



Two better than one: The complementary of different types of artificial substrates on benthic marine macrofauna studies

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ABSTRACT

Non-destructive methodologies based on the use of artificial substrates (AS) for quantitative sampling of macrofauna have been used to solve sampling problems in complex benthic environment, such as rocky reefs. The macrofauna assemblages of two different types of AS (dendritic and crevice), at two different locations were studied. The main goal was to evaluate the complementarity of dendritic and crevice AS when sampling the macrofauna associated with rocky environments, in two scenarios: within the same location and between locations. With this approach, we intend to contribute to the development of a non-destructive sampling methodology based on AS. The hypotheses tested were that i) the assemblages associated with each type of AS would differ between them and, ii) regardless of AS type, assemblages would differ between locations. Our results revealed significant differences after three months between the macrofauna assemblages from dendritic and crevice substrates in both locations and differences between locations irrespective of the AS type used. Hence, due to the high number of species that only were attracted by each type of AS and the species settlement preferences, our findings have shown the complementarity of the two types of AS (dendritic and crevice). This suggests that the mixed use of AS could be the best approach to non-destructive standard monitoring programs based on benthic marine macrofauna.

1. Introduction

Species are seldom evenly distributed in nature (Whittaker and Levin, 1977), leading to clear spatial assemblages heterogeneity at small and large spatial extents. Thus, ecological studies and environmental monitoring programs should be designed to take this problem into account (Underwood, 1992) to obtain valid biological data. In a given geographic location, species composition changes over space and time. The resulting assemblages will be a function of available space (Norderhaug et al., 2002), recruitment processes, bioengineers (Torres et al., 2015), level of perturbation, oceanographic conditions (Chemello and Milazzo, 2002; Edgar and Klumpp, 2003), and availability of colonizers (Underwood and Chapman, 2006), among other factors. Local spatial variability, which is a main source of variation during sampling on marine benthic habitats, will also influence the final result (Edgar, 1991a; Underwood and Chapman, 2006).

The implementation of the European Marine Strategy Framework Directive (MSFD; 2008/56/EC) has to deal with sampling problems to evaluate the 'Good Environmental Status' (GES). Substrate, bioengineers, and species composition are three basic descriptors on the evaluation of the GES (Danovaro et al., 2016; Rice et al., 2012). Furthermore, GES is not a synonym for unaltered Environmental Status. When variation in the status is contained within sustainable limits, it can be called GES, either because the pressures do not modify natural diversity and ecological processes or because the recovery from perturbations is so fast that guarantees the maintenance of attributes within their range of historical natural variation of the ecosystem (Rice et al., 2012). Thus, the lack of knowledge about benthic processes, and the difficulties of monitoring at the descriptor scale, make the evaluation of what constitutes the GES for sea-floor integrity a difficult task.

For monitoring purposes, benthic organisms are suitable to monitor ecological variations in marine ecosystems, as they are conspicuous, and

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with a fast response to changes (Aguado-Giménez et al., 2015; Salas et al., 2006; Van Der Linden et al., 2015). Benthic macrofauna plays a significant role in ecological interactions with other organisms like meiofauna, bacteria, plankton, and still regulates nutrient cycling in soft-sediments ecosystems (Santibañez-Aguascalientes et al., 2020). These organisms become good bioindicators for their long-life spans, limited mobility, and well-defined traits that allow local disturbances to be detected (Van Der Linden et al., 2015). However, sampling complex benthic environments, as is the case of many rocky and biogenic reefs, has serious standardization problems that can compromise unbiased procedures.

Rocky and biogenic reefs present specific difficulties for comprehensive surveys of biodiversity because of their complexity and variability. The presence of cracks, cavities, smooth and rough surfaces promote different degrees of physical heterogeneity (Baronio and Bucher, 2008; Cacabelos et al., 2016; Hutchings and Weate, 1977). Besides, the biological heterogeneity is motivated by the presence of different species of macroalgae, mussel beds, sponge mats or cnidarian banks, (Commito et al., 2008; Veiga et al., 2016; Yakovis et al., 2007). This heterogeneity affects the structure of the assemblages in a direct way (creation of new habitats, microenvironments, resources) and indirectly (alteration of the intensity of biotic processes such as predation and competition) (Underwood and Chapman, 1996). In general, equivalent systems (at the same geographic location and depth range) with large macroalgae communities or with well-developed biogenic structures, harbour a greater abundance and diversity of organisms than systems without them (Curras et al., 1993). However, these systems are relatively difficult to sample because of their heterogeneity and complexity. Thus, many questions arise about which methods are best for sampling these spatially variable communities.

Crevice-fauna includes macroinvertebrates and some fishes that use substrate cavities temporarily or permanently (Baronio and Bucher, 2008). Some are opportunistic colonizers of existing spaces whereas others are bioeroders, creating their particular cavities. All of them are a relevant part of the reef food web (Klumpp et al., 1988) for certain reef carnivores, including fishes and molluscs (Kohn and Nybakken, 1975). Monitoring the crevice-fauna assemblages is not an easy task due to the difficulty of using quantitative, replicable, and standard sampling methodologies. This unreachability explains the low level of knowledge of the fauna associated with the crevices of the substrate (Baronio and Bucher, 2008). Quadrant scraping or airlift pumps have been used as crevice-fauna sampling methods; however, these methods do not ensure real standard replicable sampling (Benson, 1989; Stanulla et al., 2016; porbjörnsson et al., 2018).

Concerning the role of the biogenic structures, on temperate rocky reefs, large native perennial macroalgae species are important elements in the marine food webs and are also considered good indicators of the ecological status of coastal habitats, since most of them are very sensitive to anthropogenic disturbances (Filbee-Dexter and Wernberg, 2018). Macroalgae are naturally abundant habitat builders for many organisms of ecological and economic importance, acting simultaneously as primary producers and bio-engineers (Christie et al., 2009; Cremades et al., 2004). Therefore, habitat-forming organisms such as perennial macroalgae are good targets for monitoring studies for benthic macrofauna (Davenport et al., 1999), as they can support stable populations that can be more sensitive to changes and integrate the impacts over long periods (Christie et al., 2009). Also, annual macroalgae have been seen as passive sampling devices as long as colonization is linked to the space available and complexity (Torres et al., 2015). Nevertheless, variations in local availability, its vertical distribution (more present in the intertidal and within the first 10 or 15 m, depending on the water turbidity), or the eventual destruction of fragile or threatened communities (Bulleri and Chapman, 2010; Firth et al., 2016), seriously constrains its over-all usage as sampling “devices”.

Standard methodologies based on the use of Artificial Substrates (AS) to macrofauna quantitative sampling have been developed (Cacabelos

et al., 2010; Carreira-Flores et al., 2020; Christie et al., 2009; Edgar, 1991a, 1991b; García-Sanz et al., 2014; Norderhaug et al., 2002). However, such methods may not sample adequately crevice-fauna (Baronio and Bucher, 2008). Therefore, the use of a methodology that can capture a representative sample of the surrounding benthic assemblages, that, i) be sensitive to changes regarding the availability of organisms in the water mass (as proxies to environmental changes), ii) be standard and able to provide not only presence/absence, but also quantitative data, is desirable but not easily reachable.

AS have been reported as a viable non-destructive alternative to traditional destructive sampling methods, provided that the structural complexity associated with the biogenic elements present is somehow mimicked by the AS (e.g. Cacabelos et al., 2010; Carreira-Flores et al., 2020; Edgar, 1991a) or provided that the crevices of the reef are mimicked by the AS (Baronio and Bucher, 2008; Plaisance et al., 2011; Zimmerman and Martin, 2004). AS have been described as a valid method to obtain quantitative and comparative data on crevice-fauna (Baronio and Bucher, 2008). Their use would make even more sense beneath the depth range of occurrence of large macroalgae, where rocky reefs are mostly colonized by crevice-fauna. AS can provide artificial habitats similar to the essential features of the natural habitat but are accessible to researchers and allow for a standardized sampling effort, enabling direct comparisons between different locations, environmental conditions, and studies (Baronio and Bucher, 2008).

Recognized worldwide monitoring programs based on AS do not have natural assemblages reference data and assume that the collected fauna is enough to record the community composition on the seafloor in a standardized way (ie. Obst et al., 2020; Pavludi et al., 2021; Ransome et al., 2017). Although these studies do not aim to obtain a perfect image of the assemblages and only aim to obtain data in a standard way, the absence of natural references in these studies is, at least, controversial. However, some studies support a successful mimicry of the natural crevices and dendritic substrates' assemblages by the AS, which is generally more successful over time (Guerra-Castro and Cruz-Motta, 2014; Yakovis et al., 2007). Even so, as a disadvantage, the effectiveness of sampling through the use of AS is not instantaneous; it takes time to allow the process of colonization by benthic organisms (Cacabelos et al., 2010; García-Sanz et al., 2014).

