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Early colonization of sessile megabenthos on electrolytic carbonated structures (Alicante's harbor, Western Mediterranean)

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ABSTRACT

Biofouling of different artificial substrates was studied to determine the differences in biofouling assemblages among different substrates. However, studies on biofouling on natural substrates like electrolytic carbonated ones are lacking. These substrates have a great potential for coral reef restoration in tropical areas and for biofilter construction. Thus, this study was developed to examine the colonization of sessile macrofouling in the port of Alicante (SE Spain, Western Mediterranean) on two types of substrates: electrolytic carbonated and bare steel (as control) over three months of immersion (October 2019–January 2020). The community diversity was studied through different biotic parameters and abundance of assemblages, and preference of organisms according to their status and functional group (active filter feeders). Univariate and multivariate analyses (PERMANOVA and SIMPER) were also applied to examine the differences between carbonate and control substrates. The carbonated substrate had a more structured community and higher abundance, recruitment, and diversity indexes than the bare steel. Moreover, filter feeders (Porifera, Bivalvia, and Ascidiacea) were more abundant, and most of them only appeared in the carbonated substrate. These results show the potential of carbonated structures as biofilters.

1. Introduction

The maritime environment has been recently exposed to novel materials, and some of which have become new substrates for marine biofouling communities (Lin and Shao, 2002; Airoldi et al., 2015; Sempere-Valverde et al., 2018). Consequently, studies on biofouling communities have also increased (Lin and Shao, 2002; Bulleri, 2005; Pierri et al., 2010; Casoli et al., 2014; Lezzi and Giangrande, 2018; Trinidad et al., 2019; Giangrande et al., 2020; Muthukrishnan et al., 2022).

Various studies noted that the development of coastal biofouling assemblages depends on the substrate type; in fact, differences in biofouling assemblages were detected within the same study site comparing naturally occurring substrates with artificial ones (Brown, 2005; Vaz-Pinto et al., 2014; Chase et al., 2016). These differences can be caused by different factors: i) spatial heterogeneity and diversity of microhabitats (Glasby et al., 2007; Megina et al., 2016); ii) surrounding water circulation (Bulleri and Chapman, 2010; Rivero et al., 2013); iii) physicochemical structures of substrates (Dafforn et al., 2009; Sempere-Valverde et al., 2018); and iv) relationship with human activities (Bulleri and Chapman, 2010; Rivero et al., 2013). These differences among substrates led to the appearance of differences in biofouling assemblages among and within artificial and natural substrates in terms of abundance, diversity, community structure, and biotic interactions (Lin and Shao, 2002; Bulleri, 2005; Bulleri et al., 2005; Tyrrell and Byers, 2007; Chase et al., 2016; Megina et al., 2016; Albano and Obenat, 2019). These differences appear in the early stages of colonization and can be maintained over time. However, the most frequent cause of differences between natural and artificial assemblages are the non-indigenous species (NIS), which are usually more abundant on artificial substrates (Airoldi et al., 2015; Chase et al., 2016; Megina et al., 2016).

There are numerous studies comparing biofouling assemblages between artificial and natural substrates (among others, Anderson and Underwood, 1994; Lin and Shao, 2002; Berntsson and Jonsson, 2003;

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Watson and Barnes, 2004; Brown, 2005; Tyrrell and Byers, 2007; Pierri et al., 2010; Sempere-Valverde et al., 2018), but there is still scarce information about the performance of electrolytic carbonated substrates (ECS). ECS formation is a consequence of the application of cathodic protection to avoid the corrosion of metals submerged in the marine environment (Cox, 1940). During this process, an electric continuous current is applied to the metal, promoting an electrolytic process in seawater; the salts dissolved in seawater precipitate around the metal (cathode), forming a hard carbonated layer made of calcium carbonate and magnesium hydroxide (Eickhoff and Shaw, 1948; Hilbertz, 1979; Hartt et al., 1984). Consequently, the formed layer has a similar composition to naturally occurring rock (Siboni et al., 2009). Hilbertz (1979) showed the potential of this material for the construction of artificial reefs, but few studies were carried out until the creation of Biorock® (Hilbertz and Goreau, 1996). Some studies performed in tropical areas with that methodology showed that it enhances the recruitment, growth, survival, and resistance of hard and soft corals against disturbances (Bakti et al., 2012; Fitri and Rachman, 2012; Goreau, 2012; Goreau and Hilbertz, 2012; Karissa et al., 2012).

Despite the fact that this methodology is not new, to our knowledge, studies on colonization over this material are scarce (apart from the ones carried out with corals, see Goreau, 2014). Additionally, the metallic structure used in all of them is connected to an electric current, allowing the continuous generation of this carbonated layer. Hence, no study has previously used a metallic structure already coated with a carbonate layer as the initial colonization material (without permanent connection to an electrical source). Thus, this study mainly aimed to examine the colonization of sessile macrofouling in the port of Alicante (Spain) on two substrate types: electrolytic carbonated and bare steel (control). The specific objectives were to: i) determine the structure of sessile macrofouling communities; ii) investigate the effects of substrates on the colonization of species according to their status (native, cryptogenic, and NIS); and iii) see whether there are significant differences in the sessile macrobenthos community between these two substrates. The abundance of filter-feeding guilds on carbonated meshes suggests its potential as a bioremediation tool in areas affected by high organic charges (aquaculture, ports, and sewage discharges).

2. Material and methods

2.1. Study site

This study was carried out in the Alicante's harbor (SE Iberian Peninsula) in the dock n° 9 (N38° 20′ 09.23″–W00° 29′ 05.81″) from October 15, 2019, to January 16, 2020. The dock is located in the outer quay of the harbor (Fig. 1) and has a muddy bottom with 8 m depth at

Table 1
Environmental parameters corresponding to the study site during the sampling period at 2 m depth.

Parameters	15/10/2019	16/01/2020
Seawater temperature (°C)	23.8	14.2
pH	8.3	8.1
Salinity (SPU)	38.9	38.4
Secchi disc (m)	4.0	3.5

