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Artificial substrates as sampling devices for marine epibenthic fauna: A quest for standardization

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ABSTRACT

In temperate regions, macroalgae are naturally abundant habitat builders for many organisms of ecological and economic importance. Hence, macroalgae are good targets for monitoring studies based on colonization processes as, through them, it is possible to sample the epifauna that uses them as habitat. Nevertheless, macroalgae collection may not be sustainable, can compromise the survival of the target macroalgae populations and destroy fragile or threatened communities.

The search for an adequate procedure that can overcome the problems related to destructive quantitative sampling of the epifauna associated with macroalgae and the development of a methodology that can be used for comparative macrofauna monitoring, regardless of the location, were the motivations for this study. The evaluation of the mobile epifauna associated with Artificial Substrates (AS) with different degrees of complexity and natural subtidal macroalgae was implemented, as a means to evaluate the viability of AS as an alternative approach for epifauna monitoring.

Cystoseira baccata and *Halidrys siliquosa* were chosen as natural macroalgae. The hypotheses tested were (1) macroalgae and AS with similar structure will support similar assemblages of mobile epifauna; (2) different complexity AS will shelter different assemblages of mobile epifauna. The results obtained after 3 and 6 months showed that AS and macroalgae, both with similar structure, supported different assemblages of mobile epifauna, differing also when the time factor is considered. Moreover, different complexity AS supported different epifaunal assemblages. Our results also show that a period of colonization of 3 months was enough to accurately discriminate locations but the 3 additional months reinforce these differences and provide more coherent results with the species colonizing natural macroalgae in both locations studied. Hence, AS could be used as a valid, replicable, standard and representative alternative tool for monitoring studies.

1. Introduction

Sampling issues have been recognized as one of the problems faced when trying to evaluate 'Good Environmental Status' (GES), as stated by the European Marine Strategy Framework Directive (MSFD; 2008/56/EC). In the scope of the GES, biodiversity and sea-floor integrity are two of the 11 descriptors that contribute to the basis of its evaluation (Danovaro et al., 2016; Rice et al., 2012). Although easily understood conceptually, the evaluation of what constitutes the GES for sea-floor integrity is not an easy task, mainly due to the scientific uncertainties about benthic processes in the oceans, and from difficulties of sampling and monitoring on scales at which the descriptor is to be applied (Rice et al., 2012).

In a given geographic location, species changes over space and time and the resulting assemblages will be a function of space available, recruitment processes, level of perturbation, oceanographic conditions and availability of colonizers, among other factors (Chemello and Milazzo, 2002; Edgar and Klumpp, 2003; Underwood and Chapman, 2006). Local spatial variability will also influence the final results and is a main source of variation during sampling (Edgar, 1991a; Underwood and Chapman, 2006). Thus, in comparative studies aiming to evaluate the role of time and/or space on the differences detected between different locations or different time moments in a given location, it is not easy to separate the effects of the natural local variability from the effects that we may be interested in, worsening the sampling problems referred above.

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For monitoring purposes, benthic and benthopelagic organisms are adequate to monitor space and time changes, as they are conspicuous, easily sampled, and with a fast response to changes. Despite its straightforward use, sampling the often complex benthic environment have serious issues regarding inter-sample variability due to the complexity of the bottom habitats that, altogether, may compromise unbiased procedures. The use of a methodology that can capture a representative sample of the surrounding benthic and benthopelagic assemblages, be sensitive to changes regarding the availability of organisms in the water mass (as proxies to environmental changes), standardized and able to provide presence/absence, but also quantitative data is desirable but not easily obtainable. Bottom physical heterogeneity, associated or not to the structural complexity of the habitat-forming organisms, is the main factor that contributes to the difficulty in assuring replicable benthic marine macrofauna quantitative sampling (Edgar, 1991a,b; Edgar and Klumpp, 2003). This is particularly true in very heterogeneous nearshore areas and is one of the methodological challenges in the study of marine benthos.

Although quantitative data may not be relevant to inventory (presence/absence) or taxonomic studies, quantitative sampling of benthic macrofauna is needed to understand changes in process and trends over time. A quantitative and standard sampling of benthic macrofauna is mandatory to understand how the abundance and diversity of species are controlled (Hauser et al., 2006), especially those associated with habitat-forming organisms, such as macroalgae (Hansen et al., 2011; Veiga et al., 2014).

In temperate rocky coastal regions, macroalgae are naturally abundant habitat builders (depending on their structural complexity, size, and chemical properties) for many organisms of ecological and economic importance, acting simultaneously as primary producers and bio-engineers (Christie et al., 2009; Cremades et al., 2004). Permanent benthic and epibenthic fauna are the primary beneficiaries of habitat generated by these bio-engineers, and its presence enhances the diversity in a given location (Perkol-Finkel et al., 2006). For many species, coastal habitats generated by bio-engineers are also crucial for some life cycle phases, as they provide shelter for eggs, larvae and juvenile forms (Gee and Warwick, 1994). Hence, habitat-forming organisms such as macroalgae are good targets for monitoring studies, especially when the bio-engineering species are perennial.

Annual macroalgae, as habitat builder organisms, can provide new uncolonized habitats each year, but they are absent during long periods. By contrast, perennial macroalgae species can support stable populations that can be more sensitive to changes and integrate the impacts over long periods (Christie et al., 2009). In this context, perennial macroalgae may be seen as passive sampling devices as long as colonization is linked to the space available and complexity (Torres et al., 2015), taking in account that the influence of the macroalgae compounds can influence the biofilm and the recruitment processes (Busetti et al., 2017; Goecke et al., 2010; van der Loos et al., 2019). Hence, macroalgae are good targets for monitoring studies based on colonization processes, mainly for benthic and hyperbenthic macrofauna that is elusive most of the time (Davenport et al., 1999). Nevertheless, variations in local availability and its distribution seriously constraint its over-all usage as sampling "devices", as well as the eventual destruction of fragile or threatened communities.