It is commonly accepted that AS are not supposed to precisely mimic natural assemblages (McGuinness, 1989), as AS are designed to represent basic features of the natural substrates, like structure, complexity, or surface (Bulleri and Chapman, 2010; Cacabelos et al., 2010). It was also reported that the same type of substrate may give different results, depending on the time of the year of the deployment (García-Sanz et al., 2014), and different kinds of substrates are colonized by different assemblages of benthic fauna (Cacabelos et al., 2010; Carreira-Flores et al., 2020; Schreider et al., 2003; Vázquez-Luis et al., 2008). Studies on the effectiveness of AS to monitor benthic assemblages have always focused on independent comparisons of dendritic (Cacabelos et al., 2010; Christie et al., 2009; Edgar, 1991a; Norderhaug et al., 2002) and crevice substrates (Baronio and Bucher, 2008; Obst et al., 2020; Yakovis et al., 2007; Zimmerman and Martin, 2004). Nevertheless, no study of the use of AS to monitor macrobenthic communities had made a direct comparison of the complementarity of using dendritic and crevice AS together. This approach could obtain more accurate data of the assemblages and species ecology in a rocky reef.

Therefore, AS appear as a simple solution, supported by scientific literature, that may overcome the main problems related to standardization and difficulties of sampling heterogeneous substrates, since they are “low cost” and easily implementable. With known features, and able to be developed with a deliberated target in mind, AS are adequate to remove the variability of sampling devices in quantitative sampling (Baronio and Bucher, 2008; Edgar, 1991a; Norderhaug et al., 2002).

The present study aims to evaluate the complementarity of dendritic and crevice AS when sampling the macrofauna associated with rocky environments, using two different scenarios as case studies: within the

same location and between two locations with different depths and macroalgae assemblages (sensu Carreira-Flores et al., 2020). By its nature, we expect that dendritic and crevice AS give rise to different ecological niches, that may promote the recruitment of complementary fauna. Two types of AS made of polyethylene plastic were designed, dendritic plastic AS provide physical support for fauna that use arborescent structures, such as macroalgae and colonial cnidarians, and a crevice plastic AS simulates the fissures of the rocky substrates and/or the complexity of the algae rhizoids, where sediments can accumulate. By considering the diversity and composition of the full invertebrate assemblage associated with both types of AS, we will test if i) the assemblages associated with each type of AS differs between them and, ii) regardless of AS type, the assemblages present in two locations can be used to discriminate them. With this preliminary study, we expect to contribute with our results to the development of a non-destructive sampling methodology for marine benthic macrofauna studies. The 2 locations were chosen as a proof of concept in order to demonstrate the usability of the methodology in the discrimination of different environmental conditions.

2. Material and methods

2.1. Study area

This study was done in the northern region of the Galician coast (NW Iberian Peninsula) at two coves of the Ría de Ferrol: Enseada de Laxe (43°27'48.8"N, 008°17'13.5"W; 3m depth) and Enseada de San Cristovo (43°27'53.8"N, 008°18'00.7"W; 11m depth) (Fig. 1), with the same water temperature pattern. The ría is subjected to a semidiurnal mesotidal regime and the tides are the main factor that controls the exchange of water with the ocean through a narrow channel, resulting in a complex current regime that determines a wide variety of sedimentary substrates (Cunha, 2017; DeCastro et al., 2003). Attending to these features, Enseada de Laxe is considered a "Semisheltered shore" and Enseada de San Cristovo a "Semiexposed shore" (Cremades et al., 2004). Enseada de Laxe is located in the medium part of the ría, and is characterized by communities of large brown sublittoral macroalgae *Treptacantha baccata* (S.G. Gmelin) S. Orellana and M. Sansón, 2019 and

Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, 2006 and seagrass beds of *Zostera* subg. *Zostera marina* Linnaeus, 1753. The Enseada de San Cristovo is located at the outer part of the ría, and is characterized by the presence of kelp forests of *Laminaria ochroleuca* Bachelot de la Pylaie, 1824 and *Laminaria hyperborea* (Gunnerus) Foslie, 1884 intercalated with *Halidrys siliquosa* (Linnaeus) Lyngbye, 1819.

2.2. Sampling design

Two types of AS made of polyethylene plastic were designed for the experiment. First, a dendritic type AS (ASMS_1 in Carreira-Flores et al. (2020)) named as D-type in this manuscript, were used to mimic a complex macroalgae species like *Treptacantha baccata*. Second, a crevice type AS (named C-type in this manuscript) was used to simulate not only the crevices and fissures of a rocky reef but also the secondary complexity of a biogenic structure (maerl, algae rhizoids, inside space of a massive sponge, coral reef ...) by inserting a PVC leaved turf inside the cavities of concrete bricks (Fig. 2).

The NW Iberia is characterized by strong seasonal upwelling events, that synchronize the larvae recruitment processes during spring and early summer (Queiroga et al., 2007; Wooster et al., 1976). Consequently, to cover up the upwelling active season and considering that the main goal of this study was to evaluate the complementarity of both types of AS, a short duration experience was envisaged and three months of colonization was considered (based on Carreira-Flores et al., 2020; Baronio and Bucher, 2008; Obst et al., 2020).

Therefore, on March 27, 2018 and at each location on the Ría de Ferrol, five D-type structures were fixed with a spacing of 60 to 43 cm between them in a concrete plate with 60x60 × 5cm (Fig. 3a). Furthermore, five C-type structures were folded within the same number of cavities in concrete bricks (40x20 × 10 cm) (Fig. 3b). All AS were deployed and recovered by scuba diving; recovery was made after three months on June 29, 2018.

Each substrate was carefully introduced in a 0.5 mm mesh bag and closed before being released from the substratum with a scraper, to prevent small motile organisms to escape. Subsequently, the mesh bag was introduced in a hermetic plastic bag (Fig. 4). In the laboratory,

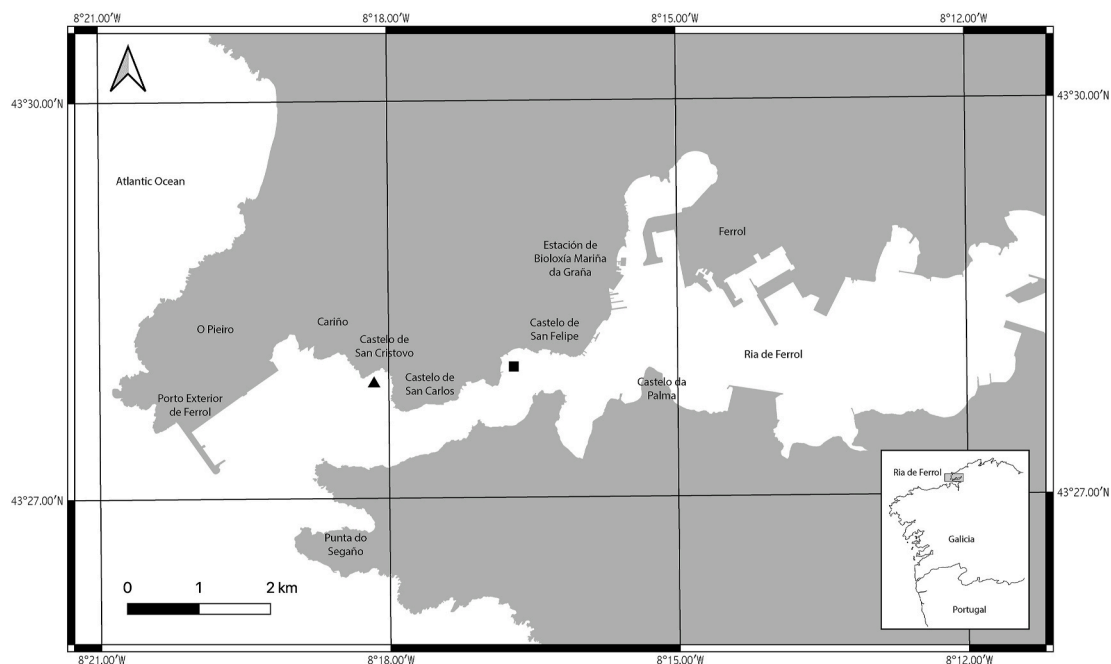


Fig. 1. Location of the two study areas in the Ría de Ferrol (Galician coast, Spain) ▲ Enseada de San Cristovo; ■ Enseada de Laxe. (Basemap: OpenStreetMap redrawn in QGIS 3.16, 2020).