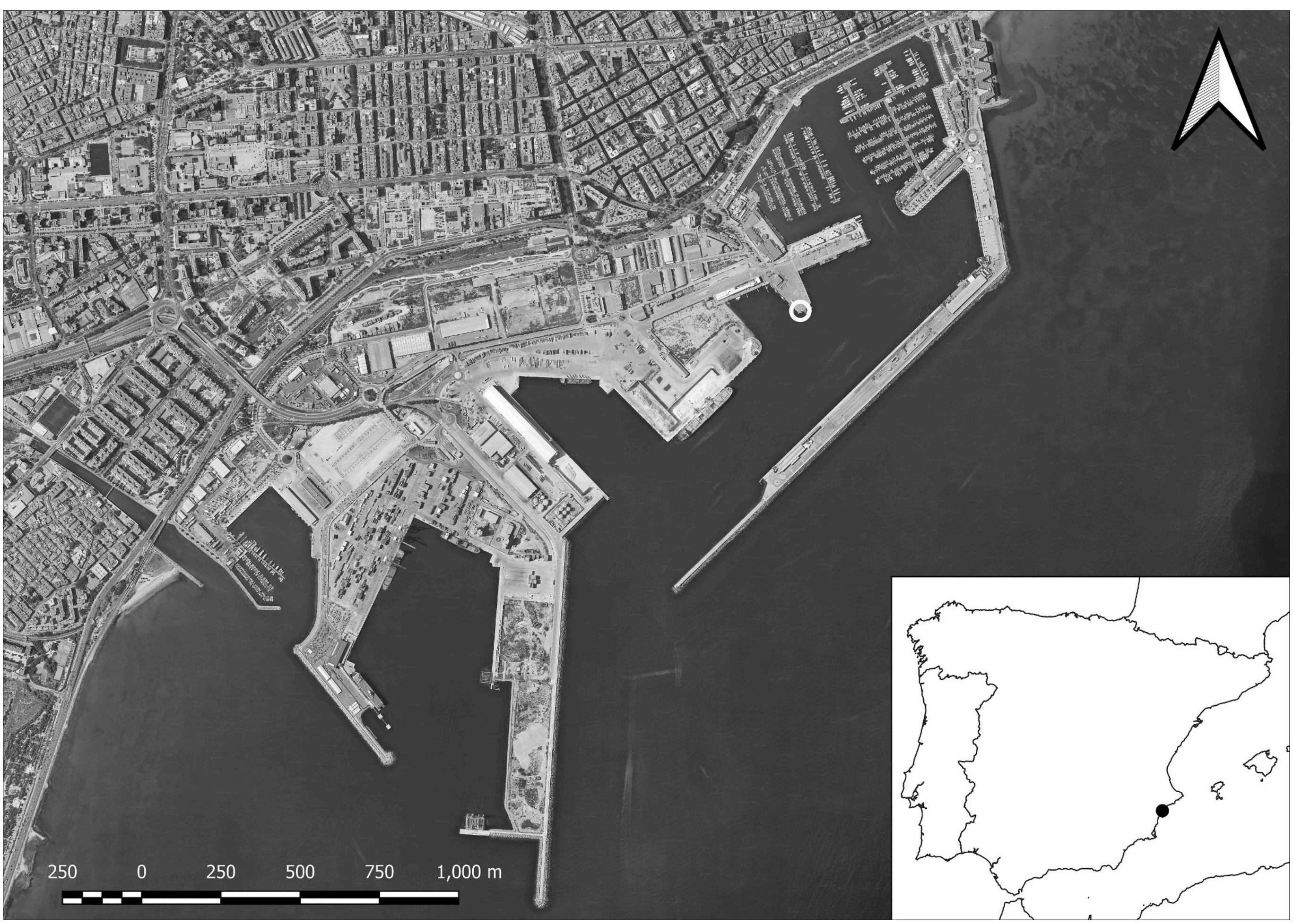

Fig. 1. Alicante's harbor location and ninth dock pier position (white circle). Images adapted from Google Earth.

the sampling site. Referring to environmental parameters, their variation is summarized in Table 1.

2.2. Sampling

For biofouling colonization, six square steel meshes with 15 cm sides were used; three of which were previously subjected to an electrolytic process, as in Hilbertz (1979), until a 0.5 mm carbonated layer was deposited around them. In this way, three bare steel meshes acted as the control treatment, whereas the other three were the carbonated treatment. The meshes were anchored in a horizontal profile, separated 20 cm from each other. The structure was located 1 m away from the dock at 2 m depth on October 15, 2019. After three months of exposure (January 25, 2020), they were manually collected, and each mesh was placed in a separate bag with seawater to avoid mixing of samples. Both sides of these meshes were photographed for further image analysis and fixed with 10 % formalin-sea water for at least 48 h. Then, the organisms were scraped and identified at the lowest taxon possible (based on World Register of Marine Species: https://www.marinespecies.org). Only sessile macrofouling taxa were used in this study. The status (native, cryptogenic, or NIS) of each identified species was also assessed by NIS inventories for the Mediterranean Sea (Zenetos et al., 2010, 2012, 2017; Ulman et al., 2017) and specialized databases (World Register of Introduced Marine Species WRiMS: http://www.marinespecies.org/introduced/, National Exotic Marine and Estuarine Species Information System NEMESIS: http://invasions.si.edu/nemesis/ and European Alien Species Information Network EASIN: https://easin.jrc.ec.europa.eu).

2.3. Data treatment

Descriptive analysis was carried out to have a holistic overview of the initial trend of colonization. Species composition was observed at high taxonomic levels (Bryozoa, Hydrozoa, Sabellidae, Serpulidae, etc.), and the most abundant species were highlighted.

To assess the effect of substrates on sessile biofouling assemblages, both univariate and multivariate analyses were performed.

The effect of substrates on community diversity was assessed with one-way ANOVA (substrate as fixed factor with two levels: control vs carbonated) over different calculated biotic parameters: species richness (S), Shannon-Wiener index (H′, e base), Pielou's evenness (J′), and abundance (N) measured in organisms/cm^2 (for solitary organisms, each organism was counted, whereas for colonial organisms, each colony was treated as a single organism). Note that all of the abundance measures given from this point were assessed in the same way.

To assess the effect of substrates on biofouling assemblages, PERMANOVA (Anderson, 2005) was performed on the matrix based on fourth root transformed abundance data of each species/taxa found on both substrates, using the Bray-Curtis dissimilarity (Clarke and Warwick, 2001). PERMANOVA was performed using the same model described for ANOVA. The SIMPER procedure was also done to observe the species/taxa that contribute more to the differences and to detect the typical ones of each substrate, and PERMDISP was conducted to test whether these differences were caused by a factor or data dispersion (Anderson et al., 2008). In addition, a univariate PERMANOVA was performed for each species/taxon determined by SIMPER in order to detect weather each one's abundance was significantly different between substrates. In these cases, a univariate PERMANOVA with Euclidean distance was used to avoid the assumption of normality of data (Anderson, 2014). In both cases, the *p*-value was assessed by Monte Carlo method as the possible permutations were scarce (Anderson, 2014).

Furthermore, to determine the effect of substrates on species colonization according to their status, two-way ANOVA was conducted for the abundance of each status, with substrate and status of introduction as fixed factors with two (control vs carbonated) and three (native vs non-indigenous vs cryptogenic) levels, respectively. These abundances were obtained by adding the abundance of every taxon belonging to each status together in each treatment (Table 2). Similarly, to see the potential of the electrolytic carbonated treatment as a biofilter material, all filter feeders belonging to Mollusca, Ascidiacea, and Porifera were added together as they are the ones with highest filtration capacity (hereafter active filter feeders) and were tested with one-way ANOVA, with substrate as a fixed factor (control vs treatment).

Prior to perform ANOVA normality and homoscedasticity of the data were checked.

Biotic parameters, PERMANOVA, and SIMPER were conducted in PRIMERv6 + PERMANOVA software (Anderson et al., 2008), whereas ANOVA was conducted in R 4.1.2 (R Core Team, 2021).