Native macroalgal communities have been damaged by the progressive increment of coastal urbanization and the construction of artificial structures like harbours or breakwaters (Bulleri and Chapman, 2010; Díaz-Agras, 2015; Díaz-Agras et al., 2010; Firth et al., 2016) and consequently, complex natural habitats were lost (Bishop et al., 2012). Moreover, native communities have been depleted by the influence of invasive species like

Sargassum muticum Yendo (Fensholt), 1955 that grows in dense stands, covering the native species (Cacabelos et al., 2013; Cremades et al., 2004; Sánchez and Fernández, 2005; Veiga et al., 2014; Wernberg et al., 2001). Therefore, for monitoring purposes, the collection of native macroalgae may not be sustainable and may compromise the macroalgae settlements in the short-term. Artificial substrates (AS) may be a viable non-destructive alternative, provided that the macroalgae structural complexity is mimicked by the AS (Cacabelos et al., 2010; Edgar, 1991a).

Sampling through the use of AS is not instantaneous and relies on the availability of organisms to colonize them. It means that some time is going to be needed to allow for the process of colonization by benthic organisms (Cacabelos et al., 2010; García-Sanz et al., 2014). It is also well known that different kinds of substrates are going to be colonized differently (Schreider et al., 2003; Vázquez-Luis et al., 2008). The same kind of substrate may also give different results, depending on the time of the year of deployment (García-Sanz et al., 2014). Nevertheless, AS appear as a simple solution that may overcome the main environmental and anthropogenic problems mentioned since they are easily implemented, and its cost may be held at reasonable values. With a known structural complexity and volume, AS are adequate to remove the variability of the sampling devices from the equation in quantitative sampling (Edgar, 1991a; Norderhaug et al., 2002).

The present study focused on the comparison between AS and two native habitat builder fucoid macroalgae reported as vulnerable to invasive species (García-Fernández and Bárbara, 2016; Wernberg et al., 2001). *Halidrys siliquosa* (Linnaeus) Lyngbye, 1819 has a flattened thallus that can growth till 2 m, originated from a robust conical disc, alternately branched in a single plane and exhibiting an axis forming a zigzag shape. It has 2 types of apical terminations of the branches: oblong air bladders, and segments ending in oblong receptacles (Moss and Lacey, 1963; Wernberg et al., 2001). *Cystoseira baccata* (S.G. Gmelin) P.C. Silva, 1952 is a macroalga that can reach a maximum of 1 metre high, with several primary axes in caespitose thalli, attached to the substratum by a conical disc or haptera. Its apex is smooth or spinous, with abundant ramification in branches, radial or distichous, sometimes with small spine-like or filiform appendages (García-Fernández and Bárbara, 2016).

The main goal of this work was to investigate the viability of using AS for epifaunal assemblages monitoring as an alternative to macroalgae collection. The assemblages of mobile epifauna associated with AS of distinct structural complexity and 2 natural subtidal macroalgae were analysed. *Cystoseira baccata* and *Halidrys siliquosa* were chosen as natural macroalgae; two different AS with different complexity were tested as alternatives. The hypotheses tested were that (1) similar structure macroalgae and AS will support similar assemblages of mobile epifauna; (2) different complexity ASMS will shelter different assemblages of mobile epifauna.

2. Material and methods

2.1. Study area

This study was carried out in the northern region of the Galician coast (NW Iberian Peninsula) at two coves of the Ría de Ferrol (Fig. 1). The ría presents a semidiurnal mesotidal regime; 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 (DeCastro et al., 2003). The main current reaches the maximum speed in the medium part of the ría, diverging into numerous low-energy secondary currents (Cunha, 2017).
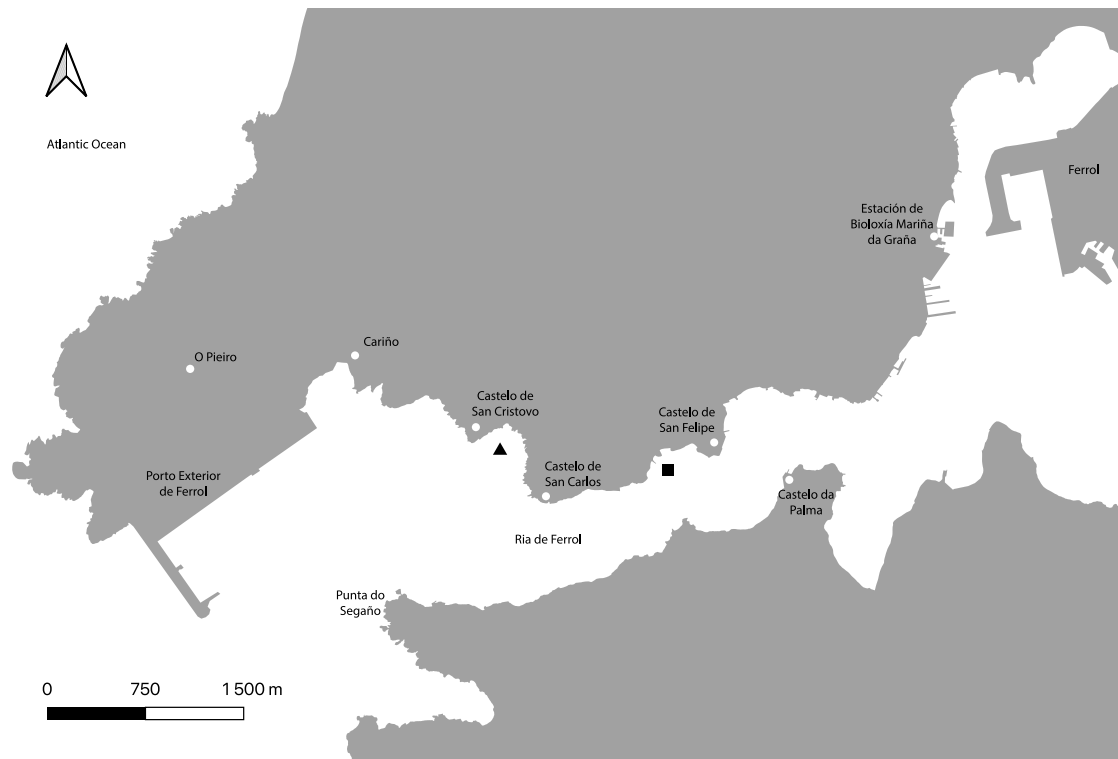
Fig. 1. ▲ Enseada de San Cristovo; ■ Enseada de Laxe.

