis of variance (PERMANOVA), including three fixed factors: "location" (two levels "inside" and "outside" of the marina), "prototype" (three levels: "Box", "CD", PVC), and "orientation" (two levels: "Up" and "Down"). The mobile organisms' assemblages were analyzed using the same approach but based on a two-way orthogonal model by excluding the factor "orientation". Data were square-root- or fourth-root-transformed if necessary and confirmed by the values of the function of PERMDISP (for testing the homogeneity of the multivariate dispersions) and further by bootstrapping the averages to see whether there was a pattern in the data. Whenever the data showed a significant interaction between the factors, it was separated accordingly for further pairwise comparisons. The estimated percentage cover for each species was used for the analysis of sessile species, while the number of individuals/1000 cm<sup>2</sup> of each taxon was used for mobile biota. Nonmetric multidimensional scaling (nMDS) was performed for graphical representation of the multivariate patterns, and taxa that contributed the most for similarities within groups were identified using SIMPER analysis. The Bray–Curtis dissimilarity was applied for multivariate analyses, whereas the Euclidean distances were used for univariate analyses. These univariate analyses were performed following the same models used for PERMANOVA [56] on the total cover and species richness, as well as percentage cover and number of NIS and native species (including species categorized as unresolved and cryptogenic, following a conservative approach, [51]) and NIS/native ratio (based on the species cover percentage). Primer version 7.0.21 with the add-on PERMANOVA was used for all the statistical analyses [57–59].

## 3. Results

### 3.1. Sessile Fauna

A total of 104 different sessile fouling taxa were found, 16% of which were classified as NIS, 47% as unresolved, 27% as cryptogenic, and 10% as native species (Table S1). In the marina environment, NIS contributed the most, on average (in percentage cover), particularly in the PVC prototype. On the open coast, the pattern of contributions was the opposite, where native species dominated, with the highest average values found in the CD prototype (Figure 3).



Table 1. Cont.

(A) sessile fraction									
Source	df	Native species				NIS			
		Cover (%)		S		Cover (%)		S	
		MS	Pseudo- F	MS	Pseudo- F	MS	Pseudo- F	MS	Pseudo- F
L	1	2046.3	<b>123.28 ***</b>	471.1	<b>44.32 ***</b>	180.9	<b>46.07 ***</b>	11.3	<b>6.59*</b>
P	2	304.7	<b>18.36 ***</b>	196.5	<b>18.48 ***</b>	85.5	<b>21.78 ***</b>	37.3	<b>21.81 ***</b>
O	1	427.2	<b>25.74 ***</b>	281.4	<b>26.47 ***</b>	360.7	<b>91.85 ***</b>	44.9	<b>26.22 ***</b>
LxP	1	127.0	<b>7.65 ***</b>	34.1	<b>3.21 *</b>	5.2	1.32	3.2	1.90
LxO	1	57.6	3.47	53.10	<b>4.99 *</b>	142.0	<b>36.16 ***</b>	8.3	<b>4.85 *</b>
PxO	2	12.2	0.74	15.9	1.50	7.4	1.88	0.7	0.41
LxPxO	2	10.4	0.63	0.8	0.08	4.5	1.15	1.7	1.01
Res	146	16.6		10.6		3.9		1.7	
Total	157								

(B) mobile fraction							
Source	df	Composition		Cover (%)		S	
		MS	Pseudo - F	MS	Pseudo - F	MS	Pseudo - F
		L	1	5197.8	<b>23.56 ***</b>	840.5	0.04
P		629.7	<b>2.86 **</b>	150630.0	6.34 *	2.5	0.85
LxP		196.0	0.89	16944.0	0.71	2.7	0.94
Res	20	220.6		23761.0		2.9	
Total	23						

L = Location; P = Prototype; O = Orientation. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