3. Results

3.1. Taxonomic overview

After three months (October 2019 to January 2020), 32 different taxa (27 to species level) were identified, of which 30 (93.8 %) appeared in carbonated meshes, and 15 (46.9 %) were in the control ones (Table 2). Bryozoa were the most diverse, while Hydrozoa were the most abundant. All taxa were more diverse and abundant in the carbonated treatment than in the steel, except for hydrozoans, which were slightly more abundant over the steel meshes (Fig. 2). Nonetheless, the abundance and species richness of different taxa had the same trend in both substrates, being Hydrozoa and Serpulidae the most abundant taxa, and Bryozoa, Mollusca, and Hydrozoa the ones with higher species richness.

Regarding species (Table 2), *Hydroides elegans* and *Obelia dichotoma* were most abundant, while *Bugulina fulva* and *Conopeum seurati* only appeared in the control treatment. Moreover, 17 species were recruited only in carbonated meshes, of which *Watersipora subtorquata* and *Hydroides dirampha* were NIS, while *Bugula neritina*, *Ciona intestinalis*, *Diplosoma listerianum*, and *Styela canopus* were cryptogenic. All Ascidiacea and Porifera species and three Bivalvia species only appeared in the carbonated treatment.

As shown in Table 2, there were 16 native species (59.3 %), 4 NIS (14.8 %), and 7 (25.9 %) cryptogenic. Regarding the differences between the two substrates (Fig. 3a), native species were significatively more abundant ($P < 0.001$; $F_{2,12} = 20.916$) than NIS and cryptogenic ones; and all of them were more abundant in the carbonated treatment ($P < 0.05$; $F_{1,12} = 5.024$).

The abundance of active filter feeders was significantly higher in the carbonated treatment ($P < 0.01$, $F_{1,4} = 22.14$). The mean abundance in the carbonated substrate was almost five times higher (Fig. 3b), and Porifera and Ascidiacea only appeared in that substrate (Table 2).

3.2. Biotic parameters

All biotic parameters were statistically higher in the carbonated substrate (S: $P < 0.01$, $F_{1,4} = 32.33$; H′: $P < 0.01$, $F_{1,4} = 57.8$; J′: $P < 0.01$, $F_{1,4} = 24.1$), except for the abundance, which was marginally significant ($P = 0.0571$, $F_{1,4} = 7.009$) (Fig. 4). In fact, species richness and Shannon-Wiener indexes stood out, as their mean values were three and two times higher for the carbonated treatment, respectively. The dispersion of values was also higher for the steel substrate, particularly the abundance.

3.3. Biofouling assemblages

The effect of substrate was statistically significant for the abundance of biofouling assemblages (PERMANOVA, pseudo-F = 4.5704, p(MC)-value $P < 0.05$). These differences (±70 %) were caused by 14 species (Table 3). However, only 9 species showed significant differences for the substrate, being *Balanus trigonus*, *Perforatus perforatus*, and *Filicrisa geniculata* typical species on the carbonated treatment; these species explained the 22 % of the total differences. Moreover, the dispersion

Table 2
Average of all recorded species for each substrate and their status of introduction. Abundance is measured in organisms/cm^2 for both solitary and colonial organisms.

Taxa	Status of introduction	Steel	Carbonated
Calcarea			
Leucetta solida (Schmidt, 1862)	Native	0.00 ± 0.00	0.01 ± 0.01
Hydrozoa			
Obelia dichotoma (Linnaeus, 1758)	Native	2.86 ± 1.48	2.91 ± 0.08
Clytia hemisphaerica (Linnaeus, 1767)	Native	0.33 ± 0.32	0.33 ± 0.32
Bougainvillia muscus (Allman, 1863)	Native	0.43 ± 0.75	0.25 ± 0.29
Sabellida			
Hydroides dirampha Mörch, 1863	NIS	0.00 ± 0.00	0.01 ± 0.01
Hydroides elegans (Haswell, 1883)	NIS	0.48 ± 0.35	1.67 ± 0.76
Parasabella langerhansi (KnightJones, 1983)	Native	0.00 ± 0.00	0.02 ± 0.02
Spirobranchus triqueter (Linnaeus, 1758)	Native	0.00 ± 0.00	0.03 ± 0.02
Cirripedia			
Amphibalanus amphitrite (Darwin, 1854)	Cryptogenic	0.04 ± 0.02	0.27 ± 0.11
Balanus trigonus Darwin, 1854	NIS	0.01 ± 0.01	0.58 ± 0.17
Perforatus perforatus (Bruguière, 1789)	Native	0.01 ± 0.01	0.28 ± 0.16
Bivalvia			
Anomia ephippium Linnaeus, 1758	Native	0.00 ± 0.00	0.01 ± 0.01
Hiatella arctica (Linnaeus, 1767)	Native	0.05 ± 0.09	0.12 ± 0.05
Mytilidae sp.	–	0.01 ± 0.01	0.21 ± 0.06
Veneridae sp.	–	0.00 ± 0.00	0.03 ± 0.03
Ostreidae sp.	–	0.00 ± 0.00	0.02 ± 0.03
Cardiidae sp.	–	0.01 ± 0.01	0.05 ± 0.03
Bryozoa			
Bugula neritina (Linnaeus, 1758)	Cryptogenic	0.00 ± 0.00	0.01 ± 0.01
Bugulina calathus (Norman, 1868)	Native	0.00 ± 0.00	0.04 ± 0.05
Bugulina flabellata (Thompson in Gray, 1848)	Native	0.00 ± 0.00	0.01 ± 0.02
Bugulina fulva (Ryland, 1960)	Cryptogenic	0.02 ± 0.02	0.00 ± 0.00
Conopeum seurati (Canu, 1928)	Native	0.01 ± 0.02	0.00 ± 0.00
Cradoscrupocellaria bertholletii (Audoiun, 1826)	Native	0.00 ± 0.00	0.04 ± 0.05
Cryptosula pallasiana (Moll, 1803)	Native	0.00 ± 0.00	0.01 ± 0.01
Filicrisia geniculata (Milne Edwards, 1838)	Native	0.01 ± 0.01	0.80 ± 0.38
Schizoporella errata (Waters, 1878)	Cryptogenic	0.03 ± 0.05	0.31 ± 0.11
Turbicellepora magnicostata (Barroso, 1919)	Native	0.01 ± 0.01	0.07 ± 0.01
Watersipora subtorquata (d'Orbigny, 1852)	NIS	0.00 ± 0.00	0.02 ± 0.02
Ascidiacea			
Ciona intestinalis (Linnaeus, 1767)	Cryptogenic	0.00 ± 0.00	0.01 ± 0.01
Diplosoma listerianum (Milne Edwards, 1841)	Cryptogenic	0.00 ± 0.00	0.03 ± 0.05
Polyclinidae sp.	–	0.00 ± 0.00	0.01 ± 0.02
Styela canopus (Savigny, 1816)	Cryptogenic	0.00 ± 0.00	0.01 ± 0.02

effect was not significant (PERMDISP > 0.05) and the dissimilarity among substrates was 51 %, but the similarity within treatments was higher in the carbonated one (78.18 % vs 56.67 %).