The Enseada de Laxe (43°27'48.8"N, 008°17'13.5"W; 3 m depth) is located at the medium part of the ría, pertains to a "Semisheltered shore" and is characterized by communities of large brown sublittoral macroalgae *Cystoseira baccata* and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, 2006 and seagrass beds of *Zostera* subg. *Zostera marina* Linnaeus, 1753 (Cremades et al., 2004). The Enseada de San Cristovo (43°27'53.8"N, 008°18'00.7"W; 11 m depth) is located at the outer part of the ría, pertains to a "Semiexposed shore" 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* (Cremades et al., 2004).

During the experiment, temperature variation followed a similar pattern at both locations. The water temperature decreased from January to March 2018 at both coves (average temperatures: Enseada de Laxe =12.27 ± 0.40 °C; Enseada de San Cristovo = 12.71 ± 0.22 °C) and increased from April to June (average temperatures: Enseada de Laxe = 14.64 ± 0.79 °C; Enseada de San Cristovo= 13.8 ± 0.52°C). In the first 3-month period, the temperature of Enseada de Laxe was lower than Enseada de San Cristovo; however, the scenario changed in the second period reaching in June the average temperature of 15.81 ± 0.77 °C in Laxe and 14.64 ± 0.58 °C in Enseada de San Cristovo.

2.2. Deployment of ASMS

Two types or Artificial substrates (Artificial Seaweed Monitoring System, ASMS) were developed: ASMS_1, commercial artificial plants made of green polyethylene plastic (IKEA, Sweeden) were used to mimic an apparently "complex" macroalgae species like *C. baccata*; ASMS_2 was created from frayed brown natural rope covered with an innocuous resin (GaiBridge-GE, Gairesa, Spain) to mimic an apparently "simple" macroalgae species like *H. siliquosa* (Fig. 2). ASMS's were deployed attached to 60 × 60 cm concrete plates within the natural settlements of natural macroalgae. Ten ASMS_1 and ten ASMS_2 were placed at each location on

the Ria de Ferrol on December 22, 2017. Temperature data loggers (TBI-32, Onset HOBO, USA) were attached to the concrete plates, recording the water temperature every 5 min, from January to June 2018.

2.3. Settlement volume and complexity

The Total Volume occupied by each alga and ASMS was estimated assuming the cone shape of the different substrates. The cone volume was calculated using the circular projection of the maximum diameter of each substrate like the basis of the cone, and the height of the substrate as the height of the cone. The Colonization Volume Available was the result of the difference between the Total Volume and the volume occupied by the mass of the substrate (displaced water volume).

To calculate the real surface of the ASMS one branch of the substrate 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 ASMS_1 has a surface of 1773 cm^2 and ASMS_2 of 849 cm^2. The available space for colonization of was 6050 cm^3 and 2804 cm^3 for ASMS_1 and ASMS_2, respectively.

The complexity of each artificial substrate was measured by the number of branches into which each secondary axis is divided, the complex substrate, ASMS_1 has 21 secondary branches divided into another 4 tertiary branches with 10 leaves, by contrast, the simplex substrate, ASMS_2 is only divided into 25 secondary branches.

2.4. Sampling strategy

Previous studies suggested that the colonization of ASMS_1 by motile epifauna occurs within 3 months after being deployed (Alves, 2017). Accordingly, 5 ASMS were recovered by scuba diving after 3 months on 27 March (T1) and another 5 after 6
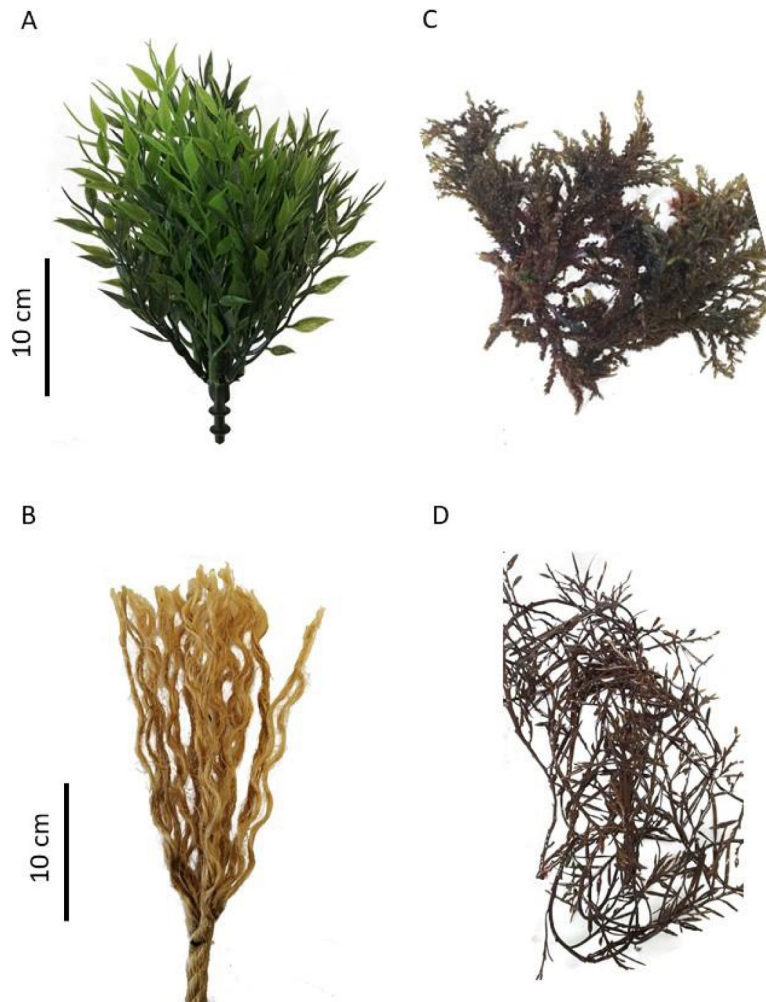
Fig. 2. Artificial Seaweed Monitoring System (ASMS), ASMS 1 (a) and ASMS 2 (b); studied natural macroalgae, *Cystoseira baccata* (c) and *Halidrys siliquosa* (d).

months on the 29 June 2018 (T2), in each location. Besides the ASMS, 5 natural *C. baccata* at Enseada de Laxe and 5 *H. siliquosa* at Enseada de San Cristovo of similar size (*C. baccata:* 22 ± 1.87 cm height, 3458.75 ± 524.51 cm^3 available colonization space; *H siliquosa*: 21.4 ± 2.073 cm height, 2305.24 ± 879.02 cm^3 available colonization space) were sampled by scuba diving at the same date to have the natural settlement reference.