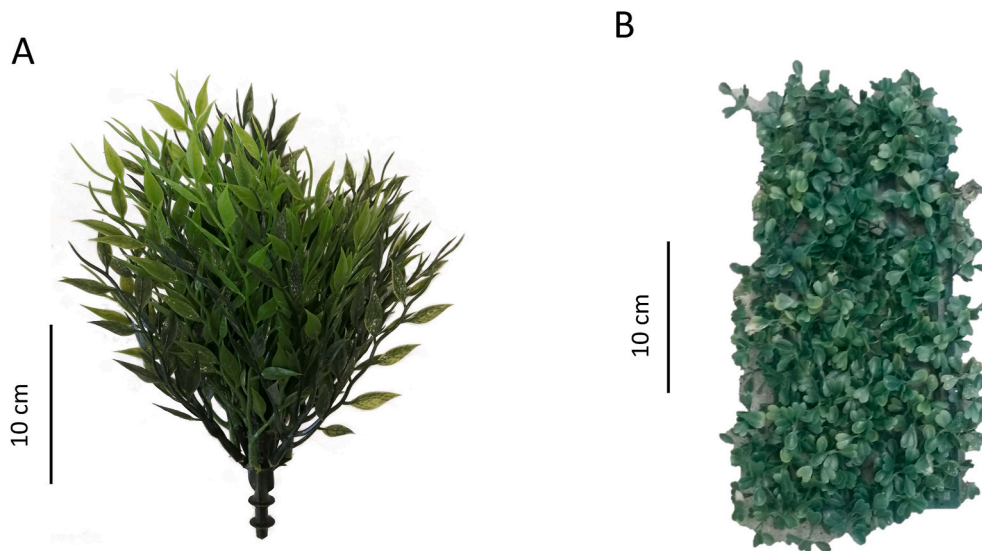


Fig. 2. Artificial substrates used in this study: dendritic, D-type (A) and crevice, C-type (B).

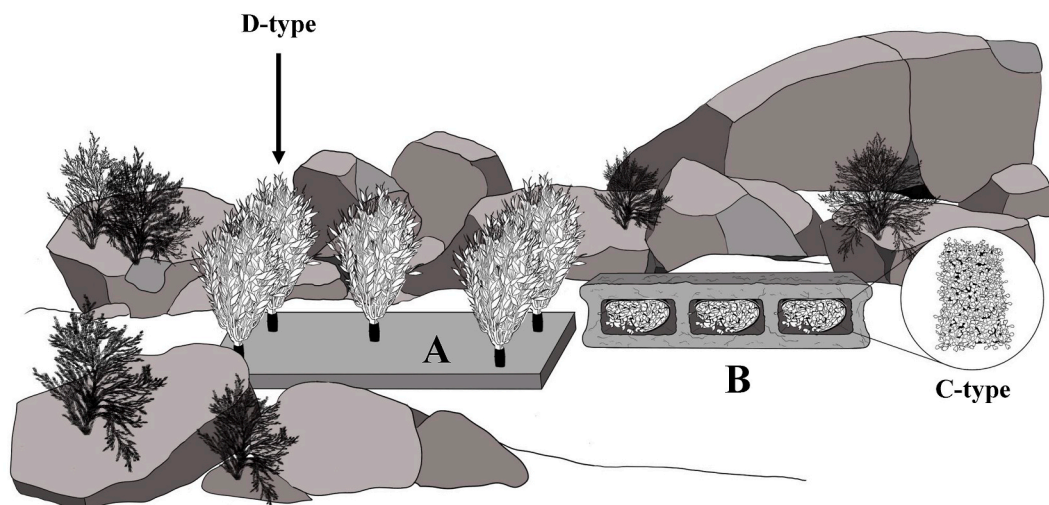


Fig. 3. Experimental deployment of D-type (A) and C-type (B) within the natural algae settlement of a rocky reef. D-type substrates were attached to a concrete plate (60x60 × 5 cm) and 5 C-type were introduced in the cavities of 2 concrete bricks (40x20 × 10 cm).

associated macrofauna was washed off in filtered saltwater by shaking each AS vigorously through 0.5 mm sieves. The macrofauna was fixed in 99% ethanol before being quantified and identified to the lowest taxonomic level feasible (usually species level).

2.3. Colonization volume and complexity

Total Volume Available of the D-type AS was estimated assuming the cone shape of the substrate, and for the C-type AS was estimated given the cubic forms of the substrate. The Colonization Volume Available was the result of the difference between the Total Volume and the volume occupied by the substrate (estimated by its displaced water volume) (Carreira-Flores et al., 2020).

To calculate the real surface of the AS, both sides of one branch of the AS was digitized, and the surface was measured with ImageJ analysis software (Rasband, 1997). The result derived from the ImageJ was multiplied by the number of branches to obtain the total value. Both types of substrates were 23 cm height, but D-type has a surface of 1773 cm² and C-type of 1326 cm². The available space for colonization was

6050 cm³ and 1000 cm³ for D-type and C-type, respectively.

2.4. Data analyses

Data were analysed using multivariate techniques to test the proposed hypotheses. The number of taxa, density of individuals, and diversity (Simpson index) of macrofaunal assemblages were calculated and plotted in R environment v 3.6.0 (Packages Vegan and Lattice) (R Core Team, 2019). Non-parametric permutational multivariate analysis of variance [PERMANOVA (Anderson, 2017);] was used to test hypotheses about differences of macrofaunal assemblages. Analyses were based on Bray-Curtis dissimilarity matrixes from square-root transformed density data to obtain a more 'balanced' view of the assemblages by reducing the influence of the most numerous taxa (Clarke and Gorley, 2001). Two hypotheses were tested: 1) the assemblages of the two types of AS would differ between them and, 2) regardless of the AS type used, the associated assemblages would reflect the local conditions (D-type assemblages of Enseada de Laxe would differ from D-type assemblages in Enseada de San Cristovo, and C-type assemblages of Enseada de Laxe

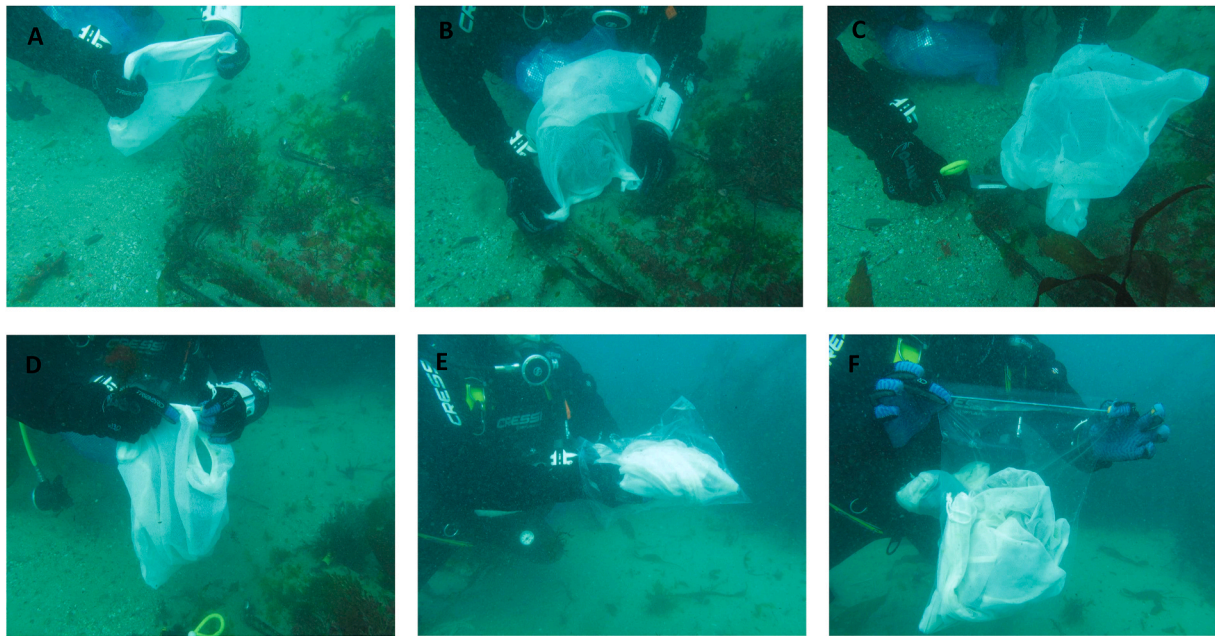


Fig. 4. D-type recovery procedure. A: mesh bag approach; B: AS coverage; C: AS release; D: mesh bag closure; E–F: mesh bag introduction into the hermetic plastic bag.

would differ from C-type assemblages in Enseada de San Cristovo). For both hypotheses, a two-way PEMAANOVA model was used considering the factors: type of substrate (fixed, 2 levels, D-type vs. C-type) and location (random, 2 levels, Enseada de Laxe vs. Enseada de San Cristovo). When appropriate, a posteriori multiple comparisons were made to test for differences between/within groups for pairs of levels of factors. The tests were based on 999 unrestricted random permutations of

data. Changes on the assemblages were visualized by non-metric multidimensional scaling (nMDS; 100 restarts) and clustering based on Bray-Curtis dissimilarity matrix on square-root transformed density data. Analyses of multivariate dispersion were also done to test for homogeneity of dispersions between the type of substrate (PERMIDISP (Anderson, 2017)). For the assemblages analysis, density data (multiplied $\times 10^3$ to obtain easy-interpretation numbers) of amphipods,