4. Discussion

The carbonated substrate had higher species richness, Pielou's evenness, and Shannon-Wiener diversity. These results agree with other studies in which substrates similar to electrolytic carbonated ones (e.g., concrete and natural rock) had higher biological diversity values than steel (Brown, 2005; Ushiama et al., 2016; Albano and Obenat, 2019). In addition, although no significant differences were found for species abundance, the results are similar to the studies of Neves et al. (2007) and Albano and Obenat (2019), in which calcareous substrates (concrete) had a higher abundance of organisms than other substrate types because concrete surfaces are similar to the natural rocks. Moreover, this also agrees with the studies of Anderson and Underwood (1994) and Ushiama et al. (2016), which showed that the steel had a lower recruitment rate than the concrete. Lin and Shao (2002) used different steels as recruitment substrates, highlighting the cathodically protected steel, which was based in the same method as our carbonated material, but the steel they used was not coated with a calcareous layer. However, there were no significant differences in species richness between their substrates. Hence, this suggests that cathodically protected steel is not enough to enhance the recruitment as the electrolytic carbonated substrate does. Moreover, regarding the species composition of each substrate assemblages, differences were found also among them. This fact agrees with other studies (Brown, 2005; Lezzi and Giangrande, 2018; Lezzi et al., 2018; Sempere-Valverde et al., 2018) in which these differences in biofouling assemblage composition appeared during the first three months of immersion. As was stated in the introduction, the development of epifaunal communities is regulated by different and complex factors, so these differences between the materials are difficult to elucidate. However, it is known that colonization process starts with the formation of a biofilm, which is a biological film made by small organisms such as diatoms, bacteria, and other microalgae (Jenkins and Martins, 2010). In this regard, roughness of the substrates appears to be an important factor that increases the colonization process of the materials for this biofilm organism (Hayek et al., 2022) and, therefore, for the following macrofouling organisms. In addition, the first stage of colonization is dependent on larval availability, substrate preferences, and inter- and intraspecific interactions (Osman, 1977). Thus, the differences found in this study, can be caused by the roughness of the material together with the physicochemical composition of it, which are the mainly differences among them. However, more specific studies are required in order to ascertain which is or are the real causes of the differences as the species composition of each assemblage are the result of a complex interaction between the substrate properties and species specific settlement criteria and propagule availability (Brown, 2005). Moreover, as a consequence of ecological succession, this difference in biofouling assemblages among substrates could disappear, as it is shown in Bulleri (2005) after 24 months and Obaza and Williams (2018), or they may be maintained, reaching different endpoint communities

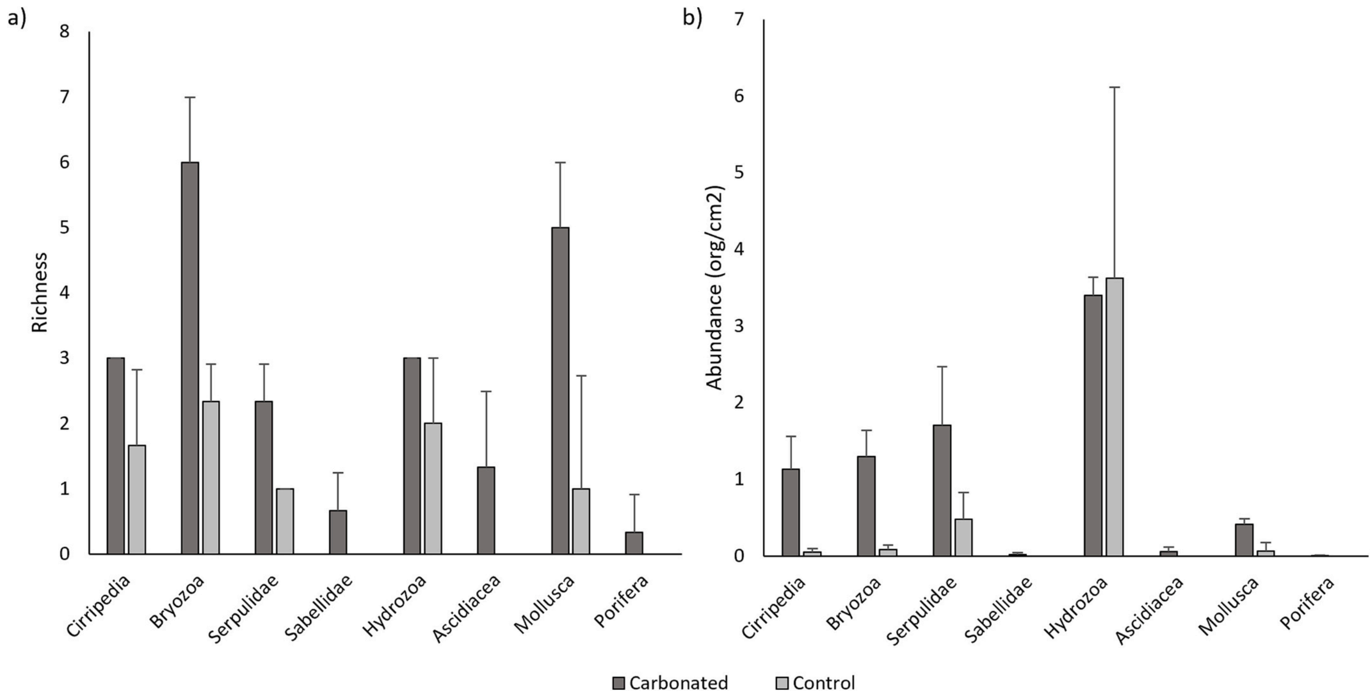

Fig. 2. Graphical representation of: a) mean species richness; and b) mean abundance of each taxa in the two substrates. Bars: standard error.