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 associated with the macroalgae to escape. In the laboratory, mobile epifauna was washed off in filtered seawater by shaking each alga 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).

2.5. Data analyses

Data were analysed through multivariate techniques to test the proposed hypotheses. The number of taxa, density of individuals and diversity (Simpson index) of epifaunal assemblages were calculated and plotted in R environment v 3.6.0 (R Core Team, 2019).

Non-parametric permutational multivariate analysis of variance [PERMANOVA; (Anderson, 2017)] was used to test hypotheses about differences of epifaunal assemblages. Two hypotheses were tested (1) ASMS and macroalgae, both with similar structure, will support similar assemblages of mobile epifauna, and

(2) different complexity ASMS will shelter different assemblages of mobile epifauna. For the first hypothesis, the factors studied were: the type of substrate (fixed, 3 levels, ASMS_1 vs. ASMS_2 vs. Natural macroalgae) and time (random, two levels, time 1 vs. time 2); for the second hypothesis were: the type of substrate (fixed, 2 levels, ASMS 1 vs. ASMS 2) 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 9999 unrestricted random permutations of data. In addition, responses of assemblages were visualized by non-metric multidimensional scaling (MDS) on the basis of Bray–Curtis dissimilarity matrix on 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). Analyses of multivariate dispersion were also done to test for homogeneity of dispersions between types of substrate [PERMDISP, (Anderson, 2017)]. 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 >=5%. Multivariate analyses were conducted using Primer v.6 (Clarke and Gorley, 2001) with PERMANOVA + add-on (Anderson et al., 2008).
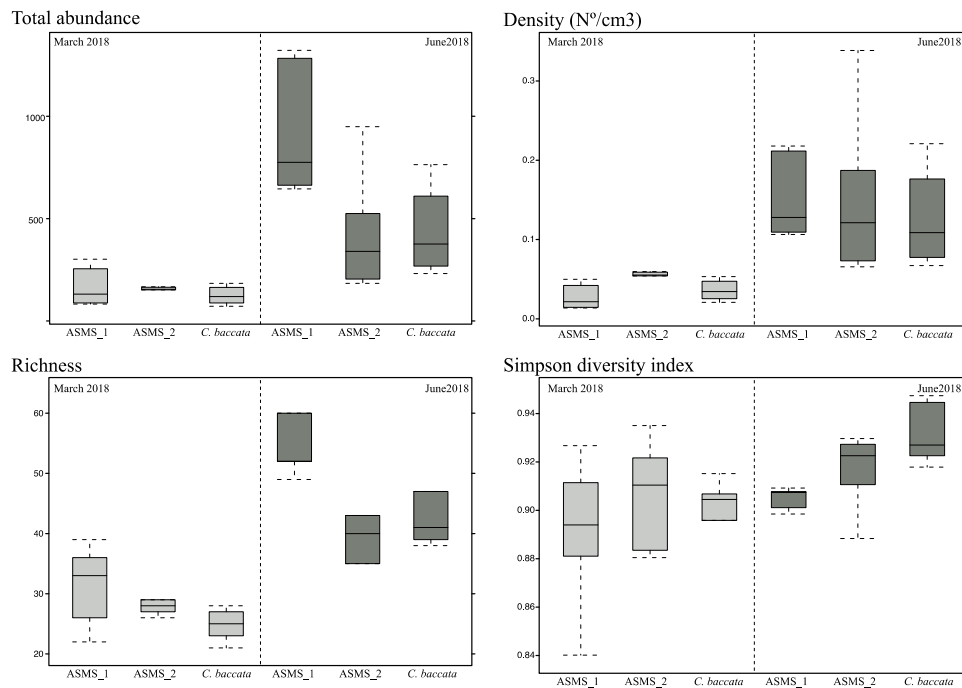
Fig. 3. Mean (+SE, n=5) of the Total abundance per substrate, Density, Number of taxa (Richness) and Diversity (Simpson index) of epifaunal assemblages associated with ASMS_1, ASMS_2 and *C. baccata* in Enseada de Laxe, time 1 (= March2018) and 2 (= June2018).
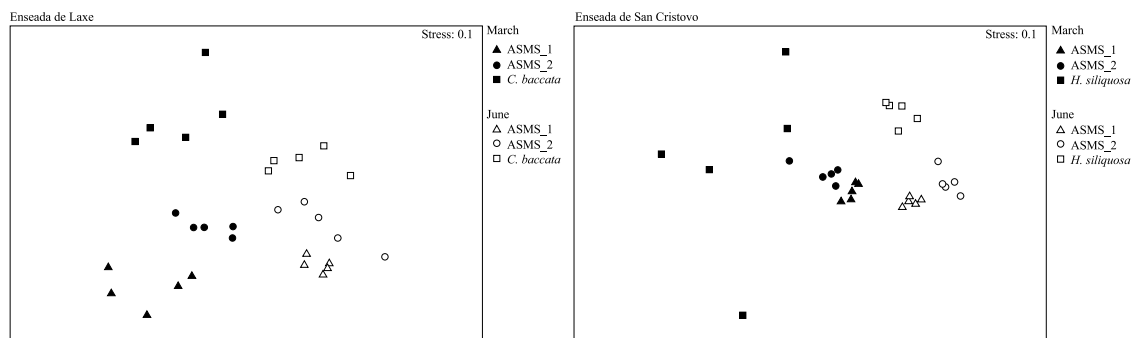

Fig. 4. Multidimensional scaling (MDS) plots of epifaunal assemblages of ASMS and natural macroalgae at Enseada de Laxe and Enseada de San Cristovo.

3. Results

3.1. Epifauna associated with artificial and natural substrates in Enseada de Laxe

The total number of individuals, density (ind/cm^3), and richness were consistently higher in June on both types of substrates (Fig. 3). In June 2018, *C. baccata* presented the highest value of diversity (Simpson index) (0.931 ± 0.01) (Fig. 3). Malacostracans (mainly amphipods and isopods) and gastropods from the order Littorinimorpha accounted for more than 75% of the total number of individuals in March. Malacostracans remaining to be the dominant group in *C. baccata* in June, while decapods were the second group in ASMS_1 and ASMS_2. Comparing each artificial substrate with *C. baccata*, ASMS_1 supported more exclusive species (24 in March and 37 in June) than that *C. baccata* (14 in March and 25 in June) and shared 34 species in March and 52 in June. Only in March ASMS_2 supported more exclusive species (14 in March

and 21 in June) than *C. baccata* (13 in March and 29 in June) and shared 35 in March and 48 in June.