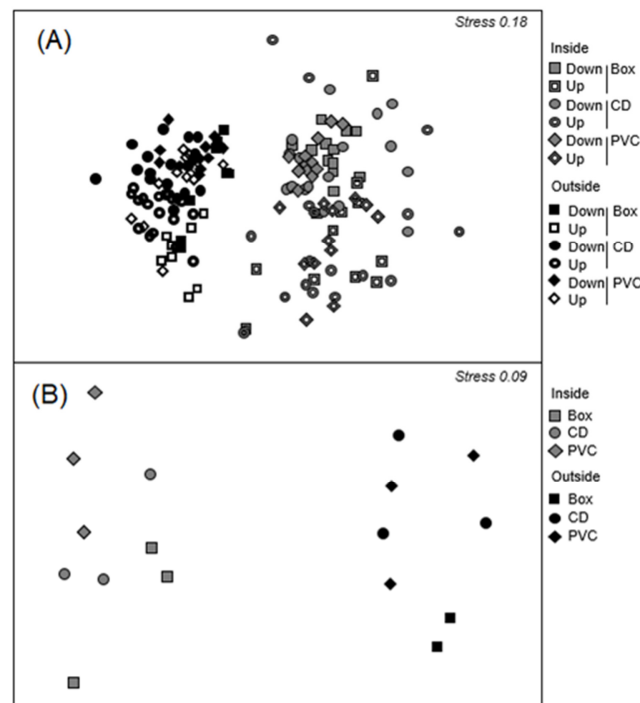


Figure 4. Nonmetric multidimensional scaling (nMDS) representing the structure of (A) sessile community among location, prototype, and orientation levels, and (B) mobile community among location, prototype, based on Bray–Curtis similarities. Data were square-root-transformed (for further details, see Table 1).



SIMPER analyses revealed *Parasmittina alba* Ramalho, Muricy & Taylor, 2011, and *Spirorbis* sp. as the species contributing most to the similarity within the community inside the marina (Table 2A), whereas *Spirobranchus triqueter* Linnaeus, 1758, and *Lithophyllum incrustans* Philippi, 1837, were the taxa that contributed most to the similarity of prototypes located outside the marina, with *S. triqueter* showing considerably lower cover in the box prototypes (Table 2A). These last two species contributed most to similarities within both orientations outside the marina, being *S. triqueter* more abundant in the down orientation and *L. incrustans* in the upper side (Table 2B). In the inner plates, *P. alba* was the species with a higher contribution to similarity within the down orientation, whereas *Spirorbis* sp. and the macroalgae *Neosiphonia sertularioides* (K.W. Nam & P.J. Kang, 2012) and *L. incrustans* were the more relevant for the upper part. Similarities within the prototypes inside the marina ranged from 21%–33%, while those located outside showed greater values (38%–46%), and similarities within the orientations inside the marina ranged from 21% to 39%, while those located outside showed greater values (42%–44%) (Table 2B).

**Table 2.** SIMPER analysis on sessile biota square-root-transformed data shows the average (Av.) taxa’s contribution, with a cut-off of 80%. Bray–Curtis similarity (Sim) for the interactions location (L) × prototype (P) and location (L) × orientation (O). Taxa were categorized as nonindigenous species (NIS), cryptogenic (C), native (N), or unresolved (U) based on literature and credited databases.