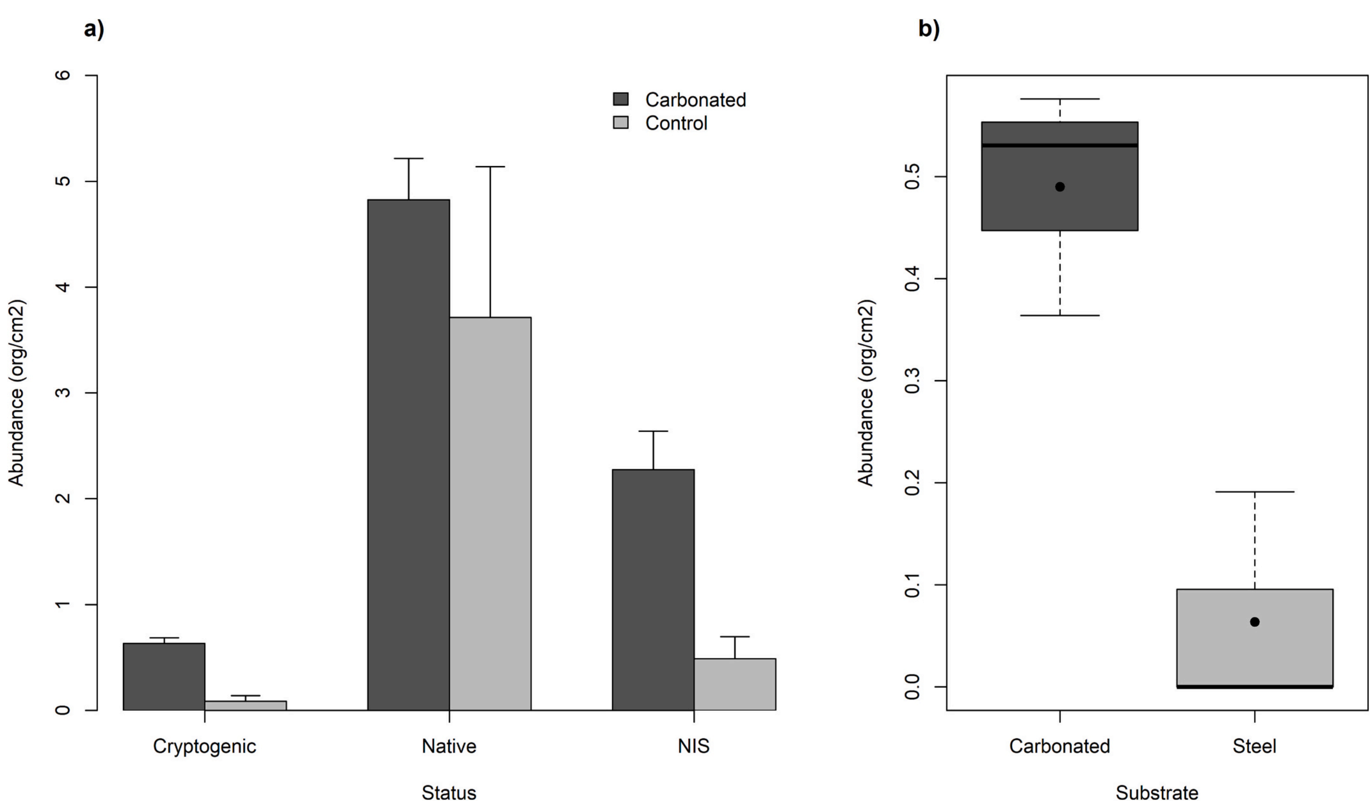

Fig. 3. Abundance of organisms in different substrates: a) according to their status; b) active filter feeders. Bars: standard error.

(Lezzi and Giangrande, 2018). Hence, it is unclear which path will these early colonizers follow during the succession process, and more immersion time is required to elucidate it.

All active filter feeder taxa appeared on the carbonate substrate with greater abundance than on steel. Among them, Ascidiacea and Porifera stand out as they have high filtration rates (e.g. *Styela* spp., Fiala-Médioni, 1978; Stuart and Klumpp, 1984; Draughon et al., 2010). Therefore, the fact that the carbonated substrate could be able to recruit this type of organisms to greater extent gives it a high potential as a material for the construction of biofilters.

Referring to typical species, cirripedes were a characteristic of the electrolytic carbonated substrate in biofouling assemblages. This

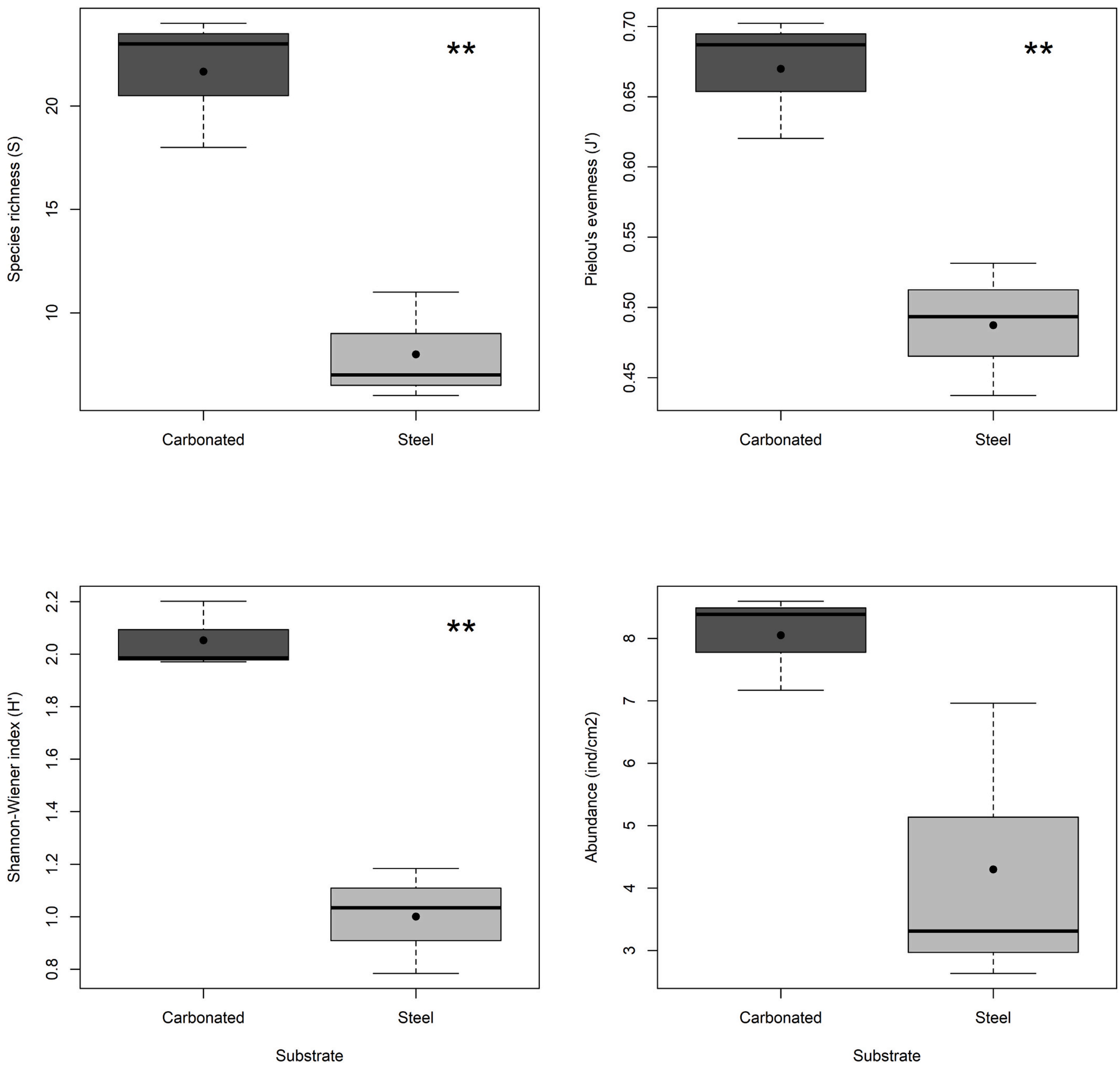

Fig. 4. Boxplot on different diversity indexes measured. Bars: standard error.

matches with Eashwar et al. (1985, 1995) and Ushiama et al. (2016), who concluded that calcareous organisms, principally cirripedes, were more abundant in cathodically protected metals and calcareous materials, respectively. Moreover, as cirripedes and many other hard-bodied organisms were more abundant on the carbonated treatment, this will result in a larger colonization surface area as their bodies could also act as one. So that, these results indicate that these differences can be intensified over time as more substrate is being generated by this type of organisms.