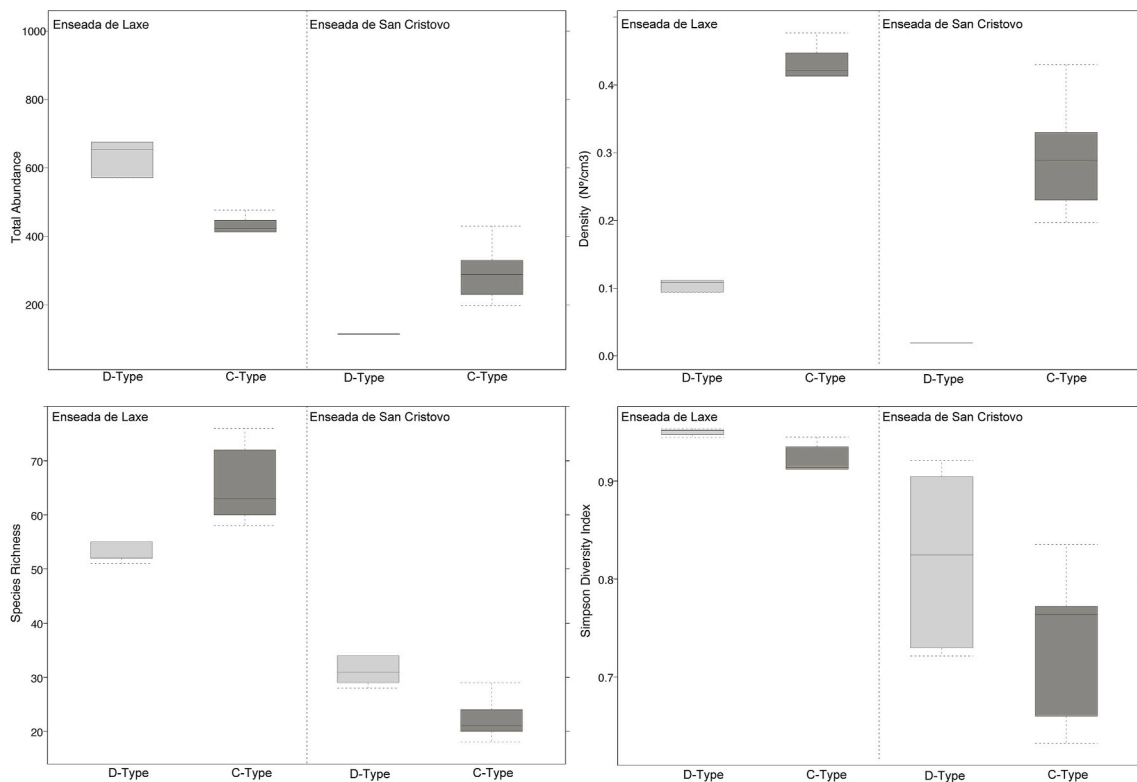


Fig. 5. Total abundance per substrate (number of individuals), Density per substrate (number of individuals/cm³), Species Richness per substrate (number of species), and Diversity (Simpson index) of macrofaunal assemblages associated with D-type and C-type at Enseada de Laxe and Enseada de San Cristovo.

gastropods, decapods and polychaetes were analysed separately, taking as relevant species the ones that contribute greater than 5% for the total abundance of each group. A separate nMDS plot based on density data was done for each major group: amphipods, gastropods, decapods, and polychaetes. The SIMPER procedure was used to identify the percentage contribution of each taxon to the Bray-Curtis dissimilarity between the averages of groups. Taxa were considered important if their contribution to percentage dissimilarity was $\geq 5\%$. Multivariate analyses were conducted using Primer v.6 (Clarke and Gorley, 2001) with PERMANOVA + add-on (Anderson et al., 2008).

3. Results

3.1. Macrofauna associated with artificial substrates in Enseada de Laxe

The total number of individuals was consistently higher in D-type (693 ± 174 individuals per substrate; available volume higher than in C-type) but the density was higher in C-type (0.41 ± 0.06 ind/cm³) (Fig. 5). C-type harbor the higher species richness (65.8 ± 7.83) but D-type shown the highest diversity value (Simpson index) (0.949 ± 0.01) (Fig. 5). Amphipods accounted for 63.73% of the total number of individuals in D-type, followed by decapods (19.25%) and isopods (3.54%). Malacostracans accounted for 74.69% of the individuals in C-type; Amphipoda (37.86%), Decapoda (28.49%), and Tanaidacea (8.34%) (Table 1). D-type and C-type shared 68 species, but D-type supported 30 exclusive species and C-type 52.

The dissimilarity value between D-type and C-type was 60.29%, being Crustacea Eucaridea [*Psidium longicornis* (Linnaeus, 1967)], Crustacea Peracarida [*Gammarella fucicola* (Leach, 1814) and *Apseudopsis latreillii* (Milne Edwards, 1828)] the main taxa responsible for this dissimilarity (39.57% according to the SIMPER analysis).

3.2. Macrofauna associated with artificial substrates in Enseada de San Cristovo

At Enseada de San Cristovo, although Simpson diversity remained higher in the D-type (0.949 ± 0.01), the total number of individuals and the density (ind/cm³) were consistently higher in C-type (295.2 ± 91.23 individuals per substrate, 0.295 ± 0.09 ind/cm³) (Fig. 5). D-type harbor the higher species richness (33 ± 6.41), and C-type had only 22.4 ± 4.27 (Fig. 5). Decapods accounted for 41.54% of the individuals in D-type, followed by amphipods (14.15%) and Gastropods from the order Littorinomorpha that accounted for 9.79%. Amphipods and decapods accounted for 87.72% of the individuals in C-type, being amphipods the ones which contributed more with 71.79% (Table 1). D-type and C-type shared 30 species, but D-type supported 37 exclusive species and C-type 24.

The dissimilarity between D-type and C-type was very high (91.79%). The amphipods from the family Gammaridae: *Gammarus oceanicus* Segerstråle, 1947, *Gammarus locusta* (Linnaeus, 1758) and *Gammarus crinicornis* Stock, 1966 and the amphipod *G. fucicola* contributed for 63.09% of the dissimilarity; the decapod *P. longicornis* contributed for 13.54% (SIMPER analysis).

Table 1

Cumulative average \pm SE (number of individuals $\times 10^3$) of the most abundant amphipods, gastropods, decapods and polychaetes in Enseada de Laxe and Enseada de San Cristovo.

Group	Enseada de Laxe						Enseada de San Cristovo					
	D-type			C-type			D-type			C-type		
Amphipoda	72.910	\pm	14.890	156.000	\pm	37.855	3.001	\pm	1.180	211.800	\pm	100.612
Gastropoda	6.793	\pm	1.133	45.000	\pm	37.901	4.155	\pm	1.834	10.800	\pm	7.791
Decapoda	22.688	\pm	9.098	118.200	\pm	52.628	8.969	\pm	6.214	47.000	\pm	37.229
Polychaeta	2.935	\pm	1.058	36.600	\pm	11.283	2.275	\pm	1.295	7.200	\pm	10.710
Total	114.394	\pm	28.849	413.200	\pm	64.360	21.204	\pm	9.240	295.200	\pm	91.262

3.3. Total assemblages associated with artificial substrates

Results of PERMANOVA analysis for the composition of assemblages showed a significant interaction between substrate and location (Table 2). Results of pair-wise tests displayed significant differences between substrates at each location (Table 3); significant differences were not influenced by the dispersion or variability among replicates (Permdisp, $p < 0.05$). The nMDS showed a clear differentiation between locations and substrates (Fig. 6). The clustering showed two main branches that correspond to the two coves and, at each location, also showed clear differences between D-Type and C-Type substrates (Fig. 7).

3.4. Analysis of relevant groups

The pair-wise tests results for amphipods, gastropods, decapods, and polychaetes assemblages showed significant differences between substrates at each location (Table 4), showing the same trend of the results of the pair wise tests of the total assemblages (Table 3). The nMDS for each group distinguished between D-Type and C-Type substrates.