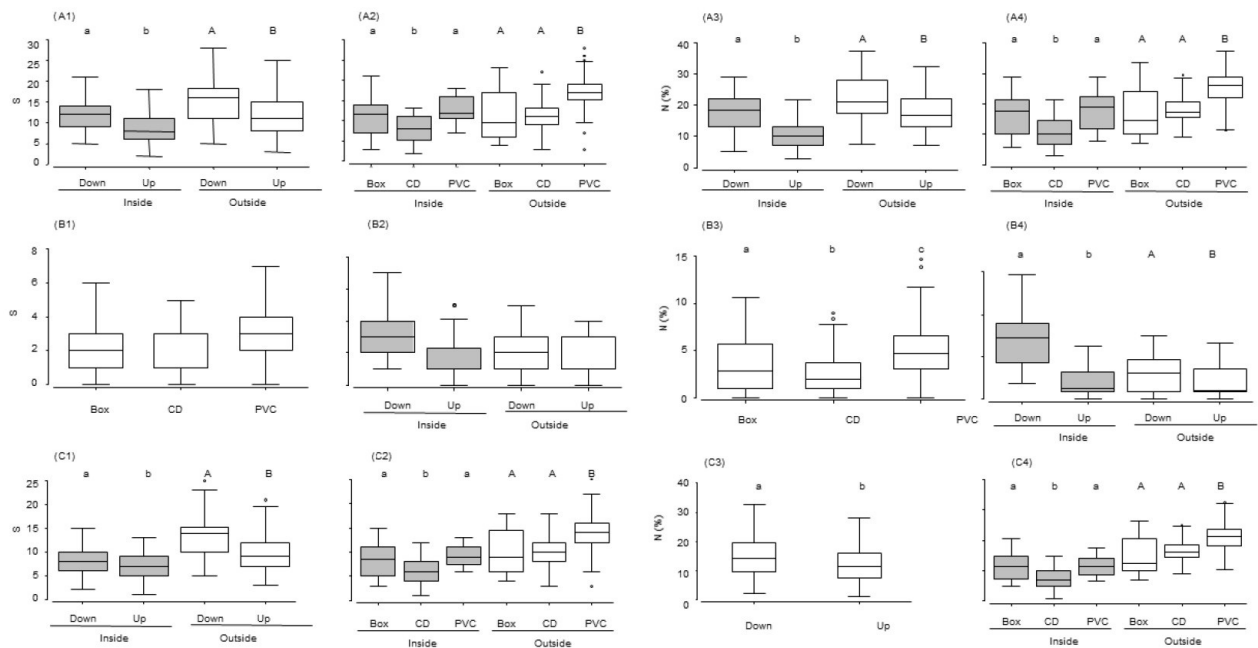
(A)		L × P				
<b>Inside</b>						
Status	Box prototype (Av. Sim = 22.61)	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
NIS	<i>Parasmittina alba</i>	13.93	6.7	0.58	29.65	29.65
U	<i>Spirorbis</i> sp.	3.24	4.23	1.42	18.71	48.36
N	<i>Salmacina dysteri</i>	2.53	2.63	0.44	11.65	60
NIS	<i>Exaíptasia diaphana</i>	0.84	1.39	0.73	6.14	66.14
C	<i>Nolella gigantea</i>	1.64	1.22	0.37	5.39	71.53
N	<i>Lithophyllum incrustans</i>	1.39	1.17	0.63	5.18	76.71
C	<i>Diplosoma listerianum</i>	1.43	0.72	0.36	3.17	79.88
U	<i>Mycale</i> sp.2	1.09	0.66	0.36	2.9	82.78
<b>CD prototype (Av. Sim = 20.62)</b>						
NIS	<i>Parasmittina alba</i>	7.91	6.09	0.59	29.54	29.54
U	<i>Spirorbis</i> sp.	2.8	4.38	0.93	21.23	50.78
N	<i>Lithophyllum incrustans</i>	1.86	2.65	0.67	12.85	63.62
N	<i>Polysiphonia sertularioides</i>	1.09	1.71	0.51	8.31	71.93
C	<i>Diplosoma listerianum</i>	3.29	1.52	0.3	7.38	79.31
N	<i>Salmacina dysteri</i>	0.54	0.94	0.34	4.56	83.87
<b>PVC prototype (Av. Sim = 32.90)</b>						
NIS	<i>Parasmittina alba</i>	15.79	9.71	0.63	29.52	29.52
U	<i>Spirorbis</i> sp.	4.8	5.55	1.32	16.86	46.38
NIS	<i>Exaíptasia diaphana</i>	1.28	3.05	1.06	9.28	55.66
N	<i>Polysiphonia sertularioides</i>	2.81	2.82	0.78	8.57	64.23
N	<i>Salmacina dysteri</i>	1.14	1.88	0.81	5.71	69.95
NIS	<i>Celleporaria inaudita</i>	1.53	1.72	0.69	5.24	75.19
N	<i>Lithophyllum incrustans</i>	0.97	1.51	0.88	4.59	79.78
U	<i>Nemoderma</i>	0.68	1.29	0.64	3.91	83.68
<b>Outside</b>						
<b>Box prototype (Av. Sim = 38.34)</b>						
N	<i>Lithophyllum incrustans</i>	18.01	25.09	1.87	65.44	65.44
N	<i>Spirobranchus triqueter</i>	2.79	4.09	0.8	10.68	76.12
U	<i>Nemoderma</i>	3.04	2.66	0.56	6.94	83.05
<b>Outside CD-prototype (Av. Sim = 42.49)</b>						
N	<i>Spirobranchus triqueter</i>	22.94	18.63	1.23	43.86	43.86
N	<i>Lithophyllum incrustans</i>	17.39	15.35	1.05	36.12	79.98
C	<i>Kirchenpaueria haleciooides</i>	2.68	2.67	1.08	6.28	86.26
<b>Outside PVC-prototype (Av. Sim = 45.92)</b>						
N	<i>Spirobranchus triqueter</i>	19.35	15.88	1.15	34.57	34.57
N	<i>Lithophyllum incrustans</i>	16.13	13.24	1.27	28.84	63.42
C	<i>Cribrilaria radiata</i>	4.78	3.46	0.89	7.54	70.96
C	<i>Kirchenpaueria haleciooides</i>	2.82	1.96	1.26	4.27	75.23
NIS	<i>Parasmittina alba</i>	2.65	1.56	0.67	3.4	78.63
C	<i>Favosipora purpurea</i>	1.41	1.32	1.12	2.87	81.5