More abundant and diverse NIS were observed on the carbonated substrate. This contrasts with other authors (Airoldi et al., 2015; Chase et al., 2016; Megina et al., 2016), who detected more NIS on steel over calcareous materials. The NIS here detected are the typical ones of port areas in the Mediterranean Sea highlighting *Balanus trigonus*, *Hydroides dirampha*, *H. elegans*, *Styela canopus* and *Watersipora subtorquata* (Airoldi et al., 2015; Ulman et al., 2017; Giangrande et al., 2020). However, this study showed an opposed trend in which NIS have a preference for the carbonated treatment, which it is supposed to act as a natural substrate. In addition, although the presence of NIS is higher in this substrate than in steel, we do not have to forget that native ones are more abundant too, so this recruitment and settlement trend of species, including the non-indigenous ones, is thanks to the similarity of this substrate to natural rock (Glasby et al., 2007; Neves et al., 2007; Siboni et al., 2009). In fact, this similarity to natural rock is also the cause of having a more diverse, stable, and complex biofouling community, as well as the reason of finding differences among substrates in the biofouling assemblages and why they are more similar within treatments.

Many artificial structures have been developed in the marine environment to address with the degradation they are suffering, being artificial reefs the most used ones as it stated in the introduction. In this regard, many studies used artificial reefs as a mitigation tool for aquaculture cages since biofouling organisms are mainly filter feeders,

Table 3
SIMPER results for the abundance of biofouling assemblages (fourth root transformed data).

Species	Average abundance (steel)	Average abundance (carbonated)	%	Cummulative %	PERMANOVA
B. trigonus	0.13	0.87	8.17	8.17	***
F. geniculata	0.25	0.93	7.40	15.57	***
P. perforatus	0.13	0.71	6.46	22.03	*
B. ramosa	0.36	0.65	6.25	28.28	0.48
S. errata	0.18	0.74	6.24	34.52	*
M. galloprovincialis	0.13	0.67	5.98	40.51	*
S. triqueter	0.00	0.41	4.49	45.00	***
H. arctica	0.21	0.58	4.47	49.47	0.15
T. magnicostata	0.13	0.52	4.29	53.75	*
B. calathus	0.00	0.32	3.62	57.37	0.12
H. elegans	0.80	1.12	3.54	60.91	*
C. hemisphaerica	0.56	0.70	3.43	64.33	0.64
C. bertholletii	0.00	0.32	3.39	67.72	0.12
A. amphitrite	0.42	0.72	3.21	70.93	***

Note: (*): $p < 0.05$; (***): $p < 0.001$.

allowing the reduction of organic matter in the system (Angel et al., 2002; Gao et al., 2008; Aguado-Giménez et al., 2011). The main material used in artificial reefs construction is concrete, as many others have negative impacts on marine habitats, although concrete is not an exception (Müllauer et al., 2015; Dennis et al., 2018; McManus et al., 2018). As it has been seen, the electrolytic carbonated substrate not only has a higher recruitment of filter-feeder organisms, but also the ones we define as active filters. In addition, it has a diverse and developed community after 3 months of immersion and it recruits more hard bodied organisms which leads to an increase of available space for other species. Furthermore, contrary to concrete, this substrate is lighter and easier to handle, so using electrolytic carbonated substrates will make the operational process easier and reduce the weight of reefs (Bohnsack and Sutherland, 1985; Baine, 2001; Burt et al., 2009), as well as it makes the process of transport and settlement cheaper. Moreover, unlike concrete, the electrolytic carbonated substrates do not have any negative impact on the marine environment as they do not release any substances from them. Taking all said into account, the potential of this material for the construction of artificial reefs for restoration of hard bottom communities and for their use as mitigation tool of aquaculture wastes and wastewater discharges is huge. In fact, as this substrate is formed in a mesh, the water circulation is higher than in other types of structures, so it will possibly enhance the filtration capacity of biofouling community settle in this material.

To conclude, during the first stages of colonization, the electrolytically carbonated substrate had higher: i) abundances (for all types of organisms and active filter feeders); ii) diversity indexes (H′ and J′) and species richness; and iii) structuration and development of biofouling assemblages. Thus, the higher diversity indexes in the carbonated substrates at the same period could indicate that this substrate needs less time than steel to have a more structured and developed community. Furthermore, being able to recruit more hard bodied organisms such as cirripedes, bivalves and solitary ascidians, will potentially increase the settlement area for new organisms, allowing the recruitment of more filter feeders which would act as biofilters. However, as it has been said, further studies and more immersion time is needed in order to clarify how the succession process will continue and if these differences among substrates and trends will sustain in time.

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CRediT authorship contribution statement

Alejandro Carmona-Rodríguez: Formal analysis, Investigation, Writing – original draft. **Carlos Antón:** Methodology, Investigation, Writing – review & editing. **Miguel Ángel Climent:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Pedro Garcés:** Methodology, Investigation, Writing – review & editing. **Vicente Montiel:** Methodology, Investigation, Writing – review & editing. **Alfonso A. Ramos-Esplá:** Conceptualization, Methodology, Investigation, 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.

Data availability

Data will be made available on request.

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References

Aguado-Giménez, F., Piedecausa, M.A., Carrasco, C., Gutiérrez, J.M., Aliaga, V., y García-García, B., 2011. Do benthic biofilters contribute to sustainability and restoration of the benthic environment impacted by offshore cage finfish aquaculture? Mar. Pollut. Bull. 62 (8), 1714–1724.

Airoldi, L., Turon, X., Perkol-Finkel, S., Rius, M., 2015. Corridors for aliens but not for natives: effects of marine urban sprawl at a regional scale. Divers. Distrib. 21 (7), 755–768.

Albano, M.J., Obenat, S.M., 2019. Fouling assemblages of native, non-indigenous and cryptogenic species on artificial structures, depths and temporal variation. J. Sea Res. 144, 1–15.

Anderson, M.J., 2005. Permutational Multivariate Analysis of Variance, vol. 26. Department of Statistics, University of Auckland, Auckland, pp. 32–46.

Anderson, M.J., 2014. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley statsref: statistics reference online, pp. 1–15.

Anderson, M.J., Underwood, A.J., 1994. Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage. J. Exp. Mar. Biol. Ecol. 184 (2), 217–236.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK (244 pp).

Angel, D.L., Eden, N., Breitstein, S., Yurman, A., Katz, T., Spanier, E., 2002. In situ biofiltration: a means to limit the dispersal of effluents from marine finfish cage aquaculture. Hydrobiologia 469 (1), 1–10.