At Enseada de Laxe, *Gammaropsis maculata* (Johnston, 1828) and amphipods from the genus *Erichthonius* were more abundant in the D-type, while the genus *Microdeutopus*, and *G. fucicola* were more abundant in the C-type (Table 5). This agrees with the SIMPER analysis: *Ampithoe ramondi* Audouin, 1826, *Ampithoe valida* Smith, 1873, *G. maculata*, *Erichthonius brasiliensis* (Dana, 1853), *Erichthonius difformis* H. Milne Edwards, 1830, *Gammaropsis maculata* (Johnston, 1828), *Aora gracilis* (Spence Bate, 1857) and *Pleonexes helleri* (Karaman, 1975) contributed to 65.86% of the similarity between D-type substrates. *G. fucicola*, *Phthisica marina* Slabber, 1769 and *Microdeutopus damnoniensis* (Spence Bate, 1856) contributed to 58.11% of the similarity between C-type. *G. fucicola*, *M. damnoniensis*, and *Microdeutopus chelifer* (Spence Bate, 1862) contributed for 47.44% of the dissimilarity between dendritic and crevice substrates. At Enseada de San Cristovo *G. maculata* is more abundant in D-type and *G. fucicola* and amphipods from the genus

Table 2

Summary of PERMANOVA results for assemblages of Artificial Substrates at Enseada de Laxe and Enseada de San Cristovo. Two-factor Permanova model based on 999 unrestricted random permutations of density data: type of substrate (fixed, 2 levels, D-type vs. C-type) and location (random, 2 levels, Enseada de Laxe vs. Enseada de San Cristovo). When the number of unique permutations were lower than 30, Monte Carlo p-values were considered *, $P < 0.05$; **, $P < 0.01$.

Source	df	MS	Pseudo-F	Unique Perms	P (MC)
Substrate	3	5187.2	2.5539	6	0.141
Location	1	11405	23.524**	998	0.001
Substrate x Location	1	2759.8	6.591**	997	0.001
Residual	16	725.46			
Total	19				
PERMDISP			P(perm):		0.001

Table 3

Results of Post-hoc pair-wise test (for **D-type**, **C-type** at Enseada de Laxe and Enseada de San Cristovo) After a two-factor Permanova model based on 999 unrestricted random permutations of density data: type of substrate (fixed, 2 levels, D-type vs. C-type) and location (random, 2 levels, Enseada de Laxe vs. Enseada de San Cristovo). *, $P < 0.05$; **, $P < 0.01$.

Groups	Enseada de Laxe		Enseada de San Cristovo	
	t	Unique Perms	t	Unique Perms
D-type, C-type	3.5152**	126	3.3702**	126
Groups	D-type		C-type	
	t	Unique Perms	t	Unique Perms
E. de Laxe, E. de San Cristovo	3.7463**	126	4.0073**	126

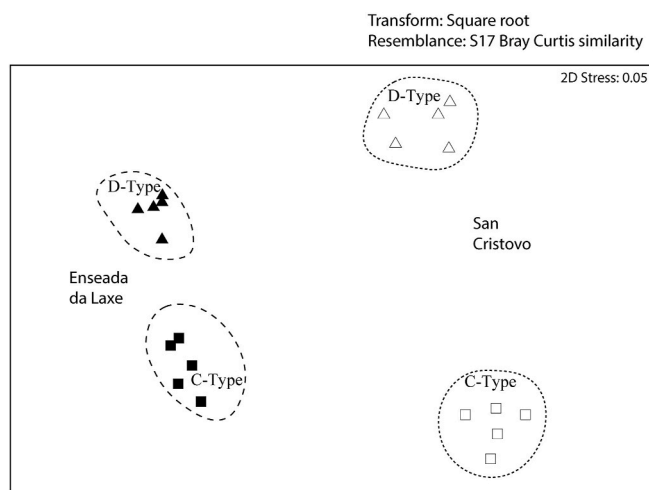


Fig. 6. Multidimensional scaling (nMDS) plot based on Bray-Curtis dissimilarity measures of macrofaunal assemblages density data of D-type and C-type at Enseada de Laxe and Enseada de San Cristovo.

Gammarus abundance is consistently higher in C-type. SIMPER analysis also showed that like at Enseada de Laxe, *G. maculata* is the species with the highest percentage of contribution (55.27%) for the similarity of the D-Type substrates; and *G. oceanicus*, *G. locusta* and *G. crinicornis* contributed to 95.07% of the similarity between C-Type substrates. *G. oceanicus*, *G. locusta*, *G. crinicornis* and *G. fucicola* contributed to 97.52% of the dissimilarity between substrate types. Between locations, the genus *Ericthonius* and *Ampithoe* were not abundant at Enseada de San Cristovo. Amphipods from the genus *Gammarus* were not found in Enseada de Laxe, *G. maculata* was the more abundant in D-type and *G. fucicola* was more abundant in C-type, in both locations (Table 5). The nMDS showed a clear differentiation between locations and substrates (Fig. 8). However, the Bray-Curtis dissimilarity measure was shorter between D-type and C-type at Enseada de Laxe; even so, the assemblages between D-type and C-type at Enseada de Laxe show significant differences (Table 4).

The total abundance of gastropods were consistently higher in C-type in both locations (Table 6), supporting exclusive species like *Onoba semicostata* (Montagu, 1803) and *Hirtomurex squamosus* (Bivona e Bernardi, 1838), and gastropods from the genus *Tritia*. At Enseada de Laxe, the genus *Steromphala* and *Bittium* were more abundant in C-type (Table 6); *Jujubinus striatus* (Linnaeus, 1758) and *Rissoa parva* (da Costa, 1778) contributed for 57.15% of the similarity of C-type (SIMPER analysis). *J. striatus*, *T. incrassata*, *R. parva*, *Pusillina inconspicua* (Alder, 1844), *Rissoa guerini* Récluz, 1843 and *Steromphala umbilicalis* (da Costa, 1778) contributed to 65.27% of the dissimilarity between D-type and C-

type. At Enseada de San Cristovo, gastropod assemblages showed the same trend as at Enseada de Laxe and the genus *Tritia* was also more abundant in C-type than in D-type. The SIMPER analysis showed also that *S. umbilicalis*, *Tritia nitida* (Jeffreys, 1867), *Tritia reticulata* (Linnaeus, 1758), and *T. incrassata* contributed to 42.67% of the dissimilarity between D-type and C-type. The distribution of gastropods followed the same trend as the amphipods, allowing for the differentiation of both location and substrates (Fig. 8).

P. longicornis and *Eualus pusiolus* (Krøyer, 1841) contributed to 82.24% of the dissimilarity between D-type and C-type at Enseada de Laxe (SIMPER analysis). In this location, the diversity of decapods is higher than at Enseada de San Cristovo. *P. longicornis* was the most common decapod in both locations and *Porcellana platycheles* (Pennant, 1777) was present only in C-type (Table 7). The nMDS showed a clear separation between D-type and C-type substrates, as well as between locations (Fig. 8).

Polychaetes from the genus *Harmothoe* and *Platynereis dumerilii* (Audouin and Milne Edwards, 1833) were the most abundant taxa at Enseada de Laxe in both substrates (Table 8), although with higher abundances in C-type, and contributing to 92.65% of the similarity in D-type and 74.71% in C-type (SIMPER analysis). At Enseada de San Cristovo, polychaetes from the genus *Platynereis* and *Harmothoe* were more abundant in D-type, *Phyllodoce lineata* (Claparède, 1870) was present only in C-type. The SIMPER analysis reinforced also the differences between AS types, giving a high value of dissimilarity (96.45%) between them. The PERMANOVA results showed no significant differences between locations ($t = 1.123$; $P(\text{perm}) = 0.123$) when considering the polychaetes group as well as the nMDS (Fig. 8). However, in the nMDS, D-type assemblages of both locations overlapped, and C-type assemblages were separated and it was only possible to distinguish locations by C-type polychaetes assemblages.

4. Discussion

Our results demonstrate the complementarity of the two AS types tested in sampling the benthic macrofauna, associated with hard substrates. This supports both hypotheses stated initially. Macrofauna assemblages collected were always significantly different between D-type and C-type at both locations. Regardless of AS type used, locations were always differentiated based on macrofauna assemblages.

One of the advantages of using AS is related to sustainability because it's a non-destructive sampling method. Previous studies have shown that AS attracts most of the elements of the mobile invertebrate fauna in the nearby area and that assemblages associated with AS are sensitive to local variation in environmental conditions (Edgar, 1991b; Russo, 1990). Our results agree with Cacabelos et al. (2010) and Carreira-Flores et al. (2020), showing a high colonization level for both AS types. For D-type, the results were similar to the ones obtained by Norderhaug et al. (2002), amphipods and gastropods were the primary colonizers, reflecting its horizontal dispersal patterns associated with its mobility capabilities. In our case, this affirmation may be extended for crevice habitats. As mentioned above, the importance of the nearby landscape in shaping the diversity and structure of the invertebrate assemblages (Jungerstam et al., 2014) by the surrounding motile macrofauna is an important driver in the AS colonization process in rocky environments (Edgar, 1991b; Norderhaug et al., 2002).