**Table 2.** Cont.

<b>(B)</b>		<b>L × O</b>				
<b>Inside</b>						
Status	<b>Down (Av. Sim = 38.67)</b>	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
NIS	<i>Parasmittina alba</i>	22.53	20.9	1.37	54.06	54.06
U	<i>Spirorbis</i> sp.	5.45	6.15	1.49	15.91	69.97
C	<i>Diplosoma listerianum</i>	5.13	3.03	0.5	7.85	77.82
N	<i>Lithophyllum incrustans</i>	1.27	1.1	0.67	2.85	80.67
<b>Up (Av. Sim = 21.15)</b>						
		Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
U	<i>Spirorbis</i> sp.	1.54	4.26	0.88	20.15	20.15
N	<i>Polysiphonia sertularioides</i>	2.46	3.28	0.64	15.49	35.64
N	<i>Lithophyllum incrustans</i>	1.67	2.71	0.71	12.8	48.45
N	<i>Salmacina dysteri</i>	1.7	2.55	0.52	12.07	60.52
NIS	<i>Exaiptasia diaphana</i>	0.91	2.47	0.7	11.67	72.19
NIS	<i>Parasmittina alba</i>	1.23	1.33	0.34	6.31	78.5
U	<i>Nemoderma</i>	1.78	0.88	0.41	4.14	82.64
<b>Outside</b>						
<b>Down (Av. Sim = 42.44)</b>						
		Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
N	<i>Spirobranchus triqueter</i>	26.04	22.24	1.3	52.39	52.39
N	<i>Lithophyllum incrustans</i>	8.61	8.19	1.29	19.3	71.69
C	<i>Cribrilaria radiata</i>	3.06	1.64	0.61	3.86	75.55
C	<i>Kirchenpaueria halecioides</i>	2.05	1.47	0.86	3.47	79.02
U	<i>Nemoderma</i>	1.81	1.22	0.59	2.88	81.9
<b>Up (Av. Sim = 44.34)</b>						
		Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
N	<i>Lithophyllum incrustans</i>	25.44	30	1.98	67.67	67.67
N	<i>Spirobranchus triqueter</i>	9.26	7.03	0.94	15.84	83.51

The univariate analyses demonstrated that the overall species richness (S) was affected by orientation, showing significantly higher values on the downward-facing plates (Figure 5A1). Additionally, the results revealed that there was an interaction between location and type (Table 1A): inside the marina, the box and PVC prototypes recruited more species than the CD prototype, whereas outside, the PVC recruited more species than the two other prototypes (Figure 5A2). Again, there was an effect of the orientation on the cover abundance (Table 1A), showing the plates oriented downwards with higher cover abundances, regardless of the other factors (Figure 5A3). The highest cover of sessile species (N) was shown in the PVC prototype, but also here, spatial variability (significant interaction L × P; Table 1A) was present. The performance of the box and PVC prototypes was similar inside, reaching higher cover abundances than the CD one, whereas outside, the PVC prototype differed and accumulated more cover of species than the other prototypes (Figure 5A4).

Furthermore, our results indicated that the settlement of the NIS and natives (including species categorized as cryptogenic and unresolved) differed: NIS richness and cover were affected by prototype and the interaction between location and orientation (Table 1A). The highest values of NIS richness and cover were found on the prototype PVC and the plates oriented downwards, but in this case, just outside for NIS richness and inside the marina for NIS cover (Figure 5B1–B4). On the other hand, the native species richness was significantly influenced by the interaction between location by prototype and location by orientation (Table 1A). There were no differences in the number and cover of the native species between the prototypes box and PVC inside and box and CD outside, reaching the maximum number of native species in the PVC outside (Figure 5C1,C4). Regarding orientation, significantly more diverse and abundant native species were recruited on the downward-facing plates compared with the plates facing upwards (although pairwise comparisons for native richness showed  $p(\text{perm}) < 0.05$  for plates located inside and  $p < 0.001$  for plates from outside, Figure 5C2,C3). On the contrary, the NIS/native ratio showed similar values for all the prototypes. However, it again demonstrated spatial variation for orientation, with significantly higher values on the downward-facing plates from the inside location (Table 1A).