Baine, M., 2001. Artificial reefs: a review of their design, application, management and performance. Ocean Coast. Manag. 44 (3–4), 241–259.
Bakti, L.A.A., Virgota, A., Damayanti, L.P.A., Radiman, T.H.U., Retnowulan, A., Sabil, A., Robbe, D., 2012. Biorock reef restoration in Gili Trawangan, North Lombok, Indonesia. In: Goreau, T.J., Trench, R.K. (Eds.), Innovative Methods of Marine Ecosystem Restoration, vol. 6. CRC Press, pp. 59–80.
Berntsson, K.M., Jonsson, P.R., 2003. Temporal and spatial patterns in recruitment and succession of a temperate marine fouling assemblage: a comparison of static panels and boat hulls during the boating season. Biofouling 19 (3), 187–195.
Bohnsack, J.A., Sutherland, D.L., 1985. Artificial reef research: a review with recommendations for future priorities. Bull. Mar. Sci. 37 (1), 11–39.
Brown, C.J., 2005. Epifaunal colonization of the Loch Linnhe artificial reef: influence of substratum on epifaunal assemblage structure. Biofouling 21 (2), 73–85.
Bulleri, F., 2005. Role of recruitment in causing differences between intertidal assemblages on seawalls and rocky shores. Mar. Ecol. Prog. Ser. 287, 53–65.
Bulleri, F., Chapman, M.G., 2010. The introduction of coastal infrastructure as a driver of change in marine environments. J. Appl. Ecol. 47 (1), 26–35.
Bulleri, F., Chapman, M.G., Underwood, A.J., 2005. Intertidal assemblages on seawalls and vertical rocky shores in Sydney Harbour, Australia. Austral. Ecol. 30 (6), 655–667.
Burt, J., Bartholomew, A., Bauman, A., Saif, A., Sale, P.F., 2009. Coral recruitment and early benthic community development on several materials used in the construction of artificial reefs and breakwaters. J. Exp. Mar. Biol. Ecol. 373 (1), 72–78.
Casoli, E., Ricci, S., Belluscio, A., Gravina, M.F., Ardizzone, G., 2014. Settlement and colonization of epi-endobenthic communities on calcareous substrata in an underwater archaeological site. Mar. Ecol. 36, 1060–1074.
Chase, A.L., Dijkstra, J.A., Harris, L.G., 2016. The influence of substrate material on ascidian larval settlement. Mar. Pollut. Bull. 106 (1–2), 35–42.
Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd edition. PRIMER-E, Plymouth.
Cox, G.C. (1940). Anticorrosive and antifouling coating and method of application. U.S. Patent No. 2,200,469. Washington, DC: U.S. Patent and Trademark Office.
Dafforn, K.A., Johnston, E.L., Glasby, T.M., 2009. Shallow moving structures promote marine invader dominance. Biofouling 25 (3), 277–287.
Dennis, H.D., Evans, A.J., Bannera, A.J., Pippa, J., Moore, P.J., 2018. Reefcrete: reducing the environmental footprint of concretes foreco-engineering marine structures. Ecol. Eng. 120, 668–678.
Draughon, L.D., Scarpa, J., Hartmann, J.X., 2010. Are filtration rates for the rough tunicate *Styela plicata* independent of weight or size? J. Environ. Sci. Health Part A 45 (2), 168–176.
Eashwar, M., Ananth, V., Paulraj, S., Subramanian, G., 1985. Biofouling studies relating to cathodic protection of some metals in seawater. Bull. Electrochem. 1 (1), 19–21.
Eashwar, M., Subramanian, G., Chandrasekaran, P., Manickam, S.T., Maruthamuthu, S., Balakrishnan, K., 1995. The interrelation of cathodic protection and marine macrofouling. Biofouling 8 (4), 303–312.
Eickhoff, A.J., Shaw, W.E., 1948. Principles of protecting metals with organic coatings. Corrosion 4 (10), 463–475.
Fiala-Médioni, A., 1978. Filter-feeding ethology of benthic invertebrates (ascidians). IV. Pumping rate, filtration rate, filtration efficiency. Mar. Biol. 48, 243–249.
Fitri, D., Rachman, M.A., 2012. Gorgonian soft corals have higher growth and survival in electrical fields. In: Goreau, T.J., Trench, R.K. (Eds.), Innovative Methods of Marine Ecosystem Restoration, vol. 9. CRC Press, Boca Raton, pp. 105–111.
Gao, Q.F., Shin, P.K., Xu, W.Z., Cheung, S.G., 2008. Amelioration of marine farming impact on the benthic environment using artificial reefs as biofilters. Mar. Pollut. Bull. 57 (6–12), 652–661.
Giangrande, A., Lezzi, M., Del Pasqua, M., Pierri, C., Longo, C., Gravina, M.F., 2020. Two cases study of fouling colonization patterns in the Mediterranean Sea in the perspective of integrated aquaculture systems. Aquacult. Rep. 18, 100455.
Glasby, T.M., Connell, S.D., Holloway, M.G., Hewitt, C.L., 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? Mar. Biol. 151 (3), 887–895.
Goreau, T.J., 2012. Marine electrolysis for building materials and environmental restoration. In: Linkov, V., Kleperis, J. (Eds.), Electrolysis, vol. 13, pp. 273–290.
Goreau, T.J., 2014. Electrical stimulation greatly increases settlement, growth, survival, and stress resistance of marine organisms. Nat. Resour. 5 (10), 527.
Goreau, T.J., Hilbertz, W., 2012. Reef restoration using seawater electrolysis in Jamaica. In: Goreau, T.J., Trench, R.K. (Eds.), Innovative Methods of Marine Ecosystem Restoration, vol. 4. CRC Press, Boca Raton, pp. 35–45.
Hartt, W.H., Culberson, C.H., Smith, S.W., 1984. Calcareous deposits on metal surfaces in seawater. A critical review. Corrosion 40 (11), 609–618.
Hayek, M., Salgues, M., Bayle, S., Souche, J.C., de Weerdt, K., Pioch, S., 2022. Influent Parameters on the Early Biocolonization of Cementitous Materials in Seawater (14th Ecocity World Summit 2022, Feb 2022, En ligne, France).
Hilbertz, W., 1979. Electrodeposition of minerals in sea water: experiments and applications. IEEE J. Ocean. Eng. 4 (3), 94–113.
Hilbertz, W. H. & Goreau, T. J. (1996). Method of enhancing the growth of aquatic organisms, and structures created thereby. U.S. Patent No. 5,543,034. Washington, DC: U.S. Patent and Trademark Office.
Jenkins, S.R., Martins, G.M., 2010. Succession on hard substrata. In: Simone, D., Jeremy, C.T. (Eds.), Biofouling, vol. 4. Blackwell Publishing Ltd, pp. 60–72.