The difficulties associated with the standard sampling of crevice fauna have resulted in a lack of knowledge of this part of the benthic assemblages, and had led that group relatively unstudied (Baronio and Bucher, 2008). Our results showed a clear differentiation between macrofauna assemblages of D-type and C-type AS, agreeing with Baronio and Bucher (2008) and Plaisance et al. (2011) that reported that AS are an effective way of sampling a wide variety of crevice-fauna. Baronio and Bucher (2008) reported who the crevice assemblages change over time and several taxa were only found after more extended periods. Hence, the 3 months colonization period possibly represents some

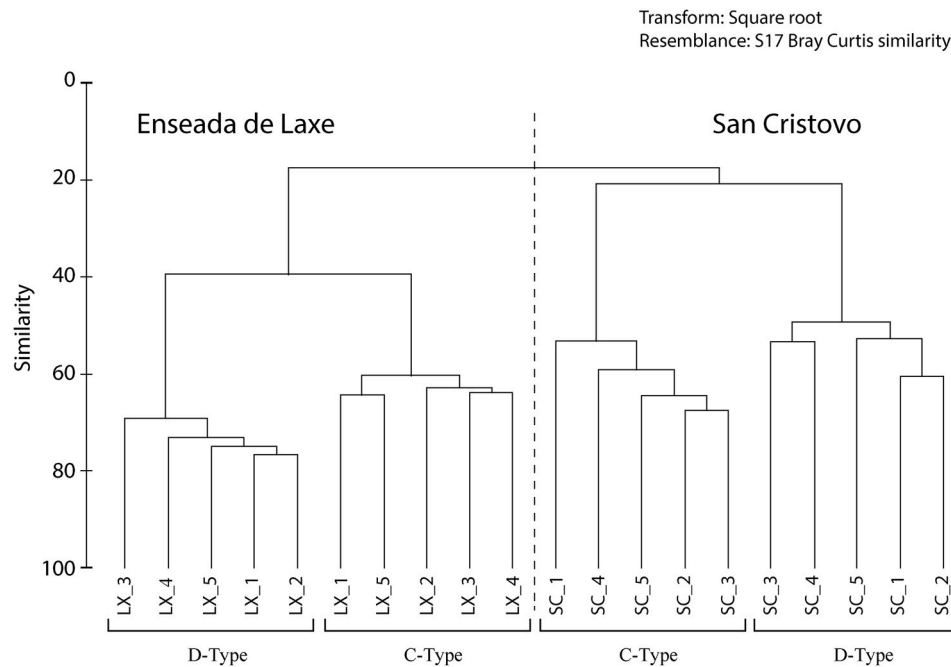


Fig. 7. Clustering (Aggregation method: Group average) based on Bray-Curtis dissimilarity measure of macrofaunal assemblages of D-type and C-type at Enseada de Laxe (LX) and Enseada de San Cristovo (SC).

Table 4

Results of Post-hoc pair-wise test for D-type, C-type separated by relevant groups. Based on the two-factor Permanova model based on 999 unrestricted random permutations of data: type of substrate (fixed, 2 levels, D-type vs. C-type) and location (random, 2 levels, Enseada de Laxe vs. Enseada de San Cristovo) *, $P < 0.05$; **, $P < 0.01$.

Groups	Amphipods				Gastropods			
	Enseada de Laxe		Enseada de San Cristovo		Enseada de Laxe		Enseada de San Cristovo	
	t	Unique Perms	t	Unique Perms	t	Unique Perms	t	Unique Perms
D-type, C-type	3.6143**	126	3.3969**	126	2.2863**	126	1.8461*	126

Groups	Decapods				Polychaetes			
	Enseada de Laxe		Enseada de San Cristovo		Enseada de Laxe		Enseada de San Cristovo	
	t	Unique Perms	t	Unique Perms	t	Unique Perms	t	Unique Perms
D-type, C-type	3.9438**	126	4.1381*	126	3.8146*	126	1.8814*	126

opportunistic colonizers of new crevices instead of mature assemblages, it may be verified with the presence of some opportunistic species like *Malacoceros fuliginosus* (Claparède, 1868) (Dauvin et al., 2017). Although many natural large crevices are relatively permanent structures (related to invertebrate life spans), others can be more ephemeral due to the effects of storms that may expose crevices that have been previously buried or filled by large sessile animals (Baronio and Bucher, 2008).

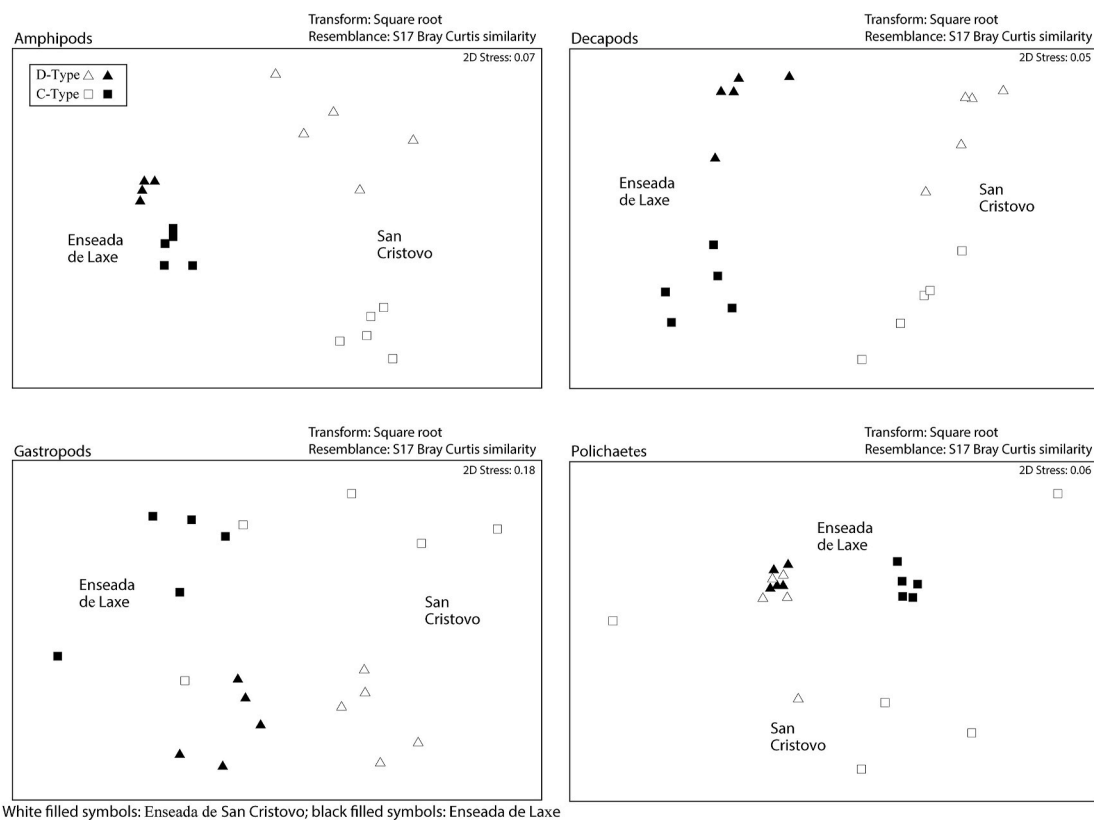
Sampling methods based on AS can differentiate between benthic macrofauna assemblages to disentangle the influence of specific factors of the sampling sites, as depth or exposure at a local scale (Cacabelos et al., 2010). They can also be used to monitor changes at a global scale (Obst et al., 2020). In this study, D-type and C-type AS supported significantly different macrofaunal assemblages between locations subjected to different environmental conditions. These differences were reflected in the density of the collected organisms, species richness, and diversity indexes. The differences can be explained either by the different depth conditions, depth can play an important role in the regulation of the AS assemblages (Balazy and Kuklinski, 2017); or by differences in the macroalgae assemblages that are going to shape the macrobenthic diversity (Jungersam et al., 2014). Enseada de Laxe is

settled by communities of *T. baccata*, *S. latissima* and *Z. marina* and Enseada de San Cristovo is characterized by the presence of kelp forest of *L. ochroleuca* and *L. hyperborea* intercalated with *H. siliquosa*. Carreira-Flores et al. (2020) reported significant differences between the assemblages associated with the populations of *T. baccata* and *H. siliquosa* of Enseada de Laxe and E. de San cristovo. Therefore, significant differences in macrofauna assemblages may also be explained by the different macroalgae communities at both locations.