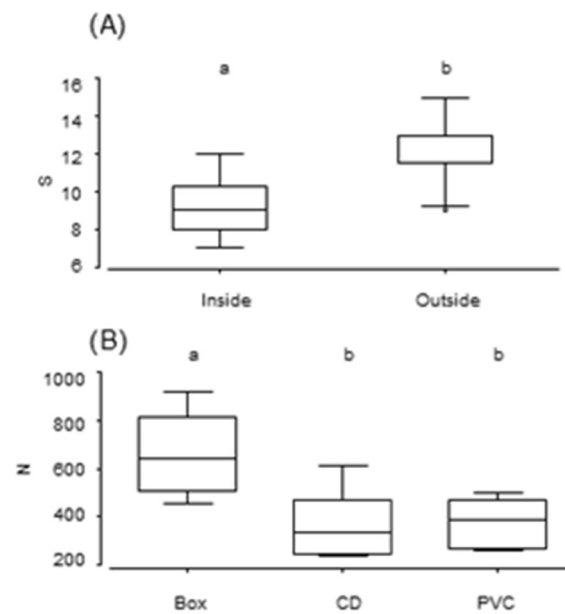
**Figure 5.** Sessile fouling number of species (S) and relative abundance (N in % cover) in each of the considered categories, (A) full sessile fouling community, (B) NIS and (C) native (A1, A3, B2, B4, and C2), show the comparison of levels for  $L \times O$  interaction, while A2 and A4, C1 and C4 show the comparison of levels for  $L \times P$  interaction. B1 and B3 show differences among prototypes, and C3 shows differences between orientations; boxplot with the first quartile, median and third quartile, minimum and maximum as whiskers and points as outliers. Different letters indicate significant differences in pairwise interactions (see Table 1).

### 3.2. Mobile Fauna

A total of 19 different taxa of mobile fauna were found, highlighting the ubiquity of the Gastropoda, Gammaridea, Bivalvia, Isopoda, Caridea, Decapoda, Caprellioidea, and Polychaeta, present in all the structures and both locations (Table S2). By contrast, several groups were almost exclusively found on the outside location, such as the Echinoidea and Sipunculida, which were only found inside the PVC and the box structure, respectively. The groups Pycnogonida, Platyhelminthes, and Gobiesciformes were only detected outside, but on all the prototypes (Table S2).

Multivariate analyses revealed that both prototype and location significantly affected the composition and structure of the mobile communities (Table 1B; Figure 4B). SIMPER analyses revealed Gammaridea as the taxa most contributing to the similarity and dissimilarity of the groups, being the more abundant inside and for all the prototypes. The taxon Isopod revealed an important contribution being the first taxon in abundance for the outside location and the second taxon for all the groups. By contrast, Mysidacea reached a relevant contribution to the mobile biota inside, being almost absent in the outside location. Especially in the PVC prototypes, Polychaeta was a taxon with a meager contribution compared with the box but maintained some representation in the CDs.

The number of taxa of the mobile fauna was mainly affected by location, with no differences among prototypes (Table 1B; Figure 6A). On the contrary, the factor prototype was most affecting the abundance of mobile fauna, with the box showing greater values (Table 1B; Figure 6B).



**Figure 6.** Mobile fauna (A) number of species (S) and (B) abundance (N equals the number of individuals per structure) in each of the considered factors, orientation and prototype (each three replicates), respectively; boxplot with the first quartile, median and third quartile, minimum and maximum as whiskers and points as outliers. Different letters indicate significant differences in pairwise interactions (see Table 1).