Karissa, P.T., Sukardi, Priyono, S.B., Mamangkey, G.F., Taylor, J.J.U., 2012. Utilization of low-voltage electricity to stimulate cultivation of pearl oysters Pinctada maxima (Jameson). In: Goreau, T.J., Trench, R.K. (Eds.), Innovative Methods of Marine Ecosystem Restoration, vol. 11. CRC Press, Boca Raton, pp. 131–139.
Lezzi, M., Giangrande, A., 2018. Seasonal and bathymetric effects on macrofouling invertebrates' primary succession in a mediterranean non-indigenous species hotspot area. Mediterr. Mar. Sci. 19 (3), 572–588.
Lezzi, M., Del Pasqua, M., Pierri, C., Giangrande, A., 2018. Seasonal non-indigenous species succession in a marine macrofouling invertebrate community. Biol. Invasions 20 (4), 937–961.
Lin, H.J., Shao, K.T., 2002. The development of subtidal fouling assemblages on artificial structures in Keelung Harbor, northern Taiwan. Zool. Stud. Taipei 41 (2), 170–182.
McManus, R.S., Archibald, N., Comber, S., Knights, A.M., Thompson, R.C., Firth, L.B., 2018. Partial replacement of cement for waste aggregates in concrete coastaland marine infrastructure: a foundation for ecological enhancement? Ecol. Eng. 120, 655–667.
Megina, C., González-Duarte, M.M., López-González, P.J., 2016. Benthic assemblages, biodiversity and invasiveness in marinas and commercial harbours: an investigation using a bioindicator group. Biofouling 32 (4), 465–475.
Müllauer, W., Beddoe, R.E., Heinz, D., 2015. Leaching behavior of major trace elements from concrete: effect of fly ash and GGBS. Cement Concrete Compos. 58, 129–139.
Muthukrishnan, T., Hassenrück, C., Al Fahdi, D., Jose, L., Al Senafi, F., Mahmoud, H., Abed, R.M., 2022. Monthly succession of biofouling communities and corresponding inter-taxa associations in the north-and south-west of the Arabian Gulf. Front. Mar. Sci. 8, 787879 https://doi.org/10.3389/fmars.2021.787879.
Neves, C.S., Rocha, R.M., Pitombo, F.B., Roper, J.J., 2007. Use of artificial substrata by introduced and cryptogenic marine species in Paranaguá Bay, southern Brazil. Biofouling 23 (5), 319–330.
Obaza, A.K., Williams, J.P., 2018. Spatial and temporal dynamics of the overwater structure fouling community in southern California. Mar. Freshw. Res. 69 (11), 1771–1783.
Osman, R.W., 1977. The establishment and development of a marine epifaunal community. Ecol. Monogr. 47 (1), 37–63.
Pierri, C., Longo, C., Giangrande, A., 2010. Variability of fouling communities in the Mar Piccolo of Taranto (Northern Ionian Sea, Mediterranean Sea). J. Mar. Biol. Assoc. U. K. 90 (1), 159–167.
R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/. URL.
Rivero, N.K., Dafforn, K.A., Coleman, M.A., Johnston, E.L., 2013. Environmental and ecological changes associated with a marina. Biofouling 29 (7), 803–815.
Sempere-Valverde, J., Ostalé-Valriberas, E., Farfán, G.M., Espinosa, F., 2018. Substratum type affects recruitment and development of marine assemblages over artificial substrata: a case study in the Alboran Sea. Estuar. Coast. Shelf Sci. 204, 56–65.
Siboni, N., Martinez, S., Abelson, A., Sivan, A., Kushmaro, A., 2009. Conditioning film and initial biofilm formation on electrochemical $CaCO_3$ deposition on a metallic net in the marine environment. Biofouling 25 (7), 675–683.
Stuart, V., Klumpp, D.W., 1984. Evidence for food-resource partitioning by kelp-bed filter feeders. Mar. Ecol. Progress Ser. Oldendorf 16 (1), 27–37.
Trinidad, C.B., Valenzuela, R.L.G., Ocampo, M.A.B., Vallejo Jr., B.M., 2019. Macrofouler community succession in South Harbor, Manila Bay, Luzon Island, Philippines during the northeast monsoon season of 2017–2018. Philippine J. Sci. 148 (3), 441–456.
Tyrrell, M.C., Byers, J.E., 2007. Do artificial substrates favor nonindigenous fouling species over native species? J. Exp. Mar. Biol. Ecol. 342 (1), 54–60.
Ulman, A., Ferrario, J., Occhpinti-Ambrogi, A., Arvanitidis, C., Bandi, A., Bertolino, M., Bogi, C., Chatzigeorgiou, G., Çiçek, B.A., Deidun, A., Ramos-Espla, A.A., Koçak, C., Lorenti, M., Martinez-Laiz, G., Merlo, G., Princisgh, E., Scribano, G., Marchini, A., 2017. A massive update of non-indigenous species records in Mediterranean marinas. PeerJ 5, e3954.
Ushiama, S., Smith, J.A., Suthers, I.M., Lowry, M., Johnston, E.L., 2016. The effects of substratum material and surface orientation on the developing epibenthic community on a designed artificial reef. Biofouling 32 (9), 1049–1060.
Vaz-Pinto, F., Torrontegi, O., Prestes, A.C.L., Álvaro, N.V., Neto, A.I., Martins, G.M., 2014. Invasion success and development of benthic assemblages: effect of timing, duration of submersion and substrate type. Mar. Environ. Res. 94, 72–79.
Watson, D.I., Barnes, D.K.A., 2004. Temporal and spatial components of variability in benthic recruitment, a 5-year temperate example. Mar. Biol. 145 (1), 201–214.
Zenetos, A., Gofas, S., Verlaque, M., Çinar, M.E., Raso, J.G., Bianchi, C.N., Morri, C., Azzurro, E., Bilecenoglu, M., Froglia, C., Siokou, I., Violanti, D., Sfriso, A., San Martín, G., Giangrande, A., Katagan, T., Ballesteros, E., Ramos-Esplá, A.A., Mastrototaro, F., Ocaña, O., Zingone, A., Gambi, M.C., Streftaris, N., 2010. Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. Mediterranean Mar. Sci. 11 (2), 381–493.
Zenetos, A., Gofas, S., Morri, C., Rosso, A., Violanti, D., García Raso, J.E., Çinar, M.E., Almogi-Labin, A., Ates, A.S., Azzurro, E., Ballesteros, E., Bianchi, C.N., Bilecenoglu, M., Gambi, M.C., Giangrande, A., Gravili, C., Hyams-Kaphzan, O., Karachle, P.K., Katsanevakis, S., Lipej, L., Mastrototaro, F., Mineur, F., Pancucci-Papadopoulou, M.A., Ramos Esplá, A.A., Salas, C., San Martín, G., Sfriso, A., Streftaris, N., Verlaque, M., 2012. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. Mediterr. Mar. Sci. 13 (2), 328–352.
Zenetos, A., Çinar, M.A., Crocetta, F., Golani, D., Rosso, A., Servello, G., Shenkar, N., Turon, X., Verlaque, M., 2017. Uncertainties and validation of alien species catalogues: the Mediterranean as an example. Estuar. Coast. Shelf Sci. 191, 171–187.