Results of PERMANOVA analysis for the composition of assemblages indicated a significant interaction between substrate and time (Table 1). Results of pair-wise tests showed significant differences between substrates at each time (Table 2), significant differences were not influenced by the dispersion or variability among replicates (Permdisp, $p > 0.05$). The MDS (Fig. 4) showed a clear differentiation between times and substrates. However, the Bray Curtis distance became shorter in June, possibly due to the stabilization of the epifaunal communities with the time.

The dissimilarity between ASMS_1 and *C. baccata* was 76.53%, being Crustacea Peracarida and Crustacea Eucaridea [*Pisidia longicornis* (Linnaeus 1967)] the taxa responsible for the 43.09% of this dissimilarity (SIMPER analysis). The amphipods *Ampithoe ramondi* Audouin, 1928 *Gammaropsis maculata* (Johnston 1828), *Ericthonius brasiliensis*) (Dana 1853), *Apocorophium acutum* (Chevreux

1908) and *Dexamine spinosa* (Montagu 1813), the isopod *Dynamene magnitorata* Holdich, 1968 and the decapod *Pisidia longicornis* were the most important taxa contributing to the dissimilarity between substrates.

The dissimilarity between ASMS_2 and *C. baccata* was 66.35%. The amphipods *Ampithoe ramondi, Gammaropsis maculata, Monocorophium sextonae* (Crawford, 1937) and *Dexamine spinosa*; the isopods *Cymodoce truncata* Leach, 1814 and *Dynamene magnitorata* were the most important taxa contributing to the dissimilarity between substrates (39.33%, SIMPER Analysis).

3.2. Epifauna associated with artificial and natural substrates in Enseada de San Cristovo

The epifaunal pattern described at Enseada de Laxe was also found in the Enseada de San Cristovo, the total number of individuals, density (ind/cm³) and richness were consistently higher in June on both types of substrates (Fig. 5). The low colonization of the macroalgae by motile macrofauna in March could explain the lowest value of diversity (Simpson index) obtained in *H. siliquosa* (0.509 ± 0.33) (Fig. 5).

Gastropods from the order Littorinimorpha and amphipods were the dominant groups in March, accounting for 70% of the total number of individuals in ASMS and 66% of the individuals in *H. siliquosa*. In June amphipods and the holoturoid *Cucumaria frondosa* (Gunnerus, 1767) account for 80% of the individuals in ASMS_2; however, amphipods and decapods account for 80% of the individuals in ASMS_1 and in *H. siliquosa,* amphipods were the dominant group followed by gastropods from the order Littorinimorpha. Comparing each artificial substrate with *H. siliquosa*, ASMS_1 supported more exclusive species (33 in March and 36 in June) than *H. siliquosa* (3 in March and 12 in June) and shared 8 in March and 34 in June. ASMS_2 also supported more exclusive species (16 in March and 27 in June) than *H. siliquosa* (3 in March and 13 in June) and shared 8 species in March and 33 in June.

Results of PERMANOVA analysis for the composition of assemblages showed a significant interaction between substrate and time (Table 1). Results of pair-wise tests revealed significant differences between substrates at each time (Table 2). Results of PERMDISP showed that the significant differences were the consequence of the community structure and variability among replicates (Permdisp, $p < 0.05$). The MDS (Fig. 4) shows that, despite the degree of dispersion in the March samples of *H. siliquosa*, there is a clear differentiation between times and substrates in Enseada de San Cristovo. Concerning the Bray Curtis distance, it became smaller in June, reflecting a possible stabilization of the epifaunal communities with time.

Dissimilarity between ASMS_1 and *H. siliquosa* was 87.24%. The amphipods *Gammaropsis maculata, Ampithoe ramondi, Dexamine thea* (Boeck, 1861)*,* Ericthonius brasiliensis, the decapod *Pisidia longicornis* and the gastropod *Rissoa lilacina* (Récluz, 1843), were the main taxa responsible for the 63.64% of dissimilarity (SIMPER analysis).

Dissimilarity between ASMS_2 and *H. siliquosa* was 86.56%, being *Cucumaria frondosa, Gammaropsis maculata, Ampithoe ramondi, Dexamine thea, Ericthonius brasiliensis* and the gastropod *Rissoa lilacina* the most important taxa contributing a 64.09% for the dissimilarity (SIMPER analysis).

3.3. Epifauna associated with artificial substrates at Enseada de Laxe and Enseada de San Cristovo

The total Number of individuals and Richness was higher in ASMS_1 than in ASMS_2, although both presented similar density values (ind/cm³) (Fig. 6). After 3 months of immersion,

amphipods, isopods, and gastropods from the order Littorinimorpha accounted for more than 80% of the total number of individuals supported by the AS at Enseada de Laxe. Amphipods and gastropods from the orders Littorinimorpha and Trochida contributed for more than the 85% of the total individuals counted at Enseada de San Cristovo. The lowest value of diversity (Simpson index) was found for ASMS_2 in Enseada de San Cristovo (0.83 ± 0.052) (Fig. 6). ASMS_1 supported more exclusive species (23 at Enseada de Laxe and 21 in Enseada de San Cristovo), than ASMS_2 (14 and 4 respectively) and shared 35 and 5 species in each location, respectively.

After 6 months of immersion, amphipods and decapods were the dominant taxa on low and mid-depth. With the exception of ASMS_2 at Enseada de San Cristovo (amphipods 46%, holoturoids 26% and decapods 4%), similar percentages were found for both substrates. The lowest values of diversity were found in Enseada de San Cristovo (ASMS_1: 0.799 ± 0.03; ASMS_2: 0.782 ± 0.07) (Fig. 6). ASMS_1 supported more exclusive species (34 at Enseada de Laxe and 23 Enseada de San Cristovo) than ASMS_2 (14 and 13, respectively) and shared 55 and 47 species in each location, respectively.