#### 4. Discussion

In this study, we deployed different prototypes inside a marina and on the open coast to evaluate their capacity to collect samples that better represent the benthic communities and the nonindigenous species. Additionally, we wanted to test the feasibility of the settling methods regarding their sampling efficiency under different environmental contexts. Overall, we found that the community composition showed strong spatial variation, that is, inside versus outside the marina. According to our results, the biofouling community contained a higher species richness outside the marina. Additionally, the sessile community composition was further influenced by orientation and then prototype. There were four main taxa that contributed most to the differences between the prototypes located inside the marina and the open coast: two native species (*S. triqueter* and *L. incrustans*), especially abundant outside; *Spirorbis* sp. (unresolved); and the NIS *P. alba*, more abundant on the plates located inside the marina. A different trend was observed with the mobile biota: they showed higher values on the outside location and were more abundant on the box prototype, independent of the location with the gammarids showing a strong dominance in the community composition.

Similarly, and going a bit further, the settlement of the NIS and native species (richness and cover) differed among the prototypes and orientation: The PVC prototype showed a higher NIS cover and richness in the sessile fraction. On the downward-facing plates, NIS and native species' cover and richness were higher compared with the upward-facing plates, although this orientation effect was stronger inside the marina. The orientation of the plates was mainly distinguished by one NIS, *P. alba*, which was prevalent on the downward-facing plates, and three native species: *S. triqueter*, *Spirorbis* sp., and *Salmacina dysteri*, (Huxley, 1855). These results were partly similar to the NIS/Native ratio, which was significantly higher on the inside location on the plates facing downwards.

Our results showed the influence of the location on species recruitment. This prevalence of NIS on fouling plates inside marinas was also observed by Gestoso et al. [46] and Tamburini et al. [36]: these studies also found a dominant cover of NIS on plates deployed inside a marina, contrasting with a higher native species cover and richness on the outside plates. Our results agreed with the taxonomic groups mainly contributing to the similarity

of the community inside the marina collected by Ferrario et al. [60] *P. alba* and spirorbids, as well as *S. triqueter* and *L. incrustans* from outside.

An explanation for the spatial variation could be that NIS tend to settle on artificial substrates, which are more commonly found inside marinas [18,53,61], or they colonize novel empty substrates more efficiently than native species. Their potential adaptation to novel habitat conditions would promote their settlement success in harbor environments during the invasion process's early stages and increase their chances of overcoming the subsequent stages (i.e., the establishment of self-sustaining populations and spread [62]). These findings also go in line with the study of Airoidi et al. [56], which further outlined the effect of exposure: in sheltered artificial habitats, the abundance of NIS was double (compared with the respective exposed habitat), whereas native ascidians preferred exposed artificial rocky habitats over sheltered ones. Another explanation could be the level of pollution: the higher level of pollution inside marinas seems to have less impact on NIS, leading to losses of sensitive species and reductions in native diversity [63]. This higher resistance, together with the higher propagule pressure recorded inside marinas (e.g., [64]), could also explain the obtained higher NIS cover versus the higher recruitment of native species documented outside the marina [60,65].

In our study, several NIS were found in both locations: *Parasmittina alba*; *Distaplia corolla* Monniot F., 1974; *Schizoporella pungens* Canu & Bassler, 1928; *Branchiomma bairdi* McIntosh, 1885; *Exaiptasia diaphana* Rapp, 1829; *Ectopleura crocea* Agassiz, 1862; *Mycale (Carmia) senegalensis* Lévi, 1952; and *Asparagopsis armata* Harvey, 1855 (Table S1), suggesting that they had spread from or to the marina of Quinta do Lorde and to other ports. They most likely arrived at the port of Funchal and spread further, that is, via recreational vessels ([21,22,64]). Shipping has been documented as the primary vector for introducing new species to Portugal's mainland and its islands, mainly to coastal ecosystems, which are more vulnerable to introductions [52]. The mechanisms of secondary spread are not well understood yet, but it is known that NIS can attach to the submerged part of shipping vessels, be transported from harbors to protected areas, and perhaps spread even further by anthropogenic activities [19,21,66]. A study by Png et al. [18] also verified the spread of NIS inside the marinas to aquaculture facilities in Madeira, and added the role and importance of ship traffic in the recruitment and dispersion of NIS.