Because of their different structure, D-type and C-type AS harbor different assemblages of benthic macrofauna. Despite the absence of a general pattern, the species distribution may be explained by their habitats preference. Suspension feeder amphipods of the genus *Ericthonius* that are more abundant in D-type, are reported as typical associated with species of macroalgae and sponges exposed to stronger water flow, providing a larger suspension feed niche (Fenwick, 1976; Hoare and Peattie, 1979). Omnivore amphipods are “specialists” of crevice rocky reefs (Chapman, 2002). In the same line, our results showed that *G. fucicola* and amphipods of the genus *Gammarus* were more abundant in C-type AS. Amphipods from the genus *Gammarus* reached their maximum values of abundance in C-type AS. Similar to our results, several studies concerning the ecology of *G. locusta* in the Atlantic Coast

Table 5Density average \pm SE ((number of individuals/cm³) $\times 10^3$) of the most abundant amphipods in Enseada de Laxe and Enseada de San Cristovo.

Species	Enseada de Laxe					Enseada de San Cristovo				
	D-type		C-type			D-type		C-type		
<i>Gammariopsis maculata</i>	9.233	\pm	4.966	3.400	\pm	1.517	0.824	\pm	0.521	0.400 \pm 0.548
<i>Aora gracilis</i>	7.321	\pm	5.724	8.800	\pm	5.848	0.099	\pm	0.090	0.200 \pm 0.447
<i>Erichthonius brasiliensis</i>	7.222	\pm	2.253	0.800	\pm	1.304	0.099	\pm	0.147	0.000 \pm 0.000
<i>Ampithoe rubricata</i>	5.540	\pm	2.292	6.800	\pm	3.421	0.099	\pm	0.147	0.000 \pm 0.000
<i>Erichthonius difformis</i>	4.419	\pm	2.493	1.200	\pm	1.304	0.000	\pm	0.000	0.000 \pm 0.000
<i>Ampithoe ramondi</i>	4.353	\pm	2.078	4.800	\pm	3.114	0.099	\pm	0.090	0.000 \pm 0.000
<i>Ampithoe valida</i>	4.320	\pm	0.674	4.000	\pm	4.062	0.066	\pm	0.090	0.200 \pm 0.447
<i>Pleonexes helleri</i>	4.254	\pm	1.362	4.000	\pm	2.739	0.132	\pm	0.215	0.000 \pm 0.000
<i>Monocorophium acherusicum</i>	3.858	\pm	1.643	8.200	\pm	5.848	0.000	\pm	0.000	0.000 \pm 0.000
<i>Gammarella fucicola</i>	0.000	\pm	0.000	52.800	\pm	9.524	0.429	\pm	0.779	21.600 \pm 33.254
<i>Microdeutopus damnoniensis</i>	0.066	\pm	0.090	11.000	\pm	9.487	0.000	\pm	0.000	0.000 \pm 0.000
<i>Microdeutopus chelifer</i>	0.099	\pm	0.221	8.400	\pm	6.229	0.000	\pm	0.000	0.000 \pm 0.000
<i>Phthisica marina</i>	2.275	\pm	0.943	8.000	\pm	2.345	0.000	\pm	0.000	0.000 \pm 0.000
<i>Dexamine spinosa</i>	2.803	\pm	0.747	2.800	\pm	1.643	0.165	\pm	0.165	0.000 \pm 0.000
<i>Gammarus oceanicus</i>	0.000	\pm	0.000	0.000	\pm	0.000	0.363	\pm	0.722	129.400 \pm 55.446
<i>Gammarus locusta</i>	0.000	\pm	0.000	0.000	\pm	0.000	0.231	\pm	0.430	37.200 \pm 15.450
<i>Gammarus crinicornis</i>	0.000	\pm	0.000	0.000	\pm	0.000	0.132	\pm	0.138	19.200 \pm 14.906
Total amphipoda	72.910	\pm	14.890	156.000	\pm	37.855	3.001	\pm	1.180	211.800 \pm 100.612

**Fig. 8.** Multidimensional scaling (nMDS) plot based on Bray-Curtis dissimilarity measure of amphipods, gastropods, decapods and polychaetes assemblages of D-type and C-type at Enseada de Laxe and Enseada de San Cristovo.

of the Iberian Peninsula showed that *G. locusta* could be found “frequently under stones between seaweeds and in sandy mud” (Den Hartog, 1964) and inhabit preferably crevices of rocky substrates with large macroalgae (Marques, 1989). Costa and Costa (1999) also reported the exponential behavior of *G. locusta* recruitment ecology.

Our results showed that *P. longicornis* was the most abundant decapod in both AS types. Accordingly, *P. longicornis* represents the major component of the biomass and abundance in the crustacean communities of Northern Europe (Robinson and Tully, 2000; Sampedro et al., 1997). Rocky substrates and other solid structures provide suitable

habitats for this decapod, which is also a less competitive feeding strategy species compared with to other species of the same Order (Sampedro et al., 1997). Finally, our results agree with Robinson and Tully (2000) who reported that the densities of this decapod are extremely high in crevices of complex rocky reefs.

In the Eastern Atlantic, a wide range of benthic macrofaunal organisms, including prosobranch gastropods, colonize artificial substrates as recruits and/or as adults (García-Sanz et al., 2012). García-Sanz et al. (2014) also showed different assemblages of gastropods between locations, based on abundance differences. In both

Table 6Density average \pm SE ((number of individuals/cm³) x10³) of the most abundant gastropods in Enseada de Laxe and Enseada de San Cristovo.

Species	Enseada de Laxe						Enseada de San Cristovo					
	D-type			C-type			D-type			C-type		
<i>Jujubinus striatus</i>	2.572	\pm	1.605	3.800	\pm	7.396	0.000	\pm	0.000	0.400	\pm	0.894
<i>Tritia incrassata</i>	1.319	\pm	0.954	3.600	\pm	2.966	0.264	\pm	0.250	2.400	\pm	3.286
<i>Rissoa parva</i>	0.594	\pm	0.445	2.600	\pm	4.219	0.297	\pm	0.411	0.400	\pm	0.548
<i>Pusillina inconspicua</i>	0.594	\pm	0.361	3.400	\pm	2.608	0.231	\pm	0.276	0.200	\pm	0.447
<i>Onoba semicostata</i>	0.000	\pm	0.000	4.000	\pm	3.391	0.000	\pm	0.000	0.400	\pm	0.894
<i>Hirtomurex squamosus</i>	0.000	\pm	0.000	3.000	\pm	3.162	0.000	\pm	0.000	0.200	\pm	0.447
<i>Bittium reticulatum</i>	0.231	\pm	0.188	2.800	\pm	3.114	0.132	\pm	0.138	0.800	\pm	1.304
<i>Steromphala umbilicalis</i>	0.132	\pm	0.138	2.800	\pm	3.564	0.594	\pm	0.276	1.800	\pm	2.049
<i>Bittium latreillii</i>	0.132	\pm	0.138	2.400	\pm	2.302	0.231	\pm	0.250	0.400	\pm	0.548
<i>Steromphala cineraria</i>	0.165	\pm	0.233	2.000	\pm	1.225	0.165	\pm	0.202	0.200	\pm	0.447
<i>Rissoa guerini</i>	0.066	\pm	0.090	0.600	\pm	0.894	0.528	\pm	0.271	0.200	\pm	0.447
<i>Setia pulcherrima</i>	0.099	\pm	0.147	1.800	\pm	1.789	0.264	\pm	0.250	0.200	\pm	0.447
<i>Tricolia pullus</i>	0.000	\pm	0.000	0.000	\pm	0.000	0.264	\pm	0.250	0.200	\pm	0.447
<i>Tritia reticulata</i>	0.000	\pm	0.000	0.800	\pm	1.095	0.066	\pm	0.090	0.800	\pm	1.304
<i>Tritia nitida</i>	0.000	\pm	0.000	0.200	\pm	0.447	0.000	\pm	0.000	0.800	\pm	0.447
<i>Tritia pygmaea</i>	0.132	\pm	0.295	2.000	\pm	1.000	0.000	\pm	0.000	0.600	\pm	1.342
Total gastropoda	6.793	\pm	1.133	45.000	\pm	37.901	4.155	\pm	1.834	10.800	\pm	7.791

Table 7Density average \pm SE ((number of individuals/cm³) x10³) of the most abundant decapods in Enseada de Laxe and Enseada de San Cristovo.

Species	Enseada de Laxe						Enseada de San Cristovo					
	D-type			C-type			D-type			C-type		
<i>Eualus cranchii</i>	8.574	\pm	2.437	4.600	\pm	2.881	0.000	\pm	0.000	0.000	\pm	0.000
<i>Pisidia longicornis</i>	7.914	\pm	5.671	90.000	\pm	40.755	8.508	\pm	6.140	44.000	\pm	35.951
<i>Eualus pusiolus</i>	3.397	\pm	1.767	9.000	\pm	2.915	0.066	\pm	0.147	0.000	\pm	0.000
<i>Hippolyte varians</i>	0.627	\pm	0.684	0.000	\pm	0.000	0.231	\pm	0.188	0.000	\pm	0.000
<i>Pagurus bernhardus</i>	0.066	\pm	0.090	0.600	\pm	0.894	0.000	\pm	0.000	0.000	\pm	0.000
<i>Porcellana platycheles</i>	0.000	\pm	0.000	1.400	\pm	1.949	0.000	\pm	0.000	3.000	\pm	1.414
<i>Macropodia linarezi</i>	0.033	\pm	0.074	0.000	\pm	0.000	0.000	\pm	0.000	0.000	\pm	0.000
Total decapoda	22.688	\pm	9.098	118.200	\pm	52.628	8.969	\pm	6.214	47.000	\pm	37.229

Table 8Density average \pm SE ((number of individuals/cm³) x10³) of the most abundant polychaetes in Enseada de Laxe and Enseada de San Cristovo.