Results of PERMANOVA analysis for the composition of assemblages showed a significant interaction between substrate and location (Table 3). Pair-wise tests revealed significant differences between locations at each time (Table 4). Results of PERMDISP showed that the significant differences were due to the community structure and variability among replicates (Permdisp, $p < 0.05$). The MDS shows a clear differentiation between locations and times (Fig. 7).

The dissimilarity between both ASMS was 65.81% after 3 months and 65% after 6 months. The isopod *Cymodoce truncata,* the gastropods *Rissoa lilacina* and *Rissoa parva* (da Costa, 1778), and the amphipods *Ericthonius brasiliensis* and *Gammaropsis maculata* were the most important taxa responsible for the dissimilarity after 3 months. The amphipods *Ericthonius brasiliensis* and *Gammaropsis maculata,* the decapod *Pisidia longicornis* and the holotoroid *Cucumaria frondosa* were the most important taxa responsible for the dissimilarity after 6 months.

4. Discussion

Results showed that the initial hypotheses were partially supported. On one hand, similar macroalgae structure and ASMS supported different assemblages of mobile epifauna, differing also when the time factor is considered. On the other hand, different complexity ASMS supported different epifaunal assemblages.

Epifaunal animals may be attracted to artificial habitats for two reasons. The habitat may be desirable by its physical nature, providing a physical refuge or a site of attachment for suspension-feeding invertebrates to rise above the seabed boundary layer, contributing to diminish the competition associated with limiting space for settlement. The habitat may also be desirable due to the associated food resources, such as encrusting diatoms, bacterial aggregates or particulate organic matter (Edgar, 1991a) or just because the space is available (Norderhaug et al., 2002). Previous studies have illustrated that AS attracts most of the elements of the mobile invertebrate fauna in their nearby area and that assemblages associated with AS are sensitive to local variation in environmental conditions (Edgar, 1991b; Russo, 1990). Our results agree with Norderhaug et al. (2002) as ASMS were primarily colonized mainly by amphipods and gastropods, reflecting its horizontal dispersal patterns, associated with its mobility capabilities.

Our results are in accordance with Cacabelos et al. (2010) showing a high colonization of the ASMS by motile epifauna. Nevertheless, the structure of the epifaunal assemblages were

Table 1
Summary of PERMANOVA results for total assemblages at Enseada de Laxe and Enseada de San Cristovo.

Source	df	Enseada de Laxe			Enseada de San Cristovo		
		MS	Pseudo-F	Unique Perms	MS	Pseudo-F	Unique Perms
Substrate	2	5187.2	1.8795	60	7814.9	1.2095	60
Time	1	11405	15.721**	9927	18214	16.863**	999
Su x Ti	2	2759.8	3.8042**	9911	6461.4	5.9823**	998
Residual	24	725.46			1080.1		
Total	29						
PERMDISP			P(perm): 0.162			P(perm): 0.001	

*, $P<0.05$; **, $P<0.01$.

Table 2
Results of pair-wise test for total assemblage at Enseada de Laxe and Enseada de San Cristovo.

Groups	Enseada de Laxe				Enseada de San Cristovo			
	March		June		March		June	
	t	Unique Perms	t	Unique Perms	t	Unique Perms	t	Unique Perms
ASMS_1, ASMS_2	2.1875**	126	2.0459**	126	1.9426*	126	2.7498**	126
ASMS_1, Natural macroalgae	2.3946**	126	3.0777**	126	2.3004**	126	3.8233**	126
ASMS_2, Natural macroalgae,	2.1955**	126	2.1747**	126	2.0207**	126	3.5235**	126

*, $P<0.05$; **, $P<0.01$.
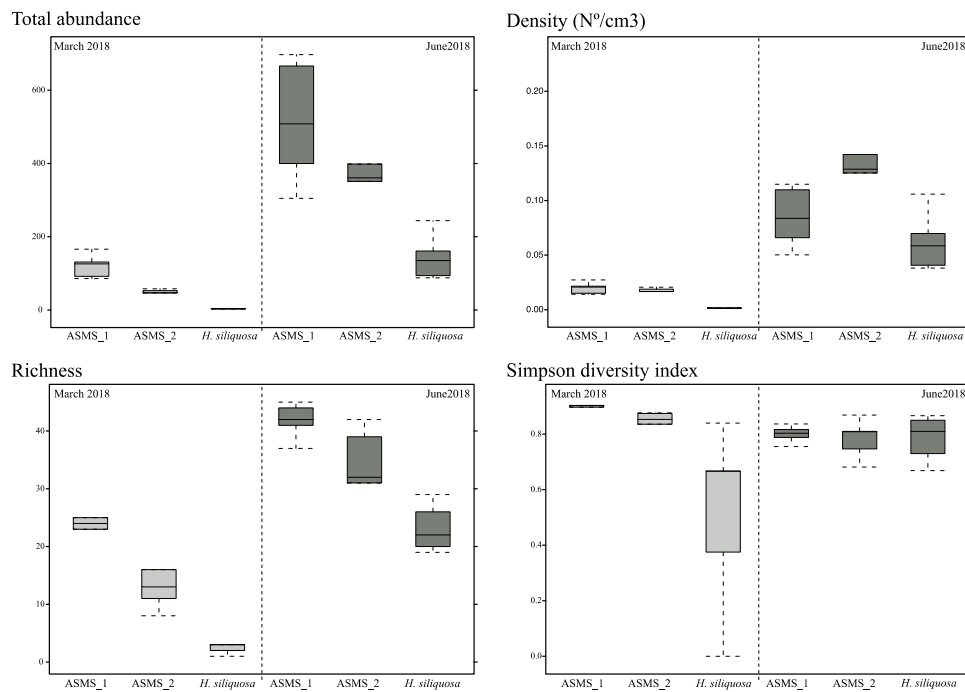
Fig. 5. Mean (+SE, n=5) Total abundance per substrate, Density, Number of taxa (Richness) and Diversity (Simpson index) of epifaunal assemblages associated with ASMS_1, ASMS 2 and *H. siliquosa* in Enseada de San Cristovo, time 1 (= March2018) and 2 (= June2018).