Our findings revealed the presence of NIS inside the marina for both orientations, particularly for the downwards-facing plates. Literature about the community differences between downward- and upward-facing plates is scarce, but as our results show, the NIS/native species cover ratio is differed, showing a significantly higher NIS/native ratio on the downward-facing plates inside the marina. This information is novel, as studies using, for instance, the SERC protocol [36] or similarly deployed PVC plates (e.g., [44]) only investigated the downward-facing sides of the plates. Studies using the Autonomous Reef Monitoring System (ARMS), taking photographs of both sides' facing plates, have not reported any significant differences in communities on the different sides of their plates [67]. As expected, the presence of light on deployed structures had important influence on fouling, with macroalgal species settling on the upward-facing plates, as they require sunlight for photosynthesis, whereas most shadow-preferring organisms on the downward-facing plates belong to the animal kingdom [68]. Among the NIS found, the majority were bryozoans and tunicates, which would explain the higher species cover of NIS on the downward-facing sides (and inside the marina). Nevertheless, as the propagule supply of the different species was unknown, the higher species cover could also be related to that.

A possible limitation of our study could be the temporal aspect. As this was a preliminary pilot study, sampling took place only once, retrieving all the structure plates at the same time. Studies show a seasonal effect on the recruitment of NIS [32,69], and more frequent samplings might lead to more conclusive results, particularly when the propagule supply is low and differs among species. Shorter and more frequent sampling events (for instance, 1–2 weeks) would therefore collect data about the start and end of the

reproductive seasons of the different species, as suggested by Ma et al. [70]. On the other hand, Rondeau et al. [71] concluded that temporal patterns do not affect that much, but spatial patterns of the community compositions of NIS do. Their study showed that the communities settle differently inside the marina and that NIS tend to accumulate mainly in the inner part (with the highest distance to the entry of the marina). As we placed our prototypes in the middle-inner part of the marina, this could, according to Rondeau et al. [71], perhaps explain our varying results or overestimate the NIS presence, as this was the part that showed the highest variation in community composition.

Regarding effectiveness, the PVC prototype showed the highest overall and native species richness and cover (more evident in outside location) for the sessile species. On the contrary, the box prototype demonstrated a higher abundance of mobile fauna, but not a higher number of taxa. The apparent sessile species' preference for the PVC prototype could be promoted by the sanded PVC plates, which were rougher and, therefore, easier to settle on for sessile species that usually prefer hard and rough surfaces [72,73]. The CDs were also sanded, but this procedure was less effective, and they tended to maintain a smoother surface and be potentially underneath the lower recruitment values. In the case of the box prototype, with only one species' entrance, the lower species richness and cover detected could derive from less favorable conditions for settling biofouling species, that is, more difficult access to the PVC plates. Although some previous studies have documented poor efficiency of the PVC plates compared with other passive monitoring methods ([32]), our study seems to confirm their particular ability to recruit the sessile fraction of the benthic community. Settlement plates have been extensively used in the marine realm as monitoring devices, especially in marinas and harbors (e.g., [37,74]). The different prototypes tested here also affected the settlement of the NIS, possibly altering the invasion process, with PVC collecting greater NIS cover and richness. Moreover, these plates are usually used to assess the sessile benthic community, although some recently conducted trials focused on sampling mobile biota (e.g., [62]).

On the other hand, for the mobile fauna, the box prototype was revealed to be more efficient. However, it is possible to overestimate the abundances of some particular groups (i.e., Gammaridae) compared with the CD and PVC prototypes. In particular, the CD prototype maintained the best balance between the counts of mobile individuals and the fouling species richness documented, providing better performance. With our prototypes, mobile fauna could also be easily monitored, which is crucial for the early detection of NIS introductions and the management of biological species, as mobile biota also have a strong potential to impact the recipient ecosystem [29]. Therefore, as the prototype can affect the patterns of species recruitments, specifically by showing different abilities to characterize the sessile and mobile fraction, the selection of the passive system should be considered accordingly for the purpose of the monitoring program.

## 5. Conclusions

In conclusion, and as a general recommendation from our study, a combination of different features from the prototypes seemed to be the best option for sampling the whole benthic community. In particular, a mixture of the PVC prototype with the design of the CD one could rise as the best affordable option to monitor local diversity adequately in both enclosed and open coast environments.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse11020264/s1>. Table S1: Sessile taxa were recorded in the different prototypes inside and outside the marina. The presence of organisms is shown by the dots "•". Taxa were categorized as nonindigenous species (NIS), cryptogenic (C), native (N), or unresolved (U) based on literature and credited databases; Table S2: Mobile fauna recorded in the different prototypes inside and outside the marina. The presence of organisms is shown by the dots "•"; Figure S1: The CD prototype deployed at the marina, fixed with a rope to the pontoon (A), and all three prototypes deployed at the open coast (B).

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