Species	Enseada de Laxe						Enseada de San Cristovo					
	D-type			C-type			D-type			C-type		
<i>Platynereis dumerilii</i>	2.110	\pm	0.957	12.000	\pm	2.449	0.627	\pm	0.394	0.000	\pm	0.000
<i>Harmothoe</i> sp	0.330	\pm	0.387	5.000	\pm	3.391	0.462	\pm	0.411	0.000	\pm	0.000
<i>Harmothoe imbricata</i>	0.264	\pm	0.147	2.600	\pm	0.548	0.363	\pm	0.357	0.200	\pm	0.447
<i>Spirorbis spirorbis</i>	0.132	\pm	0.215	5.400	\pm	7.797	0.495	\pm	1.016	0.000	\pm	0.000
<i>Notomastus latericeus</i>	0.000	\pm	0.000	2.800	\pm	2.683	0.000	\pm	0.000	0.000	\pm	0.000
<i>Malacoceros fuliginiosus</i>	0.000	\pm	0.000	1.200	\pm	0.837	0.033	\pm	0.074	2.600	\pm	4.722
<i>Malacoceros tetracerus</i>	0.000	\pm	0.000	0.400	\pm	0.548	0.066	\pm	0.147	2.000	\pm	3.937
<i>Malacoceros vulgaris</i>	0.000	\pm	0.000	0.200	\pm	0.447	0.066	\pm	0.147	0.800	\pm	1.789
<i>Neanthes nubila</i>	0.033	\pm	0.074	0.000	\pm	0.000	0.033	\pm	0.074	0.400	\pm	0.894
<i>Phyllodoce lineata</i>	0.000	\pm	0.000	0.000	\pm	0.000	0.000	\pm	0.000	0.400	\pm	0.894
Total polychaetes	2.934872	\pm	1.058322	36.6	\pm	11.28273	2.27535	\pm	1.295125	7.2	\pm	10.70981

locations, the results showed that carnivorous species of the genus *Tritia* are present in higher proportion in C-type, being *T. nitida*, *T. pygmaea* and *H. squamosus* exclusive of C-type. This agrees with [Crisp \(1978\)](#), [Moran \(1985\)](#) and [Morton \(2006, 2003\)](#) who reported that gastropods from the family Nasariidae and Muricidae occupy keystone positions on rocky shores and present a lie-in-buried-repose behavior, usually in the substrate's crevices.

The presence of stabilized sediments beneath rocks or inside the reef cavities seems favorable to some species ([Le Hir and Hily, 2005](#)). C-type were partially clogged by sandy sediments, explaining the presence of deposit feeders polychaetes of the genus *Malacoceros* in both locations. At Enseada de San Cristovo the low density of the genus *Malacoceros* in

D-type AS agrees with their opportunistic behavior ([Dauvin et al., 2017](#)). At Enseada de Laxe, polychaetes of the genus *Malacoceros* and *Notomastus latericeus* Sars, 1851, are exclusive of C-type AS. This result reinforces the complementarity of both AS types when the main goal is to obtain the maximum accurate knowledge of the benthic assemblages.

If only D-type AS had been used, the information of 52 (E. Laxe) and 24 (E. de San Cristovo) species would have been lost. If only C-type AS had been used, 30 species in Enseada de Laxe and 37 in Enseada de San Cristovo would have been lost. According to [Glasby \(1999\)](#), the position on the water column can determine the community composition. In the same way, the type of substrate can influence the water fluxes conditioning the macrofauna settlement. Those species discrepancies between

AS may be due to the differences in hydrodynamic conditions caused by the substrate's top shape that affect the recruitment process (Eckman, 1983). On the one hand, the dendritic shape of D-type is designed to capture macrofauna in the same way as habitat-forming species. On the other hand, the crevice shape of C-type is designed to support stability and less water flux and, consequently, accumulating more sediment and making them more suitable to the crevice-fauna.

The main problem of using any type of AS as a sampling methodology is that the effectiveness of the sampling is not instantaneous; it takes time to allow for the development of the process of colonization by benthic organisms. Hence, the standardization of these periods to get comparable data between experiences is a critical point to consider in monitoring programs. Similar results were found in Carreira-Flores et al. (2020), who reported that regardless of the type of substrate, 3 months seems enough to differentiate between macrofaunal assemblages of different locations. Other authors also used a standard 3-month period of deployment to get macrofaunal data through AS (Baronio and Bucher, 2008; Obst et al., 2020). Contrarywise, other methodologies deploy AS in the field, from few days (Norderhaug et al., 2002; Zimmerman and Martin, 2004) to one month (Cacabelos et al., 2010), to several months (Zimmerman and Martin, 2004) or various years (Pearman et al., 2018; Ransome et al., 2017). Classically, monitoring studies based on AS to record the community composition on the seafloor in a standardized way are not concerned about the period/timing of deployment but assuring the same temporality in their experimental designs. Thus, the deployment time must be standardized from the very beginning to get an accurate, comparable picture of the benthic assemblages (Ransome et al., 2017), taking into account the particular characteristics (seasonality, upwelling events, latitude...) of the target assemblages.

The most significant benefit of using AS over sampling natural habitats is the standard sampling method (Carreira-Flores et al., 2020; Plaisance et al., 2011). Habitat builder organisms like macroalgae or gorgonians of different sizes collected in the field or crevice-fauna sampled by scraping quadrants or aspiration cannot be easily standardized. AS with a known structural complexity and volume are adequate to remove the variability of the sampling devices from the equation of quantitative sampling. Additionally, the use of AS for monitoring purposes does not compromise the algal settlements, vulnerable habitat builder species, or the rocky reef and can be used globally as they are not limited by the natural lifecycle or any distribution range nor specific features (eg. type of stone and complexity).

Implementing a standard D-type and C-type AS can give complementary information about how benthic diversity is controlled and may be used to contribute to better evaluate the 'Good Environmental Status' (GES). This methodology, based on the use of both AS types to discriminate species habitat preferences can be complementary to ARMS (Autonomous Reef Monitoring Structures) (Obst et al., 2020; Pearman et al., 2018; Ransome et al., 2017), since the design of ARMS is structurally simpler than C-type and is not targeted to the fauna that prefers substrates with high structural complexity. Eventually, ARMS will be colonized by algae or other dendritic organisms and will capture the same organisms as C-type, but at a more considerable time cost.

This study complements and reinforces the strengths of the standard methodology proposed by Carreira-Flores et al. (2020) for benthic monitoring studies that can be used regardless of the geographic location. The comparative study between D-type and C-type AS clearly showed different macrofauna assemblages and the benefits of an integrated approach using the 2 types of AS as sampling devices.

Several globally distributed monitoring programs based on crevice AS that use the same sampling methodology in many different marine conditions (ie. Obst et al., 2020; Pavloudi et al., 2021; Ransome et al., 2017), were never validated with control sampling of the natural assemblages (e.g. by scraping or using airlifts). In the future, we aim to validate the use of C-type AS with natural crevices sampling control. Besides, this standard methodology should be replicated in different periods and with a greater number of replicas per location, to improve

the understanding of benthic assemblages dynamics and to reinforce the present results.

5. Conclusions

- (1) Dendritic and crevice habitats support different macrofauna assemblages
- (2) The complementary use of both AS types was clearly demonstrated in the time period studied;
- (3) Even in a three months sampling period, significant differences between locations were detected;
- (4) The standardization of the sampling strategy allows for the easy comparison between locations, with different depth and macroalgae.
- (5) Non-destructive sampling units that combine dendritic and crevice AS is a more inclusive approach to monitor benthic assemblages than the use of separated ecology assemblages data.

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Marine Environmental Research.

Authorship contributions:

- **Diego Carreira-Flores:** Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing.
- **Regina Neto:** Methodology, Data curation.
- **Hugo Ferreira:** Data curation.
- **Edna Cabecinha:** Supervision, Writing - review & editing.
- **Guillermo Díaz-Agras:** Conceptualization, Methodology, Data curation, Supervision, Writing - original draft, Writing - review & editing.
- **Pedro T. Gomes:** Conceptualization, Methodology, Data curation, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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