significantly different from those of the studied natural macroalgae. Most of the fucoids are perennial, hence, *C baccata* and *H. siliquosa* harbour stable assemblages of invertebrates, characterized by lesser motility compared with the high motility fauna associated with habitats of shorter duration (Christie et al., 2009). Also, biofilms are considered to be key drivers for the settlement and the subsequent colonization by epifaunal macrofauna in many studies (Patil and Anil, 2005; Qian et al., 2007), epifaunal assemblages could be directly reliant on host plant tissue or a mature microflora assemblages mediated by chemical compounds released by the host (Busetti et al., 2017) The differences

between natural and AS could be related to the limited time that AS were deployed in comparison with natural perennial macroalgae (Cacabelos et al., 2010). These differences could be due to the fact that ASMS only collected the surrounding more motile epifauna from the substrate or from the annual macroalgae (Norderhaug et al., 2002), not having enough time to collect the less motile epifauna associated with the perennial macroalgae. Besides, the biofilm can be an important driver for the assemblages settlement and the duration of the experiment may not be enough to reach a mature biofilm or it just may be different from the natural macroalgae biofilm. Taking into account the

Table 3

Summary of PERMANOVA results for assemblages of Complex substrates and Simplex substrates.

Source	df	March			June		
		MS	Pseudo-F	Unique Perms	MS	Pseudo-F	Unique Perms
Substrate	1	3191.2	1.1262	6	2635.7	1.1734	6
Location	1	13543	19.31**	9935	4533.9	9.9609**	9925
Su x Lo	1	2833.7	4.0406*	9952	2246.3	4.9352**	9939
Residual	16	701.31			455.16		
Total	19						
PERMDISP			P(perm): 0.01			P(perm): 0.004	

*, $P<0.05$; **, $P<0.01$.

Table 4

Results of pair-wise test for ASMS_1 and ASMS_2.

Groups	March				June			
	ASMS_1		ASMS_2		ASMS_1		ASMS_2	
	t	Unique Perms	t	Unique Perms	t	Unique Perms	t	Unique Perms
Laxe, San Cristovo	2.6628**	126	4.1382*	126	3.2707**	126	2.9047**	126

*, $P<0.05$; **, $P<0.01$.

observed pattern when comparing March and June samples, the 6 months in the field might be not enough for the ASMS to reach a stable community similar to the natural macroalgae. Thus, if the immersion time had been longer, the assemblages could have been significantly similar with natural macroalgae assemblages.

ASMS_1 and ASMS_2 were considered as presenting different complexity because of the high branch divisions of the polyethylene plastic substrate, in contrast with the absence of divisions in the resinous substrate. Our results showed that both ASMS supported significant different epifaunal assemblages, differing among locations and in the total number of collected organisms. ASMS_1 captured more organisms because it has approximately twice the volume of the ASMS_2, but Density of individuals (ind/cm^3) showed quite similar values for both substrates. These results seem to agree with previous studies, as the relationship between diversity and abundance have been correlated with the habitat complexity, increasing the values of diversity and abundance of marine organisms with the complexity of the habitat (Hauser et al., 2006; Hull, 1997; Torres et al., 2015). Nevertheless, the positive relationship between the structural complexity of macrophytes, as well as with the abundance and diversity of their associated fauna, have not been always found (Cremona et al., 2008; Russo, 1990). Veiga et al. (2014) referred that the structure of epifaunal assemblages could be the result of the interactions of several significant but weak drivers. Our results suggest that for the studied ASMS the available volume for colonization is indeed the main factor regulating macrofauna settlement and more space available reflects higher richness and density values. Greater complexity is reflected primarily in the space available and this may be the important factor regulating settlement of the surrounding macrofauna.

ASMS macrofauna assemblages showed significant differences when comparing the two studied locations at the Ria de Ferrol after 3 and 6 months of deployment. These differences can be explained by the different depth conditions as, in accordance with Balazy and Kuklinski (2017), depth can play an important role in the regulation of the assemblages. Our results also show that a period of colonization of 3 months was enough to accurately discriminate locations but the 3 additional months corroborate these differences. As above mentioned, the importance of the surrounding 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 greatest benefit of using ASMS over sampling natural habitats is the standardization between replicates. Algal thalli of different sizes collected in the field cannot be simply standardized as a quotient with plant biomass, surface area or volume available. Artificial macroalgae with a known structural complexity and volume, are adequate to remove the variability of the sampling devices from the equation in quantitative sampling. Additionally, the use of ASMS for monitoring purposes do not compromise the algal settlements and ASMS can be used globally as they are not limited by the natural lifecycle and distribution range of the macroalgae.

Several types of AS designed to sample macrofauna have been implemented such as the ARMS initiative (Autonomous Reef Monitoring Structures) that mimic the structure of coral reefs (Zimmerman and Martin, 2015). Nevertheless, these kinds of AS do not adequately sample all sorts of macrofauna and are aimed preferably to cavity associated animals. Mobile epifauna, such as amphipods and other suspension feeder organisms, a priori, are poorly sampled by these devices. The implementation of a standard ASMS which mimics habitat-forming macroalgae can give complementary information about how the benthic diversity is controlled and can be used in association with cavity AS such as ARMS. This strategy may considerably contribute to better evaluate the 'Good Environmental Status' (GES). Furthermore, our results showed that comparable conclusions can be obtained using ASMS_2 instead of ASMS_1. Less laboratory work, faster results, no use of polyethylene plastic, neutral surface and a lower impact in the natural communities making it a better choice for implementation in future studies.

This study is only the first step to find a standard methodology for benthic monitoring studies that can be used regardless of the geographic location and complementing the already used ones, such as ARMS. The comparative study between ASMS
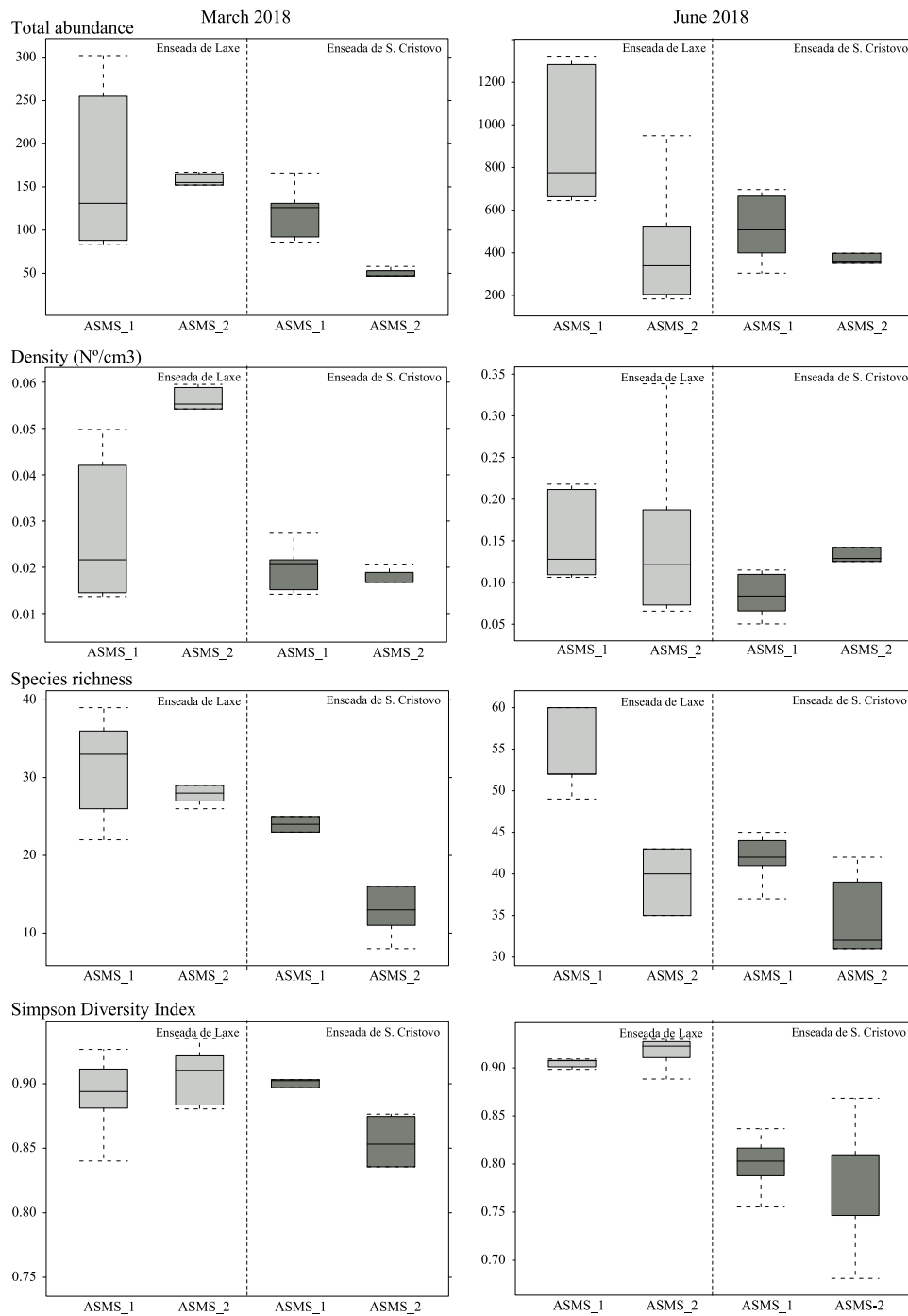
Fig. 6. Mean (+SE, n=5) Total abundance, Density, Species Richness (Number of taxa), Diversity (Simpson index) of epifaunal assemblages associated with ASMS 1 and ASMS 2 at Enseada de Laxe and Enseada de San Cristovo.

and ARMS is still on-going by our team and the results to be obtained may show if the expected differences are real. Anyway, the ASMS_2 approach is far less expensive than ARMS_1 and the use of polyethylene is reduced. ASMS_2 have also the advantage of being covered by an innocuous resin, therefore a neutral colonization surface is guaranteed. The characterization of the biofilm compared with macrofaunal assemblages in other locations and times must be tested and the assemblages on other natural substrates must be compared with the ones captured by AS, such as macroalgae and the natural rocky substrates, sampled by other methods (ex: scrapping or by using airlifts).

5. Conclusions

(1) Significant differences were found between the macrofauna assemblages colonizing ASMS and macroalgae but the differences seem to decrease over time;

(2) Significant differences were found between the macrofauna assemblages colonizing ASMS_1 and ASMS_2; also, significant differences were found when the location factor is taken into account;

(3) A 3 month period of colonization is enough to capture the diversity of epifauna by the ASMS that allows for the differentiation of locations, regardless of the substrate type, in the time interval of this study;
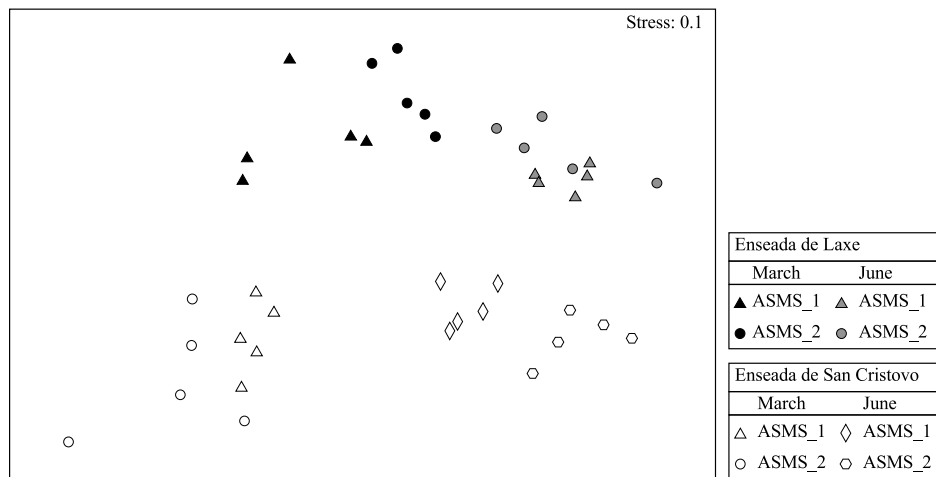
Fig. 7. Multidimensional scaling (MDS) plot of epifaunal assemblages of ASMS_1 and ASMS_2 at Enseada de Laxe and Enseada de San Cristovo.

(4) ASMS_2 has the advantage over ASMS_1 of being more eco-friendly and less work-intensive and of being closer to the studied natural assemblages;

(5) ASMS can be a valid standard replicable tool for monitoring purposes.

CRediT authorship contribution statement

Diego Carreira-Flores: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Regina Neto:** 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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